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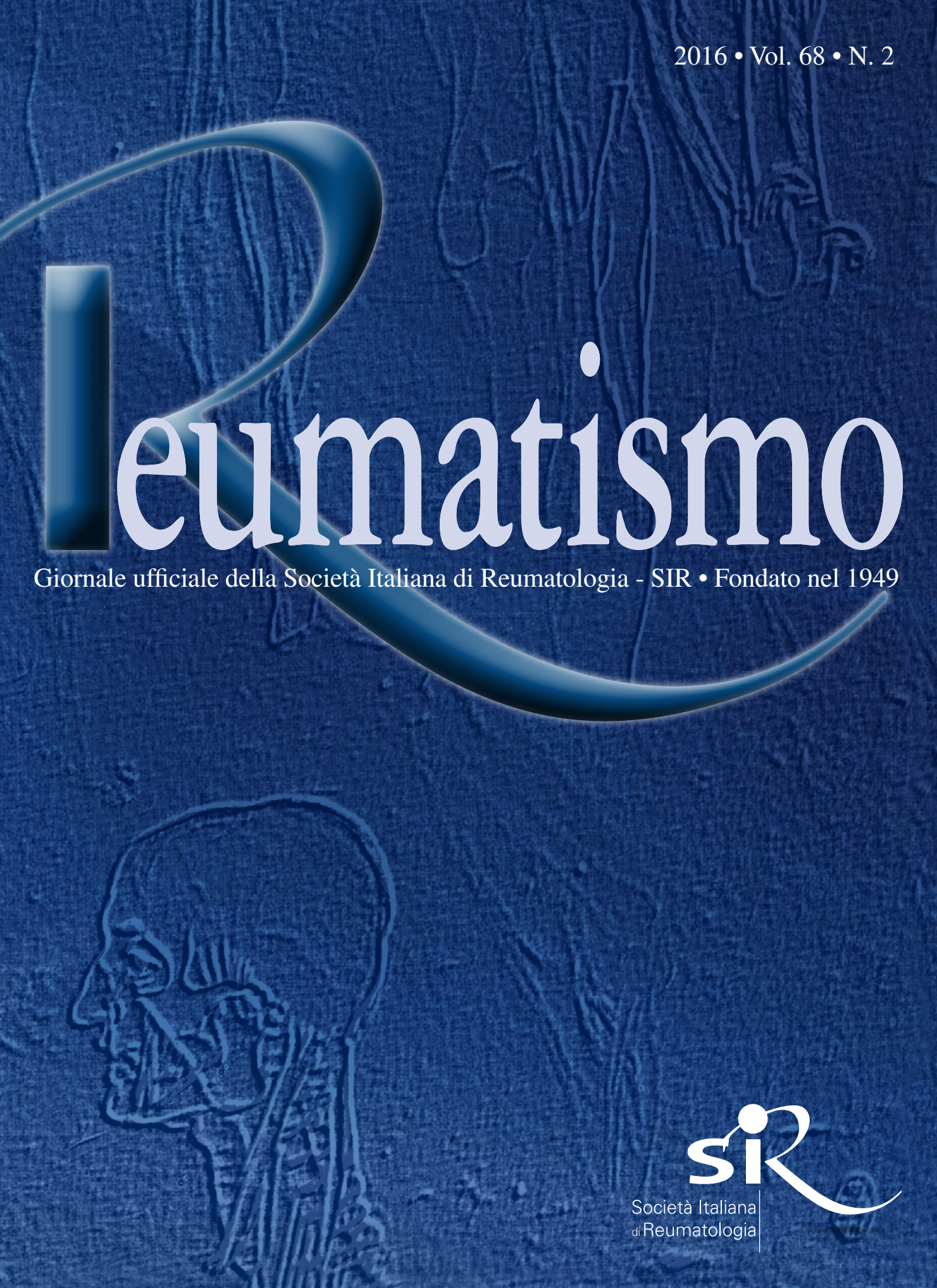

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New approved drugs for psoriatic arthritis

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SUMMARY

Psoriatic arthritis (PsA) is a chronic inflammatory disease that possibly leads to structural damage and to a reduction of joint function and poor quality of life. Treatment of PsA has changed since its introduction of anti-TNF drugs, which have shown to reduce the symptoms and signs of the disease and slow the radiographic progression. However, recently, the discovery of new pathogenic mechanisms have made possible the development of new molecules that target pro-inflammatory cytokines involved in skin, joint and entheseal inflammation. New drugs like ustekinumab, secukinumab and apremilast inhibit interleukin axis and intracellular pathways and showed their efficacy and safety in randomized clinical trials. These drugs have been recently approved for the treatment of PsA and included in the new EULAR and GRAPPA treatment recommendations. The aim of this paper is to briefly review the clinical trials that led to their approval for PsA.

Key words: *Psoriatic arthritis; treatment; ustekinumab; secukinumab; apremilast.*

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INTRODUCTION

Psoriatic arthritis (PsA) is a chronic inflammatory disease characterized by the association of musculoskeletal involvement and psoriasis with a variable clinical course (1) and potentially associated to functional disability and poor quality of life (1, 2). The introduction of tumor necrosis factor (TNF) inhibitors dramatically changed the outcome of PsA patients.

Data coming from over ten years of experiences with randomized clinical trials and observational studies showed the efficacy of anti-TNF in all PsA domains (peripheral arthritis, axial involvement, enthesitis, dactylitis and extra-articular manifestations) and in reduction of radiographic progression (3, 4).

These agents proved to have significantly better responses than placebo, with American College of Rheumatology (ACR) 20 improvement criteria of 51-59% for TNF inhibitors *vs* 9-24.3% for placebo over 12-24 weeks of treatment (5). Clinical and laboratory indices showed similar favorable outcomes for all of anti-TNF drugs: in two indirect comparison meta-analyses, adalimumab, etanercept, golimumab, and infliximab, showed no important differences in the effectiveness and safety (6, 7). In this scenario, despite improved therapeutic benefits with TNF inhibitors, an unmet need remains the disease control in patients who are non-responders. In recent years, the understanding of the immunologic processes in the pathogenesis of disease led to the development of new terapies for PsA, based on the discovered cell pathways and cytokines involved. T-helper (Th) cells producing interleukin (IL)-17 (Th17 cells), seem to play a pivotal role in chronic inflammatory conditions and are stimulated by IL-23, which is highly expressed in psoriatic plaques, synovium and enthesis. Furthermore, other molecules such as phosphodiesterase (PDE) 4, seem to have a relevant role in the activation of immune cells and in the cytokines production. Blocking these cytokines and cellular pathways is now possible using biotechnological drugs and small molecules that were recently approved for the treatment of PsA. The aim of this paper is to briefly review the new drugs for the treatment of PsA.

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BLOCKING INTERLUKIN-12/23 AND INTERLUKIN-17 AXIS: USTEKINUMAB AND SECUKINUMAB

IL-12 is a heterodimer formed by a 35-kDa light chain (p35) and a 40-kDa heavy chain (p40). The two-receptor chains for IL-12 (12Rβ1 and IL-12Rβ2) are expressed mainly by activated T cells and natural killer cells but also on other cell types, such as dendritic cells (DCs) and B-cell lines. Similar to other pro-inflammatory cytokines, the production of IL-12 is regulated by different exogenous and endogenous stimuli: bacteria and material from microorganisms (including intracellular parasites, fungi, double-stranded RNA, bacterial DNA and CpG-containing oligonucleotides) are inducers of IL-12 production by macrophages, monocytes, neutrophils and DCs. These products engage Toll like receptor on phagocytes and DCs and thus lead to IL-12 production. IL-12 seems to play an important role in host innate response to bacteria, viruses and fungi and is responsible for the activation of Th1 response (8). The p40 heavy chain associates not only with IL-12 p35 to form IL-12, but also with another molecule, p19, to form the heterodimeric cytokine IL-23 (9). IL-23 binds to a receptor that is formed by IL-12Rβ1 and a new second chain, IL-23R. IL-12 and IL-23 play an important role in the pathogenesis of psoriasis and PsA: mutations in both IL-23 receptor and *IL-12* gene were associated with the susceptibility to psoriasis, inflammatory bowel disease and PsA, (10, 11) and, furthermore, IL-12 and IL-23 are essential for the induction and maintenance of the Th1/Th17 immune response, that are the two major phenotypes present in PsA and psoriasis (12). IL-23 activates Th17, which produces IL-17, a potent pro-inflammatory cytokine, activating DCs to produce IL-12, hence stimulating Th1. Moreover, IL-23 is essential for the proliferation and terminal differentiation of CD4+ Th17 T cells, maintaining IL-17 production, and ultimately driving the pathogenicity of these cells in multiple autoimmune models (12, 13). Recently, it has been shown that IL-23 is essential in enthesitis and acts on previously unidentified IL-23 receptor (IL-23R)+, on entheseal resident T cells, stimulating IL-17 expression and leading to specific IL-23 dependent inflammation in an animal model (14). IL-17 family includes six members (IL-17A-F) and there are several studies suggesting a role for IL-17A signaling in the pathogenesis of PsA. Polymorphisms associated with susceptibility to PsA are present in genetic loci involved in IL-17 signaling, such as IL-12B and TRAF3IP2. Levels of IL-17 receptor A (IL-17RA) and IL-17-positive T cells are elevated in synovial fluid and psoriatic plaques of patients with PsA. Of note, patients with spondyloarthritis, including PsA and ankylosing spondylitis, show higher levels of circulating Th17 cells in respect to rheumatoid arthritis patients (15). IL-17 has also been involved in both inflammation and bone remodeling in a murine model of spondyloarthritis: abundant in synovial fluids, IL-17 stimulated osteoclastogenesis in an osteoblast-dependent manner. Furthermore, IL-17 stimulated bone resorption in combination with TNF in fetal mouse long bones and induced the expression of the receptor activator of nuclear factor kappa-B ligand (the osteoclast differentiation factor) in osteoclast-supporting cells (16). In humans, IL-17 and TNF seem to be the two major cytokines involved in the structural damage of affected joints. On this basis, the inhibition of IL-12/23 and IL-17 axis proved to be effective in several autoimmune diseases, such as rheumatoid arthritis, psoriasis, multiple sclerosis and spondyloarthritis.

Ustekinumab

Ustekinumab is a fully human IgG1κ monoclonal antibody that binds to the common p40 subunit shared by IL-12 and IL-23, and it is the first non anti-TNF biologic approved for the treatment of PsA. Ustekinumab therapy rapidly decreased expression of a variety of pro-inflammatory cytokine codifying genes in psoriatic skin lesions including *p19*, *p40*, and *IL-17A* (17, 18). Ustekinumab demonstrated efficacy in the treatment of chronic plaque

psoriasis. Furthermore, ustekinumab 45 or 90 mg was superior to etanercept over a 12-week period in patients with psoriasis (19). In PsA, two-phase 3 studies (PSUMMIT 1 and 2) reported the efficacy and safety of ustekinumab in the treatment of all manifestations of the disease. In PSUMMIT 1, 615 naïve to anti-TNFα patients with active PsA were randomly assigned to placebo, 45 mg ustekinumab, and 90 mg ustekinumab. At week 24, a significantly higher proportion of patients in the ustekinumab groups than in the placebo group achieved an ACR20, ACR50 and ACR70 response (42.4, 24.9 and 12.2% respectively for ustekinumab 45 mg). Furthermore both ustekinumab dosages showed efficacy in improving quality of life [reduction of both health assessment questionnaire disability index (HAQ) and short form-36] in respect to placebo (20). In PSUMMIT 2 trial, patients with PsA previously exposed to TNF inhibitor were also enrolled. In this study more ustekinumab-treated patients (43.8% combined) than placebo-treated patients (20.2%) achieved ACR20 at week 24. ACR50 (P<0.05), HAQ improvement (P<0.001), and psoriasis area and severity index (PASI) 75 (P<0.01) also showed statistically significant differences. The extension study through week 52 showed that all benefits from ustekinumab were maintained. Of note, clinical responses tended to be lower among patients previously exposed to anti-TNF compared with anti-TNF-naïve patients (21). The numbers of patients with adverse events (including serious adverse events) and the types of events were similar across treatment groups in both studies and no deaths, opportunistic infections, cases of tuberculosis, or malignancies were reported (21). Ustekinumab treatment was generally safe and well tolerated in the two randomized studies with low number of injection site reactions. Recently, the analysis of the largest registry of ustekinumab-treated patients [the 2014 psoriasis longitudinal assessment and registry (PSOLAR)] on over 12,000 psoriasis patients identified no increased risk of malignancy, major adverse cardiovascular events, serious infection, or mortality (22). Ustekinumab significantly inhibits radiographic progression and joint damage in patients with active PsA: data coming from PSUMMIT 1 and 2 showed that, at week 24, significantly higher proportions of ustekinumab-treated (91.7%) than placebo-treated (83.8%; P=0.005) patients demonstrated no radiographic progression, as defined by change in total PsA-modified van der Heijde score from baseline (23). Clinical and radiographic benefits from ustekinumab treatment were maintained throughout 2 years of observation in patients enrolled in PSUMMIT 1 (24). Furthermore, ustekinumab treatment shows efficacy in all PsA clinical features. In PSUMMIT 1 there was a significant reduction in the number of patients with active enthesitis and dactylitis in respect to placebo and data also show a bath ankylosing spondylitis disease activity index 20, 50 e 70% result significantely higher in reducing the disease activity of patients with axial involvement (21).

Secukinumab

Secukinumab is a fully human IgG1κ monoclonal antibody that selectively binds to IL-17A cytokine and inhibits its interaction with the IL-17 receptor. Anti-IL-17A drug secukinumab showed to be superior to ustekinumab (CLEAR study) (25) and etanercept (FIXTURE study) (26) by PASI 90 and 75 response in patients with psoriasis, with a similar rate of adverse events. Anti-IL-17A drug secukinumab was also tested in two-phase 3, double-blind, placebo-controlled studies. In the FUTURE 2 study, adults (aged ≥18 years old) with active PsA were randomly allocated in a 1:1:1:1 ratio to receive subcutaneous placebo or secukinumab 300, 150, or 75 mg once a week from baseline and then every 4 weeks from week 4. A significantly higher proportion of patients achieved an ACR20 at week 24 with secukinumab 300 mg [54% of patients; odds ratio *vs* placebo 6.81, 95% confidence interval (CI)=3.42-13.56; P<0.0001], 150 mg (51% of patients; 6.52, 95% CI=3.25-13.08; P<0.0001), and 75 mg (29% of patients; 2.32, 95% CI=1.14-4.73; P=0.0399) *vs* placebo (15% of patients).

ACR50 was reached by 35% of patients in both secukinumab 300 and 150 mg groups and in 18% of patients in secukinumab 75 mg group at week 24. Up to week 16, the most common adverse events were upper respiratory tract infections (4, 8, 10 and 7% with secukinumab 300, 150, 75 mg, and placebo, respectively) and nasopharyngitis (6, 4, 6 and 8%, respectively). Serious adverse events were reported by 5, 1, and 4% of patients in the secukinumab 300, 150, and 75 mg groups, respectively, compared with 2% in the placebo group. No deaths were reported (27). In the FUTURE 2 study, responses in anti-TNF naive and anti-TNF treated subjects were sustained through week 52, with an ACR20 response rate of 68.7 and 54.5% respectively (28). Moreover, resolution of enthesitis and dactylitis was found in 69.2 and 65.9% of patients at week 52. The authors reported that subcutaneous secukinumab 300 and 150 mg improved the signs and symptoms of PsA, suggesting that secukinumab is a potential future treatment option for patients with this disease (27). Furthermore secukinumab significantly inhibits radiographic progression in peripheral joints in respect to placebo at week 24. Sustained inhibition of radiographic progression was observed through week 52 (29). FUTURE 1 study confirmed the efficacy of secukinumab, however some concern remains about the risk of infections and cardiovascular diseases and long term studies are needed (30).

INHIBITION OF PHOSPHODIESTERASE 4

PDEs are the enzymes that hydrolyze and degrade cyclic adenosine monophosphate (cAMP) (31). PDE4 is a cAMP PDE widely expressed in hematopoietic cells (*e.g.*, myeloid, lymphoid), non-hematopoietic cells (*e.g.*, smooth muscle, keratinocyte, endothelial), and sensory/memory neurons (32). The evidence for the PDE4 role in inflammatory response derives from different observations. It has been demonstrated that lipopolysaccharide selectively induces PDE4B2 mRNA expression in human circulating monocytes and PDE4A4 and PDE4B2 were detected at higher levels in peripheral blood monocytes of smokers (so with a possible continuous inflammatory stimulation) compared with non-smokers (33). Monocytes and macrophages are the main producers of the pro-inflammatory cytokine TNF whose levels decreased with PDE4 inhibition (34) and different studies show that production of TNF, IL-2, IL-4, and IL-5 and the proliferation of T lymphocytes are all dependent from PDE4 activity and, moreover, overexpression of PDE4 leads to an augmented inflammatory cytokines production (35). IL-12 production in macrophages, which is important for the differentiation of Th 1 cells, is also regulated by PDE4 (36). These evidences show that PDE4 is a key-enzyme in inflammatory response. On this basis, PDE4 inhibitors were proposed as therapy in different immune mediated diseases, including PsA.

Apremilast

Apremilast is a small molecule and a selective inhibitor of PDE4. It binds to the catalytic site of the PDE4 enzyme, thereby blocking cAMP degradation. Apremilast demonstrated to inhibit IL-2, IFNγ, IL-8, TNF production and different T-cell-derived cytokines *in vitro* (37). The efficacy and safety of apremilast in the treatment of psoriatic plaque were evaluated in two randomized phase 3 trials with comparable design. In ESTEEM 1 and ESTEEM 2, patients were randomized 2:1 to receive apremilast 30 mg twice daily or placebo for 16 weeks. The proportion of patients achieving a PASI-75 response was significantly greater ($P<0.0001$) in the apremilast-treated group than in the placebo group in both studies (38, 39). In PsA patients, four trials evaluated the efficacy and safety of apremilast. The PALACE 1 trial evaluated the efficacy and safety of apremilast in patients with active PsA with previous use of biologic therapy (40). In this trial, 504 patients were randomized to placebo, apremilast 20 mg twice daily, or apremilast 30 mg twice daily. At week 24, placebo treated patients were re-randomized to either the apremilast 20 mg arm or the apremilast 30 mg

arm. Of the 504 randomized patients prior use of a biologic was reported in 24% of patients. The primary efficacy endpoint was the proportion of patients achieving the ACR20 response at week 16, with significantly more patients achieving this endpoint in the apremilast 20 mg group (31%, P=0.0140) and in the apremilast 30 mg group (40%, P=0.0001) compared with placebo-treated patients (19%) (40). In this study, significant improvements in other secondary endpoints at week 24 were also noted with apremilast therapy (ACR50, ACR70 and physical functioning). Study discontinuation, because of adverse events, was comparable among groups (6% for apremilast 20 mg, 7% for apremilast 30 mg, and 5% for placebo) (40). The most frequently reported adverse events with apremilast were largely mild to moderate and dose-dependent. These included diarrhea, reported by 11 and 19% of patients in the apremilast 20 and 30 mg groups, respectively (*vs* 2% for placebo), and nausea, reported by 10% of apremilast 20 mg patients and 19% of apremilast 30 mg patients (*vs* 7% for placebo). These events presented early and were self-limiting, accounting for few study discontinuations. The 52-week results of the PALACE 1 trial demonstrated that in those patients who continued treatment with apremilast, treatment efficacy was mantained; ACR20 responses of 63 and 55% were reported in the apremilast 20 mg and apremilast 30 mg groups, respectively (41). Furthermore, apremilast was efficient in reducing the Maastricht ankylosing spondylitis enthesitis score, while none of the two doses significantly reduced C reactive protein levels and dactylitis score in respect to placebo at week 24 (40). No information was available regarding the efficacy of apremilast in axial disease or about the possibility to achieve a state of disease remission; however PALACE 2, 3 and 4 studies are still ongoing and will provide information on these aspects. On these bases, apremilast has been approved for the treatment of psoriasis and PsA.

