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HIGHLIGHTS

Mediterranean Journal of Rheumatology June 2018 Issue Highlights

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Mediterr J Rheumatol 2018;29(2):65-6

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In this issue of MJR there are interesting case reports, research protocols and reviews.

Panagopoulos et al.[1] reviewed the role of microRNAs (miRNAs) in the pathogenesis of osteoarthritis (OA) and their potential role as therapeutic targets in this disease. miRNAs are small, single-stranded non-coding RNAs that regulate gene expression at the post-transcriptional level.

Chikanza et al.[2] use a case of a woman with Adamantiades-Behcet disease (ABD), who subsequently developed monoclonal gammopathy of unknown significance (MGUS) to speculate on the mechanisms of development of MGUS and ABD. ABD shares features of autoimmune disease and autoinflammatory disease and the authors elaborate that an early event in B cells, such as IgH translocation, may make them sensitive to growth factors, such as interleukin(IL)-6 which is raised in ABD. Also, the CD56 marker, increased in ABD T cells, is also increased in MGUS plasma cells.

Patients with antiphopsholipid syndrome (APS) may develop angina and myocardial infarction. In a research protocol, Tektonidou et al.[7] will utilize stress perfusion cardiac magnetic resonance in asymptomatic patients with APS to detect myocardial ischemia.

Tsalapaki et al.[8] in a 5-year prospective protocol will study disease course, comorbidities, treatment efficacy and outcome in giant cell arteritis in Greece.

O'Brien et al.[9] in a research protocol will examine longitudinal relationships between sedentary behavior (defined as waking behavior characterized by ≤ 1.5 metabolic equivalents, METS) while in a sitting, reclining or lying position, or light intensity physical activity(1.6-<3.0 METS) with health outcomes in rheumatoid arthritis.

Calcified chest lymph nodes in a patient with systemic sclerosis (SSc) is a rare finding in SSc. Yet, as Angelopoulou et al.[6] pointed out, this finding as well SSc may well be a consequence of silica exposure.

Migkos et al.[5] reported on two patients with Sjogren's syndrome (SjS) who developed polymyositis and inclusion body myositis and identified another 24 cases of SjS with inflammatory myopathies in the literature.

Venetsanopoulou et al.[3] reported on a patient with ankylosing spondylitis who developed systemic sclerosis (SSc) and scleroderma renal crisis. The co-existence of ankylosing spondylitis and SSc is rare.

Patients on immunosuppressants are susceptible to various infections. In this issue, Kostopoulos et al[4] described a patient with RA treated with steroids, methotrexate and adalimumab developed Orf disease, also known as ecthyma contagious; a rare self-limited disease caused by a DNA virus of the parapoxvirus group and transmitted to humans from goats and sheep.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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REVIEW

The Involvement of MicroRNAs in Osteoarthritis and Recent Developments: A Narrative Review

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ABSTRACT

Background: Osteoarthritis (OA) is the most common chronic joint disease and it may progressively cause disability and compromise quality of life. Lately, the role of miRNAs in the pathogenesis of OA has drawn a lot of attention. miRNAs are small, single-stranded, non-coding molecules of RNA which regulate gene expression at post-transcriptional level. The dysregulation of the expression of several miRNAs affects pathways involved in OA pathogenesis. **Objective**: The purpose of this article is to review the literature on the involvement of miRNAs in the pathogenesis of OA and the implications on its diagnosis and treatment. **Materials and Methods**: An extensive electronic literature search was conducted by two researchers from January 2008 to August 2017. Titles and abstracts of papers were screened by the authors for further inclusion in the present work. Finally, full texts of the selected articles were retrieved. **Results**: Abnormally expressed miRNAs enhance the production of cartilage degrading enzymes, inhibit the expression of cartilage matrix components, increase the production of proinflammatory cytokines, facilitate chondrocyte apoptosis, suppress autophagy in chondrocytes and are involved in pain-related pathways. miRNAs are also incorporated in extracellular membranous vesicles such as exosomes and participate in the intercellular communication in osteoarthritic joints. **Conclusion**: Ongoing research on miRNAs has potential implications in the diagnosis and treatment of OA. Their different levels in peripheral blood and synovial fluid between OA patients and healthy population makes them candidates for being used as biomarkers of the disease, while targeting miRNAs may be a novel therapeutic strategy in OA.

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ABBREVIATIONS

3'-UTR: 3'-untranslated region
ADAMTS5: ADAM Metallopeptidase with Thrombospondin Type 1 Motif 5
ATG14: Autophagy related 14
ATG2B: Autophagy related 2B

ATG3: Autophagy related 3
ATG5: Autophagy related 5
Bax: BCL2 associated X, apoptosis regulator Bcl-2 B cell leukemia/lymphoma 2
DR6: Death Receptor 6
FOXO3: Forkhead box O3
GABARAPL1: GABA type A receptor associated protein like 1
GAS5: Growth arrest-specific 5
GWAS: Genome Wide Association Studies
HDAC-4: Histone Deacetylase 4
HDL: High Density Lipoprotein
HIF-1α: Hypoxia-Inducible Factor 1 alpha
HMGB1: High Mobility Group Box 1
IGFBP5: Insulin Like Growth Factor Binding Protein 5
IL-6: Interleukin 6
IL-8: Interleukin 8
iNOS: Nitric Oxide Synthase
IκBα: Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
KPNA3: Karyopherin Subunit Alpha 3
LAMP2: Lysosomal associated membrane protein 2
MAP1LC3: Microtubule-associated protein 1 light chain 3 beta
MCPIP-1: Monocyte Chemoattractant Protein–Induced Protein 1
miRNAs: Micro RNAs
MMP13: Matrix Metalloproteinase 13
MMPs: Matrix Metalloproteinases
mRNA: Messenger RNA
NF-κB: Nuclear Factor-κB
NO: Nitric Oxide
NSAIDS: Nonsteroidal Anti-Inflammatory Drugs
OA: Osteoarthritis
RALA: RAS like proto-oncogene A
SIRT1: Silent Information Regulator 1
SMAD3: SMAD family member 3
SOX9: SRY-box 9
TGF-β: Transforming Growth Factor-β
TGF-β: Transforming Growth Factor-β
TNF-α: Tumor Necrosis Factor alpha
ULK1: Unc-51 like autophagy activating kinase 1
VEGF: Vascular Endothelial Growth Factor

INTRODUCTION

Osteoarthritis (OA) is the most common chronic arthropathy and is characterised by failure of damaged cartilage to repair itself, synovial inflammation and changes in the subchondral bone. Increased production of cartilage-degrading enzymes (Matrix Metalloproteinases, aggrecanases) by articular chondrocytes, insufficient synthesis of cartilage matrix components (collagen type II, aggrecan) and increased chondrocyte apoptosis lead to gradual cartilage loss. Pain and stiffness are the main clinical features of OA. Loss of movement and function are features of more severe disease, resulting in a worse quality of life.[1] The etiology of OA is complex and not fully understood yet. It involves genetic and environmental factors, such as joint injury, obesity and aging.[2]

According to epidemiological and family-based genetic studies, genetic factors seem to be responsible for a significant proportion of OA susceptibility. Heritability has been estimated to be 39-79% depending on the affected joint, gender and severity of diseases. In addition, these studies have shown that OA is a complex polygenic disorder – multiple risk loci contribute to OA heritability, each of which accounts for a small proportion of it.[3] During the last decade, large Genome Wide Association Studies (GWAS) have identified 17 genetic loci for OA,[4-14] but these risk loci do not fully account for OA heritability. Epigenetic modifications may be responsible for OA heritability that remains unexplained by OA genetics. Epigenetics include heritable mechanisms, such as DNA methylation, histone modifications and microRNAs, which regulate gene expression without changes to the DNA sequences.[15-16] MicroRNAs (miRNAs) have attracted a lot of attention lately, since they have the potential to be used as biomarkers or as novel therapeutic agents. Numerous studies have shown that miRNA expression is altered in OA and these alterations possibly contribute to OA pathogenesis. The purpose of this article is to review the literature on the role of miRNAs in the pathogenesis of OA and its implications on diagnosis and treatment of this disorder.

MicroRNAs

MicroRNAs (miRNAs) are small, single-stranded, non-coding RNAs, consisting of 20-25 nucleotides, whose role is post-transcriptional regulation of gene expression. MiRNAs are partially complementary and bind to the 3'-Untranslated Region (3'-UTR) of their target messenger RNA (mRNA). They then inhibit the translation of their target mRNA or cause its degradation. Thus, miRNAs inhibit the expression of their target gene at post-transcriptional level.[17] Concerning the synthesis of miRNAs, primary miRNA is transcribed in the nucleus from its gene (**Figure 1**). Afterwards, ribonuclease Drosha and protein DGCR8 process the primary miRNA to precursor miRNA. The precursor miRNA is transferred to the cytoplasm and ribonuclease Dicer processes it to mature miRNA. The passenger strand of the miRNA is ejected and degraded and the other strand – the mature miRNA – is loaded to protein Argonaute (Ago). The mature miRNA interacts with proteins Ago and GW182, binds to its target mRNA and inhibits its translation.[18]

miRNAs are encoded by DNA sequences which are found in the genome either as separate miRNA genes or within the introns of other genes. Over 3% of hu-

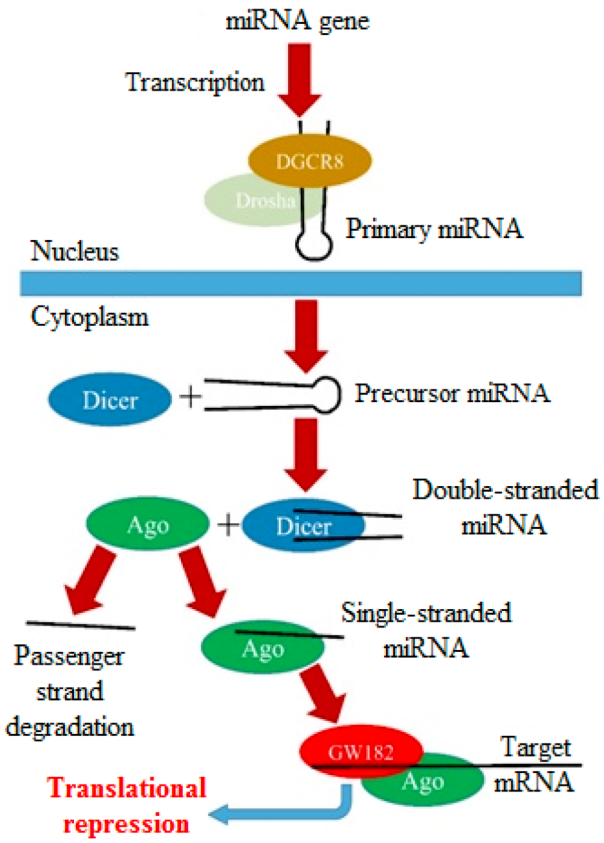

Figure 1. The synthesis of miRNAs and their mechanism of action (adjusted from Miao et al. 2013) (Miao, Yang et al. 2013).
Figure 1 has been redesigned and created from scratch based on the work of Miao et al. 2013, using the Microsoft Office ® PowerPoint software.[20]

man genes have been found to contain miRNA-coding sequences, while the expression of 40-90% of human protein-coding genes is regulated by miRNAs.[19] The expression of a protein-coding gene may be regulated by more than one miRNA and each miRNA may regulate the expression of several target genes.[17] miRNAs participate in many biological procedures, such as cell differentiation, proliferation and apoptosis, and they are involved in several diseases, including cancer, viral infections and autoimmune diseases.[18]
Apart from binding to their target mRNAs and inhibiting their translation, miRNAs may be packaged and transferred extracellularly by three different ways: (a) incorporated in extracellular membranous vesicles (exosomes, shedding vesicles and apoptotic vesicles), (b) bound to lipoproteins, like High Density Lipoprotein (HDL), and (c) bound to RNA-binding proteins, like Argonaute-2 and nucleophosmin-1. These miRNAs are secreted via exocytosis, they may be received by other cells via endocytosis and regulate their gene expression. Thus, miRNAs participate in intercellular communication.[20-21]

The Role of MicroRNAs in OA
A remarkable number of studies have been published during the last few years about the expression of different miRNAs in osteoarthritic cartilage and subchondral bone. Most of these studies examine the expression of miRNAs targeting genes known to participate in the pathogenesis of OA. For this purpose, we have summarized in **Table 1** the miRNAs found to be dysregulated in OA and their target genes.

miR-140 in OA
One of the most studied miRNAs in OA is miRNA-140 (miR-140). The expression of miR-140 in chondrocytes increases during their differentiation, suggesting that it is probably a regulator of the differentiation of these cells. In osteoarthritic cartilage the expression of miR-140 is reduced in comparison to healthy cartilage.[22-23] Target genes of miR-140 include ADAMTS5 (ADAM Metallopeptidase with Thrombospondin Type 1 Motif 5), MMP13 (Matrix Metalloproteinase 13), IGFBP5 (Insulin Like Growth Factor Binding Protein 5) and RALA (RAS like proto-oncogene A).[22,24-26] ADAMTS5 and MMP13 are proteinases that mediate the degradation of several components of cartilage matrix and might play an important role in OA pathogenesis.[22,24] IGFBP-5 (Insulin-like Growth Factor Binding Protein 5) is also involved in OA pathology by modulating the availability of IGF-1 in the joint.[25] RALA (RAS like proto-oncogene A) is a small GTPase that downregulates the transcription factor SOX9 (SRY-box 9). SOX9 is a master regulator of cartilage development and it enhances the production of cartilage matrix components. Downregulation of RALA by miR-140 results in upregulation of SOX9.[26] Concerning the expression of miR-140, the cytokine Interleukin-1β (IL-1β), a key player in OA pathogenesis, inhibits the expression of miR-140 by chondrocytes,[22,24] while the transcription factor SOX9 enhances its expression (27). Moreover, the transcription factor SMAD3 (SMAD family member 3), a mediator of Transforming Growth Factor-β (TGF-β), downregulates miR-140 expression by articular chondrocytes.[28] Therefore, Interleukin-1β (IL-1β) and Transforming Growth Factor-β (TGF-β) inhibit the expression of miR-140 in chondrocytes of osteoarthritic cartilage, resulting in increased expression of ADAMTS5, MMP13, IGFBP5 and RALA and degradation of articular cartilage matrix.[22,24-26,28] In addition, targeted deletion of miR-140 in mice resulted to OA-like changes of articular cartilage, while overexpression of miR-140 in cartilage protected it from antigen-induced arthritis, enhancing

Table 1: MicroRNAs dysregulated in OA and their target genes.

MicroRNA	Target gene	Reference
miR-9	MCPIP1	Makki, Haseeb et al. 2015[54]
	PTRG	Song, Kim, Chun et al. 2013[80]
miR-15a-5p	VEGFA	Chen et al. 2017[81]
miR-16-5p	*SMAD3*	Li L et al. 2015[48]
miR-21	*GDF5*	Zhang et al. 2014[82]
	GAS5	Song et al. 2014[60]
miR-23a-3p	*SMAD3*	Kang et al. 2016[47]
miR-24	*INK4A*	Philipot et al. 2014[39]
miR-26a	iNOS	Rasheed et al. 2016[36]
	KPNA3	Yin et al. 2017[34]
miR-26b	KPNA3	Yin et al. 2017[34]
miR-27b	*MMP13*	Akhtar et al. 2010[37]
miR-29	Smad, NFκB, and canonical Wnt signaling	Le et al. 2016[83]
miR-30a	*ADAMTS5*	Ji, Xu, Zhang et al. 2016[45]
miR-30b	ERG	Li, Yang et al. 2015[84]
miR-33	CCL2	Wei et al. 2016[85]
miR-33a	*SMAD7*	Kostopoulou et al. 2015[86]
mir-34a	SIRT1	Yan et al. 2016[56]
miR-98	-	Wang GL et al. 2016[87]
miR-105	Runx2	Ji, Xu, Xu et al. 2016[44]
miR-122	*IL1A*	Yang et al. 2015[88]
miR-125b	*ADAMTS4*	Matsukawa et al. 2013[46]
miR-127	OPN	Tu et al. 2016[89]
miR-130	*TNFA*	Li ZC et al. 2015[51]
miR-139	MCPIP1	Makki and Haqqi, 2015[53]
	EIF4G2, IGF1R	Hu et al. 2016[90]
miR-140	*ADAMTS5*	Miyaki et al. 2009,[22] Miyaki et al. 2010[29]
	IGFBP5	Tardif et al. 2009[25]
	MMP13	Liang et al. 2012,[24] Liang et al. 2016[91]
miR-142-3p	HMGB1	Wang X et al. 2016[49]
miR-146	*MMP13*	Yamasaki et al. 2009[30]
	MMP13, ADAMTS5	Li et al. 2011[31]
	SMAD4	Li et al. 2012[33]
miR-148	*COL10A1*, MMP13, ADAMTS5	Vonk et al. 2014[43]
miR-149	*TNFA*, *IL1B*, *IL6*	Santini et al. 2014[52]
miR-155	*ULK1*, *MAP1LC3*, *ATG14*	D' Adamo et al. 2016[59]
miR-181	PTEN	Wu et al. 2017[92]
miR-210	*DR6*	Zhang et al. 2015[50]
	HIF-3α	Li Z et al. 2016[38]
miR-222	HDAC-4	Song, Jin et al. 2015[40]
miR-335	-	Tornero-Esteban et al. 2014[93]
miR-370	SHMT-2	Song, Kim et al. 2015[41]
miR-373	MECP-2	Song, Kim et al. 2015[41]
miR-381a-3p	IkBα	Xia et al. 2016[55]
miR-483-5p	*Matn3*, *Timp2*	Wang et al. 2017[77]
miR-488	*ZIP8*	Song, Kim, Lee et al. 2013[42]
miR-558	*COX2*	Park et al. 2013[94]
miR-634	PIK3R1	Cui et al. 2016[95]

Each microRNA is reported with its respective target genes and the bibliographical reference where the information was obtained.

the hypothesis of miR-140 participating in OA pathogenesis.[29] The basic interactions of miR-140 and its target genes is presented in **Figure 2**.

miR-146 in OA

Another miRNA that has been studied in OA is miR-146. Its expression is increased in osteoarthritic cartilage during the early stages of the disease and it gradually de-

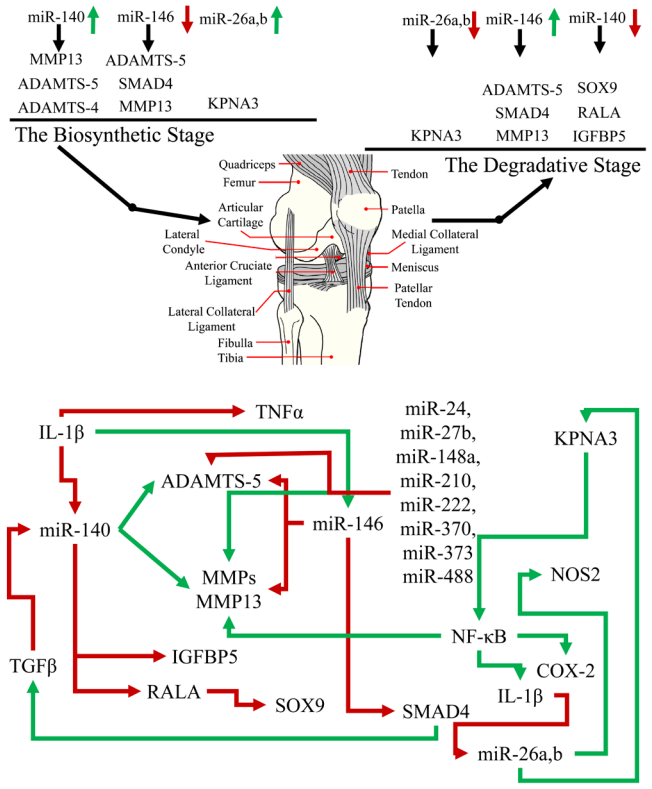

Figure 2. Schematic representation of basic miRNAs and their target genes. It is interesting that similar miRNAs could manifest a stimulatory as well as an inhibitory role in OA. Depending on their expression levels miRNAs play a respective role, either as OA stimulatory or OA inhibitory factors. In the lower sub-figure, the basic interactions are presented between miRNAs and their target genes (**Legend:** in the upper sub-figure, green arrows indicate up-regulation and red arrows indicate down-regulation. In the lower sub-figure, green arrows indicate stimulation or activation and red arrows indicate inhibition).

creases as OA progresses. The target gene of miR-146 is MMP13 and the expression of this miRNA is upregulated by IL-1β. Thus, it seems that miR-146 is a negative feedback regulator of MMP13 and it possibly plays a protective role in OA cartilage.[30] Indeed, miR-146 inhibits IL-1β-induced MMP13 and ADAMTS5 production by chondrocytes and IL-1β-induced suppression of collagen type II and aggrecan, which are components of the cartilage matrix.[31] miR-146 also inhibits IL-1β-induced TNF-α upregulation in OA cartilage.[32] Moreover, miR-146 downregulates the expression of SMAD4, a transcription factor that is a mediator of TGF-β. Thus, upregulation of miR-146 in OA chondrocytes downregulates SMAD4, reduces cellular responsiveness to TGF-β and induces chondrocyte apoptosis. Downregulation of SMAD4 also leads to an increase in the expression of Vascular Endothelial Growth Factor (VEGF), which contributes to inflammation and pathological angiogenesis in OA.[33] Furthermore, altered expression of miR-146 appears to play a role in pain-related pathways in OA. miR-146 is downregulated in dorsal root ganglia and in the dorsal horn of the spinal cords of rats with osteoarthritic pain. miR-146 decreases the expression of pain modulators that enhance pain perception, such as Tumor Necrosis Factor-α (TNF-α), Interleukin-6 (IL-6), Interleukin-8 (IL-8), COX-2 and iNOS, in astrocytes. Thus, it seems that downregulation of miR-146 in the central and peripheral nervous system of the rat OA model mediates osteoarthritic pain.[31] The basic interactions of miR-146 and its target genes is presented in **Figure 2**.