CONCLUSIONS

Anti-TNF therapy showed its efficacy and safety in different rheumatic diseases and now a state of remission or low disease activity are achievable targets even in spondyloarthritis in general (42, 43) and in PsA (44-46). However, about 40% of patients lack to respond to TNF inhibitors. Fortunately, the treatment of PsA is rapidly evolving: beyond anti-TNF therapy, emerging novel therapies that target new molecules are rising. The discovery of the role of Th17 cells, the understanding of the role of the cytokines production together with the pathways involved in immune system activation, have made possible the develop-

Table I - Summary of efficacy of new approved drugs for psoriatic arthritis (data from randomized controlled trials).

		ACR20	ACR50	ACR70	PASI75	HAQ (mean change from baseline)
Ustekinumab 45 mg	24-week evaluation	42.4%	24.9%	12.2%	57.2%	–0.25
Ustekinumab 90 mg	24-week evaluation	49.5%	27.9%	14.2%	62.4%	–0.25
Secukinumab 150 mg	24-week evaluation	51%	35%	Not provided at 24 weeks	48%	Not provided at 24 weeks
Secukinumab 300 mg	24-week evaluation	54%	35%	Not provided at 24 weeks	63%	Not provided at 24 weeks
Apremilast 20 mg	24-week evaluation	26.4%	14.7%	5.5%	17.6%	–0.21
Apremilast 30 mg	24-week evaluation	36.6%	19.9%	10.6%	21%	–0.26

ACR, American College of Rheumatology; PASI, psoriasis area and severity index; HAQ, health assessment questionnaire.

ment of new drugs effective in treating PsA. Some of these agents are now available and their effectiveness on the various component of the disease seems to be similar in terms of ACR20 response (Table I) (47). Ustekinumab, secukinuamb and apremilast have been approved for PsA and, therefore, have been included in the recent EULAR update 2015 (48) and GRAPPA 2015 (49).

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The negative bone effects of the disease and of chronic corticosteroid treatment in premenopausal women affected by rheumatoid arthritis

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SUMMARY

Osteoporosis is a well-known extra-articular complication in rheumatoid arthritis (RA). The chronic corticosteroid treatment, the functional impairment associated with RA and the disease itself appear to be the most relevant determinants. Most of the previous studies involved postmenopausal women, in whom the estrogenic deficiency might amplify the negative effect towards bone of both RA and corticosteroid therapy. We decided to evaluate bone health in a cohort of premenopausal RA patients. The study population includes 47 premenopausal women attending our outpatient clinic for RA and twice as many healthy age-matched control women selected from the hospital personnel. The bone density at the spine and femoral neck were significantly lower in patients with RA as compared with controls. When spine bone mineral density (BMD) values were adjusted for the cumulative glucocorticoid (GC) dose alone and for the cumulative GC dose plus body mass index (BMI) the mean differences between two groups decreased but they remained statistically significant. We found no difference when the spine BMD was adjusted for cumulative GC dose, BMI and health assessment questionnaire. The difference in femoral neck BMD remained statistically significant also after all the same adjustments.

In conclusion, our study shows that a BMD deficiency is frequent also in premenopausal women affected by RA, especially at femoral site and that the main determinants of this bone loss are not only the disease-related weight loss, corticosteroid therapy and functional impairment, but also the systemic effects of the disease itself.

Key words: *Rheumatoid arthritis; glucocorticoid therapy; premenopausal women.*

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INTRODUCTION

Osteoporosis is a well-known extra-articular complication in rheumatoid arthritis (RA) (1, 2) and an increased risk of fractures has been clearly documented in patients with RA (1-7). The pathogenesis of the decreased bone mineral density (BMD) values at the hip, spine and total body reported in a number of studies (1, 4, 8-12) is multifactorial (2, 13-15). Chronic glucocorticoid (GC) treatment, together with the functional impairment associated with RA appear to be the most relevant determinants (4, 6, 14, 16), even though the disease itself may play a relevant role too (17, 18). Recently, RA has been taken into account as an independent risk factor in the assessment of fracture risk (19, 20).

In all previous studies, the study population was almost invariably made of postmenopausal women where the estrogenic deficiency might amplify the negative effect of RA and corticosteroid therapy on skeletal health.

Indeed, all the major scientific societies recommend a specific treatment in postmenopausal women receiving GC therapy particularly when they are affected by RA (21).

The aim of our study was to compare bone mass and mineral metabolism in a cohort of premenopausal women and in a group of age-matched healthy women.

MATERIALS AND METHODS

The study population includes 47 premenopausal women (last menses less than 40 days before the index visit) attending to our outpatient clinic for RA and twice as

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many healthy age-matched control women selected form the hospital personnel. All patients fulfilled the revised 1987 American College of Rheumatology (22).

We evaluated: mobility activities of daily living score (23), Steinbrocker functional state (1 to 4) (24), erythrocyte sedimentation rate, rheumatoid factor, anti-cyclic citrullinated peptide and routine biochemistry. Additional information included: disease onset, extra-articular manifestations, smoking and alcohol intake habits, treatments specific for RA and osteoporosis.

Vitamin D supplements, taken during the previous year, were carefully evaluated and expressed as mean daily dose even when vitamin D was taken intermittently. Exposure to sunlight from March to September was quantified as <20 min, 20 to 40 min and >40 min daily. Daily intake of calcium was assessed by a simplified validated questionnaire (25). In all subjects body weight and height (Harpender stadiometer) were assessed and the body mass index (BMI = kg/m^2) calculated.

Individual aliquots of serum samples were collected from September to December 2008 from each woman and kept at –70°C for the measurements of serum intact parathyroid hormone and 25 hydroxy-vitamin D [25(OH)D] by commercial ELISA kits (IDS Co., Bolden, UK) with inter-assay coefficient of variations ranging from 5 to 15%.

A Hologic Delphi instrument executed the dual-energy X-ray absorptiometry (DXA) evaluation of the BMD at the lumbar spine and femoral neck. The *in vivo* coefficient of variation was 0.7% at the spine and 1.2% at the hip. The BMD values were expressed both as absolute values and as T-scores. T-score is the number of standard deviations below the average for a young adult at peak bone density. The World Health Organization has defined the following categories based on bone density in white women (26):

1) normal bone: T-score better than –1;
2) osteopenia: T-score between –1 and –2.5;
3) osteoporosis: T-score less than –2.5;
4) established (severe) osteoporosis includes the presence of a non-traumatic fracture.

The differences between control women and patients for continuous values were tested by unpaired t-test. ANCOVA was used to test significance for BMD values adjusted for all potential interfering variable. Statistical analysis was realized by SPSS version 11 (SPSS Inc., Chicago, IL, USA).

RESULTS

The baseline main features of the two groups are reported in Table I.

We found no difference in biochemical markers of bone metabolism. Levels of 25(OH)D lower than 20 ng/mL (27) was found in 38.3% (18/47) and 38.1% (37/94)

Table I - Main features of the two groups.

		No.	Mean±SD	P
Age (years)	Controls RA	94 47	41.9±5.9 42.2±5.7	NS
BMI (Kg/m2)	Controls RA	94 47	24.1±4.4 22.6±3.2	<0.05
25 OH Vit D (ng/mL)	Controls RA	94 47	23.1±9.2 24.1±11.0	NS
CTX (ng/mL)	Controls RA	94 47	0.29±0.14 0.31±0.14	NS
PTH (pg/mL)	Controls RA	94 47	26.8±10.9 23.7±11.6	NS
Smoking subjects	Controls RA	94 47	19/94=20.2% 9/47=19.1%	NS

SD, standard deviation; RA, rheumatoid arthritis; BMI, body mass index; CTX, bone turnover marker; PTH, serum intact parathyroid hormone; NS, not significant.

Table II - Disease related variables in the 47 patients affected by rheumatoid arthritis.

	Mean±SD	Minimum	Maximum
Disease duration (years)	9±7	2	25
Steinbrocker's classification	1.6±0.7	1	4
HAQ	0.6±0.6	0.00	2.62
Number of swollen joints	2.4±3.6	0	20
ESR (mm/h)	28.1±22.4	4	97
Prednisone equivalent daily dose (mg)*	5.3±1.7	2	8
Months of corticosteroid therapy*	28.1±33.7	6	127
Cumulative dose (mg prednisone equivalent)*	776±917	150	4500

SD, standard deviation; HAQ, health assessment questionnaire; ESR, erythrocyte sedimentation rate.
*Relative only to the 37 patients on corticosteroid therapy.

of the patients and controls, respectively (thus confirming what was already found in different rheumatic diseases (28) (data not shown). Mean BMI was statistically lower in RA patients (P<0.05).

The main disease related findings of the 47 patients affected by RA are listed in Table II.

Steroid treatment was underway in 37 of 47 patients while 10 subjects never received treatment with corticosteroids. The features of steroid therapy relative only to the 37 patients on corticosteroid therapy were (Table II): prednisone equivalent mean daily dose (mg) 5.3±1.7 mg, mean duration of corticosteroid therapy 28.1±33.7 months and mean cumulative dose 776±917 mg (prednisone equivalent).

The bone densities at spine and femoral neck were significantly lower in patients with RA as compared with controls.

The proportion of patients with osteopenia and osteoporosis at the spine were 32 and 11% in patients *vs* 17 and 0% in controls. The corresponding proportion of patients with osteopenia and osteoporosis at the femoral neck were 26 and 13% in patients *vs* 4 and 0% in controls respectively.

As compared with control group, the relative hazard to be osteopenic or osteoporotic was 3.61 [95% confidence interval (CI)=1.64 to 7.95] and 13.97 (95% CI=4.37 to 44.61) at spine and femoral neck respectively.

The BMD differences were 7.9 and 16.9% at the spine and the femoral neck respec-

Table III - Comparison of spine and femoral bone mineral density data expressed as absolute values in rheumatoid arthritis and in control groups with and without adjustment for cumulative corticosteroid dose, body mass index and health assessment questionnaire.

Spine	RA	Controls	Diff%	P
Not adjusted	965.8±131.1	1048.8±118.8	–7.9	<0.001
Adj. for CORT	981.5±134.4	1041.0±130.0	–5.7	<0.02
Adj. for CORT+BMI	983.9±132.9	1040.0±128.9	–5.4	<0.03
Adj. for CORT+BMI+HAQ	1029.0±153.3	1020.0±136.7	0.0	NS
Femoral neck	**RA**	**Controls**	**Diff%**	**P**
Not adjusted	760.3±175.7	915.0±112.1	–16.9	<0.001
Adj. for CORT	788.6±145.4	903.5±136.7	–12.7	<0.001
Adj. for CORT+BMI	792.7±137.5	901.8±128.9	–12.0	<0.001
Adj. for CORT+BMI+HAQ	814.9±163.9	892.9±140.6	–8.7	<0.03

RA, reumatoid arthritis; CORT, corticosteroid; BMI, body mass index; HAQ, health assessment questionnaire; NS, not significant.

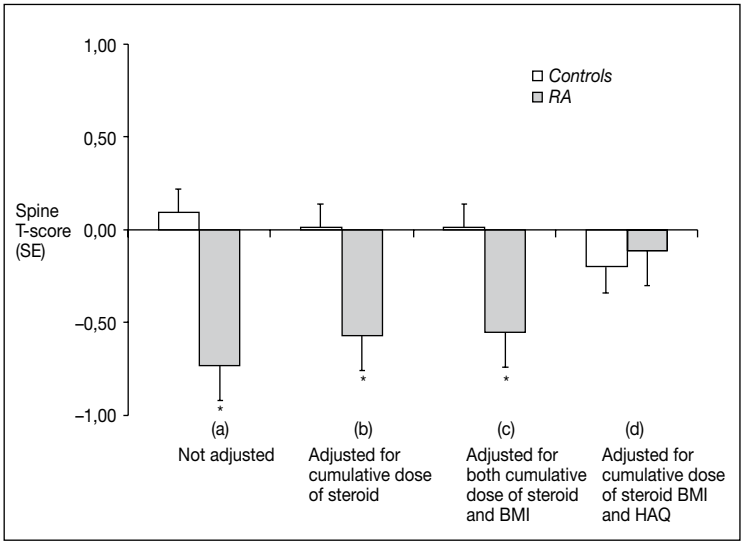

Figure 1 - Mean spine T-score values (±SE) both un-adjusted and adjusted (see figure for adjusting variables) in patients with reumatoid arthritis and controls. *P<0.05 vs control subjects.

tively (Table III). When spine BMD values were adjusted for the cumulative GC dose alone and for the cumulative GC dose plus BMI the mean differences decreased to 5.7 and to 5.4% respectively but they remained statistically significant (Table III; Figure 1). No difference was found when spine BMD was adjusted for cumulative GC dose, BMI and health assessment questionnaire (HAQ) (Table III; Figure 1).

The difference in femoral neck BMD remained statistically significant also after all adjustment (cumulative GC dose alone, cumulative GC dose plus BMI, and cumulative GC dose plus BMI plus HAQ) although their extents gradually decreased from 16.9 to 8.7% (Table III; Figure 2).

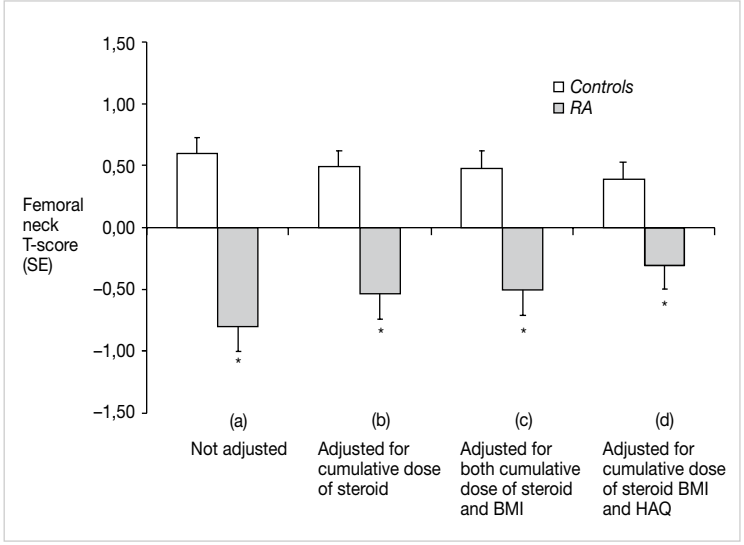

Figure 2 - Mean femoral neck T-score values (±SE) both un-adjusted and adjusted (see figure for adjusting variables) in patients with reumatoid arthritis and controls. *P<0.05 vs control subjects.

DISCUSSION AND CONCLUSIONS

In this study we have shown that that in premenopausal women with RA both spine and hip BMD values are significantly lower than in age-matched controls and that this difference is maintained at the hip when the BMD values are adjusted for GC therapy and the indices of disease activity. This suggests that the disease itself is responsible of significant bone loss, particularly at skeletal sites made of predominantly cortical bone.

An association between RA and low BMD values has been reported in a number of studies but a proper controlled study has been carried out only by Gough and colleagues (2). In this study Gough and his colleagues performed a DXA prospective evaluation (at 12 and 24 months) in a group of patients with RA (disease duration less than 2 years) and controls showing that RA patients tended to lose bone significantly faster than controls at all sites.

All these studies, however, included a large proportion of post-menopausal women, in whom estrogen deficiency might amplify the negative effect of RA and corticosteroid therapy on bone metabolism.

Hämäläinen and colleagues (29) studied the changes in BMD in 74 premenopausal women with RA during a two-year follow-up with DXA-BMD measurements in the lumbar spine and left femoral neck. The patients with RA treated with prednisone had lower BMD values than those without this treatment present at the start of the follow-up. Mean BMD decreased significantly in both lumbar spine and femoral

neck only in the RA group treated with corticosteroids.

Tourinho and colleagues (30) studied 78 premenopausal patients with RA and 39 controls and showed a significantly lower lumbar spine BMD in the patients. Lumbar spine osteopenia correlated with *no physical activity at work* status, low body weight, and duration of GC therapy.

In our study too, an important determinant of low BMD values was BMI, which was significantly lower in patients than in controls (22.6±3.2 *vs* 24.1±4.4 kg/m^2). Body weight and BMI are among the most relevant determinants of BMD values (31) and our finding should be evaluated in the context of the strong relationship between BMI with disease activity (32, 33).

We found no differences in biochemical markers. The same high prevalence of vitamin D deficiency (25(OH)D levels lower than 20 ng/mL) was found in 2 groups: 38.3% (18/47) in RA patients *vs* 38.1% (37/94) in controls.

No differences in bone turnover markers were shown and these are important data, given that a large proportion (37/47: about 80%) of RA patients were in steroid treatment.

The crude BMD reductions in RA patients *vs* controls were statistically significant (by 8.3 and 17.1% at spine and femoral neck respectively) (Table III; Figures 1 and 2).

The RA patients were slimmer as likely effect of chronic inflammation and disease and we decided to adjust BMD values by BMI (ANCOVA) (Table III; Figures 1 and 2). The differences remained significant and only slightly decreased after this adjustment and this fact shows that the BMD reduction is due only in small part to disease related weight loss.

The corticosteroid therapy is known as a very important cause of bone mass reduction (34, 35). Of our 47 patients, 37 were on GC therapy whereas only 10 patients never assumed steroids. BMD values were then adjusted (ANCOVA) for both the cumulative GC dose and BMI (Table III; Figures 1 and 2). The differences with the control group decreased to 5.4% (P<0.03) and 12.1% (P<0.001) at the spine and hip, but remained statistically significant.

This fact also proves that in premenopausal women the BMD defect in RA is not due at all to corticosteroid therapy. Finally, we wanted see the weight of disability on RA osteoporosis. The BMD data were also adjusted for HAQ-DI as well as for BMI and cumulative GC dose (Table III; Figures 1 and 2). The adjusted difference of BMD values *vs* control subjects remained statistically significant (P<0.03) even if only at the femoral neck (8.7% difference).

Thus, it seems that the cumulative dose of GC, the effect of the disease on weight and the disability are together able to justify the densitometric deficit at the lumbar spine (cancellous bone). On the contrary, at the femur (cortical bone), the correction for those parameters (however relevant) is able to decrease but not to eliminate the densitometric gap when compared to controls. A possible further cause might be, in our opinion, the disease itself, which may be able to cause negative effect on the cortical bone not only locally, but also in a systemic manner (17).

The main results of our study show that a BMD defect is also a frequent feature in premenopausal women with RA and that this defect is more evident at femoral site. This fact confirms a preferential effect of RA on cortical bone (18).

Indeed, the role of the cumulative dose of GC, BMI and HAQ is demonstrated by the observation that, after the correction of data concerning BMD for these parameters, the differences decrease (femur) or nullify (lumbar spine). However, the persistence of a difference at the femur supports the hypothesis that also the disease itself can justify the BMD deficit at the densitometry.

Conflict of interest: the authors declare no potential conflict of interest.

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Spine and sacroiliac joints on magnetic resonance imaging in patients with early axial spondyloarthritis: prevalence of lesions and association with clinical and disease activity indices from the Italian group of the SPACE study

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SUMMARY

Our aim was to determine the prevalence of spine and sacroiliac joint (SIJ) lesions on magnetic resonance imaging (MRI) in patients with early axial spondyloarthritis (axSpA) and their correlation with disease activity indices.

Sixty patients with low back pain (LBP) (≥3 months, ≤2 years, onset ≤45 years), attending the SpA-clinic of the Unità Operativa Complessa Reumatologia of Padova [SpondyloArthritis-Caught-Early (SPACE) study], were studied following a protocol including physical examination, questionnaires, laboratory tests, X-rays and spine and SIJ MRI. Positive spine and SIJ MRI and X-rays images were scored independently by 2 readers using the SPARCC method, modified Stoke ankylosing spondylitis spine score and New York criteria. The axial pain and localization of MRI-lesions were referred to 4 sites: cervical/thoracic/lumbar spine and SIJ. All patients were classified into three groups: patients with signs of radiographic sacroiliitis (r-axSpA), patients without signs of r-axSpA but with signs of sacroiliitis on MRI (nr-axSpA MRI SIJ+), patients without signs of sacroiliitis on MRI and X-rays (nr-axSpA MRI SIJ-).