miR-26a and miR-26b in OA

The role of miR-26a and miR-26b in the pathogenesis of OA has been recently studied. The expression of miR-26a and miR-26b is significantly downregulated in cartilage from osteoarthritic joints, while the target gene of these miRNAs has been found to be the one encoding Karyopherin Subunit Alpha 3 (KPNA3). KPNA3 is a mediator of Nuclear Factor-κB (NF-κB) pathway which binds to NF-κB and facilitates its translocation from cytoplasm to nucleus.[34] It is suggested that the NF-κB pathway might play a significant role in OA pathogenesis, since it induces production of proinflammatory cytokines, Cyclooxygenase-2 (COX-2) and metalloproteinases (MMPs), which result in joint inflammation and degradation of joint cartilage.[34,35] Therefore, downregulation of miR-26a and miR-26b in OA cartilage results in upregulation of KPNA3 and NF-κB and production of MMPs and COX-2.[34] In addition, activation of NF-κB pathway negatively regulates the expression of miR-26a, implying a reciprocal inhibition between miR-26a and NF-κB. Moreover, obesity, a well known risk factor of OA, induces the activation of NF-κB, resulting in downregulation of miR-26a expression.[35] On the other hand, another target gene of miR-26a is NOS2, which encodes inducible Nitric Oxide Synthase (iNOS). In OA, activation of iNOS in chondrocytes results in Nitric Oxide (NO) overproduction leading to chondrocyte apoptosis, cartilage degradation and inhibition of matrix synthesis. Thus, IL-1β downregulates miR-26a expression in OA chondrocytes through NF-κB activation, resulting in upregulation of iNOS expression, overproduction of NO and cartilage damage.[36]

MicroRNAs involved in cartilage matrix degradation and joint inflammation in OA

Dysregulation of the expression of several miRNAs in OA results in increased production of cartilage matrix degrading enzymes (MMPs, ADAMTS proteases). Downregula-

tion of miR-24, miR-27b, miR-148a, miR-210, miR-222, miR-370, miR-373 and miR-488 in OA cartilage leads directly or indirectly to an increase in the production of MMPs,[37-43] while downregulation of miR-30a, miR-105, miR-125b and miR-148a results in overproduction of ADAMTS proteases.[43-46] Moreover, upregulation of miR-16-5p and miR-23a-3p leads to upregulation of MMPs and ADAMTS proteases and downregulation of matrix components (type II collagen, aggrecan).[47-48]

Besides, other miRNAs are involved in the production of proinflammatory cytokines like TNF-α, IL-1, IL-6 and IL-8 in OA. Downregulation of miR-142-3p and miR-210 in chondrocytes of OA cartilage leads to overexpression of High Mobility Group Box 1 (HMGB1) and Death Receptor 6 (DR6) respectively. As a result, the NF-κB signaling pathway is activated and the production of TNF-α, IL-1 and IL-6 is increased.[49,50] Downregulation of miR-130a and miR-149 in OA chondrocytes results also in an increase in the production of TNF-α, IL-1 and IL-6.[51-52] Upregulation of miR-9 and miR-139 in chondrocytes of OA cartilage downregulates the expression of Monocyte Chemoattractant Protein–Induced Protein 1 (MCPIP-1), thus promoting IL-6 expression and the apoptosis of chondrocytes.[53,54] Moreover, upregulation of miR-381a-3p in OA chondrocytes inhibits IκBα (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha), resulting in an increase of the production of TNF-α, COX-2, iNOS, IL-6 and IL8.[55]

MicroRNAs involved in apoptosis in OA

Aberrant expression of miRNAs facilitates the apoptosis of articular chondrocytes, thus enhancing the degradation of articular cartilage. The involvement of miR-9,[54] miR-26a,[36] miR-139[53] and miR-146[33] in increased apoptosis of chondrocytes in osteoarthritic cartilage has already been discussed. Another miRNA involved in chondrocytes apoptosis in OA is miR-34a. The expression of miR-34a is upregulated in human articular chondrocytes isolated from OA patients. The target gene of miR-34a encodes Silent Information Regulator 1 (SIRT1), a deacetylase playing a crucial role in the prevention of cell apoptosis. The upregulation of miR-34a in osteoarthritic chondrocytes results in downregulation of SIRT1 leading to upregulation of pro-apoptotic protein Bax (BCL2 associated X, apoptosis regulator), downregulation of anti-apoptotic protein Bcl-2 (B cell leukemia/lymphoma 2) and promotion of cell apoptosis.[56]

miR-210 is also involved in increased apoptosis of osteoarthritic chondrocytes. The expression of miR-210 is inhibited in OA chondrocytes, resulting to upregulation of its target gene DR6. Increased expression of DR6 leads to increased activation of the NF-κB signaling pathway and facilitates the apoptosis of the chondrocytes.[50] In addition, miR-222 is downregulated in OA chondrocytes, resulting in increased expression of Histone Deacetylase 4 (HDAC-4) and increased cell apoptosis.[40] Besides, miR-195 is overexpressed in peripheral blood of patients with OA.[57] The target gene of miR-195 encodes Hypoxia-Inducible Factor 1 alpha (HIF-1α). *In vitro* study in chondrocyte cell cultures has shown that overexpression of miR-195 results in downregulation of HIF-1α and in increased apoptosis of chondrocytes.[58]

MicroRNAs and autophagy in OA

Autophagy is a cell response to stress, in which cytoplasmic organelles and macromolecules are degraded by lysosomes and then recycled in order to support cellular metabolism and survival. Aging and age-related diseases, including OA, are related to reduced autophagy. Lately, several studies have been published about the involvement of microRNAs in reduced autophagy in OA.[59-61]

miR-155 is upregulated in human osteoarthritic cartilage and takes part in reduced autophagy in OA chondrocytes. Bioinformatics predict that miR-155 targets the autophagy-related genes ATG3 (autophagy related 3), GABARAPL1 (GABA type A receptor associated protein-like 1), ATG5 (autophagy related 5), ATG2B (autophagy related 2B), LAMP2 (lysosomal associated membrane protein 2) and FOXO3 (forkhead box O3). Recent *in vitro* study confirmed that miR-155 downregulates the expression of ATG3, GABARAPL1, ATG5 and FOXO3 in human articular chondrocytes, as well as the expression of other autophagy-related genes (ULK1 [unc-51-like autophagy activating kinase 1], MAP1LC3 [microtubule-associated protein 1 light chain 3 beta] and ATG14 [autophagy-related 14]), resulting in inhibition of autophagy. D' Adamo et al. conclude that miR-155 inhibits autophagy in chondrocytes and is partially responsible for defective autophagy in OA.[59]

miR-21 is another miRNA whose dysregulated expression leads to decreased autophagy in OA. Its target gene is GAS5 (Growth arrest-specific 5), which stimulates cell apoptosis and suppresses autophagy. The expression of miR-21 is decreased in osteoarthritic chondrocytes, resulting in upregulation of GAS5, increased apoptosis and suppressed autophagy. Besides, GAS5 downregulates miR-21, implying a reciprocal interplay between miR-21 and GAS5. Furthermore, when miR-21 was injected in osteoarthritic joints of a mouse OA model, it reduced cartilage destruction, whereas intra-articular injection of an inhibitor of miR-21 worsened cartilage destruction.[60]

On the other hand, increased expression of miR-146 seems to have a protective effect in osteoarthritic cartilage by promoting chondrocytes autophagy. Zhang et al. studied the effect of hypoxia, a pathogenetic mechanism contributing to OA development, on the expression of miR-146 and autophagy in chondrocytes. They demonstrated that hypoxia induces HIF-1α (Hypoxia-inducible factor-1α) in chondrocytes, which upregulates the expression of miR-146a. Upregulated miR-146a suppress-

es Bcl-2, an autophagy inhibitor, resulting in promotion of autophagy. Zhang et al. conclude that miR-146a plays probably a protective role in OA by enhancing chondrocyte autophagy.[61]

Profiling multiple microRNAs expressed in osteoarthritic tissues

The aforementioned studies have examined the expression of one or a few miRNAs, which target a gene or a pathway that is already known to participate in the pathogenesis of OA. On the other hand, during recent years, other studies have used high-throughput methods, such as hybridization microarrays and next generation RNA-sequencing, in order to examine the profile of multiple miRNAs expressed in the cartilage and subchondral bone of osteoarthritic joints and compare it to healthy controls.[32,62-68] A summary of the respective studies is presented in **Table 2**. Although these studies have some results in common, such as the downregulation of miR-140, most of their results do not overlap. There are plenty of reasons for this variety of results. Some studies measured miRNA expression in fresh samples of cartilage, subchondral bone or synovial fluid from osteoarthritic joints, while other studies used cultured chondrocytes from OA cartilage. Moreover, different studies used different sets of microarrays in order to examine the miRNA expression, while one study used next generation RNA-sequencing. In addition, sample size was small and there were no adjustments for confounding factors, such as age, gender or obesity. However, these studies have identified a lot of new miRNAs and genes that potentially participate in OA pathogenesis. Further studies will investigate the role of these miRNAs in OA and reveal novel pathogenetic mechanisms related with them.

Extracellular Vesicles and microRNAs IN OA

miRNAs may be packaged in extracellular vesicles such as exosomes, secreted from the cell that produces them and transferred to another cell, regulating thus the gene expression of the latter.[20] In OA, miRNAs in exosomes are altered and these alterations seem to get involved in OA pathogenesis. Recent study demonstrated that the expression of several miRNAs was altered in exosomes contained in synovial fluid derived from osteoarthritic joints compared to normal joints.[69] In another study,

Table 2. High-throughput methods used in OA literature.

Study	Experimental material	Number of samples	Methodology	Results
Jones et al. 2009[32]	Cartilage and subchondral bone from OA vs normal joints	4/4	Microarrays (157 miRNAs)	47 differentially expressed miRNAs
Iliopoulos et al. 2008[64]	Cultured chondrocytes from OA vs normal cartilage	33/10	Microarrays (365 miRNAs)	11 differentially expressed miRNAs
Swingler et al. 2012[67]	Discovery: Cultured chondrocytes Validation: Cartilage from OA vs normal joints	10/10	Discovery: Microarrays Validation: RT-PCR	39 miRNAs differentially expressed during chondrogenesis 2 miRNAs differentially expressed in OA vs normal cartilage
Diaz-Prado et al. 2012[63]	Cultured chondrocytes from OA vs normal cartilage	6/4	Microarrays (723 miRNAs)	7 differentially expressed miRNAs
Tornero-Estaban et al. 2015[68]	Cultures of bone marrow mesenchymal stem cells from OA patients vs controls	10/10	Microarrays (754 miRNAs)	246 differentially expressed miRNAs
Crowe et al. 2016[62]	Discovery: Cartilage from OA joints Validation: Cartilage from OA vs normal joints	11/6	Discovery: Next generation RNA-sequencing Validation: RT-PCR	60 new miRNAs expressed in OA cartilage 3 differentially expressed miRNAs
Li YH et al. 2016[65]	Synovial fluid from late-stage vs early-stage OA joints	4/4	Microarrays (752 miRNAs)	7 differentially expressed miRNAs
Rasheed et al. 2016[66]	Cultured chondrocytes from OA cartilage, stimulated or not with IL-1β	Unknown	Microarrays (1347 miRNAs)	36 differentially expressed miRNAs

Studies that used high-throughput methods in order to examine the profile of multiple miRNAs expressed in cartilage, subchondral bone and synovial fluid of osteoarthritic joints.

Kato et al. used IL-1β to stimulate synovial fibroblasts and examined the effect of exosomes derived from the stimulated synovial fibroblasts on articular chondrocytes. IL-1β is a key player of OA pathogenesis mediating synovial inflammation and cartilage degradation. Kato et al. demonstrated that exosomes from IL-1β-stimulated synovial fibroblasts upregulated the expression of degrading enzymes MMP13 and ADAMTS5 in articular chondrocytes and downregulated the expression of cartilage matrix components (type II collagen and aggrecan). They also showed that the expression of 50 miRNAs was dysregulated in exosomes derived from IL-1β-stimulated synovial fibroblasts compared with non-stimulated synovial fibroblasts.[70] In addition, Nakasa et al. showed that exosomes derived from IL-1β-stimulated OA cartilage upregulated the expression of MMP13, IL-1β, TNF-α and COX-2 in OA synovium.[71] Thus, miRNAs packaged in exosomes participate in OA pathogenesis by mediating cell to cell communication in osteoarthritic joints.

MicroRNAs as Biomarkers in OA

miRNAs may be detected in peripheral blood and synovial fluid incorporated in extracellular vesicles or bound to lipoproteins and RNA-binding proteins.[20,21] The stability of miRNAs in circulation[72] and their different levels between patients with OA and healthy population offer the opportunity of using these molecules as biomarkers for this disease. Murata et al. showed that plasma levels of miR-16 and miR-132 differentiated OA patients from healthy controls, since they were significantly lower in the former. Moreover, synovial fluid concentrations of miR-16, miR-146a, miR-155 and miR-223 were significantly lower in patients with OA compared to patients with rheumatoid arthritis and could differentiate those two groups of patients. In the same study, Murata et al. discovered that there was no correlation between plasma and synovial fluid miRNA levels, implying different origins for them, and then demonstrated that synovial membrane is the main source of synovial fluid miRNAs.[72] In another study, Borgonio Cuadra et al. compared plasma levels of 380 miRNAs between OA patients and healthy subjects and found 12 miRNAs that were overexpressed in the plasma of OA patients (miR-16, miR-20b, miR-29c, miR-30b, miR-93, miR-126, miR-146a, miR-184, miR-186, miR-195, miR-345, miR-885-5p).[57] Recently, Withrow et al. demonstrated that the concentration of miR-7-5p and miR-200c-3p in exosomes derived from synovial fluid was significantly higher in OA patients in comparison to healthy subjects.[69] Moreover, Okuhara et al. have shown that peripheral blood mononuclear cells express significantly higher levels of miR-146a, -155, -181a, and -223 in OA patients compared to healthy population.[73] Furthermore, in an interesting study, Beyer et al. investigated the possibility of using plasma miRNA levels in order to predict the development of severe knee and hip OA. They discovered that lower plasma levels of let-7e were associated with severe knee and hip OA requiring total joint arthroplasty.[74] Therefore, although results are limited and sometimes contradicting, miRNAs have the potential of being used as biomarkers for OA. Their stability, ease of measurement and different expression in the blood and synovial fluid of OA patients offer the opportunity of using them to predict the prognosis or even measure disease activity or predict response to treatment. However, more studies are needed for this to become possible.

Therapeutic Potential of microRNAs in OA

Current treatment of OA includes drugs such as Non-steroidal Anti-Inflammatory Drugs (NSAIDS) for alleviating symptoms and total joint arthroplasty in cases of severe OA. There are no drugs that halt the progress of the disease, like disease-modifying drugs do in rheumatoid arthritis.[1] MiRNAs represent a promising target for the treatment of OA. A remarkable number of miRNAs participate in the pathogenesis of OA. Inhibition of these miRNAs with antisense oligonucleotides (anti-miRs) or administration of miRNAs that silence genes participating in OA pathogenesis could be a novel approach for arresting the progress of OA. An advantage of this approach is that synovial joints are an isolated environment and intra-articular administration of miRNAs would not have systemic effects. However, an important issue is the delivery method of the miRNAs or the anti-miRs. Several solutions have been proposed, including extracellular vesicles (exosomes), nanoparticles and antibodies.[75]

An example of targeting miRNAs for the treatment of OA is the inhibition of miR-34a. The upregulation of miR-34a in osteoarthritic chondrocytes results in inhibition of SIRT1, leading to increased cell apoptosis.[56] Abouheif et al. demonstrated that silencing of miR-34a with oligonucleotides of antisense miR-34a inhibited chondrocytes apoptosis in rat chondrocyte cultures treated with IL-1β.[76] Yan et al. recently examined the results of suppressing miR-34a in rats with OA. Oligonucleotides of antisense miR-34a (anti-miR-34a) were cloned into a lentivirus vector and the lentiviral vectors were injected into the osteoarthritic joints of the rats. Intra-articular injection of lentiviruses encoding anti-miR-34a ameliorated cartilage destruction of the OA joints.[56]

miR-483-5p is upregulated in articular cartilage from OA patients and it targets and downregulates matrilin 3 (Matn3) and tissue inhibitor of metalloproteinase 2 (Timp2). Matn3 is a protein of the cartilage matrix and Timp2 is an inhibitor of cartilage degrading metalloproteinases. Wang et al. recently studied the results of silencing miR-483-5p in an experimental OA mouse model. Lentiviruses encoding oligonucleotides of antisense miR-483-5p (anti-miR-483-5p) were injected in the OA joints and it was demonstrated that anti-miR-384-5p

attenuated cartilage damage and loss and inhibited the formation of fibrous cartilage.[77]

miR-140 is one of the most studied miRNAs in OA. Karlsen et al. studied the protective effect of miR-140 in an *in vitro* model of OA. They transfected miR-140 into IL-1β-treated articular chondrocyte and mesenchymal stem cell cultures and they demonstrated that miR-140 upregulated the synthesis of cartilage matrix components and downregulated the production of cartilage degradation enzymes.[78] In a recent study, Tao et al. used exosomes in order to transfer miR-140 into osteoarthritic joints in a rat OA model. They acquired exosomes rich in miR-140 by transfecting mesenchymal stem cells (MSCs) with lentivirus encoding miR-140 and by isolating the exosomes derived from the miR-140-overexpressing-MSCs. They first transfected articular chondrocytes with miR-140-exosomes and showed that miR-140 downregulated RALA and upregulated SOX9, aggrecan and collagen type II. Then they injected miR-140-exosomes into osteoarthritic joints of rats and demonstrated that miR-140 reduced the damage of the articular cartilage in comparison to the control group.[79]

CONCLUSIONS

Multiple studies demonstrate that miRNAs potentially play an important role in the pathogenesis of OA. The dysregulation of their expression affects several pathways involved in OA pathogenesis. Dysregulated miRNAs increase the expression of cartilage degrading enzymes by articular chondrocytes, decrease the production of cartilage matrix components, facilitate chondrocyte apoptosis and inhibit autophagy in chondrocytes, thus contributing to cartilage damage. They are also involved in the production of proinflammatory cytokines and the induction of joint inflammation, as well as in pain-related pathways in OA. In addition, miRNAs are incorporated in extracellular membranous vesicles such as exosomes and transferred from one cell to another. Thus, they participate in the communication between synoviocytes and articular chondrocytes in osteoarthritic joints, enhancing the production of degrading enzymes and cytokines by these cells.

The role of miRNAs in OA still remains to be elucidated. Yet, based on the available data and the overall role of miRNA molecular machinery, it is possible to gain some insight on their participation in OA. Hence, there are some general concepts governing miRNA physiology. Their role depends mainly on the target gene. This means that if a gene has an enhancing or suppressive effect on a certain physiological procedure, the down- or up-regulation of the respective miRNA signifies the opposite effect. For example, in the case of miR-146 we have mentioned that it decreases the expression of pain modulators that enhance pain perception, such as TNF-α, IL-6, IL-8, COX-2 and iNOS, in astrocytes. Thus, it seems that *downregulation* of miR-146 in the central and peripheral nervous system signals the *upregulation* of the target genes. In other words, the genes that mediate pain are inhibited by miR-146. Further on, although it is known that miRNA expression and binding to target-genes is linked to gene negative regulation, the only way to determine miRNA role is through experimental validation; this varies from one pathophysiological condition to another.

Besides, it seems that dysregulation of miRNA expression in OA is both a consequence of upstream events (such as increased production of proinflammatory cytokines) and a consequence of negative feedback from downstream events. For example, as mentioned above, increased production of the proinflammatory cytokine IL-1*β* in OA results in the downregulation of miR-26a and miR-140. On the other hand, downregulation of miR-26a in OA leads to upregulation of KPNA3 and activation of NF-κB pathway. In turn, the activated NF-κB pathway negatively regulates miR-26a (negative feedback).

Moreover, the protective or harmful role of miRNA in OA is a subject of intensive discussion. As aforementioned, several miRNAs have been reported to have a protective role in OA, such as miR-140 or miR-146, yet at the same time several other miRNAs are reported to play a negative role in OA, such as miR-155 and miR-195.

On the other hand, different expression of miRNAs in peripheral blood and synovial fluid between OA patients and healthy population, their stability in body fluids and the ease of their measurement creates the potential of utilizing them as biomarkers of the disease. Besides, next generation RNA-sequencing will facilitate the identification of new miRNAs in order to be used as biomarkers. In the future, miRNAs may be used as biomarkers of disease activity or as predictors of prognosis or of response to treatment. However, further studies are still needed in this direction.

Furthermore, targeting of miRNAs is a potential novel therapeutic strategy in OA. Inhibition of miRNAs contributing to OA pathogenesis or administration of miRNAs silencing genes participating in OA pathogenesis has been studied in animal OA models. Intra-articular injection of anti-miRs and of miRNAs has been proved successful in animals. However, delivery of these molecules in OA joints remains an issue. Exosomes may be an option for OA treatment, but further research is necessary.

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AUTHORS' CONTRIBUTIONS

PKP: collected literature, drafted the manuscript. **GIL**: drafted the manuscript, proof-read the manuscript, gave final permission for submission.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REVIEW

Association of Monoclonal Gammopathy of Undetermined Significance with Behcet's Disease: A Review of Shared Common Disease Pathogenetic Mechanisms

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ABSTRACT

An association between a number of chronic inflammatory rheumatic diseases and Monoclonal Gammopathy of Undetermined Significance (MGUS) has been reported. To date no cases of Behcet's disease (BD) and MGUS have been documented. BD sits at the interphase of auto-inflammatory and chronic auto-immune disease spectrums. Alterations in the cellular and cytokine microenvironments can promote a pro-inflammatory state in which persistent antigenic stimulation and cellular proliferation can progressively induce cytogenetic abnormalities which could perturbate plasma cell functions such as seen in MGUS. Herein, we present a rare case of a woman presenting with BD who subsequently developed MGUS. Pathogenetic mechanisms that could potentially contribute to development of both conditions, are reviewed and demonstrate that this disease association is not coincidental but is an evolutionary association driven by shared common disease pathogenetic mechanisms.