The median age at LBP onset was 29.05±8.38 years; 51.6% of patients showed bone marrow edema (BME) in spine-MRI and 56.7% of patients in SIJ-MRI. Signs of enthesitis were found in 55% of patients in the thoracic district. Of the 55% of patients with BME on spine-MRI, 15% presented presented a negative SIJ-MRI. There was a significant difference between these cohorts with regard to the prevalence of radiographic sacroiliitis, active sacroiliitis on MRI and SPARCC SIJ score.

The site of pain correlated statistically with BME lesions in thoracic and buttock districts. Since positive spine-MRI images were observed in absence of sacroiliitis, we can hypothesize that this finding could have a diagnostic significance in axSpA suspected axSpA.

Key words: *Axial spondyloarthritis; early onset spondyloarthritis; disease activity; clinimetric indices spine; sacroiliac joints.*

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INTRODUCTION

Spondyloarthritis (SpA) is a group of chronic inflammatory rheumatic diseases that share overlapping features and that can be divided into two main groups: axial SpA (axSpA) and peripheral SpA (pSpA) (1-3). Thanks to the development of biotechnological drugs, significant progress has been made in the treatment of axSpA making early diagnosis and treatment even more relevant. Rheumatologists are making every effort to assess disease activity not only with the intent of initiating treatment as soon as possible but also to monitor patients' response to therapy. In accordance with the revised New York criteria, conventional radiographs of the sacroiliac joint (SIJ) are frequently used to detect sacroiliitis, a typical expression of radiographic axSpA (r-axSpA) or of ankylosing spondylitis (AS) (4). This method has however proved to be inadequate to diagnose a patient with suspected

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early axSpA, as it is able to detect only structural damage, which is a characteristic of advanced disease stage (1, 4). Magnetic resonance imaging (MRI), which can detect both inflammatory lesions and structural damage of the SIJ, can be used in addition to radiographs when SpA is suspected (5-8). In patients with early-onset axSpA without evidence of radiographic sacroiliitis, MRI can, in fact, detect inflammatory lesions before bone damage becomes visible. As it has been seen that these patients respond quickly and effectively to anti-tumor necrosis factor-α drugs (3, 9-11), it has become urgent to identify early stages of the disease in order to begin appropriate treatment as early as possible.

The Assessment of SpondyloArthritis International Society (ASAS) has recently established classification criteria for patients with suspected axSpA, including those with and without radiographic sacroiliitis (5). As far as imaging is concerned, the ASAS criteria require the presence of sacroiliitis on a MRI or conventional radiography in addition to at least one of the signs of SpA for patients with chronic low back pain (LBP) with age at onset ≤45 years. Positive SIJ MRI scans were defined by the ASAS/Outcome Measures in Rheumatology MRI working group (OMERACT) as the presence of inflammatory lesions such as bone marrow edema (BME) which is highly suggestive of SpA (8).

Whether structural SIJ lesions should be added to this definition and if structural and inflammatory spinal lesions could contribute to detecting axSpA remains to be established (12). Inflammatory spinal lesions on MRIs may nevertheless occur in the absence of SIJ involvement (13). Spinal MRIs in AS patients have uncovered abnormalities in this district even before they are noted on plain radiographs (14). The introduction of fat-suppression sequences has allowed the visualization of lesions within bone marrow that may be obscured on MRI by marrow fat. These lesions include BME adjacent to vertebral endplates at the attachment of the annulus fibrosus to the vertebral rim and at the insertion of anterior and posterior longitudinal ligaments, both within the facet joints. Since there is evidence that spondylitis may also occur prior to or even without sacroiliitis, it was considered important to define the characteristics of a spinal MRI considered positive for inflammation.

The ASAS/OMERACT working group thus set out to define spinal MRIs positive for inflammatory lesions (spondylitis) and structural changes (fat deposition) (12). It is also unknown whether the localization of lesions is correlated to the site of axial pain.

Imaging of the thoracic spine, which is often involved in axSpA, has not yet been taken into consideration when structural damage is being evaluated (15-17). The goal of this study is to determine the prevalence of spine and SIJ lesions on MRI and their correlation with disease activity indices in patients with early axSpA included in the SpondyloArthritis Caught Early (SPACE) Italian cohort.

MATERIALS AND METHODS

Patients

Patients who were at least 16 years old, suffering from inflammatory LBP (≥3 months, ≤2 years, onset <45 years) of unknown origin and referred to a rheumatologist were included in the Italian section of the SPACE study.

This is an ongoing observational cohort study, which was originally launched at the Leiden University Medical Centre in January 2009. In March 2012 the SpA Study Group of the Rheumatology Unit at the University of Padua was involved in the SPACE study. Eligible patients underwent physical examinations, laboratory tests, SIJ and spinal plain radiographs and MRIs, following a standardized protocol. The patients also completed questionnaires on disease activity, physical functioning, pain, and disease-related impairment. Axial pain and MRI lesions were localized in 4 sites: in the cervical/thoracic/lumbar spine and the SIJ. An experienced rheumatologist made the diagnosis of axSpA.

In order to meet the ASAS criteria (5), it was necessary to verify if the patients had MRI evidence of active inflammatory lesions of the SIJ with definite BME, which is highly suggestive of sacroiliitis. After the X-rays and MRI images were read, the patients were divided into three cohorts: those with early signs of r-axSpA, those without signs of r-axSpA but with signs of active sacroiliitis on MRI (nr-axSpA MRI SIJ+), those without signs of sacroiliitis on MRI and plain radiograph (nr-axSpA MRI SIJ-). Only baseline (t0) data were used in these analyses. At that time point, all the patients were being treated with non-steroidal anti-inflammatory drugs. No patients were treated with synthetic or biological disease modifying anti-rheumatic drugs. The local medical ethical committee approved the study and informed consent was obtained from all patients at study inclusion.

Magnetic resonance imaging assessments

SIJ and spinal MRIs were performed at baseline using a 1.5T scanner Magnetom Harmony (Siemens AG Medical Solutions, Munich, Germany) with phased-array surphace coil, acquiring T1-weighted turbo spin echo (T1TSE; TR 550/TE 10) and short-tau inversion recovery (STIR; TR 2500/TE60) sequences.

The coronal oblique and sagittal views of the SIJ and spine were in 4 mm slice thicknesses.

The images were analyzed independently by two expert radiologists trained in scoring MRIs in accordance with the ASAS definition and the SPARCC scoring system (18, 19). If the two readers scored positive, the image was scored accordingly. All readers were blinded for clinical and laboratory data, and for the results of the other imaging methods.

The mean scores were calculated using those of both of the readers. It was performed intra and inter-observer reliability.

All of the inflammatory lesions typical of SpA were graded using the SPARCC scoring system: the SIJ is graded positive if ≥1 BME lesion highly suggestive of SpA is visible on ≥2 consecutive slices or if several BME lesions highly suggestive of SpA are visible on a single slice (8).

The presence of only synovitis, enthesitis, or capsulitis without BME is not sufficient for a positive reading.

According to the SPARCC scoring method, the presence of an increased signal corresponding to BME lesions on SIJ-MRIs should be noted on 6 consecutive coronal slices selected as representing the synovial compartment of the SIJ.

The left and right SIJ MRIs were divided into quadrants for a total of 8 per coronal slice. Each quadrant was assessed and evaluated for the presence (scored 1) or absence (scored 0) of BME. To each coronal slice per SIJ was given an additional score of 1 for the presence of an intense signal and an additional score of 1 for a deep lesion, defined as a homogeneous, unequivocal increase in a signal 1 cm from the articular surface.

The maximum possible score across 6 slices was 48 for the presence of BME, 12 for intense edema, and 12 for deep edema, for a maximum possible total score of 72 (18). Structural lesions on SIJ-MRIs were also evaluated. According to the ASAS/OMERACT MRI group, BME and fatty lesions on spinal MRIs are considered when they are visible on ≥2 consecutive slices, while the presence of ≥1 slice is enough for structural lesions (erosions, sindesmophytes) (12). For the spine, the 6 most severely affected disco-vertebral units (DVUs) were selected and each was divided into 4 quadrants, with each quadrant assessed for the presence (scored 1) or absence (scored 0) of BME. Each quadrant was scored on 3 consecutive sagittal slices per DVU, yielding a maximum possible score of 12 per DVU for BME. Each sagittal slice per DVU was given an additional score of 1 for the presence of an intense signal and an additional score of 1 for a deep lesion, defined as a homogeneous, unequivocal increase in STIR signal >1 cm from the vertebral end plate. The maximum possible score for all 6 DVUs was 72 for the presence of BME, 18 for intense edema, and 18 for deep edema, for a maximum possible total score of

108 (19). The structural lesions on spinal MRIs were also evaluated.

Radiographs assessments

Lateral view radiographs of the cervical and lumbar spine and anterior-posterior view radiographs of the pelvis were taken. The images were obtained with a Philips vertical bucky, with a focus-film distance of 140 cm, film size of 18×43 cm. The images were read independently by two expert trained musculoskeletal radiologists, blinded for patients' characteristics, clinical outcome and for the results of the other imaging methods. The mean scores were calculated using those of both of the readers. It was performed intra and inter-observer reliability.

The modified Stoke ankylosing spondylitis spine score (mSASSS) scoring method modified by Creemers was used (16). According to this method, lateral views of the anterior vertebral corners (VCs) of the cervical (lower border of C2 to upper border of T1) and lumbar (lower border of T12 to upper border of S1) segments (a total of 24 VCs) are scored for the presence of erosions and/or sclerosis and/or squaring (1 point), syndesmophytes (2 points) and bridging syndesmophytes (3 points). The total score ranges from 0 to 72 (16). Evaluation of the SIJ was based on the New York criteria (4), with scores ranging from 0 to 4 (0=no change, 1=look slightly faded edge joint, pseudo-widening or narrowing of the rhyme, mild subchondral sclerosis, 2=irregular margin joint with images of erosion, shrinkage of rhyme, subchondral sclerosis evident, 3=erosions and subchondral sclerosis evident with initial synostosis, 4=complete ankylosis).

Clinical evaluation, questionnaires and laboratory tests

The clinical evaluation focused on an examination of the spine, SIJ and entheses, using the Bath ankylosing spondylitis metrology index (BASMI) and the Maastricht ankylosing spondylitis enthesitis score (MASES) as a guide. The patients' disease activity and physical functioning were assessed using self-reported questionnaires and composite indices: the Bath ankylosing spondylitis disease activity index (BASDAI), the Bath ankylosing spondylitis functional index (BASFI), the ankylosing spondylitis disease activity score (ASDAS), the visual analogue scale (VAS pain), the VAS night pain scale, the VAS disease activity, the Bath ankylosing spondylitis patient global score (BASG1), the BASG2, the health assessment questionnaire (HAQ).

Patients' erythrocyte sedimentation rate (ESR) (normal range 0-15 mm/h) and high sensitivity C reactive protein (hsCRP) (normal range 0-6 mg/L) were also evaluated.

Statistical analyses

Odds ratio (OR) was used to assess the association at t0 between the site of axial pain and the localization of the inflammatory and structural lesions on the spinal and SIJ MRIs. Cohen's Kappa was used for intra and inter-observer reliability. The Kruskal Wallis (ANOVA) was used to compare at t0 the following indices: clinical (BASMI, MASES), serological (ESR, hsCRP), functional (BASFI, HAQ, BASG1, BASG2, VAS pain, VAS night pain, VAS disease activity), disease activity (BASDAI, ASDAS) indices and imaging score (mSASSS, NY score, SPARCC SIJ and SPARCC 6-DVU). A P value <0.05 was considered significant.

RESULTS

One hundred percent of the 60 patients studied fulfilled the ASAS criteria for axSpA; 23 (38.3%) patients were diagnosed as having r-axSpA, 17 (28.3%) as having nr-axSpA MRI SIJ+ and 20 (33.3%) as having nr-axSpA MRI SIJ–.

The median age at LBP onset was 29.05±8.38 years, 45% were male, 38.3% of the patients were HLA-B27+; there was axial involvement in 34 (55.7%) patients and axial/peripheral involvement in 27 (44.3%) patients. High prevalence of psoriasis and heel enthesitis (respectively 34.4% and 80.3%) were noted. Other patient characteristics and SpA features are outlined in Table I.

Table I - Baseline characteristics of the 60 patients studied [Italian section of the SpondyloArthritis Caught-Early (SPACE) study].

Age of onset back pain, mean (±SD)	**29.05 (±8.38)**
Male, n (%)	26 (43.3%)
Duration (months) back pain, mean (±SD)	12.62 (±5.85)
Only axial involvement, n (%)	34 (56.7%)
Axial and peripheral involvement, n (%)	27 (43.3%)
HLA-B27 positive, n (%)	22 (36.7%)
Positive family history of SpA, n (%)	29 (48.3%)
IBP, n (%)	60 (100%)
Peripheral arthritis, n (%)	26 (43.3%)
Psoriasis, n (%)	21 (35%)
Dactylitis, n (%)	15 (25%)
Heel enthesitis, n (%)	48 (80%)
Uveitis, n (%)	4 (6.7%)
IBD, n (%)	8 (13.3%)
Preceding infection, n (%)*	2 (3.3%)
Good response to NSAIDs, n (%)	58 (96.7%)
Elevated CRP/ESR, n (%)	33 (55%)
Cervical pain, n (%)	39 (65%)
Thoracic pain, n(%)	28(46.6%)
Buttock pain, n (%)	49 (81.6%)
Alternating buttock pain, n (%)	26 (43.3%)
Morning stiffness, n (%)	57 (95%)
Night pain, n (%)	58 (96.7%)
Sacroiilitis MRI, n (%)°	32 (53.3%)
Sacroiliitis X-ray, n (%)#	23 (38.3%)

SD, standard deviation; HLA-B27, human leukocyte antigen; SpA, spondyloarthritis; IBP, inflammatory back pain; IBD, inflammatory bowel disease; NSAID, non-steroidal anti-inflammatory drugs; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; MRI, magnetic resonance imaging. *Balanitis, urethritis or cervicitis; °sacroiilitis MRI according the Assessment of SpondyloArthritis International Society/European League Against Rheumatism (ASAS/EULAR); #Sacroiliitis X-ray according New York criteria.

Of the studied patients, 65%, 46.6%, 100%, and 81.6%, respectively, complained about cervical/thoracic/lumbar/buttock pain.

All spine and SIJ on MRI images were evaluated by two expert radiologists and the inter-observer reliability was respectively good to moderate (kappa 0.73 for inflammatory lesions and 0.58 for structural lesions on spine MRI) and good to moderate (kappa 0.78 for inflammatory lesions and 0.60 for structural lesions on SIJ MRI). The inter-observer reliability for all X-rays images was good (kappa 0.79 for spine radiological lesions and kappa 0.77 for SIJ radiological lesions). The intra-observer reliability was moderate to good for all spine and SIJ images on X-rays and MRI (mean kappa-coefficients between 0.59 and 0.80 for SIJ). Forty-two patients (70%) presented structural and/or inflammatory lesions on SIJ-MRIs at t0 (on the right SIJ in 60% of the patients and on left one in 53.3% of the patients). BME lesions were present in 34 (56.7%) patients (50% on the right SIJ and 38.33% on the left one). Structural lesions on SIJ-MRI were present in

Table II - The prevalence of inflammatory and structural lesions at baseline in three cohorts.

	r-axSpA	nr-axSpA MRI SIJ+	nr-axSpA MRI SIJ-
Total number of patients	23 (38.3%)	17 (28.3%)	20 (33.3%)
SIJ total lesions on MRI	22 (95.6%)	17 (100%)	3 (15%)
BME lesions	18 (81.7%)	17 (100%)	0 (0%)
Sclerosis lesions	13 (56.5%)	2 (11.8%)	3 (15%)
Fatty lesions	5 (21.7%)	2 (11.8%)	1 (5%)
Erosive lesions	5 (21.7%)	1 (5.9%)	0 (0%)
Spine total lesions on MRI	19 (82.6%)	14 (82.3%)	10 (50%)
BME lesions	16 (69.6%)	10 (58.8%)	6 (30%)
Enthesitis lesions	12 (52.2%)	8 (47.1%)	5 (25%)
Fatty lesions	6 (26.1%)	4 (23.5%)	3 (15%)
Sclerosis/syndesmophytes lesions	6 (26.1%)	4 (23.5%)	4 (20%)
Erosive lesions	1 (4.3%)	2 (11.8%)	2 (10%)

r-axSpA, patients with signs of radiographic sacroiliitis; nr-axSpA MRI SIJ+, patients with no signs of radiographic sacroiliitis but with signs of sacroiliitis on magnetic resonance imaging; nr-axSpA MRI SIJ-, subjects with no signs of sacroiliitis neither on MRI not on X-rays; SIJ, sacroiliac joints; BME, bone marrow edema.

22 (36.7%) patients (26.7% on the right SIJ and 30% on the left one). Thirty-three (55%) patients presented inflammatory and/or structural lesions on the spinal MRI at t0. BME lesions at the anterior corner of the spine were present in 51.6% (18.3, 30 and 31.7%, respectively, in the cervical/thoracic/lumbar regions). Structural spine lesions were present in 35% (17, 13, 17%, respectively, in the cervical/thoracic/lumbar regions). Signs of enthesitis were found in 35 (58.3%) patients: at the level of the cervical spine in 5% of the patients, of the thoracic spine in 55% of the patients, at the lumbar spine in 6% of the patients.

The 9 (15%) patients with inflammatory lesions on spinal MRIs showed no abnormalities on the SIJ ones, while 12 (20%) patients without active sacroiliitis on SIJ MRIs did not presented lesions on the spinal MRIs.

The prevalence of inflammatory and structural lesions at t0 in the three cohorts (r-axSpA, nr-axSpA MRI SIJ + and nr-axSpA MRI SIJ) are outlined in Table II. An increased prevalence of structural lesions on SIJ MRI that was found in the r-axSpA patients with respect to the other two cohorts would support the presence of sacroiliitis involvement on standard X-rays. R-axSpA and nr-axSpA MRI SIJ+ patients had more inflammatory and structural spinal lesions with respect to the nr-axSpA MRI SIJ-patients (Table II).

The OR between the site of pain and the localization of BME lesions was, respectively, 20.78 [confidence interval (CI):0.39-11.05; P=not significant (NS)], 163.93 (CI:3.31-81.28; P=0.0006), 0.34 (CI:0.01-17.91; P=NS) for the cervical/thoracic/lumbar spine areas and 304.88 (CI: 1.71-546.56; P=0.0203) for the buttocks.

The association of pain with structural lesions on the MRIs of the same site was not significant except for the buttock pain/structural SIJ MRI lesions (OR=70.1; CI: 0.84-58.40; P=0.0122). The association between thoracic pain and enthesitis of the thoracic district was found to be significant (OR=32.69; CI: 1.096-9.748; P=0.0336). Clinical and disease activity indices and imaging scores at t0 in the three cohorts are outlined in Table III.

The ANOVA test uncovered a significant difference in the prevalence of radiographic sacroiliitis, active sacroiliitis on MRI and the SPARCC SIJ score in the three cohorts. No differences in the clinical and disease activity indices between the groups were found.

Table III - Clinical and disease activity indices and imaging scores at baseline in the 60 patients studied and in the three cohorts.