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INTRODUCTION

Inflammatory rheumatic diseases (RD) and neoplastic disorders can be associated in a number of ways. However, although the association of RD with a pre-malignant state such as monoclonal gammopathy of unknown significance (MGUS) has been recognised in a number of chronic inflammatory autoimmune conditions, no such link has been described in patients with Behcet's disease.

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MGUS is a CD19-, CD45- and CD56+ plasma cell disorder associated with an asymptomatic monoclonal paraproteinaemia and has a 1% average annual progression rate to multiple myeloma (MM).[1-3] MGUS, like smoldering multiple myeloma (SMM) and MM, is a neoplastic disease that retains many of the phenotypic properties of healthy CD138+, CD19+, CD45+, CD56- plasma cells. However, MGUS plasma cells are immunophenotypically indistinguishable from those in SSM and MM, maintain low proliferation rates and can evolve into MM or Waldenström's macroglobulinemia or AL amyloidosis or a lymphoma. There is a genomic instability associated with IgH translocations or hyper-diploidy and chromosome 13 deletion in MGUS. Its cause is unknown.

MGUS has been associated with a number of RDs: Sjogren's syndrome (SS), Systemic Lupus Erythematous (SLE), Rheumatoid arthritis (RA) and spondyloarthropathies.[2,3] It has been suggested that this association is

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not coincidental and could arise from persistent antigenic stimulation and cytogenetic abnormalities due to chronic, persistent pro-inflammatory microenvironment milieu-induced deleterious epigenetic modifications.[4] There are no reports in the literature of an association between MGUS and BD. Given the relatively high prevalence of MGUS in the general population, the reported associations may be coincidental.[5] However, a population-based study reported that inflammatory conditions and autoimmune diseases including rheumatic diseases, but not Behcet's disease (BD), are significantly associated with an increased risk of developing MGUS.[6]

Behcet's disease (BD) is a multi-systemic chronic auto-inflammatory disorder exhibiting varied clinical characteristics which include the classic triad of recurrent orogenital aphthosis, uveitis, and cutaneous lesions. Nervous system, visual loss, gastrointestinal, vascular and musculoskeletal involvement may occur.[7] From a pathophysiologic perspective, it sits at the interphase between chronic auto-inflammatory and chronic autoimmune inflammatory disease, but the exact pathophysiologic mechanisms have not been fully elucidated. In BD, a pro-inflammatory state is maintained by the adaptive immune system in response to environmental and auto-antigenic factors which trigger an overexpression of pro-inflammatory cytokines and vasculitis. BD lacks the classical chronic autoimmune inflammatory disease features, such as autoantibody production, but interestingly, immunosuppressants have proven effective treatments of the condition. A unifying hypothesis has been proposed suggesting that innate and adaptive immune pathways are aberrantly integrated in BD through mechanisms such as neutrophil activation, T-cell derived chemokines and HLA-B51-associated immune reactions. Although an infectious agent probably triggers the innate-derived inflammation, bacterial persistence or autoantigen-activated antigen-presenting cells may sustain the adaptive responses.[8]

The aim of this review is to examine the association of MGUS with BD, in light of the rare case of a patient with BD who developed MGUS reported here, and to discuss the potential pathogenic mechanisms. This BD-MGUS association can provide a basis for molecular exploration of the aetiopathogenetic mechanisms of these two ill understood conditions and may offer some insights into the development of targeted therapies.

CASE-BASED REVIEW

A 43-year-old unemployed woman, living with her husband and three children (one with Down's syndrome) presented with arthralgia of the knee and hip joints as well as back pain. She repeated episodes of oral ulcerations, iritis, erythema nodosum and a rapidly progressive facial rash from 1993 when she was 23 years old. She also had constipation and bloating but denied shortness of breath or chest pain. She had no clinical stigmata of SLE. She also has a history of recto-colonic prolapse which was treated with colectomy. Her pathergy skin test was positive. She was diagnosed with BD which was managed with Colchicine; subsequently Anakinra, then Infliximab which induced SLE that resolved on cessation of infliximab therapy. She also tried Methotrexate and a Beclomethasone inhaler for flares of oral ulcers. The course of her illness has been one of remission and relapses.

In 2015, she was diagnosed with asymptomatic MGUS and tests performed did not indicate progression to myeloma. Serum electrophoresis showed raised IgA = 9.11g/L associated with an IgA paraprotein band, with normal IgG and IgM levels of 6.9 and 1.46g/l respectively. IgG4 levels were normal. She had normal FBC, adjusted calcium, creatinine, albumin, ESR = 8mm/Hr and CRP = <5 mg/l. Her RF, anti-CCP, ENA, ANA and anti-sDNA antibody tests were negative. HIV, Hep C and Hep B tests were also negative. Her Vitamin D_3 levels and thyroid, cortisol and prolactin profiles were normal. She is HLA-B57.01 positive.

DISCUSSION

We review and report here for the first time the association of BD with MGUS development. There has been a report of a patient receiving hydroxyurea who developed chronic myeloid leukaemia (CML) and three other CML cases who also developed BD whilst on IFN-α therapy.[9] The link between RDs and MGUS has been investigated with population-based studies reporting a significant association.[5,6] No associations of MGUS and BD have been reported in the medical literature. BD and MGUS are both complex diseases with poorly understood pathogenetic and pathophysiological mechanisms. The mechanisms involved in both conditions probably arise from shared immunological aberrations and overlap in pathogenetic pathways. One hypothesis is the shared role of chronic antigen stimulation in the aetiopathogenesis of both diseases. Autoinflammatory perturbations in BD could act as a trigger for MGUS development via epigenetic modifications in part. A multi-step view of autoimmune disease pathogenesis indicates that tolerance checkpoints exist and that the genes and molecular pathways that underpin these mechanisms overlap with those involved in tumour suppression.[10] Elimination or disruption of these pathways can therefore potentially result in the development of both diseases in tandem or sequentially.

Immunogenetic and Cytokine Response Perspectives

BD straddles the interphase between autoinflammatory and autoimmune diseases. HLA-B*51 alleles are implicated in the immunogenetics of BD.[11] Of the more than 89 different subtypes of HLA-B51, HLA-B5101 is the

major sub-allele associated with BD in most populations studied to date. In Middle Eastern, Italian, Spanish, Greek, Turkish, and German patients, BD is strongly associated with HLA-B5108. Several other HLA class I and II alleles including HLA-A26, HLA-B15, HLA-B5701, HLA-B2702, HLA-B3901, HLA-B52, HLA-B56, Cw1, Cw14, Cw15, Cw16, HLA-DRB104, and HLA-DRB107 have been shown to be linked to BD in other populations. The overall effect of these genes which influence the adaptive and innate immunity systems, is to alter the T-cell repertoire, inducing polarisation towards the Th1/Th17 profile in BD.[12]

Individual amino acid residues located on HLA-B51 molecules are associated with disease and are located in the antigen binding regions mediating peptide binding and interactions between CD8 lymphocytes, Natural killer (NK) cells and MHC class I (MHC-1) molecules.[12] Studies have also shown an association between BD and the MHC Class I polypeptide-related sequence A (MICA) allele 009. MICA are genes found on MHC-1 region in the chromosome which code for proteins expressed on cells such as endothelium and fibroblasts. However, the significance of this association is not known; this association could just be linkage disequilibrium between MICA and HLA-B51,[13] while the recent Genome-Wide Association Study (GWAS) did not find an independent association.[14]

A recent GWAS has demonstrated that expression of risk alleles in BD leads to defects in cytokine gene expression that results in decreased expression of the anti-inflammatory cytokine IL-10, which can down-regulate the expression of pro-inflammatory cytokines such as TNF α, IL-6 and IL-12; inhibition of the costimulatory coupling activities of macrophages on T cell and NK cells; and the expression of disease associated variants of IL-23 gene (this regulates Th17 cell development).[14] IL-12 and IL-23, which are both crucial in Th17-associated pathology such as BD, share the receptor subunit p40 which can be targeted by the monoclonal antibody (Ustekinumab). This biodrug is effective in disease amelioration.[15] It could therefore be postulated that this drug could also be a potential effective therapy for BD.

Pro-inflammatory cytokine IL-6 is essential for the growth of human B lymphocytes and myeloma cells, whilst TNFα plays a role in the pathogenesis of plasma cell dyscrasias.[16] Serum IL-6 levels are significantly elevated in MGUS compared to controls.[17] Similar observations have been made for TNFα and IL-8. However, the levels of TNFα and IL-8 were not significantly associated with a higher probability of malignant transformation as previously reported.[18] Furthermore, Zheng et al. found no association between polymorphisms of IL-6, TNFα, and MGUS.[16] Serum IL-6 and TNFα have been shown independently, to be significantly elevated in patients with BD when compared to health controls, and their levels correlate with BD disease activity.[17]

IL-10 levels are decreased in BD whilst the expression of IL-6 and TNFα are upregulated. Raised levels of IL-6 might promote aberrant plasma cell growth, which may play a role in the pathophysiology of MGUS by promoting the growth and survival of myelomatoid cells. A potential unifying early event (e.g., IgH translocation) may render B cells vulnerable to proliferative stimuli such as IL-6. IL-6 plays an essential role in plasma cell disorders and more importantly progression to Multiple Myeloma (MM).[18]

IL-6 induced stimulation and proliferation of cells requires signal transduction mediated by the STAT (Signal Transducers and activators of transcription) (STAT1 and STAT3) and MAPK (mitogen-activated protein kinase) pathways.[19] In inflammation, a positive feedback autocrine loop exists in fibroblasts with upregulated STAT4 leading to sustained IL-6 transcription.[20] Whether fibroblasts are involved in BD or MGUS pathogenesis remains to be determined.