Disease activity indices and imaging scores	Cohort 1 r-axSpA, n=23	Cohort 2 nr-axSpA MRI SIJ+, n=17	Cohort 3 nr-axSpA MRI SIJ–, n=20	P*	Total=60 pts
ESR (mm/h), mean (SD)	17.57 (14.45)	18.41 (12.75)	18.95 (21.83)	10	18.27 (16.61)
hsCRP (mg/L), mean (SD)	2.54 (2.79)	1.56 (1.71)	2.76 (3.76)	ns	2.11 (2.50)
HLA B27, n(%)	11 (47.8)	9 (52.9%)	3 (15%)	ns	23 (38.3)
BASMI, mean (SD)	0.74 (1.01)	1.06 (1.35)	0.75 (0.97)	ns	1.83 (1.09)
MASES, mean (SD)	2.87 (2.39)	4.35 (2.69)	3.6 (2.37)	ns	3.51 (2.51)
BASFI, mean (SD)	16.22 (23.96)	24.74(23.19)	15 (15.73)	ns	18.23 (21.37)
HAQ, mean (SD)	0.32 (0.52)	0.48 (0.61)	0.38 (0.35)	ns	0.38 (0.49)
BASG1, mean (SD)	3.26 (2.93)	4.24 (2.73)	3.85 (3.36)	ns	3.73 (3.01)
BASG2, mean (SD)	4.78 (2.78)	5.47 (2.79)	5.05 (2.98)	ns	5.07(2.82)
VAS pain, mean (SD)	3.70 (3.01)	4.06 (3.07)	4.55 (3.27)	ns	4.08 (3.08)
VAS disease activity, mean (SD)	3.39 (2.86)	4.36 (3.30)	4.5 (3.55)	ns	4.03 (3.21)
VAS pain night, mean (SD)	3.61 (3.63)	3.71 (3.57)	3.8 (3.44)	ns	3.07 (3.49)
BASDAI, mean (SD)	38.02 (25.58)	53 (25.61)	46.38 (23.75)	ns	45.05 (25.33)
ASDAS, mean (SD)	2.45 (0.79)	2.7 (0.86)	2.50 (0.87)	ns	2.53(0.81)
Sacroiliitis X-ray,° n (%)	23 (100%)	0 (0)	0 (0)	<0.001	25 (41.7)
Sacroiilitis MRI,# n (%)	17 (73.9%)	17 (100%)	0 (0)	<0.001	32 (53.3)
mSASSS, mean (SD)	3.26 (3.21)	3.53 (4.86)	3 (3.73)	ns	3.25 (3.84)
Score SIJ, mean (SD)	1.30 (0.63)	0 (0)	0 (0)	ns	0.5 (0.75)
SPARCC spine, mean (SD)	7.26 (12.33)	5.18 (15.52)	2.05 (3.49)	ns	4.93 (11.44)
SPARCC SIJ, mean (SD)	15.35 (16.57)	12.53 (9.15)	0 (0)	<0.001	9.43 (13.10)

r-axSpA, patients with signs of radiographic sacroiliitis; nr-axSpA MRI SIJ+, patients without signs of r-axSpA but with signs of sacroiliitis on magnetic resonance imaging; nr-axSpA MRI SIJ–, patients without signs of sacroiliitis on MRI and X-rays; ESR, erythrocyte sedimentation rate; SD, standard deviation; hsCRP, high sensitive C-reactive protein; ns, not significant; HLA-B27, human leukocyte antigen; BASMI, bath ankylosing score metrology index; MASES, Maastricht ankylosing spondilities enthesitis score; BASFI, bath ankylosing spondylitis functional index; HAQ, health assessment questionnaire; BASG1, bath ankylosing spondylitis patient global score 1; BASG2, bath ankylosing spondylitis patient global score 2; VAS, visual analogue scale; BASDAI, bath ankylosing spondylitis disease activity index; ASDAS, ankylosing spondylitis disease activity score; mSASSS, modified stoke ankylosing spondylitis spine score; SIJ, sacroiliac joints; SPARCC, Spondyloarthritis Research Consortium of Canada. *P analysis of variance (Kruskal-Wallis) a t0: P<0.05; °sacroiliitis X-ray according New York criteria; #sacroiilitis MRI according the Assessment of SpondyloArthritis International Society/European League Against Rheumatism (ASAS/EULAR).

DISCUSSION

Modern imaging technology of the pelvis and spine has become a crucial tool for diagnosing, classifying and monitoring axSpA. The traditional radiograph is currently used in clinical practice to evaluate structural bone changes which are an expression of advanced pathological processes such as sacroiliitis in AS. Radiographic sacroiliitis, which was included in the New York criteria (1984) (4), has long been taken into consideration. Over the past two decades, with the advent and development of new imaging techniques, efforts have been made to diagnose AS and axSpA at ever-earlier stages before structural damage has occurred in order to enhance treatment efficacy (1, 3, 5, 7). MRI, which can detect inflammatory lesions and signs of active processes even before structural bone damage has occurred, is widely used in clinical practice to evaluate patients with LBP and suspected axSpA (8, 12). A positive MRI of the SIJ has recently been included in the ASAS classification criteria, whose definition was based exclusively on the presence of inflammation of SIJ (5, 7, 8, 20). Some studies (12, 21, 22) have recently taken into consideration structural and inflammatory lesions that can be seen on spinal MRIs. The type

of injury most frequently observed in axSpA is BME of the anterior vertebral corners, which is an expression of anterior osteitis (19). Another type of lesion that is detected is the replacement of vertebral angles with adipose tissue (fatty lesions), which seems to be less specific and occur later in SpA (23, 24). The presence of BME at the posterior vertebral corners appears to be highly specific for this disease, but its use as a diagnostic criterion has been limited by low sensitivity. Just as the ASAS/OMERACT MRI study group (8, 12), we analyzed the prevalence and type of inflammatory and structural lesions in a cohort of patients with early stage axSpA. The prevalence of BME lesions in MRIs of the SJI was high in our study, but it was even higher than the prevalence of structural lesions, thus underlining the peculiarity of this method in the visualization of inflammatory findings in comparison to morphological abnormalities. A significant prevalence of BME lesions in the anterior vertebral corners was also observed on MRIs of the spine. Consistent with results reported in previous studies (23-25), the finding highlights the importance of the involvement of the spine from the very first phase of the inflammatory process in axSpA. A high prevalence of other inflammatory signs on MRI linked to enthesitis especially in the thoracic spine were found in our patients suggesting involvement of this district in the early stages of axSpA. From the data reported in the literature (8, 12), it is unclear whether the location of lesions on MRI is also associated to the site of axial pain. A significant association between the site of pain and BME lesions in the thoracic and buttock district was noted in our patients. This result seems to indicate that the location of axial pain (thoracic and buttock pain) could be used as a specific predictor of the presence of inflammatory lesions on MRI. The association between the site of pain and the localization of MRI lesions is less striking with regard to structural damage, which was significant only for buttock pain and structural lesions on MRIs of the SIJ. Several studies (12, 13, 26, 27) have systematically evaluated the concomitant use of spinal and pelvis MRI in patients with suspected SpA and in healthy subjects. One of these (13) demonstrated that the simultaneous evaluation of the spine and the SIJ using MRI can lead to higher diagnostic accuracy. Other authors who have not confirmed this finding (27) sustain that the combined use of spinal and pelvis MRI only moderately increases the diagnostic value in patients with suspected nr-axSpA, because of the inclusion of false positives. In fact, other rheumatic diseases involving the spine such as Scheuermann's disease, spondylodiscitis, erosive osteochondrosis and other degenerative diseases of the intervertebral disc, may show similar patterns on MRI (12). Even the appearance of multiple structural lesions (at least three), just as fatty lesions, increases the probability of axSpA (28), although, according to other studies, the prevalence of these forms of lesions tends to increase with aging and can be present even in healthy individuals or patients affected with other spinal degenerative diseases. Patients with nonspecific LBP and healthy subjects may have some signs suggestive of SpA such as fatty lesions on spinal MRIs (12, 28). According to Weber and colleagues (27) using a MRI of the SIJ alone is less sensitive but more specific than the combined use of the spinal and pelvis MRI, while inclusion of a spinal MRI leads to increased sensitivity and reduced specificity.

The cost and time necessary to carry out MRIs cannot, in any case, be ignored. Nine of the patients (15%) with inflammatory lesions on spinal MRIs showed no abnormalities on SIJ MRIs, while 12 (20%) patients with sacroiliitis on MRI did not present lesions on spinal MRIs. Our data would seem to indicate that the use of spinal MRIs together with SIJ MRIs can add additional, relevant information during both the diagnostic process and the therapeutic follow-up. The current study investigated if there were any differences in the clinical indices of disease activity commonly used in clinical practice in relation to the presence or absence of signs

of sacroiliitis on plain radiographs and on MRIs. Although a significant difference was found in the three cohorts with regard to the prevalence of sacroiliitis on MRIs and X-rays and on the SPARCC SIJ score, we did not find any differences in clinical and disease activity indices. Higher indices were not found in the patients with active sacroiliitis on MRI with respect to those without inflammatory changes in the SIJ or with initial signs of radiographic sacroiliitis. This result may depend on both the early stages of axSpA and the small sample size. In fact, several studies (29-34) have reported higher values of clinical, functional and disease activity indices in r-axSpA, in patients with disease duration of several years with respect to subjects with nr-axSpA. Future studies could examine if there is any correlation between the levels of these indices and the presence/absence of active sacroiliitis in large patient cohorts.

CONCLUSIONS

It has become increasingly urgent to detect axSpA in its earliest stages in order to initiate treatment as early as possible. MRI can detect inflammatory lesions and signs of active disease process even before structural bone damage has occurred. Pelvis MRI has recently been included in the ASAS classification criteria whose positivity is defined on the basis of the exclusive presence of inflammatory signs of SIJ. It remains a matter of debate whether the inclusion of inflammatory and structural lesions of spinal MRIs in the ASAS classification criteria could help to identify patients with suspected axSpA. A high prevalence of inflammatory lesions on MRIs of the SJI and of the spine (anterior osteitis and enthesitis) were found in our patients. As inflammatory lesions on spinal MRIs can occur in the absence of SIJ involvement, the use of spinal MRIs together with SIJ MRIs may add additional, relevant information to the diagnostic process, especially with regard to nr-axSpA patients without signs of sacroiliitis on MRI. A standard radiograph of the pelvis continues to be a crucial step in the diagnostic investigation in patients with suspected axSpA, especially in those with a longer history of symptoms. Studies on the involvement of the thoracic spine, which has until now never been considered by methods scoring spinal structural damage, are warranted. A significant involvement of the thoracic region was noted in the patients studied.

Conflict of interest: the authors declare no potential conflict of interest.

Contributions: ML, manuscript drafting, data analyzing, acquiring and interpreting; RR and LP, study conceiving and design, data processing and manuscript drafting. ST and CLC, performing of spine and pelvis X-rays and MRI, images reading; FO, statistical analysis, data analyzing and interpreting. PF and AO, data acquiring. All the authors made substantive intellectual contributions to the study, reviewed the article, and gave the final approval of the version being submitted.

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Evaluation of Th9 lymphocytes in peripheral blood of rheumatoid arthritis patients and correlation with anti-tumor necrosis factor therapy: results from an *in vitro* pivotal study

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SUMMARY

The aim of this study was to determine the prevalence of T helper 9 (Th9) lymphocytes in rheumatoid arthritis (RA) patients and to identify a possible association between the percentage of Th9 and the discontinuation of a biological treatment with an anti-tumor necrosis factor (TNF) (infliximab).

We collected peripheral blood mononuclear cells (PBMCs) from 55 consecutive RA outpatients and 10 healthy controls. Among RA patients, 15 were not receiving any immunosuppressive drug, 20 were successfully treated with infliximab and 20 discontinued infliximab because of adverse events or inefficacy and were treated with other biological agents. PBMCs were cultured with/without infliximab 50 mg/L for 18 h, and the percentage of Th9 cells was assessed by means of flow cytometry. Th9 lymphocytes were identified as interferon gamma, interleukin (IL)4-, IL17-, IL9-secreting cluster of differentiation 4 (CD4)+ T cells.

Cytometric analysis revealed no significant decrease in the percentage of Th9 cells after infliximab exposure in any of the groups, although it was lower in healthy controls than RA patients either before and after the infliximab stimulation assay.

Th9 cells are IL-9-secreting T helper lymphocytes whose role in RA is still poorly known. IL-9 levels are increased in RA patients, in whom this cytokine plays a crucial role. Th9 cells are the major producers of IL-9, and their prevalence is higher in RA patients than in healthy subjects; however our experiment *in vitro* does not demonstrate an association between Th9 lymphocytes and the response to infliximab. Further studies are required to evaluate the real involvement of Th9 population in the immunogenicity of anti-TNF agents.

Key words: *Infliximab; immunogenicity; Th9 lymphocytes; rheumatoid arthritis.*

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease affecting up to 1% of worldwide population with a progressively disabling course. RA is characterized by chronic synovitis of peripheral joints, mainly ruled by the uncontrolled activation of cells belonging to the adaptive immunity.

Cluster of differentiation 4 (CD4)+ T lymphocytes play a central role in the induction and in the maintenance of the disease, due to their complex interplay with dendritic cells, macrophages, and B-lymphocytes. According to the cytokine milieu, CD4+ T lymphocytes may differentiate into distinct phenotypes, each of which plays a precise role in inducing, tuning and repressing the immune response. Over the past twenty years, new acquisitions in the immunologic field led to the definition of five distinct T helper (Th) cell populations: Th1 cells mainly secreting interferon gamma (IFNγ-), Th2 cells mainly secreting interleukin (IL)-4, Th17 cells secreting IL-17 and involved in autoimmune diseases, Th22 cells secreting IL-22 and Th9 cells secreting IL-9 (1). A counterpart of T cells with repressive properties, named T regulatory cells (Tregs), was also described. In RA, T helper lymphocytes mostly give rise to Th2 or Th17 responses (2). Th9 are a novel pool of T

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helper lymphocytes, almost implied in allergic diseases. There is little knowledge about the role of Th9 in RA. Indeed, IL-9 is increased in serum and synovial fluid of RA patients. A recent study demonstrated that Th9 cells are responsible for the degree of lymphoid organisation in the synovial membrane of RA patients, being also related to the titre of anti-cyclic peptide antibodies (ACPAs) (3). However, due to their peculiar property of orchestrating both Th17 and Tregs responses, Th9 cells own a more complex function in the immunological network at the basis of RA. Furthermore, no study has still attempted to clarify the implication of these cells in the response to a biological treatment with an anti-tumor necrosis factor (TNF) agent. In a previous study on RA patients (4) we demonstrated that the stimulation *in vitro* of peripheral blood mononuclear cells (PBMCs) with infliximab could induce a paradoxical activation of Th1 and Th17 lymphocytes while repressing T regulatory cells in those patients who had failed the treatment with infliximab. Accordingly, we hypothesized that Th9 lymphocytes may vary in response to infliximab, conditioning the final outcome of the therapy. The primary objective of our study was therefore to evaluate the prevalence of Th9 lymphocytes in the peripheral blood of RA patients compared with a group of matched healthy controls.

The secondary objective was to detect the association between the percentages of Th9 cells and the clinical response to a TNF-blocker (infliximab), following a stimulation test *in vitro*.

MATERIALS AND METHODS

We enrolled 55 consecutive RA outpatients diagnosed according to the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) 2010 criteria (5). Patients were recruited from our previous study (4). This cohort included 15 subjects affected by early arthritis not concurrently treated with immunosuppressive drugs, 20 patients successfully treated with infliximab, 20 patients who had swapped to biologic drugs other than infliximab due to adverse events or inefficacy, and a matched control group of 10 healthy subjects.

Demographic characteristics are resumed in Table I. Patients were concomitantly treated with prednisone ≤10 mg/day (at a stable dose within 4 weeks since blood sampling); methotrexate ≤15 mg/week, sulfasalazine ≤3 g/day, hydroxychloroquine ≤400 mg/day, all provided that were administered at stable dose in the 6 weeks before the blood sample. In the group of non responder, the patients were treated with intravenous (i.v.) abatacept 10 mg/kg every 4 weeks (13 subjects), i.v. tocilizumab 8 mg/kg every 4 weeks (5 subjects), subcutaneous (s.c.) etanercept 50 mg once a week (1 subject), or s.c. certolizumab pegol 200 mg every other week (1 subject), as second (8 patients), third (8 patients) or fourth biologic line (4 patients). Patients with concomitant infections, atopic dermatitis, haematological disorders, treated with leflunomide or cyclosporine, or receiving vaccinations in the previous 2 months were excluded. The study was approved by a local Ethical Committee and all the procedures were performed in accordance with the Declaration of Helsinki.

After giving an informed consent, peripheral blood from each subject was collected into Vacutainer tubes containing EDTA (Becton Dickinson; Rutherford, NJ, USA). PBMCs were isolated by centrifugation on lymphocyte separation medium (Cedarlane Laboratories, Burlington, NC, USA). The number and viability of PBMCs were determined by an automatic cell counter, ADAM-MC (Digital-Bio, NanoEnTek Inc., Seoul, Korea). PBMC viability was typically >98%.

Cell cultures were performed in RPMI 1640 plus penicillin, streptomycin, l-glutamine and 10% pooled Human AB Serum [all from Euroclone, Siziano (PV), Italy]. PBMCs, at a concentration of 1×10^6 cells/mL were incubated for 18 h with culture medium alone or in the presence of 50 µg/mL infliximab (Remicade, Janssen Biologics, Leiden, Netherlands), or 50 µg/mL recombinant Human IgG Fc (R&D Systems, Minneapolis, MN, USA). Pulse-width mod-

Table I - Demographic characteristics of the study population.

Variables	Healthy controls	Treatment-naïve RA patients	RA patients responding to IFX	RA patients not responding to IFX
No.	10	15	20	20
Mean age±SD, years	43.9±8.3	54.8 ±16.2	61.3±12.2	57.0±12.2
Mean disease duration±SD, years	/	2.3±3.9	13.4±7.2	18.1±9.5
F/M	4/6	12/3	16/4	15/5
ACPA+	/	5	15	15
RF+	/	7	11	15
ANA+	/	2	12	17
Anti-dsDNA Ab+	/	0	3	2
Anti-ENA Ab+	/	0	1	3
ACLA/LAC+	/	0	1	2
Prednisone (2.5-10 mg/day)	/	/	8	14
Methotrexate (5-15 mg/week)	/	/	20	9
Hydroxychloroquine (200-400 mg/day)	/	/	3	5
NSAIDs	/	14	As needed	As needed

RA, rheumatoid arthritis; IFX, infliximab; SD, standard deviation; F, females; M, males; ACPA, anti-citrullinated-protein antibodies; RF, rheumatoid factor; ANA, anti-nuclear antibodies; anti-dsDNA, anti-double stranded DNA antibodies; anti-ENA, anti-extractable nuclear antigen antibodies; ACLA, anticardiolipin antibodies; LAC, lupus anticoagulant; NSAIDs, non-steroidal anti-inflammatory drugs.

ulation (lectin from *Phytolacca americana*; 1 μg/mL; Sigma-Aldrich, St. Louis, MO, USA) was used as a positive control to evaluate the responsiveness of PBMCs. To facilitate co-stimulation, 1 μg/mL anti-human CD28 (R&D Systems) was added to the cell cultures. Brefeldin A (10 μg/mL; Sigma-Aldrich) was added after the first three hours in order to inhibit cytokine secretion.

The percentage of Th9 lymphocytes was determined by flow cytometric analysis. Th9 lymphocytes were identified as IFNγ-, IL-4-, IL-17-, IL-9 secreting CD4+ T cells (6).

A concentration of infliximab of 50 μg/mL was chosen after setting a titration test with increasing concentrations of the drug and on the basis of the infliximab median serum concentrations one hour after infusion (peak serum concentration: 39.9-219.1 μg/mL) (7).

The following monoclonal antibodies (mAbs) were used: CD4 PE-Cy7, IFNγ (Beckman Coulter, Milan, Italy), IL17 PerCP-Cy5.5 (Biolegend, San Diego, CA, USA), IL-9 APC and IL-4 PE (R&D Systems). PBMCs were incubated 15 minutes with the mAbs for the detection of cell surface antigens and fixed with 1% paraformaldehyde (PFA). Then cells were permeabilized with Saponin (Sigma) and stained with the antibodies for the detection of intracellular cytokines. Following a 45-minute incubation in ice, the cells were fixed with 1% PFA.