Risk alleles rs897200, rs7564070 and rs7572482 in BD are associated with raised expression of STAT4 gene and upregulation of the Th17 pathway. STAT4 gene encodes the transcription activator and signal transducer STAT4, which is activated by proinflammatory cytokines such as IL-12 and plays a role in T-cell maturation.[21] STAT4 has been identified through GWAS as a disease susceptibility loci shared in several immune diseases such as BD, RA and SS.[12] STAT5, an anti-apoptotic transcription activator which acts directly on the IL-17 gene to limit IL-17 transcription, is in a balance with STAT3 (pro-inflammatory promoting IL-17 production) and variation in the levels of cytokines activating these factors determine the outcome of the activities of Th17 cells.[15]

The overlap of susceptibility genes therefore, suggests an overlap of pathophysiogenetic mechanisms. SS, SLE, RA and spondyloarthropathies have been associated with MGUS.[4] However seropositive rheumatic arthritis is strongly associated with MHC class II whilst seronegative diseases and BD show an association with MHC class I.[22]

Immune System Interaction Considerations

$CD8^+$ T cells, T regulatory (Treg) and Th17 cells are implicated in BD pathogenesis. CD8+ and $CD56^+$ T cells are increased in the peripheral blood and aqueous humour of BD patients with uveitis.[23] These cells produce IFN-γ in active disease which upregulates and augments their cytolytic tissue destructive effects.[24] The cytolytic and effector functions of CD8+ cells correlate with CD56 cellular expression.[25] Chronic antigen stimulation induces CD56 expression on cytotoxic T-lymphocytes in BD.[25] This surface marker is also expressed by aberrant CD56+ plasma cells in MGUS which act as clonal cells.[1] The CD8+ T cell population in patients with MGUS is significantly expanded with a high degree of clonality.[26] These expansions were attributed to chronic antigen stimulation. Such shared immune pathways might

explain the development of MGUS in our BD patient.
The disease alleles in BD are in antigen binding regions which mediate interactions between CD8+ and MHC class I molecules. An abnormal response to antigen stimulation could promote a Th1 dominant microenvironment with enhanced CD8+ T cell cytotoxic effects due to excessive IFN-γ production. We propose that chronic antigen stimulation may sustain this response in BD and this coupled with dysregulated cytokine microenvironment -induced cytogenetic abnormalities, could initiate and/or maintain proliferation of aberrant clonal CD56+ and CD8+ T cells which promote MGUS.
BD patients with active and untreated disease have high blood circulating levels of Th17 cells and low levels of Treg cells mediated by IL-21 which correlates with disease activity.[27] IL-21 is produced by $CD4^+$ T-cells.[12] Activated Th17 cells produce IL-17 under the influence of IL-6 produced after antigenic (extracellular pathogens) activation of the innate immune system, which upregulates adhesion molecule expression on endothelial cells.[28] Therefore, enhanced Th17 cellular activity plays a role in the vascular inflammation and thrombosis in BD.
There is a complex functional antagonism between Treg and Th17 cells in chronic inflammation. However, studies on the role of Treg cells in BD have produced conflicting results.[27,29] Tregs have an essential role in peripheral tolerance and the preservation of self-tolerance.[30] The clinical manifestation of BD support decreased numbers and function of Tregs in active disease.
A balance between Treg and Th17 is essential for maintaining anti-tumour immunity, and IL-6 plays a pivotal role in regulating this balance. The role of Treg in neoplastic disease is also plagued by contradictory results as seen in BD. A similar reduction of Treg has been reported in peripheral blood from patients with MGUS.[30] However, other studies have shown no significant difference in comparison to normal controls.[31] An increase in Th17 cells has been demonstrated in the bone marrow of patients with multiple myeloma but not in those with MGUS.[32]
Although there is limited research into the Th1/Th2 ratio in myeloma patients, a study to determine the clinical significance of this ratio found an insignificant increase in the ratio in MGUS patients.[33]

Environmental Perspectives

Immunogenetics provide insight into the means of predisposition but does not completely account for incidence of BD. There is interestingly, a significant carriage rate of HLA-B51 in healthy individuals.[34] Chronic antigen stimulation or abnormal response to antigen stimulation has been implicated in the pathogenesis of both BD and MGUS.[12,35] BD commonly begins in oral mucosa, and oral lesions increase after dental work, with reports of antibacterial therapy decreasing symptoms; supporting the potential role of microbial flora possibly mediated by non-specific T-cell hyperactivity against the ubiquitous antigens.[8] Expression of risk alleles of chemokine receptor and H antigen encoding genes alter the body's response to microbial pathogens increasing BD risk.[12]
High levels of Heat shock proteins (HSP60) have been reported in BD and RA, where they can act as a danger signal of abnormal antigen presentation.[34] HSPs are synthesised in response to cell exposure to non-specific stimuli such as infection or trauma. HSP60 acts as a ligand for Toll-Like-Receptors (TLR), stimulating inflammatory cytokines (IL12, IL6, TNFα) release which can boost adaptive Th1 immune responses. Subsequent activation of the innate adaptive immunity fits in with the clinical spectrum of BD.[36] Therefore, the pathogenesis of BD can putatively be viewed as an antigen-driven immune response superimposed on the primed state present in predisposed individual, induced by heat shock proteins or other non-specific antigens.[37]
The racial predisposition in MGUS could indicate the influence of environmental factors rather than genetics.[18] The pathogenesis of MGUS can on the other hand be hypothesised to be an abnormal response to antigenic stimulation, mediated possibly by aberrant expression of toll-like receptors and overexpression of IL-6 bioactivity.[35]

Epigenetics

The role of epigenetics in the pathogenesis of BD and its association with MGUS could explain in part the contribution of the environment to the pathogenetic mechanisms. MicroRNAs which are short noncoding RNAs that negatively regulate gene expression by acting post-transcriptionally, have been implicated in BD.[38] They are crucial in modulating cellular processes such as proliferation and apoptosis. MicroRNA-155 is significantly decreased in dendritic cells from BD patients whilst IL-6 and IL-17 production are increased.[39] DNA methylation - an epigenetic mechanism through which methyl groups are added to DNA molecule causing genes to switch off or on - were identified by a recent genome-wide study as playing a role in the epigenetic remodelling of cytoskeleton-related genes involved in the pathogenesis of BD.[40] Dysregulation of these genes could underpin increased leukocyte migration observed in BD. In comparison to health controls, 283 and 125 differentially methylated sites were identified in monocytes and CD4+ T-cells from BD patients. Interestingly, treatment reversed these methylation differences with complete restoration of a normal profile in many cases.[40]
GWAS has shown changes in DNA methylation in MGUS also, with aberrant demethylation occurring in Cytosine-phosphodiester-Guanine (CpG) islands and differential methylation occurring in genes involved in cell proliferation and cell cycle.[41] A microRNA microarray study has reported upregulation of microRNA with oncogenic

functions, such as miR-21 in MGUS, which could promote plasma cell transformation by blocking apoptosis. Upregulation of miR-17 downregulates a gene (SOCS-1) which play a critical role in inhibiting IL-6 growth signalling.[42] Epigenetic therapy targeting the distinctive signatures in MGUS, myeloma and BD inflammatory cells could therefore have therapeutic potential.

Therapeutic Implications

BD and MGUS have shared and specific pathogenetic mechanisms which are important targets for current and emerging therapies. Potential therapeutic targets such as biologics are avenues for further exploration and for development of personalised therapy. Cytokine profiling (e.g. elevated levels of 1L-1β, 1L-12, 1L-17) can aid in the identification of which biologicals are potential treatments based on the cytokine profile of a disease. Microarray gene profiling will enable assessment of gene expression patterns that may help identify which patients can respond to certain targeted therapy such as epigenetic therapy. There is also a role in monitoring to detect changes in cytokine or epigenetic profiles as a measure of disease remission or as a signal to change to a better suited therapy.

CONCLUSION

Chronic antigen stimulation in genetically predisposed individuals leads to a sustained inflammatory response in BD leading to perturbation of anti- and pro-inflammatory cytokine and T-cell balances. The resultant inflammatory microenvironment may promote the development of aberrant clonal plasma cells leading to MGUS. Epigenetic contributions can perpetuate proliferative and anti-apoptotic mechanisms, promoting disease development. A more developed understanding of the disease pathogenetic mechanisms linking BD and MGUS may provide further insights into the aetiopathophysiology and create options for new and targeted therapeutics in BD and possibly MGUS.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DISCLOSURES

Informed consent was obtained from the patient for publication of this Case report.

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CASE REPORT

Patient with ankylosing spondylitis and scleroderma renal crisis

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ABSTRACT

We report a 56-year-old man with a history of ankylosing spondylitis and systemic scleroderma. The patient had been diagnosed with ankylosing spondylitis 20 years ago and had been receiving treatment with NSAIDs and anti TNFα drugs. He referred to our rheumatology department for Raynaud's phenomenon, arthralgias and weight loss. Physical examination revealed stiffness of the skin with difficulty in pinching (mainly at lower extremities, from knee to ankle). Soon after his first visit to our department, he developed renal scleroderma crisis with abrupt increase in blood pressure, decline in renal function, and microangiopathic haemolytic anaemia in accordance with positive antinuclear autoantibodies and positive anti-topoisomerase I antibody (anti-Scl70). This is one of the few reports in the literature of coexistence of ankylosing spondylitis and systemic scleroderma. A genetic correlation seems to be an explanation in some patients who carry one or two susceptibility alleles to both diseases. Thus, this might be the case of a 'genetic trap' in which distinct genes are cooperating to favour the susceptibility to two different HLA-associated systemic autoimmune diseases.

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Keywords: Ankylosing spondylitis, Systemic Scleroderma.

A 56-year-old man with a known history of ankylosing spondylitis (AS) was referred to our rheumatology department for Raynaud's phenomenon, arthralgias and body weight loss (~ 10% BMI). The patient had been receiving treatment for 10 years with an anti-TNFα agent. Six months before, due to his new symptoms, 200mg of hydroxychloroquine and 10mg of prednisolone per day were added. However, the patient showed no improvement and visited the external rheumatology department of the Clinic of Pathological Physiology of the Laikon General Hospital for a second opinion.

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According to his medical history, he had ankylosing spondylitis that started with inflammatory back pain 20 years ago, with radiographic findings of sacroiliitis and spinal involvement (**Figures 1a, 1b**) and a positive HLA-B27 test. He had been receiving treatment with NSAIDs and anti TNFα drugs (adalimumab that was switched 6 months before to etanercept). He also suffered from depression and he had been treated with citalopram and alprazolam. He was a smoker (30 years, 1 pack a day), a social drinker and he had free family history.