Lymphocyte population was gated based on the basis of forward and side scatter properties, and further gated for CD4 expression; at least 20,000 events were acquired within the CD4 gate. The samples were acquired using a Gallios flow cytometer and data were analysed using Kaluza software (both Beckman Coulter).

As data were normally distributed, procedures were based on parametric analyses. Comparisons between the different groups were performed using unpaired Student's *t* test for unequal variances with a two tailed P value. Significance was set at $P<0.05$. Statistical analysis was performed using GraphPad Prism Software (GraphPad Software, San Diego, CA, USA).

RESULTS

Our cohort included 20 consecutive RA patients (16 females; mean age 61.3±12.2 years; mean disease duration 13.4±7.2 years) successfully treated with infliximab 3 mg/kg every eight weeks, and 20 consecutive RA patients (15 females; mean age 57.0±12.2 years; mean disease duration 18.1±9.5 years) who discontinued infliximab due to inefficacy (11 cases) or adverse events (9 cases). Fifteen patients in both the two groups were positive for ACPAs, and respectively 11 responders and 15 non-responders were positive for rheumatoid factor (RF). During infliximab treatment, we detected anti-nuclear antibodies (ANAs) in 12 responders and 17 non-responders without any clinical involvement (Table I). The patients in both treated groups concomitantly received the allowed disease-modifying antirheumatic drugs and corticosteroids.

Cytometric analysis revealed no significant decrease in the percentage of Th9 cells after infliximab exposure in any of the groups, but percentage of Th9 cells was lower in healthy controls than RA patients both before and after the stimulation assay with infliximab (Figure 1).

The higher frequency of Th9 cells in the patients was not associated with higher levels of anti-nucleus auto-antibodies or other auto-antibody subsets, or with a higher likelihood of experiencing an adverse event or lack of efficacy on infliximab treatment.

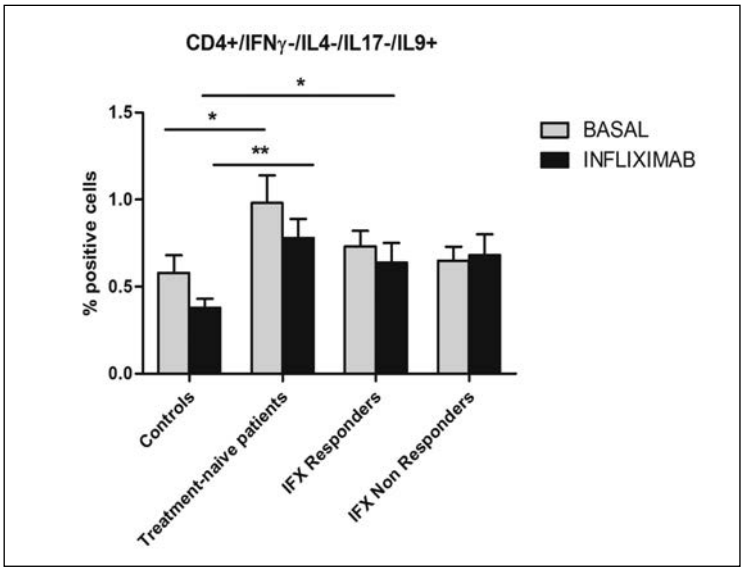

Figure 1 - The percentage of gamma interferon (IFNγ)-, interleukin (IL)4-, IL17-, IL9-secreting CD4+ T cells in rheumatoid arthritis patients and healthy controls under unstimulated and infliximab (IFX)-stimulated conditions. Mean values ± standard error. *P<0.05; **P<0.01 (Student's *t* test).

DISCUSSION

In our work we aimed firstly to evaluate the percentage of Th9 lymphocytes in peripheral blood of RA patients. Secondly, we performed an immune-stimulation assay *in vitro* in order to detect a possible relationship between Th9 cells and the outcome of a biologic therapy (infliximab). Th9 cell, discovered in 2008 by Veldhoen and colleagues, are a T helper cell subset developing from primary naïve T helper lymphocytes or from primed T helper 2 lymphocytes in presence of IL-4, TGFβ, OX40 and PU-1 (8). The main cytokine secreted is IL-9, but, *in vitro,* these cells may also produce IL-10, IL-17, IL-21 and IL-22 (9). It has been demonstrated that Th2 cells, Th17 cells, Treg cells, innate lymphoid cells, mast cells, natural killer cells are capable of releasing large amount of IL-9 (10). However, Th9 cells are a distinct class of CD4+ T lymphocytes, identified by a peculiar set of transcriptional factors (PU.1, IRF-4). The role played by Th9 cells has been investigated *in vivo* and *in vitro*. Th9 lymphocytes seem to be involved in the immunological responses underlying parasitic infections as well as allergic diseases (11, 12). A consistent number of reports has evidenced that these cells are highly represented in the airways of asthmatic subjects as well as in the skin of people suffering from atopic dermatitis (13, 14). Recent reports on melanoma tumor models have evidenced that Th9 lymphocytes enhance the intra-tumor expression of chemokines and their receptors such as CCL20 and CCR6, thus promoting the recruitment of dendritic cells and anti-tumor CD8+ T lymphocytes (15).

The role of Th9 cells in rheumatic diseases is less characterized. According to some

studies, IL-9 may contemporarily favor the differentiation of Th17, acting like IL-6 or IL-21 in the presence of TGFβ, and inhibit the apoptosis of Treg cells, by enhancing the expression of STAT5. Mice knock-out for *IL-9R* gene develop a more aggressive form of experimental autoimmune encephalomyelitis when compared to wild types, mainly due to an over-expansion of Th1 lineage (16). Similar results were obtained in a recent report that analysed the cytokine interplay in human skin samples and murine models of atopic dermatitis, finding an indirect regulation of IL-9 on IFNγ expression (17). On the other hand IL-23 and IL-21 represent inhibiting cytokines for the development of Th9 lineage. IL-9 mRNA and IL-9R have been found significantly increased in gut specimens from ulcerative colitis patients, where this cytokine may activate neutrophils and epithelial cells, delaying ulcers healing (18). In the gut, Th9 cells are involved in the defence against parasitic infections and an increase in this T helper subset may represent a link between dysbiosis and autoimmune inflammatory bowel diseases (11). One study on connective tissue diseases reported that IL-9 serum levels were increased in systemic sclerosis but not in systemic lupus erythematosus or dermatomyositis, being inversely correlated with the degree of lung fibrosis (19). This result may be interpreted in the light of the pleiotropic effects of IL-9, promoting at the same time the expansion of Treg and Th17 lymphocytes (Figure 2) (20).

To our knowledge, there are actually few studies on the involvement of IL-9 in RA. One study analysed by multiple suspension array the serum cytokine profile of 6 RA patients undergoing rituximab treatment after failing a previous therapy line with infliximab. The authors found increased levels of IL-9 at baseline and at follow-up times in responder patients and concluded that IL-9 may be considered as a predictive marker of response to rituximab (21). Another

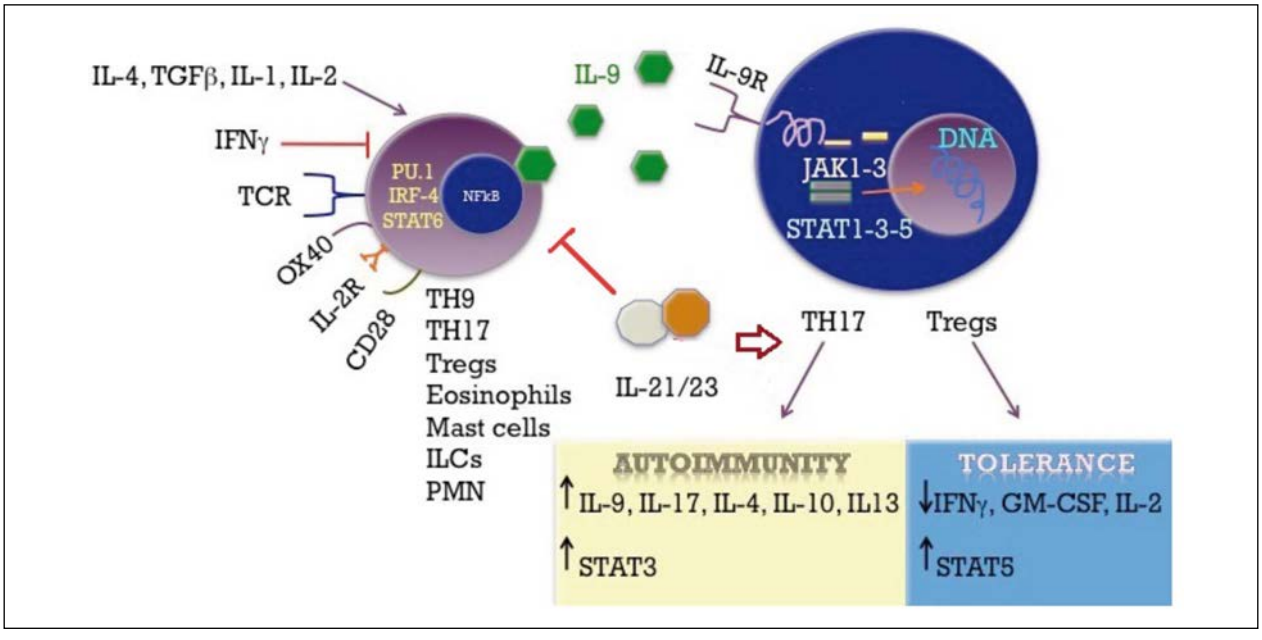

Figure 2 - The network of interleukin (IL)-9 and IL-9 producing cells. Th9 lymphocytes are induced following the stimulation with IL-4, TGFβ, IL-1, IL-2 and the interaction of TCR, OX40 and CD28 with their ligandos; on the contrary, IFNγ, IL-21 and IL-23 inhibit their differentiation. Th9 cells express specific transcriptional factors (PU.1 and IRF-4) that favor the activation of NFkB. Beyond Th9 lymphocytes, Th17 cells, T regulatory cells, eosinophils, mast cells, innate lymphoid cells (ILCs) and polymorphonucleates (PMN) may produce IL-9. IL-9 interacts with its receptor (IL-9R), composed by a specific α- and a common γ chain, thus activating an intracellular pathway that promotes the transcription of specific genes involved in survival of either Th17 (implied in autoimmunity) and T regulatory cells (implied in immune-tolerance).

study on 44 subjects showed an increased serum concentration of IL-9 in first-degree relatives of RA patients, which was associated to a higher ratio of auto-antibodies positivity, such as RF or ACPAs (22). A recent report on RA, psoriatic arthritis (PsA) and osteoarthritis (OA) patients showed an augmented concentration of IL-9 in blood and synovial fluid of RA and PsA subjects than in OA ones. Furthermore the treatment of magnetically sorted synovial and blood CD3+ T cells with recombinant IL-9 induced their expansion only in RA and PsA samples, underlining the hyper-expression of *IL-9R* on cells coming from patients suffering from autoimmune arthritis (23). Finally, a recent histological study on synovial membrane biopsies from RA patients evidenced an augmented expression of Th9 lymphocytes in the most aggressive forms of disease and an association between these cells and the organisation of the lymphoid centres or the synthesis of ACPAs (3). In line with these data, our results showed an increased percentage of Th9 cells in the peripheral blood of RA patients when compared to healthy controls, but no difference was noticed before and after the incubation of PBMCs *in vitro* with infliximab. A limit of the study was that we did not sorted Th9 lymphocytes according to the specific transcriptional factor profile (PU.1 or IRF-4), perhaps including other T CD4+ subsets in the pool of Th9 producing cells.
However, these data may also reflect the bipolar effects of these cells, favoring at the same time the survival and the activation of Th17 and Treg lymphocytes. Due to their ambivalent behavior, Th9 cells would be therefore not suitable for predicting the response or the failure to a specific treatment in RA patients. Figure 2 resumes the complexity of the role of Th9 cells played in the immune network.

CONCLUSIONS

In conclusion, IL-9 levels are increased in RA patients. Th9 cells are the major producers of IL-9, and their amount is higher in RA patients than in healthy subjects; however our experiment *in vitro* does not demonstrate an association between Th9 lymphocytes and the response to infliximab. IL-9 producing cells restore a balance between tolerance and inflammation and, according to our results, cannot be considered a reliable marker in predicting the response to anti-TNF agents. However our study has some limits and the actual knowledge about the biologic effects of these cells in RA is still incomplete and requires further investigations.

Conflict of interest: the authors declare no potential conflict of interest.

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Real-world experiences of folic acid supplementation (5 *versus* 30 mg/week) with methotrexate in rheumatoid arthritis patients: a comparison study

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SUMMARY

The objective of this study was to compare the tolerability of methotrexate in two different regimes of folic acid (FA) supplementation in rheumatoid arthritis (RA). We performed a multicenter, cross-sectional observational cohort study on 240 RA patients with 120 patients each in 5 mg of FA weekly and 30 mg of FA weekly supplementation.

There were no significant differences for side effects (14.2 versus 22.5%, P=0.523) and discontinuation of methotrexate (3.6 versus 13.3%, P=0.085). RA patients given 5 mg of FA weekly supplementation had a lower disease activity score 28 compared to 30 mg of FA weekly supplementation [3.44 (1.10) versus 3.85 (1.40), P=0.014].

FA supplementation of 5 mg per week and 30 mg per week was associated with similar tolerability of methotrexate in RA patients.

Key words: *Folic acid; methotrexate; rheumatoid arthritis; disease activity score 28; treatment regimes.*

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INTRODUCTION

Methotrexate (MTX) is the anchor treatment for rheumatoid arthritis (RA), whether as a monotherapy or as a combination therapy (1, 2). However, MTX is associated with significant number of side effects, which can lead to discontinuation of treatment. It has been reported that mild toxicity occurred in about 60% of patients, and roughly 7 to 30% of patients discontinued MTX therapy within the first year of treatment because of toxicity (3). MTX side effects range from gastrointestinal (nausea, vomiting, abdominal pain), stomatitis, transaminitis, bone marrow suppression and alopecia.

The current major guidelines suggest folic acid (FA) supplementation in MTX treated RA patients to reduce side effects of MTX and to improve drug survival. In patients with RA treated with MTX, administration of 5 mg of folic or folinic acid/week is recommended, separating the intake of it from MTX by 24 h (4). The recently published FOLVARI Study concluded that there was no additional benefit (or harm) of a higher dose of folic acid (30 mg/week) over a usual dose (10 mg/week) in a clinical trial setting (5). A recent review suggests that low dose FA (≤7 mg per week) can reduce gastrointestinal side effects, hepatic dysfunction and discontinuation of MTX treatment (3). On the other hand, another study has shown that the use of FA supplementation may reduce the efficacy of MTX and increases the dosage requirement of MTX (6). There is no consensus for the optimal dosing or administration of FA supplementation in MTX treated RA patients. Therefore, the search for the optimal dosing and administration of FA supplementation can potentially benefit RA patients receiving MTX therapy.

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The aim of our study is to compare the tolerability of MTX in RA patients assuming 5 mg versus 30 mg of FA weekly in a real world setting. Specifically, we compared the side effects of MTX (gastrointestinal side effects, hepatic dysfunction, pulmonary fibrosis, infection and others) and discontinuation of MTX between the 2 folic acid regimes in real-world settings.

MATERIALS AND METHODS

Study design

We conducted a cross-sectional observational cohort study of 240 patients with a diagnosis of RA who received treatment at Sarawak General Hospital, Miri Hospital and Tuanku Ja'afar Hospital. There were 120 patients each in 5 mg of folic acid weekly and 30 mg of folic acid weekly supplementation. In Sarawak General Hospital and Miri Hospital, the practice of folic acid supplementation was 5 mg weekly. Patients were given folic acid 5 mg one day after they took their MTX. In Tuanku Ja'afar Hospital, folic acid supplementation was 30 mg weekly. Patients were given folic acid 5 mg daily except on the day they took their MTX (total 30 mg/week). This study was approved by Malaysian Research Ethnics Committee.

Sample size

The study was designed to have statistical power of 0.8 and P-value of 0.05. Since there were no previous studies that directly compared these 2 doses of FA supplementation, we used the estimated incidence of gastrointestinal side effects for MTX with folic acid supplementation and placebo, which was 9 and 29.2% (7). Thus, to detect a 20% difference for our primary outcome, we needed 54 patients in each arm of our study.

For the second outcome of discontinuation of MTX, we used the MTX discontinuation rate due to toxicity, which ranges from 7-30% (3). In order to detect a 23% difference, we needed 44 patients in each arm of our study. There was no missing data in our population. Hence, a sample size of 240 was adequately powered for the study.

Patients

We recruited 240 consecutive RA patients who fulfilled inclusion and exclusion criteria during their clinic visit from April 1 to June 30, 2014. Data collection was stopped once 120 patients were recruited in each group. Patients were included into the study if they were diagnosed with RA based on the 1987 revised American College of Rheumatology (ACR) criteria (8), more than 18 years old and received MTX with FA supplementations in their treatment regimes. We excluded patients who had history of gastrointestinal disease (peptic ulcer disease, gastritis, gastroesophageal reflux syndrome), liver disease (viral hepatitis, autoimmune hepatitis and alcoholic liver disease), hematological disease causing cytopenias, mixed connective tissue disorder and overlap syndrome.

Data collection

We collected demography data and clinical data of RA based on face-to-face interviews and case notes review. Clinical data on RA included date of diagnosis, treatment regimes (current dose of prednisolone, other disease modifying anti rheumatic drugs (DMARDs) and biologic DMARDs, non-steroidal anti-inflammatory drugs, other analgesics, anti-emetics, H2-anatagonists and proton pump inhibitors) and IgM rheumatoid factor. Dosages of FA and MTX were identified from the patient's prescription. Adherence to FA and MTX were quoted as good or poor based on patient's self report of adherence. Adherence rate of 80% was used as a cut off point for good adherence. Data on MTX included the date of starting, date of discontinuation, reason for discontinuation, current dose, and side effects [including methotrexate intolerance severity score (MISS)].

MISS is a validated questionnaire to determine the severity of gastrointestinal side effects of MTX (9). It consists of 4 domains: abdominal pain, nausea, vomiting and behavioral symptoms. Symptoms are assessed by after MTX, anticipatory (before taking MTX) and associative (thinking of MTX). Symptoms were graded as 0 (no symptom), 1 (mild symptom), 2 (moderate

symptom) and 3 (severe symptom). It has a sensitivity of 88% and specificity of 80% in diagnosing MTX intolerance with a cut-off score of ≥6, including at least 1 anticipatory, associative or behavioral symptom. Assessment of disease activity of RA was scored using disease activity score 28 (DAS 28), a continuous measure consisting of the number of tender and swollen joints in a 28 joint count, erythrocyte sedimentation rate and patient global health as measured on a visual analogue scale of 100 mm (10). DAS 28 was applied by physicians and rheumatologists trained in performing DAS 28 assessment.

Statistical analysis

Statistical analysis was performed using IBM® SPSS® Statistics version 20 (IBM Corp., Armonk, NJ, USA). Quantitative variables were examined for normal distribution prior to statistical analysis. Normal quantitative variables were compared using independent Student's t test and results were expressed as mean (standard deviation). Qualitative variables were compared using chi-square test and results were expressed as number (percentage). When the conditions of validity of the chi-square test were not met, it was replaced by the Fisher's exact test. The significance level was set at 5% for all tests used.

RESULTS

Baseline characteristics of patients

Baseline characteristics are summarized in Table I. There was no significant difference in the baseline characteristics between the 2 folic acid regimes except for higher percentage of prednisolone usage (65.8 versus 20.8%, P<0.001) and lower usage of biologic DMARDs (0 versus 7.5%, P=0.002) in 5 mg folic acid weekly group. There was no significant difference in the mean dose for methotrexate (mg/week) between the 2 groups [13.54 (4.03) versus 13.81 (4.24),

Table I - Baseline characteristics.