On physical examination, Raynaud's phenomenon was observed in both hands, with joint pain in all limbs and stiffness of the skin with difficulty in pinching (mainly at lower extremities, from knee to ankle). The rest of the physical examination and vital signs were normal. Laboratory tests were without pathological findings, with only an increase of ESR (57mmHg). Due to the signifi-

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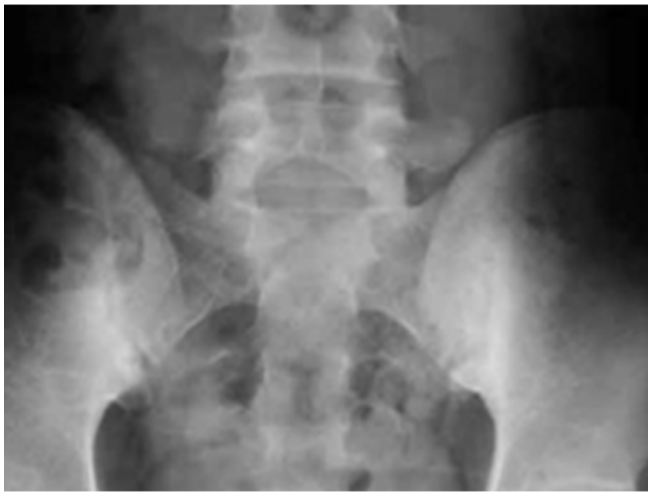


Figure 1a. Sacroiliac joint radiography demonstrating bilateral sacroiliitis

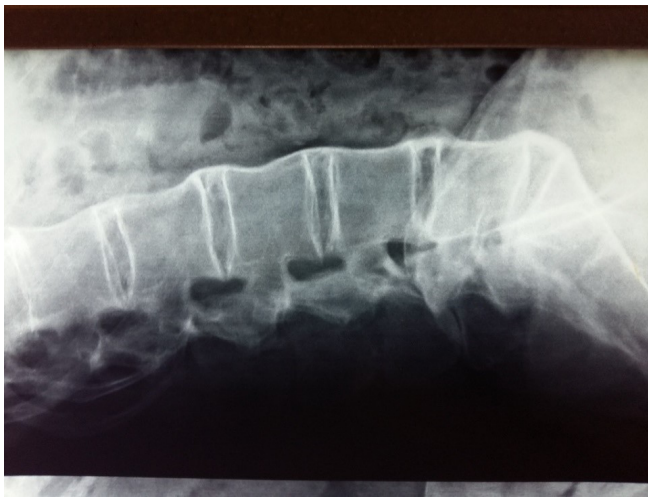
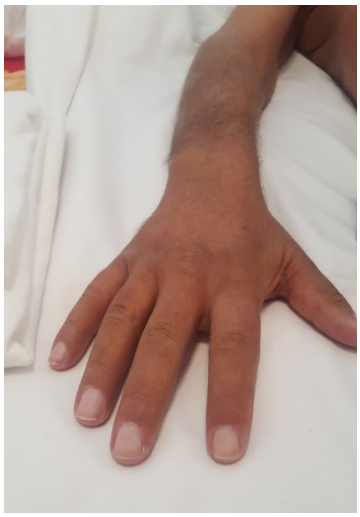


Figure 1b. Lumbar spine X-ray with marginal syndesmophytes

cant weight loss and his smoking, a chest and upper/lower abdominal CT scan was requested. The results were without abnormal findings. Further evaluation for Raynaud's phenomenon and stiffness of the skin included serum protein electrophoresis, hepatitis control and immunological tests (antinuclear autoantibodies [ANA], antibodies to Extractable Nuclear Antigens [ENA], rheumatoid factor, anti-topoisomerase I antibody [anti-Scl70] and cryoglobulins [CRYO]). While waiting for the results, hydroxychloroquine was discontinued, prednisolone was reduced to 7.5 mg per day and, as he suffered from arthralgias, 15mg of methotrexate and 5mg of folate per week were added.

After one month, on his scheduled appointment the patient presented with an exacerbation of his symptoms. In particular, he had arthralgias, dyspnoea and orthopnoea. Physical examination revealed an extension of skin hardening (trunk, upper and lower extremities) (**Figure 2a, 2b**), unexplained velcro-like inspiratory movements and increased blood pressure (200/100 mmHg). Laboratory tests were performed immediately, and rapid decline of renal function was revealed (Creat: 6.78 mg / dl, Urea: 75mg / dl), with microangiopathic haemolytic anaemia (Ht 29.3%, Hb 8.1 gr/dl, MCV 91) and increased inflammation markers (CRP 100 mg/dl, TKE 47 mmHg). The immunological tests were positive for ANA (1/1280 fsp),



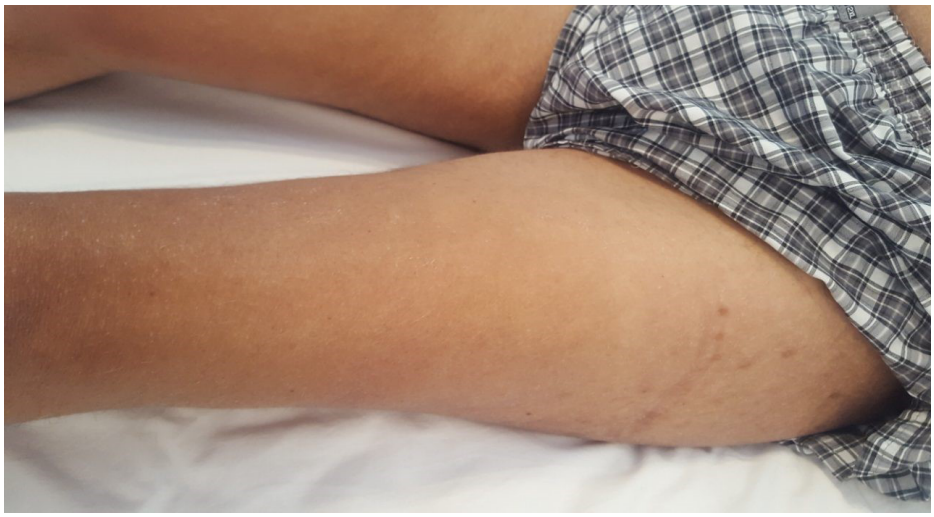


Figure 2a, 2b. Stiffness of patient skin with difficulty in pinching

Anti-SSA/ Ro and Anti-Scl-70. As he had rapidly deteriorating renal function, the patient was admitted to the clinic for further investigation and management.
Based on his clinical picture and the abrupt increase in blood pressure, decline in renal function, microangiopathic haemolytic anaemia and, in accordance with the positive immunological tests, a diagnosis of scleroderma renal crisis was made. The patient was treated with an angiotensin converting enzyme inhibitor (captopril) in order to obtain a gradual adjustment of blood pressure. At the same time, he began haemodialysis sessions. However, he presented thrombocytopenia and leukopenia due to captopril and his treatment switched to an angiotensin II receptor blocker (valsartan). His blood pressure was successfully reduced to normal levels, but his creatinine remained high and he continued on haemodialysis. Currently, 1 year after his initial admission to the hospital, the patient remains on chronic haemodialysis and is treated for AS with a new biologic agent (secukinumab, a human interleukin-17A antagonist) at a dose of 150mg per month with satisfactory results and a BASDAI improvement.

DISCUSSION

We report the case of a patient who had AS and developed diffuse systemic sclerosis (Ssc). Someone could suggest that scleroderma occurrence may be associated with our patient's previous treatment. There are several factors have been identified as possible triggers of Ssc. These include drugs (e.g., vitamin K, cocaine, penicillamine and some chemotherapeutic agents), and chemicals (e.g., silica, pesticides, aliphatic hydrocarbons). However, the exact role of TNF-α drugs remains controversial. In vitro studies have shown that TNFα inhibits the activity of fibroblasts, acts as a potent inducer of metalloproteinases and thus, inhibition of TNF-α could worsen fibrosis.[1,2,3] On the other hand, inhibition of TNF-α seems to improve fibrosis in animal experimental models.[4] Clinical studies have showed that, in some cases, anti-TNFα therapy worsened the fibrotic cellulitis and had infectious complications.[5] Also, development of morphea, a localized scleroderma lesion, has been reported in patients treated with anti-TNFα drugs,[6,7,8] suggesting a rare paradoxical side effect. However, in a small series of cases, anti-TNFα drugs are beneficial for the treatment of Ssc by improving arthritis and reducing the modified Rodnan score for skin (mRSS).[9] Eventually, could this be a case of AS coexistence with Systemic Scleroderma? It is well known that AS rarely coexists with other inflammatory joint diseases. Cases of AS have been reported in patients who had systemic lupus erythematosus,[10] mixed connective tissue disease,[11] or Behçet's disease.[12] AS and Ssc rarely occur in combination.[13,14] This is one of the few reports in the literature of coexistence of the two diseases. A genetic correlation seems to be an explanation. Particularly, there are reports of association of AS and SSc in the presence of HLA specificities known to be associated with these two diseases: HLA*B27, associated with susceptibility to AS; and HLA*B35, DRB1*11, DRB1*15, DRB3, DQB1*03, and DQB1*06, associated with SSc in different populations.[15] Thus, this might be the case of a 'genetic trap' in which distinct genes are cooperating to favour the susceptibility to two different HLA-associated systemic autoimmune diseases. Hopefully, future research will identify the environmental factors that trigger the expression of both diseases in the same patient.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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CASE REPORT

Orf disease in a patient with rheumatoid arthritis

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ABSTRACT

Orf disease is a viral infection, affecting patients being involved in the care of livestock either professionally or habitually. It is also known as ecthyma contagious, contagious pustular dermatitis or infectious euphoria being a rare zoonotic disease caused by an epitheliotropic DNA virus from the parapoxvirus group. We report a case of Orf disease affecting the hand of a patient with rheumatoid arthritis (RA) on treatment with methotrexate and adalimumab, an anti-tumor necrosis factor biological agent. The patient was successfully treated with doxycycline, while immunosuppressive treatment was discontinued.

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Keywords: Orf disease, rheumatoid arthritis, immunosuppression.

ABBREVIATIONS

RA:	Rheumatoid arthritis
CRP:	C-reactive protein
ESR:	Erythrocyte sedimentation rate
WBC:	White blood cells
PCR:	Polymerase chain reaction
DMARDs:	Disease Modifying Anti Rheumatic Drugs

INTRODUCTION

Orf disease is a viral disease affecting mainly farmers being involved in the care of livestock. The disease is a zoonotic disease being transmitted from animal to animal by contact. It affects people who are in contact with animals, either professionally or habitually; the case of sheep being mostly reported in the literature. It is a self-remitting disease.

Nowadays, rheumatoid arthritis (RA) is treated by immunosuppressive agents, particularly anti-TNF biologics. Patients may become immunosuppressed and they may develop infections. The case of a farmer with RA on treatment with immunosuppressants, who developed Orf disease which was successfully treated, is described.

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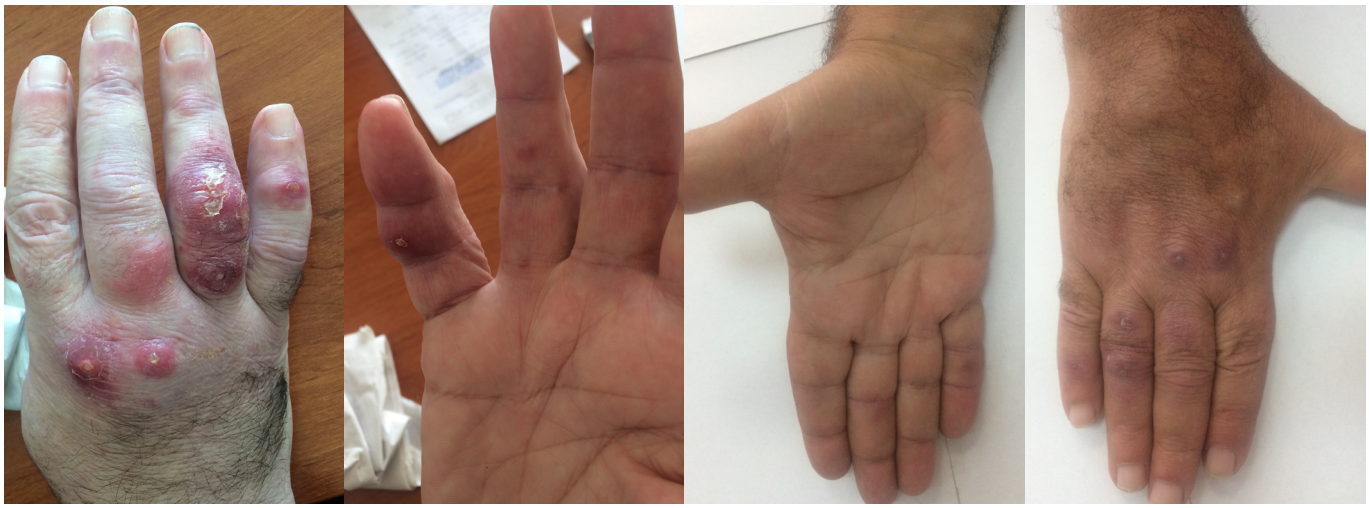
CASE REPORT

A 65-year old man presented to the outpatient clinic complaining of severe pain in his right hand, which developed over the course of two months and progressively deteriorated. The patient had a 3-year history of anti-CCP positive and RF positive RA. The clinical examination revealed a swollen and warm hand with tender and erythematous nodules. These lesions exhibited spontaneous outflow of serous fluid. The lesions evolved after exposure to livestock. The patient was a farmer and had a farm in which he looked after sheep. The first lesion started as a blister on the palmar surface of the fifth finger of the right hand, with new lesions gradually expanding to the dorsal surface (**Figure 1**). Body temperature and vital signs were normal. Physical examination revealed absence of tender and swollen joints, with RA being in remission.