	5 mg 1 day after MTX n=120	5 mg daily except on the day of MTX n=120	P-value
Age, mean (SD), years	52 (11.6)	53 (11.4)	0.282*
Female gender, n (%)	101 (84.2)	100 (83.3)	0.861°
Use of NSAIDs, n (%)	66 (55.0)	63 (52.5)	0.698°
Use of anti-emetics, n (%)	1 (0.8)	1 (0.8)	1.00#
Use of H2-Antagonists, n (%)	7 (5.8)	5 (4.2)	0.554°
Use of PPI, n (%)	16 (13.3)	12 (10)	0.421°
Use of analgesics, n (%)	26 (21.7)	23 (19.2)	0.631°
Adherence to FA, n (%)	117 (97.5)	111 (92.5)	0.076°
Adherence to MTX, n (%)	116 (96.7)	109 (90.8)	0.062°
MTX dose, mean (SD), mg/week	13.54 (4.03)	13.81 (4.24)	0.612*
Prednisolone, n (%)	79 (65.8)	25 (20.8)	<0.001°
Other DMARDs, n (%)			0.759°
None	43 (35.8)	37 (30.8)	
Hydroxychloroquine	26 (21.7)	32 (26.7)	
Sulfasalazine	15 (12.5)	15 (12.5)	
Leflunamide	11 (9.2)	9 (7.5)	
Sulfasalazine + HCQ	17 (14.2)	21 (17.5)	
Leflunamide + HCQ	8 (6.6)	5 (4.2)	
Sulfasalazine + Leflunamide	0 (0)	1 (0.8)	
Use of Biologic DMARDs, n (%)	0 (0)	9 (7.5)	0.002#

MTX, methotrexate; SD, standard deviation; NSAIDs, non-steroidal anti-inflammatory drugs; PPI, proton-pump inhibitors; FA, folic acid; DMARDs, disease modifying anti rheumatic drugs; HCQ, hydroxychloroquine. *Independent Student's t test; °Chi-square test for independence; #Fisher's exact test.

Table II - Tolerability of methotrexate.

	5 mg 1 day after MTX n=120	5 mg daily except on the day of MTX n=120	P-value
MISS score ≥6, n (%)	0 (0)	5 (4.2)	0.060*
Side effects of MTX, n (%)			0.523°
No	103 (85.8)	93 (77.5)	
Gastrointestinal	12 (10)	18 (15)	
Hepatitis	3 (2.5)	5 (4.2)	
Pulmonary	1 (0.8)	1 (0.8)	
Infection	1 (0.8)	3 (2.5)	
Discontinuation of MTX, n (%)	8 (6.7)	16 (13.3)	0.085°

MTX, methotrexate; MISS, methotrexate intolerance severity score. *Fisher's exact test; °Chi-square test for independence.

P=0.612, 95% confidence interval (CI) –1.323, 0.781].

Tolerability of methotrexate

The tolerability of MTX was summarized in Table II. There was no significant difference noted on the side effects (gastrointestinal, hepatitis, pulmonary and infection) of MTX (14.2 versus 22.5%, P=0.523). MTX intolerance measured by MISS score of ≥6 was not significantly different between the 2 groups (0 versus 4.2%, P=0.060). There was no significant difference on the discontinuation of MTX (6.7 versus 13.3%, P=0.085). Analysis of tolerability of MTX according to baseline char-

Table III - Logistic linear regression model for tolerability of methotrexate.

Variables	β (ΣE)	OR	95% CI	P-value
Gastrointestnal side effects of MTX				
Age	–0.049 (0.018)	0.952	0.919, 0.986	0.006
Female gender	0.278 (0.547)	1.321	0.452, 3.860	0.611
Dose of folic acid 30 mg per week	–0.716 (0.480)	0.489	0.191, 1.252	0.136
Dose of mehotrexate	–0.005 (0.050)	0.995	0.902, 1.098	0.927
Usage of prednisolone	–0.180 (0.483)	0.836	0.324, 2.153	0.710
Usage of biologic DMARDs	0.834 (1.131)	2.304	0.251, 21.151	0.461
Any side effects of MTX				
Age	–0.038 (0.016)	0.963	0.934, 0.993	0.015
Female gender	0.653 (0.434)	1.921	0.821, 4.496	0.132
Dose of folic acid 30 mg per week	–0.682 (0.409)	0.506	0.227, 1.127	0.095
Dose of mehotrexate	–0.030 (0.042)	0.970	0.893, 1.055	0.480
Usage of prednisolone	–0.110 (0.412)	0.896	0.400, 2.007	0.789
Usage of biologic DMARDs	–0.226 (0.786)	0.798	0.171, 3.724	0.774
Discontinuation of MTX				
Age	–0.025 (0.020)	0.975	0.938, 1.014	0.205
Female gender	0.990 (0.513)	2.691	0.984, 7.362	0.054
Dose of folic acid 30 mg per week	–0.877 (0.545)	0.416	0.143, 1.211	0.108
Dose of mehotrexate	0.029 (0.055)	1.030	0.924, 1.146	0.596
Usage of prednisolone	–0.175 (0.532)	0.840	0.296, 2.384	0.743
Usage of biologic DMARDs	–0.192 (0.906)	0.825	0.140, 4.873	0.832

SE, standard error; OR, odd ratio; CI, confidence interval; MTX, methotrexate; DMARDs, disease modifying anti-rheumatic drugs.

Table IV - Efficacy of methotrexate.

	5 mg 1 day after MTX n=120	5 mg daily except on the day of MTX n=120	P-value	95% CI
DAS-28 score, mean (SD)	3.44 (1.10)	3.85 (1.40)	0.014*	-0.741, -0.084
TJC, mean (SD)	1.71 (3.86)	3.41 (4.82)	0.003*	-2.811, -0.589
SJC, mean (SD)	1.52 (2.40)	1.49 (2.05)	0.919*	-0.540, 0.599
VAS, mean (SD)	26 (18)	37 (23)	<0.001*	-1.598, -0.535

MTX, methotrexate; CI, confidence interval; DAS-28, disease activity score 28; SD, standard deviation; TJC, tender joint count; SJC, swollen joint count; VAS, visual analogue scale. *Independent Student's t test.

acteristics showed younger patients were more likely to have gastrointestinal side effects of MTX [odds ratio (OR)=1.05, P=0.006] and any other side effects of MTX (OR=1.04, P=0.015). Other baseline characteristics did not influence the tolerability nor discontinuation rate of MTX as shown in Table III.

Disease activity

Disease activity of our RA patients was measured by the parameters of DAS28 score, tender joint count (TJC), swollen joint count (SJC) and visual analogue scale (VAS), as presented in Table IV. RA patients given 5 mg of folic acid weekly supplementation had a lower DAS28 score compared to those given 30 mg of folic acid weekly supplementation [3.44 (1.10) versus 3.85 (1.40), P=0.014, 95% CI –0.741, –0.084]. There was also a lower TJC (1.71 versus 3.41, P=0.003, 95% CI –2.811, –0.589) and lower VAS (26 versus 37, P<0.001, 95% CI –1.598, –0.535) in folic acid 5 mg weekly group. SJC was not significantly different between the 2 groups (1.52 versus 1.49, P=0.919).

In view of the presence of multiple confounding factors that can potentially affect DAS28 score, multiple linear regression was calculated to predict DAS28 score based on dose of folic acid, dose of prednisolone, dose of methotrexate and usage of biologics (Table V). A significant regression model was found with dose of folic acid, dose of prednisolone and dose of methotrexate, F (3, 236) =19.600, P<0.001, adjusted R2=0.189. There was no significant association for the usage of biologics (P=0.557). Usage of 30 mg of folic acid weekly was associated with 0.693 increment of DAS28 score (P<0.001, 95% CI 0.360, 1.025).

DISCUSSION

Our study showed that both 5 and 30 mg FA weekly supplementation was well tolerated and efficacious in MTX-treated RA patients in clinical settings. There was no significant difference for side effects and discontinuation of MTX in both arms. Our findings were consistent with findings from other studies that showed FA supplementation reduced side effects and toxicities of MTX and increased MTX drug survivals (3, 6). The finding was also consistent with another study that showed no differences in the tolerability and discontinuation of MTX in both high and low dose of folinic acid

Table V - Multiple linear regression model for disease activity score 28.

	Adjusted b* (95% CI)	Standard error	β	t-stat	P-value
Constant	1.788 (1.245, 2.331)	0.276		6.486	<0.001
Methotrexate dose	0.091 (0.054, 0.128)	0.019	0.288	4.840	<0.001
Prednisolone dose	0.112 (0.059. 0.165)	0.027	0.276	4.195	<0.001
Usage of folic acid	0.693 (0.360, 1.025)	0.169	0.266	4.106	<0.001

The dependent variable was DAS-28 score. Adjusted R2=0.189; the model reasonably fits well; model assumptions are met; there is no interaction between independent variables, and no multicollinearity problem). *Adjusted regression coefficient.

supplementation (11). The FOLVARI study showed that both 10 and 30 mg weekly FA supplementation had the same tolerability and MTX drug survival rates. Previous study by Morgan and colleagues showed that low dose FA (5 mg/week) and high dose FA (27.5 mg/week) had the same MTX toxicity (11). Compared to that study, our study population had a higher dose of MTX (median 15 versus 7.5 mg/week). Higher dose of FA supplementation (30 or 27.5 mg/week) probably has no additional benefit on the tolerability and discontinuation of MTX.
A prospective study of 434 patients conducted by van Ede and colleagues showed that there was a higher MTX dose in RA patients receiving FA than placebo (18 and 14.5 mg/week) (12). However, our study did not show significant difference in the average MTX dose. The authors concluded that FA decreased the efficacy of MTX and higher dosages of MTX were needed for the same clinical response. Alternatively, it was postulated that co-administration of FA may allow the use of higher dose of MTX to achieve a better disease control before side effects were encountered (13, 14).
The efficacy of MTX was not reduced in RA patients receiving low dose FA supplementation (≤7 mg per week) compared to placebo (3). However, post hoc analysis study done by Khanna and colleagues showed a significant reduction of MTX efficacy in RA patients receiving folic acid compared to placebo (6). There is no consensus regarding guidelines for dose and frequency of FA supplementation in RA patients receiving MTX (14). Our study showed that FA supplementation at 5 mg per week had a lower DAS28 score compared to FA supplementation at 30 mg per week. Patients receiving 30 mg of FA weekly supplementation had a lower efficacy of MTX than 5 mg FA weekly supplementation in clinical setting. This is in contrast to the finding of the FOLVARI study that showed that both 10 and 30 mg weekly FA supplementation had similar efficacy data.
The dosage of MTX in our study was higher than previous studies that showed no difference in the efficacy of MTX-treated patients receiving folic acid. The median MTX dose in the previous studies was 7.5 mg/week (11, 15).
These studies were done during the time that higher dosage of MTX was not commonly used. Post hoc analysis study by Khanna and colleagues using average MTX dose of 12 mg/week showed a reduction of MTX efficacy in patients receiving folic acid supplement than in those assuming placebo (6). The study by van Ede and colleagues showed a higher final MTX dose was required in patients receiving folate supplement than placebo (12). All recent studies of FA supplementation in RA did not include high dose FA supplementation as this regime has fall out of favor and no longer practiced in most Western countries (3).
Interestingly, we found that younger patients were more likely to have side effects of MTX compared to older patients. Further study on the relationship of age with side effects of MTX would be able to clarify this finding.
The results from our current study should be interpreted with caution, as it was a cross-sectional observational study with selection bias, recall bias and poor recovery of old data. The baseline characteristics for both arms of our study were similar except for usage of prednisolone and biologic DMARDs.
The higher usage of prednisolone in 5 mg of FA weekly can potentially lower the DAS28 score. However, multiple regression analysis after adjusting confounding factors for dose of prednisolone, dose of MTX, and usage of biologics showed significant association between dose of folic acid and DAS28 score, with increment of 0.693 in DAS28 score for patients using 30 mg of folic acid per week. The second limitation in our study is the validity of the measurement of disease activity. Our study only collected data at one time point; hence we were unable to compare the current DAS28 score to baseline DAS28 score. A randomized-controlled trial comparing the different doses of folic acid supplementation will be able to provide more substantial clinical evidence.

CONCLUSIONS

FA supplementation of 5 and 30 mg per week was associated with similar tolerability of methotrexate in RA patients. However, folic acid supplementation of 30 mg per week was associated with lower efficacy of methotrexate compared to folic acid supplementation of 5 mg per week in real world settings.

Conflict of interest: the authors declare no potential conflict of interest.

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Idiopathic granulomatous mastitis with erythema nodosum and polyarthritis

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SUMMARY
A 25 year-old woman presented with a painful mass in the left breast, polyarthritis and erythema nodosum. Fine needle aspiration cytology led to a diagnosis of granulomatous mastitis. Oral prednisolone rapidly improved the arthritis and the erythema nodosum. Granulomatous mastitis is a very rare, chronic inflammatory disease and only ten patients with granulomatous mastitis with erythema nodosum and polyarthitis have been described.

Key words: *Erythema nodosum; Rheumatology; Arthritis.*

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INTRODUCTION

Idiopathic granulomatous mastitis (IGM) is a rare benign inflammatory disease of the breast, commonly affecting women in the reproductive age group. Patients typically present with a firm breast mass, associated with inflammation of the overlying skin. Nipple retraction, peau d'orange-like changes, and axillary adenopathy may be present. Extramammary manifestations of IGM are extremely rare. We report a rare association of IGM with erythema nodosum and polyarthritis in a 25 year-old female, who was successfully treated with systemic glucocorticoids.

CASE REPORT

A 25 year-old woman presented with pain and swelling in the left breast for one month with yellowish discharge from nipple. Three weeks later she developed bilateral pain, swelling and redness of ankles, knees, elbows, wrists and proximal interphalangeal joints with low-grade fever. She also noticed painful red nodules in the anterior aspect of both legs and forearm. Examination showed tender axillary lymphadenopathy, erythematous palpable discrete nodules over both limbs and forearm and a swelling of 5×4 cm in the inner upper quadrant of the left breast, which was tender and firm. Hemoglobin was 10.5 gm% (normocytic normochromic), total leucocyte count 12,700 cells/mm3, with 79% neutrophils, platelet 3.2 Lakhs/mm3 and erythrocyte sedimentation rate 64 mm/1st hr. Renal function tests, liver function tests, and urinaysis were all normal. Her blood culture revealed no growth and chest radiography was normal. Fine needle aspiration cytology of the breast swelling showed collection of epitheloid cells, multinucleated giant cells and non-caseous granuloma (Figure 1).

No organisms were seen on gram, and acid-fast bacilli stainings. Fungal, mycobacteria tuberculosis (TB) and atypical mycobacteria cultures were negative. Tuberculin skin test was negative.

Anti-citrullinated cyclic peptide, antinuclear antibody, P and C antineutrophil cytoplasmic antibodies were all negative. Serum angiotensin converting enzyme level and serum calcium were normal. Causes of granulomatous breast disease, such as TB, fungal infections, sarcoidosis, Wegener granulomatosis were ruled out. Thus, a diagnosis of IGM with erythema nodosum and polyarthritis was made and she was started on oral corticoste-

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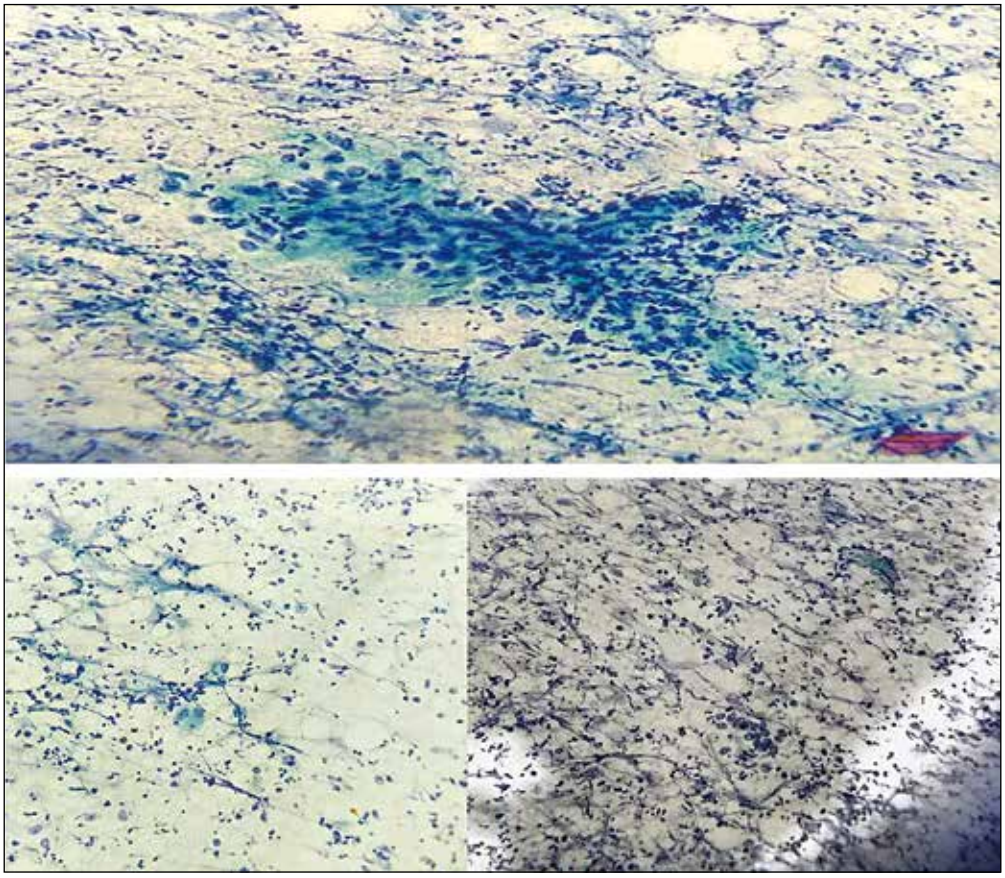

Figure 1 - Photomicrograph of the breast tissue showing a non-caseous granuloma composed of epithelioid cells and multinucleated giant cells (Papanicolaou stain 40x).

roids. When she returned for review after one month she was symptom-free and a follow-up ultrasonogram of the breast showed a significant decrease in size of the breast lump.

DISCUSSION AND CONCLUSIONS

IGM is a very rare disease that was originally reported by Kessler and Wolloch in 1972 (1). It is a rare benign inflammatory disease of women in the reproductive age group that may clinically mimic breast carcinoma (2). The diagnosis is by exclusion of other causes of granulomatous inflammation by special stains in tissue sections and by microbiological investigation.

Granulomas in the breast are caused by a wide variety of diseases, all present in less than 1% of the breast biopsies (3). Two types of IGM are identified: idiopathic and specific. Specific granuloma may be seen in TB, mycotic infections parasitic infestations and sarcoidosis (4). The distribution of granulomas, caseation necrosis, fungal special stains and serological tests help to differentiate these conditions. The presence of an exclusively granulomatous inflammatory reaction with neither caseous necrosis nor any specific organism and the presence of numerous epithelioid cells as well as the multinucleated Langerhans-type giant cells, neutrophils, lymphocytes, and stromal cells will confirm the diagnosis of IGM (5).

The mechanism of development of IGM is believed to involve the following sequence: ductal epithelial damage, transition of luminal secretions to the lobular connective tissue, local inflammation in connective tissue, macrophage and lym-

phocyte migration to the region, and local granulomatosis inflammatory response (6). However, the trigger factor in the development of epithelial damage has not been clarified. Autoimmunity, pregnancy, lactation, hyperprolactinemia, oral contraceptive use, local trauma to the breast, smoking, are believed to be trigger factors in IGM etiology (7).

Polyarthritis and erythema nodosum are rare systemic manifestations of granulomatous mastitis. In 1987, Adams and colleagues reported the first case of granulomatous mastitis complicated by arthritis and erythema nodusum (8). Although cases of granulomatous mastitis complicated solely by erythema nodusum are occasionally reported, as far as we know, only ten other cases of granulomatous mastitis complicated by both arthritis and erythema nodosum have been previously reported. Response of IGM to steroids (9), and the occurrence of extramammary involvement such as erythema nodosum or arthritis support an autoimmune component in the etiology.

In endemic TB regions like our country, a painful breast mass with systemic manifestations like erythema nodosum and arthritis is suggestive of a diagnosis of breast TB. Diagnosis should be confirmed by histopathological findings, culture as well as molecular detection of mycobacterium TB using polymerase chain reaction.

Complete resection or open biopsy and corticosteroid therapy are the choice of treatment for IGM. Our patient was started on oral steroids and had marked improvement in her symptoms and reduction in the size of breast lump.

Conflict of interest: the authors declare no potential conflict of interest.

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Isolated oculocutaneous sarcoidosis in a teenage male: a rare case report

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SUMMARY

Sarcoidosis is an inflammatory disease with multisystem involvement characterized by the presence of non-caseating granulomas. It can affect virtually every organ of the body, with lung involvement being most common occurring in >90% of patients. Other organs affected are skin, eye and liver. Skin involvement is common, affecting 25-35%. Here we present a rare case of a 15 year-old male with isolated oculocutaneous sarcoidosis without systemic involvement.

Key words: *Sarcoidosis; Non-caseating granulomas; Oculocutaneous granulomatosis.*

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INTRODUCTION

Sarcoidosis is a multisystem granulomatous disorder of unknown etiology characterized by presence of non-caseating granulomas. The main organ affected is the lung. 30% of patients have extra pulmonary sarcoid with involvement of lymph node, liver and spleen. Sarcoidosis has variable prevalence worldwide ranging between 30 and 60 per 100,000 people. It mainly affects young adults and is uncommon to be diagnosed in patients younger than 18 years.

CASE REPORT

A 15 year-old male patient, non-smoker, non-alcoholic, vegetarian, presented with chief complaints of skin lesions on the dorsal aspect of both hands since 2 months that were reddish in color, not associated with pain, ulcerations or photosensitivity. There was no history of fever, cough with or without expectoration, night sweats, loss of appetite or weight, shortness of breath, weakness, joint pains, hair fall, oral ulcer, dry eyes, photophobia, watering eye or decreased vision.

Skin examination revealed erythematous patches on the dorsum of the hands, which were multiple, well defined, soft, flat, with smooth surface, and variable size varying from 1.5×1.5 to 4×4 cm (Figure 1A). On laboratory investigations complete hemogram, renal and hepatic functions were within normal limits.

Erythrocyte sedimentation rate was 20 mm/hr and of 1st hour and Mantoux test was negative. Further biochemical investigations revealed corrected serum calcium of 9.1 mg/dL, serum phosphate 3.5 mg/dL and serum alkaline phosphatase 83 U/L. Serum acetylcholine esterase (ACE) level was elevated with an absolute value of 140 U/L (8-65 U/L). Anti-nuclear antibodies and other autoimmune disease markers like antineutrophil cytoplasmic antibodies (c ANCA, p ANCA), antibodies to double-stranded DNA (anti dsDNA), anti SS-A(Ro) antibodies and anti SSB(La) antibody were negative. Chest X ray, ultrasound of the abdomen, pulmonary function test and echocardiography were normal.

Slit lamp examination of the eye showed granulomatous iridocyclitis, mutton fat precipitates, iris nodules with posterior synechiae (Figure 1B). Fundus examination was normal. High-resolution computed tomography (HRCT) and contrast enhanced computed tomography of the chest and magnetic resonance of the brain were also normal (Figure 2A, B). On bronchoalveolar lavage (BAL) CD4:CD8 ratio was

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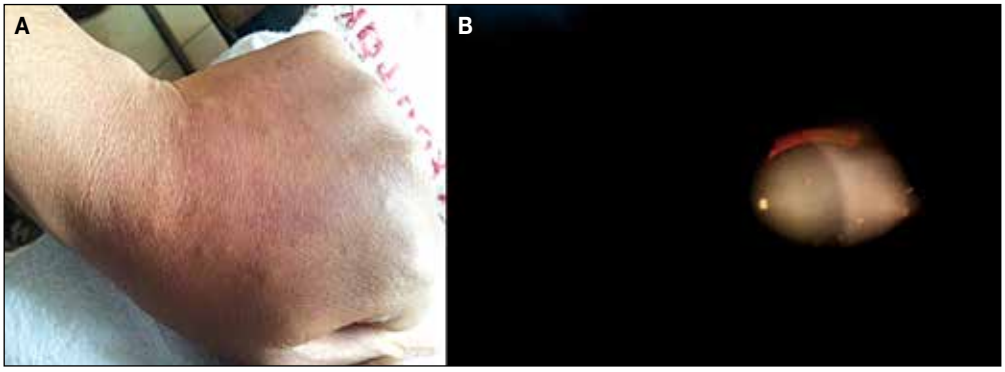

Figure 1 - A) Multiple, well defined, soft, flat, smooth surface, variable size (from 1.5×1.5 to 4×4 cm), erythematous lesions and patches on dorsum of hand; B) slit lamp examination of eye.

<2 ruling out any pulmonary involvement. Skin biopsy revealed mildly atrophic epidermis with upper dermis showing mild perivascular lympho-mononuclear infiltrates and deep dermis revealed naked non-caseating epithelioid cell granulomas, which consist of aggregates of epithelioid histiocytes, giant cells and mature macrophages without significant lymphocytic infiltrate and absence of Langerhans giant cells. Staining for acid-fast bacillus was negative (Figure 3).

The patient was treated with topical steroids for skin and ocular lesions. After one month of therapy there was partial improvement in skin lesions and the iridocyclitis was in healing phase. The patient was continued on therapy and kept on regular follow up to monitor for other systemic involvement.

DISCUSSION

Sarcoidosis is a multisystem disorder of unknown etiology characterized by presence of non-caseating granulomas. It occurs worldwide with highest prevalence in the Scandinavian population. The highest annual incidence of sarcoidosis has been observed in northern European countries (5 to 40 cases per 100,000 people) (1). It commonly affects young adults with a second peak in incidence occurring around age 60. It mainly involves lung and up to 30% patients present with extra pulmonary sarcoidosis (2, 3). Lesions of sarcoidosis

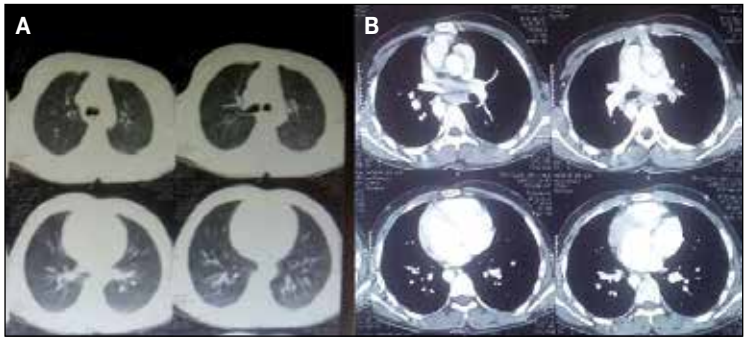

Figure 2 - High-resolution computed tomography and contrast enhanced computed tomography of the chest.

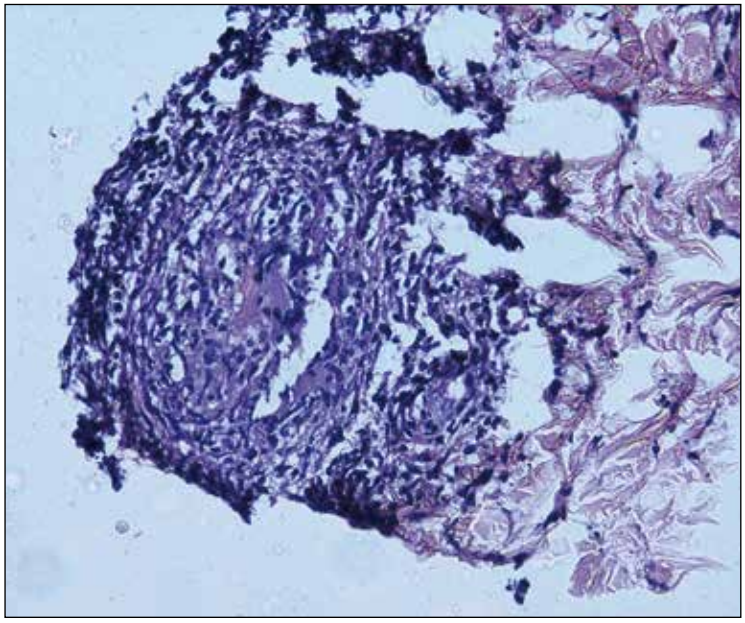

Figure 3 - Acid-fast bacillus test of a skin biopsy.

manifest in the lung in form of bilateral hilar lymphadenopathy, peribronchial thickening and reticulo-nodular changes. Patient can be asymptomatic, detected incidentally on routine X ray or they may present with chronic dry cough. The most common skin lesions are erythema nodosum, plaques, maculopapular eruptions, subcutaneous nodules, and lupus pernio (4). Skin lesions are often overlooked, as these are chronic and not painful.

The most common involvement of the eye is anterior uveitis, but retinitis and pars planitis can also occur. Chronic anterior uveitis is usually associated with iris nodules. Although Busacca nodules are true granulomatous lesions found on the iris, a Koeppe nodule is a granulomatous lesion found only on the pupillary margin. It may become the nidus for posterior synechiae (2). These patients can present with photophobia, increased lacrimation and blurred vision or they can be asymptomatic even with active inflammation. Such patients are at risk of losing their vision if early diagnosis is not made. Other organs are less commonly involved in sarcoidosis. Pathologically, sarcoidosis is characterized by formation of non-caseating granulomas in the affected organ, which are formed as a result of activation of cellular immune response to antigen exposure.

The exact etiology behind sarcoidosis is unknown but there are hypotheses suggesting a role for environmental factors initiating an immunological response in a genetically susceptible individual. Some viral and bacterial infections like mycobacteria, Epstein-Barr virus and human herpesvirus-8 are implicated.

The initial inflammatory response is an influx of T helper cells associated with an accumulation of activated monocytes. Using the HLA-CD4 complex, antigen-presenting cells present an unknown antigen to the helper T cell, which leads to their activation. T-helper cells produce chemokines and cytokines, which cause amplification of cellular immune response and migration of mononuclear phagocytes and other inflammatory cells into the tissues (5-7).

The diagnosis of sarcoidosis is based mainly on clinical characteristics, radiological findings, histopathology and exclusion of other non-caseating granuloma forming conditions (8).

Certain criteria have been proposed to support the diagnosis of definite or probable ocular sarcoidosis in a patient with known sarcoidosis (9). On the other hand, like in our case, patients may present with ocular findings compatible with sarcoidosis but no symptoms from extra ocular disease. In this situation, several groups have tried to identify features that support ocular sarcoidosis, including roentgenographic findings on HRCT or cellular elements in the BAL. However, the gold standard for definitive diagnosis remains biopsy confirmation. This approach has been supported by one retrospective case-controlled study of Japanese patients (10). Hematological and biochemical tests may show normal erythrocyte sedimentation rate, anemia, lymphocytopenia, mildly increased liver enzymes, hypercalcemia and increased serum angiotensin enzyme levels. Serum chitotriosidase is a sensitive and reproducible biomarker of sarcoidosis as well as a reliable bio indicator of disease severity and is increased in over 90% of patients with active disease (11).

The diagnosis is further supported by the histological appearance of the non-caseating epitheloid cell granulomas in affected organs or tissues. Schaumann bodies, which contain calcium oxalate crystals, are found in 48 to 88% of the patients. Asteroid or satellite bodies are interesting occasional findings seen in less than 2 and 9% of the cases (12).

Cutaneous lesions of sarcoid are often missed because they mimic many common skin conditions like lupus vulgaris, psoriasis, lichen planus etc. Cutaneous sarcoidosis has been reported rarely from India with a much lesser incidence in childrens (13). Similarly isolated ocular involvement is very uncommon in sarcoidosis. Ocular lesions, if missed, can cause severe visual impairment or even blindness in the absence of adequate treatment. Uveitis precedes the non-ocular signs of sarcoidosis

in approximately 30%. So a high degree of suspicion is necessary for diagnosing this condition because early diagnosis and intervention can prevent significant morbidity and mortality. These patients are highly likely to develop other organ involvement so they must be kept on regular follow up for timely detection and intervention as required.

The optimal treatment of sarcoidosis depends on the location of the disease and severity of involvement. The mainstay of treatment is corticosteroids. For refractory cases, methotrexate has been found to be effective. Ocular and cutaneous lesions are usually treated with topical steroids and have good prognosis with treatment. Systemic steroids are indicated in optic neuritis and topical treatment resistant posterior uveitis.

Our patient presented with characteristic findings on eye examination along with confirmation on skin biopsy and biochemical evidence of increased ACE levels after exclusion of tuberculosis. The present case demonstrated an isolated oculocutaneous sarcoid without pulmonary involvement in a teenage male, which has not been reported the literature. These cases, later on, can progress to involve respiratory system thus leading to increased morbidity, hence highlighting the issue of repeated follow-up to prevent pulmonary and ophthalmological complications.

CONCLUSIONS

Sarcoidosis is a great emulator of many dermatological diseases since it has a variable presentation. Great awareness about this presentation of sarcoidosis helps in early diagnosis as most of these patients progress to have systemic involvement giving us an opportunity to instate early therapy.

Conflict of interest: the authors declare no potential conflict of interest.

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Possible relationship between certolizumab pegol and arrhythmias: report of two cases

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SUMMARY

It is still unknown whether there is an association between the use of certolizumab pegol (CZP) in rheumatic patients and the onset of cardiac arrhythmias. We describe the cases of two patients with rheumatoid arthritis (RA) treated with CZP as the first-line biological drug and methotrexate (MTX), who developed an arrhythmic event. The first was a 60-year-old, hypertensive male smoker, the second a 66-year-old dyslipidemic female non-smoker. Both were diagnosed as having RA in 2010, and started treatment with MTX plus CZP. The first patient developed undatable atrial fibrillation, which was resistant to pharmacological treatment and electrical cardioversion. The second patient developed an atrial flutter, which was treated with a beta-blocker. In both cases, we set a cautious interval between two consecutive administrations of CZP and, in the first case, also reduced the dose of MTX without any worsening of RA activity. Although many studies have shown that tumor necrosis factor (TNF)-alpha plays a pathogenetic role in inducing an arrhythmogenic substrate that is apparently rescued by anti-TNF drugs, there is still a lack of conclusive data. We suggest caution in any patient developing a cardiac event (including rhythm disorders) during treatment with a conventional or biological disease-modifying anti-rheumatic drug.

Key words: *Anti-TNF drugs; heart diseases; rheumatoid arthritis; arrhythmia.*

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INTRODUCTION

The anti-tumour necrosis factor-alpha (anti-TNFα) agents approved for the treatment of rheumatoid arthritis (RA) and spondyloarthritis (SpA) are biological drugs with well-characterized safety and efficacy profiles. Certolizumab pegol (CZP) is a pegylated antigen-binding fragment of a humanized monoclonal antibody against TNFα that is licensed for use in patients with RA or SpA (1, 2). Because of its molecular structure, CZP rapidly reaches inflamed joints and provides symptomatic relief from the time of its first administrations (3).

TNFα plays a crucial role in remodelling acutely or chronically damaged heart tissue. However, administering an anti-TNFα agent in patients with cardiac disorders may be simultaneously useful and detrimental as cases of precipitating heart failure and arrhythmias have been reported during treatment with infliximab, etanercept and adalimumab, whose use is therefore contraindicated in patients with New York Heart Association class III and IV heart failure. No data have yet been published about the risk of CZP-induced arrhythmia, which is described as being an uncommon side effect in drug data sheets and pharmacovigilance reports.

We describe two cases of cardiac arrhythmias occurring during treatment with CZP as the first biological line in two RA patients.

CASE REPORTS

Case #1

The first patient was a 60-year-old, hypertensive male smoker whose hypertension had been treated with an angiotensin-converting enzyme inhibitor, a thiazide diuretic (enalapril/hydrochlorothiazide 20/12.5 mg/day), a calcium channel antagonist (lacidipine 4 mg/day) and a beta-blocker (bisoprolol 1.25 mg/day) from the age of

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42 years. He began experiencing peripheral arthralgias in October 2010 and was referred to our center. At the first visit the patient had both wrists and knees tender and swollen and laboratory tests revealed the presence of anti-citrullinated peptide antibodies and rheumatoid factor. A diagnosis of early RA (4) was made and treatment with methotrexate (MTX) 15 mg/week associated with prednisone 5 mg/daily was promptly prescribed, which led to remission lasting for nearly two years. However, in July 2012, the patient experienced an arthritic flare [8 swollen joints, 9 tender joints, erythrocyte sedimentation rate (ESR) = 22 mm 1st h, C-reactive protein (CRP) = 3.4 mg/L, disease activity score on 28 joints (DAS28) = 4.97] and due to the partial efficacy of MTX, after a careful screening for the use of anti-TNF drugs, CZP 200 mg every other week was added in November 2012.

The combined treatment led to a rapid improvement in clinical and laboratory parameters, although it had less effect on auto-antibody titres.

The baseline clinical examination revealed a bradycardic rhythm probably related to the use of the beta-blocker and, in October 2013, about one year after starting CZP, he was admitted to the Emergency Department because of an undatable episode of atrial fibrillation (AF) that failed to respond to anti-arrhythmic treatment with amiodarone and flecainide. Echocardiography revealed left atrial dilatation (48 mm) and moderate mitral and tricuspid valve insufficiency.

The patient immediately started treatment with an oral anti-coagulant (warfarin) and, after three attempts at electrical cardioversion in January, February and March 2014, sinus rhythm was eventually restored. Treatment with amiodarone 100 mg/day was continued until April 2015, when it was stopped by the patient, and AF re-occurred in May 2015.

Meanwhile, given the clinical remission of RA and the potential risk of using an anti-TNF agent during a cardiac event, the interval between CZP injections was extended to three weeks in May 2014 (when the dose of MTX was lowered to 7.5 mg/week) and then to four weeks in October of the same year.

The patient is currently still arrhythmic (slow heart rate AF) and receiving a treatment with bisoprolol 1.25 mg/day and apixaban 10 mg/day. His anti-hypertensive treatment has been maintained, and the continued treatment with MTX 7.5 mg/week and CZP 200 mg every 28 days is still ensuring good control of the rheumatic disease (DAS28=1.79 in June 2015).

Case #2

The second patient was a 66-year-old non-smoker female, who had diet-treated dyslipidemia since 2001. In March 2010, she was diagnosed as having seropositive RA and treatment with MTX 15 mg/week was started. Concomitantly, she reported periodic extra-systoles and anxiety symptoms that were treated with bromazepam as needed. Echocardiography performed in 2010 for a suspected organic heart disease only revealed mitral valve prolapse.

As RA disease activity was only partially controlled (7 swollen and tender joints, ESR=58 mm 1st h, CRP=5.3 mg/L, DAS28=5.62), CZP 200 mg every other week was added in June 2012. This was fairly successful clinimetrically, although there was little change in her auto-antibody titres. In December 2013, an increase in liver enzyme levels led to a reduction in the dose of MTX (7.5 mg every 10 days) and, in May 2015, hydroxychloroquine 200 mg/day was added.

In June 2015, she attended the Emergency Department because of the onset of tachyarrhythmia, which proved to be an atrial flutter. A new echocardiography examination revealed initial left atrial dilatation, and treatment with an oral anticoagulant plus heparin and a beta-blocker (carvedilol 3.125 mg/day) was prescribed.

At the time of her last visit to our centre (June 2015), RA was in remission (DAS28=2.50); the treatment with conventional disease modifying antirheumatic drugs (DMARDs) was maintained, whereas the administration of CZP was tapered to one injection every 21 days. The

patient is currently waiting to undergo radio-frequency ablation of the arrhythmogenic focus.

DISCUSSION

TNFα plays a crucial role in cardiac tissue remodeling. The myocardiocytes of patients affected by heart failure express TNFα receptors and may also synthesize TNFα which induces the activation of nuclear factor kappa-light-chain-enhancer of activated B cells and favors myocardiocyte proliferation and apoptosis, the deposition of extracellular matrix, and the up- or down-regulation of metalloproteases (MMP). It also increases the amount of intracytosolic calcium ions and the production of nitric oxide (5). The systemic inflammation occurring during the course of rheumatic diseases can also affect cardiac tissue and generate histological changes that increase the risk of arrhythmias. There is much evidence to support the pathogenetic role of cytokines such as TNFα and interleukin (IL)-6 in AF (6-8), and systemic inflammation, increased CRP levels and a pro-coagulant state are also risk factors for AF-related thromboembolism (9). In animal models, by binding to TNFRI and TNFRII on atrial myocardiocytes and fibroblasts, TNFα may increase the deposition of extracellular matrix, reduce the expression of connexin-40, and increase the release of MMP, thus finally contributing to atrial remodeling (10).

The use of anti-TNFα agents can be useful in reducing the inflammatory burden and restoring endothelial function, but may simultaneously interfere with left ventricular remodeling or induce the complement-dependent cytotoxicity or antibody-dependent cell-mediated cytotoxicity of myocardiocytes. The neutralization of TNFα induced by administering the monoclonal antibody infliximab to *Trypanosoma cruzi*-infected mice restores cardiac inflammation by increasing the level of IL-10 while the decrease in IL-17 concentrations reduces the deposition of fibronectin, and thus prevents the risk of arrhythmias (11).

TNFα also seems to affect the ion-potential currents of myocardiocytes. Experiments on mice treated with TNFα have highlighted a reduced intake of sarcoplasmic Ca2+, possibly related to the nitrosylation of RyR2 channels (12, 13). This reduction in Ca2+ levels may be responsible for depressed cell contractility and the development of aberrant sparks or hump-like depolarizations, which are risk factors for the occurrence of an arrhythmic event. Patients with ankylosing spondylitis or inflammatory bowel diseases often have a prolonged QT interval (14), which, according to some authors, can be restored by treatment with infliximab (15). Neither of our patients had alterations of the QT interval, as emerged by an electrocardiographic test performed at baseline and after 12 month of treatment wit CZP. Murine models have shown that TNFα may promote exercise-induced atrial remodeling and favor the occurrence of AF, both of which can be prevented by the use of etanercept (16). Moreover, the use of infliximab in RA patients seems to improve echocardiographic findings of diastolic dysfunction and left atrial volumes within the first three months of treatment (17).

A recent review of arrhythmic events in RA patients found an increased risk of sudden cardiac death (often related to malignant ventricular arrhythmias, a prolonged QT interval frequently associated with increased CRP levels, and AF) in comparison with the general population; some of these anomalies may be normalized by treatment with the biological agent tocilizumab (18). The higher incidence of arrhythmic events can be attributed to the detrimental effects of systemic inflammation on the myocardium and the triggering of the autonomous sympathetic system.

Few published reports have highlighted an association between the risk of developing cardiac arrhythmia and the concomitant treatment with a TNFα blocker (19-21), and this association seems to be stronger in RA patients with a prolonged QT interval (22). Accordingly, trials of anti-TNF agents during cardiac failure or post-infarction have not led to any conclusive findings and were stopped prematurely (23).

To the best of our knowledge, there are no

published case reports of arrhythmia during treatment with CZP that, like other anti-TNF drugs, acts by blocking the remodeling effects of TNFα. Although the apoptosis of myocardiocytes is less intense because of the absence of crystallizable fragments, it can be sustained by the activation of the pro-apoptotic pathway via TNFRI and TNFRII, or by means of the mechanism of reverse signaling, which has been demonstrated to be highly active during CZP therapy.

The safety of CZP has been tested in clinical trials and pre-pivotal placebo-controlled studies involving more than 4000 RA patients, and analyses of aggregate data and the information arising from post-marketing surveillance have classified the occurrence of arrhythmias (including AF) as uncommon (24). Furthermore, only three cases of tachycardia were reported in the CZP arm of the CERTAIN trial (25).

Both of our patients had a history of altered sinus rhythm (extrasystoles and bradycardia), and the concomitant use of MTX even at medium-low doses of 7.5-15 mg/week may have contributed to the development of arrhythmias, an association that is supported by some published alerting reports (26). Similarly, the concomitant use of hydroxychloroquine in patient #2 may have been an aggravating factor, even though there are only rare reports of severe heart conduction disorders (bundle branch block/atrioventricular block), during treatment with hydroxychloroquine, with complete remission after its discontinuation (27).

CONCLUSIONS

Our two RA patients experienced cardiac arrhythmias during combined CZP and MTX treatment. The interference of anti-TNF agents with the physiological processes governing the correct function of the sinus node or heart conductance apparatus is still a matter of debate. However, given the number of reports of cardiac events developing during anti-TNF treatment, patients with RA and concomitant cardiac risk factors (*e.g.*, extrasystoles, hypertension, or valvulopathies) should be referred to a cardiologist and assessed before and during treatment. In both cases, the development of the arrhythmic event may be explained by a predisposing organic condition and/or the use of conventional and biologic DMARDs that may have acted as a trigger. Once RA remission has been achieved, consideration should be given to tapering the dose or increasing the interval between two consecutive administrations of the TNFα-blocker. Reducing the conventional DMARD burden may be an alternative choice.

Conflict of interest: the authors declare no potential conflict of interest.

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Critical finger ischemia and myocardial fibrosis development after sudden interruption of sildenafil treatment in a systemic sclerosis patient

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SUMMARY

Systemic sclerosis (SSc) is a connective tissue disease frequently associated with Raynaud's Phenomenon (RP). Among possible pharmacological treatments, phosphodiesterase 5 inhibitors are considered in cases of severe non -responsive RP. We present the case of a male SSc patient wh presented with critical finger ischemia and concomitant appearance of myocardial fibrosis after sudden interruption of sildenafil treatment.

Key words: *Systemic sclerosis; critical ischemia; phosphodiesterase 5 inhibitors; cardiac magnetic resonance imaging; myocardial fibrosis.*

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A sixty-year-old Caucasian man first presented to our center in December 2010 complaining of a 25 years history of Raynaud's phenomenon (RP), skin thickening of fingers and face, esophageal reflux and history of digital ulcerations; he was on atorvastatin and allopurinol for hypercholesterolemia and hyperuricemia, respectively.

On examination he presented calcinosis on the left elbow, sclerodactily with modified Rodnan skin score of 4/51 and facial telangiectasia. The positivity of anti-nuclear and anti-centromere antibodies and a *late* scleroderma pattern on nail fold videocapillaroscopy (1) supported the diagnosis and classification of systemic sclerosis (2). Internal organ baseline assessments excluded pulmonary involvement on chest high resolution computed tomography and lung function tests, while morphologic and functional cardiac abnormalities were ruled out on echocardiography and cardiac magnetic resonance imaging (MRI). The latter showed also absence of delayed contrast medium enhancement. Due to previous intolerance to calcium-channel blockers and refusal to intravenous iloprost, he was started on sildenafil 20 mg three times a day for non-controlled RP.

The treatment was beneficial until April 2011, when he stopped all oral medications for an episode of viral gastroenteritis; two days after he presented to emergency room with a critical ischemia of the right index finger: arterial doppler ultrasound showed completely absent blood flow in the second and third phalanx of the finger.

He was admitted to our unit and started on continuous intravenous iloprost and low molecular weight heparin, with rapid progressive improvement of the ischemic problem. A further arterial Doppler ultrasound showed a recovery of blood flow and repeated cardiac MRI showed delayed gadolinium enhancement at the inferior septum-ventricular junction (Figure 1). This suggested the presence of myocardial fibrosis with non-coronary distribution (patchy fibrosis) and without inducible

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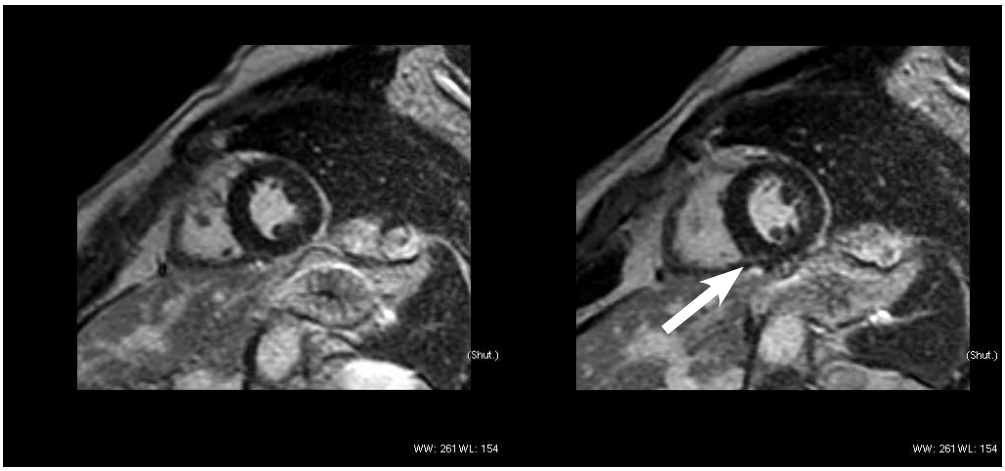

Figure 1 - Baseline (left) and follow up (right) cardiac magnetic resonance imaging with gadolinium enhancement studies images, the latter showing the appearence of delayed gadolinium enhancement (white arrow).

heart ischemia on perfusional miocardial single positron emission tomography later performed (3).

The patient was discharged with all previous medications plus prescription for monthly iloprost infusion and daily low dose aspirin.

To our knowledge, this is the first report of acute peripheral ischemic attack after sudden interruption of phosphodiesterase-5 inhibitors chronic vasodilating treatment (4), which led not only to ischemic finger suffering but also to tissue myocardial modifications (5, 6). In our case, cardiac MRI was useful in detecting early myocardial involvement.

MRI was previously suggested as a screening tool to analyze non-symptomatic scleroderma patients and identify subclinical involvement (7-9). According to the previously proposed hypothesis of *myocardial RP* (10), we believe that the development of myocardial fibrosis could have been the effect of a sufferance of epicardial coronaries due to a prolonged vasospasm (11). The possibility of these two events being caused by a sildenafil withdraw rebound effect was never reported in previous clinical trials and therefore remains unproven.

Conflict of interest: the authors declare no potential conflict of interest.

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Severe psoriatic dactylitis: when psoriasis involves beyond the skin

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To the Editor:

Psoriatic arthritis (PsA) is a distinct form of seronegative inflammatory arthritis occurring in 7-42% of patients with psoriasis (1). It is considered a multifactorial disease that may exhibit different clinical phenotypes such as peripheral arthritis, axial disease, dactylitis and enthesitis (2). Dactylitis is a hallmark manifestation of PsA and a key feature for its diagnosis, which occurs in 30 to 50% patients, commonly in early stages (3). Evidence is increasing that PsA patients may have a significant functional impairment, reduced quality of life and long-term disability (4), and thus recently guidelines have arisen for management of dactylitis (5).

We report an example of a farmer with a severe psoriatic dactylitis with impact on his work productivity and activity due this condition and who presented a satisfactory clinical response only after switching to a biologic agent.

A 57 year-old male presented with a 2 year history of swelling and pain in the dystal and proximal interfalangeal joints of third, fourth and fifth fingers in both hands associated with morning stiffness. Past medical history revealed hypertension controlled with daily enalapril 20 mg and a 30-year history of palmoplantar psoriasis treated with weekly methotrexate 20 mg and topical high-potency steroids. Clinical examination revealed severe dactylitis in digits (*sausage fingers*) associated with ungueal hyperkeratosis and onycholysis (Figure 1). Despite the therapy, erythematous scaly plaques of psoriasis were present in palms and soles. The patient was a farmer and he was unable to work due to symptoms for almost one year.

Although it was originally considered a benign condition, PsA is now recognized as a serious, debilitating, progressive and destructive disease with a significant psychological, social and functional impact. The most prevalent form of PsA is the asymmetric oligoarticular arthritis, which involves small peripheral joints, particularly the distal interphalangeal joints. Thus, disability in the workplace is very common in population with severe PsA (6), and it has a significant effect on the patient's quality of life and financial status. Classification criteria for PsA have set the specificity of dactylitis as one of the most discriminative musculoskeletal manifestation (7, 8), and for yet unclear reasons, the risk for developing erosions in PsA seems to be higher in patients with dactylitis in comparison with those who show only arthritis (9).

Traditionally, therapeutic approaches are not based on well-designed trials, but on empirical experiences. Physicians use drugs such as non-steroidal antinflammatory drugs, local corticosteroid injections, metotrexate, cyclosporine and azathioprine (10). However, controlling symptoms cannot be achieved in all cases and the introduction of anti-tumor necrosis factor (TNF)-alpha therapies is required. Treatment with TNF-alpha inhibitors may result in not only substantial improvements in signs and symptoms of skin and nail diseases, but also improvements in all distinct sites of joint diseases such as axial arthritis, enthesitis and dactylitis (10). Since metotrexate was not effective in our patient, it was discontinued and azathioprine 1 mg/kg was initiated. Three months later, the symptoms were barely controlled and at this point, infliximab 5 mg/kg was started (at 0, 4, 8, and 12 weeks) and the morning stiffness and pain decreased dramatically after the first infusion. With the second infusion, the swelling disappeared. At this

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point, azathioprine was discontinued and a maintenance therapy based only on infliximab was established with infusions every 8 weeks. The patient is back at work and no relapse has been observed during the two years of follow-up. This case highlights the notable impact of dactylitis on work place and quality of life in psoriatic patients and the efficacy of the TNF-alpha therapies in cases where outcomes are not satisfactory with classic drugs. Although efficacy of anti-TNFs seems to be independent of synthetic disease-modifying anti-rheumatic drugs, suggesting only a minor role of combination therapy in PsA (11), we decided to keep the azathioprine in combination with infliximab until the swelling was completely resolved.

The limited data on dactylitis researches warrants the need for randomized trials, with psoriatic dactylitis as a primary outcome, to determine a valid, reliable, and clinical algorithm for PsA patients with dactylitis in order to achieve prolonged remission and prevention of irreversible bone damage.

Conflict of interest: the author declares no potential conflict of interest.

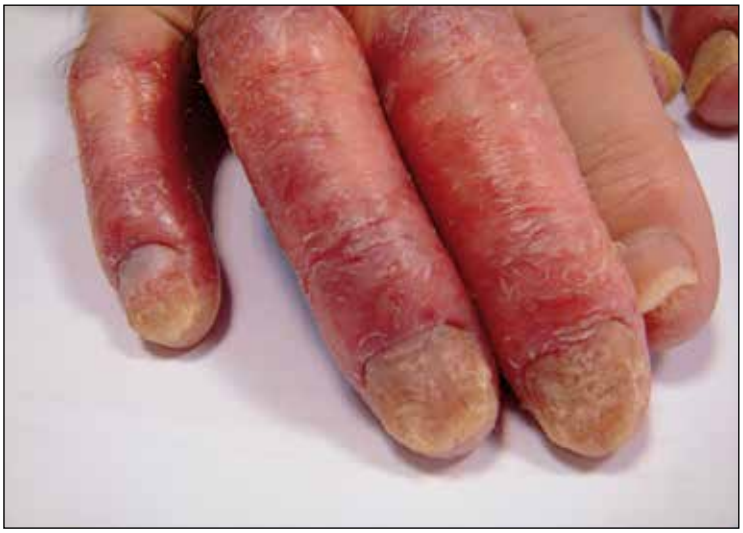

Figure 1 - Dactylitis involving the third, fourth and fifth fingers of the right hand in both distal and proximal interfalangeal joints associated with skin and ungueal affections. Scaly and erythematous psoriatic plaques are observed with onycholysis and ungueal hyperkeratosis.

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Efficacy of high-dose methylprednisolone pulses in a child with noninfectious persistent pleuropericarditis revealing systemic juvenile idiopathic arthritis

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To the Editor,

The combination of persistent polyserositis and fever in children might be referred to systemic juvenile idiopathic arthritis (sJIA), a distinct variant among all forms of pediatric arthritides, as it may be regarded as an acquired autoinflammatory disorder (1).

An 11-year-old boy was hospitalized due to fever started 7 days before, unresponding to ibuprofen and ceftriaxone, with neutrophil leukocytosis (12,810/mm3, N 82.1%), increased C-reactive protein [141 mg/L, normal values (n.v.) <5] and hyperferritinemia (1128 ng/mL, n.v. 21-275). A relevant left pleural effusion combined with a circumferential pericardial effusion was revealed by chest X-ray film and echocardiography, performed due to anterior chest pain and progressive dyspnea. Tachycardia and paraphonic heart tones were also evident. Two days later a chest computed tomographic scan revealed copious bilateral pleural and pericardial effusions. Furosemide was started and ibuprofen (30 mg/kg/day in four doses) continued. After 1 week, due to persistent chest pain, irregular dyspnea and massive left pleural effusion, thoracentesis was performed, removing 400 cc. from patient's left pleural space. The pleural fluid was sterile with low pH, poor cell count, and low glucose level. The child remained still febrile with different fever spikes during the day and tachycardia (135 beats/min) due to his pericardial effusion, though with no clear signs of heart tamponade. Extensive laboratory testing for tuberculosis, infectious, and autoimmune systemic diseases was unrevealing. After 5 days bone marrow aspirate was negative for primary hematological disorders and macrophage activation syndrome, and the boy underwent 2 courses of intravenous immunoglobulins (1 g/kg/day) for two days, without any improvement of his serositis. Fever spikes (higher than 39°C) still persisted in combination with diffuse severe joint pains, and diagnosis of sJIA was postulated after one further week.

High-dose pulsed methylprednisolone (30 mg/kg/day) was then intravenously administered for three consecutive days, followed by prednisone (2 mg/kg/day). Pleural effusion and fibrinous pericardial effusion disappeared completely after 5 days. Prednisone was then slowly tapered in 4 weeks, and naproxen (15 mg/kg/day in two doses) administered for further 6 months. No pleural or pericardial sequelae were observed.

Children with apparently refractory noninfectious polyserositis can be framed in the setting of sJIA, but intravenous immunoglobulins have given conflicting results in these children, differently from other rheumatologic diseases, such as Kawasaki disease (2). Very few cases of pediatric patients with persistent pleural and pericardial disease treated with pulsed methylprednisolone have been reported, mostly in the case they showed macrophage activation syndrome, an ominous complications of sJIA (3).

A study carried out in 1998 assessed the outcome of high-dose methylprednisolone pulses in children with sJIA, reporting clinical improvement only in a minority of

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cases (4). However, methylprednisolone pulses were unsuccessful in a 7-year-old boy with a 4-month history of sJIA who presented a fibrinous pericarditis: this patient improved only after intravenous immunoglobulins (5).

Another previously healthy 5-year-old boy developed pleural and pericardial effusions with cardiac tamponade, as the initial presentation of sJIA, requiring placement of a pericardial drain, but did not respond to pulsed methylprednisolone, as tamponade recurred shortly thereafter; subsequently, the same child required high-dose intravenous immunoglobulin, infliximab, and anakinra to obtain the remission of the pericardial disease (6). Systemic inflammation in sJIA has been associated with dysregulation of the innate immune system, suggesting that its autoinflammatory mechanisms might respond to interleukin-1 inhibition: many recent data suggest that early cytokine blockage might abrogate chronic features of the disease and open a potential window of opportunity in the care of children with sJIA.

Overall outcome data of patients with pleuropericarditis treated with high-dose methylprednisolone pulses are not simple to analyze, especially in children: multicenter trials should be attempted to confirm that high-dose methylprednisolone pulses could be effective on sJIA-related pleuropericarditis before considering more invasive procedures.

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