Figures 1-4: Orf disease. Nodular lesions of the dorsal (1) and palmar (2) surface of the right hand two months after the eruption. Healed lesions with scarred tissue two months after the discontinuation of immunosuppressive drugs (3,4).

The patient was examined by a dermatologist, and childblains were diagnosed. Local medication was initiated, and amoxicillin with clavulanic acid was administered for a period of 20 days. However, the lesion did not improve. The immunosuppressive treatment for RA consisted of methotrexate 12.5mg orally weekly, methylprednisolone 4mg daily and adalimumab 40mg subcutaneously every other week. Other medications included amiodarone and dicumarol for atrial fibrillation, lorazepam and enalapril for arterial hypertension, and calcium plus vitamin D, as the patient was on long-term treatment with corticosteroids. Initial laboratory testing showed mild leukocytosis (WBC 11,000 x 10^9/L) with elevated markers of inflammation (ESR 42mm/h and CRP 1.9mg/dL). Serological testing for Brucella species, hepatitis viruses and tuberculin skin test were all negative. Ziehl-Neelsen stain and cultures of the fluid for common bacteria, fungi and mycobacteria were negative.

A new dermatological assessment was made, and the lesions were described as target-like papillary nodules with exudative fluid and erythematous margins.

The diagnosis of Orf disease in an immunocompromised patient was made in a patient with a history of occupational exposure to sheep. Subsequently, all immunosuppressive drugs were discontinued except methylprednisolone, which was increased from 4 to 6mg in order to keep RA in remission. In parallel, surgical debridement of the inflamed cutaneous area was performed. Ten days after the aforementioned interventions, the patient's hand improved. Concurrently inflammatory markers improved, ESR and CRP being 31 mm/h and 0.5 mg/dL, respectively. Two months later, the nodules improved, leaving a scar at the initial sites of inflammation (**Figure 2**). RA remained in remission, and methotrexate was reintroduced at the initial dose, while methylprednisolone was tapered to 4mg daily.

DISCUSSION

Orf disease, also known as ecthyma contagious, contagious pustular dermatitis or infectious euphoria, is a rare zoonotic disease caused by an epitheliotropic DNA virus from the parapoxvirus group.[1] Carrier animals (goats and sheep) transmit the virus to humans through direct contact, especially during milking from infected breasts.[2] Transmission also occurs indirectly from the environment after inoculation of infected materials on plants.[2] Areas of skin or mucosal discontinuation are entrance gates where the virus is locally multiplied, creating the characteristic lesions of the disease.[1] They tend to appear on the hands and the fingers, and, rarely, on the face, nostrils, tongue, eyelids and perianally.[1]

The first phase of the disease is characterized by the appearance of a small papule one week after exposure to the virus. In the second phase, the lesions enlarge and take on an iris-like shape with a central red nodule, a surrounding white circle and an erythematous exterior margin. In the third phase, the lesions enlarge rapidly and have an exudative appearance. The fourth phase is regenerative, when the lesion takes on black spots and exhibits a thin crust. The fifth phase is papillomatous, with very small papillomas that can be seen on the lesion. The last phase is regression.[1]

Contact with sheep or goats, a history of work in the slaughtering industry, clinical appearance and epidemiologic data are important for diagnosis which is made clinically, based on history of contact with infected animals or environment, accompanied by the typical nodular target-like lesions. The diagnosis is confirmed only in

severe cases with histopathologic studies, electron microscopy and virus isolation by PCR.[3] Differential diagnosis includes herpetic whitlow, cowpox, milker's nodule, cat-scratch disease, anthrax, tularemia, deep fungal infections, tuberculosis, atypical mycobacterial infections, syphilitic chancre, sporotrichosis, keratoacanthoma, pyogenic granulomas and malignant tumors.[4] In our patient, there was no need for further examinations, as the clinical findings and the history of exposure were typical. The disease usually follows a benign course and is self-limited within 4-8 weeks, making medical and surgical interventions unnecessary.[5] In immunocompromised patients, the disease may be persistent, requiring medical treatment such as antiviral therapy (cidofovir), corticosteroid use, cryotherapy and surgical resection because of the risk of serious secondary infections.[5-8]

In the case described herein, the patient suffered from RA and was treated with immunosuppressive drugs, namely methotrexate, adalimumab and corticosteroids. This treatment approach led to remission of RA, but was the cause for a potentially self-limiting disease to become chronic. For this reason, patients should be monitored regularly and, in case of infection, the administration of synthetic and biological DMARDs should be modified or discontinued, the infection being treated simultaneously.[9] In the case of our patient, discontinuation of immunosuppressive drugs was enough for the cure of Orf disease.

CONCLUSION

Detailed history and clinical examination are vital for the diagnosis and treatment of atypical or uncommon infections such as Orf disease in immunosuppressed patients. Orf disease is benign and self-limiting, but the administration of immunosuppressive therapy can lead to chronicity with the risk of secondary bacterial infection. Awareness of the disease and a high index of suspicion is important in order to avoid inappropriate treatment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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CASE REPORT

Sjögren Syndrome Associated with Inflammatory Muscle Diseases

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ABSTRACT

Objectives: Sjögren's syndrome (SS) is a chronic autoimmune inflammatory disorder characterized by diminished lacrimal and salivary gland function that may affect multiple organ systems. The association of SS with inflammatory myopathies (IM), a group of diseases characterized by chronic inflammation of striated muscle and skin has been infrequently described. **Methods:** We present two cases diagnosed with SS who developed IM. We have also conducted a review of the English literature to depict all available clinical evidence on the clinical association of SS with IM. **Results:** Two female patients diagnosed with SS developed polymyositis (PM) and inclusion body myositis (IBM) respectively. The literature review identified 24 cases with coexistence of the two autoimmune conditions (SS and IM). Twenty-two patients were females and two males. Eight patients were diagnosed with IBM, 15 were diagnosed with PM and 1 with dermatomyositis. All patients had biopsy proven IM. **Conclusions:** There is evidence of clinical association of primary SS and IM especially with IBM and PM. Patients with SS and symptoms of muscle weakness should be investigated for associated IM.

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INTRODUCTION

Sjögren Syndrome (SS) is a chronic autoimmune inflammatory disease that primarily affects the exocrine glands and has also a variety of extraglandular manifestations.[1-6] SS can occur as a distinct disease entity (primary SS) or in the context of an underlying autoimmune rheumatic disease (secondary SS) mainly rheumatoid arthritis, systemic lupus erythematosus, scleroderma and others.[7-10] Inflammatory myopathies (IM) is a heterogeneous group of disorders characterized by chronic inflammation of striated muscle and skin. They share features of skeletal muscle weakness and elevated serum levels of muscle enzymes.[11,12] Muscle involvement is a frequent clinical

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manifestation in SS, expressed mainly with myalgias and muscle weakness. Frank IM is less common.[13-15] In this report we present two cases of primary SS associated with IM with a literature review.

METHODS

We present two cases of female patients diagnosed with Sjögren syndrome who were followed up regularly in the Rheumatology Department of the University Hospital of Ioannina. The patients developed muscle weakness and were additionally diagnosed with IM. We also reviewed the English language literature using PubMed database and the following index terms: SS, IM, inclusion body myositis (IBM), Polymyositis (PM), Dermatomyositis (DM) and myositis in order to indentify cases of SS associated with IM. We recorded the epidemiological features of the patients found in the literature including age, gender, age of diagnosis of SS and IM and their serological profile. All patients had to have positive muscle biopsy.

CASE 1

A 70-year-old white female with a past medical history of arterial hypertension and sinus tachycardia was diagnosed with SS in 2002. The diagnosis was based on sicca symptoms (dry eyes and dry mouth), as well as positive objective sicca parameters for dry eyes (Schirmer's I and Rose Bengal tests), positive antinuclear antibodies (ANA) at a titer of 1/320 with a fine speckled pattern, positive anti-Ro52 and minor salivary gland biopsy compatible with SS. The patient presented also arthralgias, thus she was treated with hydroxychloroquine (HCQ) 200mg/day. In August 2014 the patient complained of proximal and distal muscle weakness. The laboratory findings showed elevated serum muscle enzymes: creatine kinase (CK) 556 IU/L (normal range: 40-190 IU/L), lactate dehydrogenate (LDH) 371 U/L (normal range: 115-230 U/L). The transaminases (AST 32 IU/L, ALT 27 IU/L) and aldolase (3,8 IU/L) were in normal range. To rule out occult malignancy, a chest computed tomography and an ultrasound of the abdomen were performed and did not reveal significant findings. An electromyogram showed diffuse atrophy and loss of muscle fibers.
Muscle biopsy showed findings compatible with IBM (**Figure 1**). The patient started treatment with methylprednisolone 48mg/day with tapering and subsequently methotrexate (MTX) 15mg/week was added as a sparing agent of steroids. In May 2015 a new electromyogram revealed improvement of muscle findings which correlated with her clinical and laboratory improvement. To date the patient continues therapy with low doses of steroids (4mg/day) and MTX (15mg/week).

CASE 2

A 67-year-old white female was diagnosed in 2002 with SS based on arthralgias, sicca symptoms (dry eyes and dry mouth), positive ocular signs (Schirmer's I and Rose Bengal tests), positive ANA at a titer of 1/320 with fine speckled pattern. In addition, minor salivary gland biopsy was positive for SS. In January 2015, she presented to our outpatient clinic with progressive proximal muscle weakness, inability to climb stairs and elevated muscle enzymes. Her past medical history included diabetes mellitus and dyslipidemia. She was treated with HCQ, atorvastastin, metformine, saxagliptin and pilocarpine. Her family doctor had discontinued HCQ and atorvastatin 10 months ago, when abnormal laboratory findings and clinical symptoms had begun. The patient was admitted to our clinic for further investigation. Physical examination revealed mild decrease of muscle strength of biceps and iliopsoas (4/5). Laboratory evaluation showed elevation of CK: 5004 IU/L, AST: 121 IU/L (normal range: 10-35 IU/L), ALT: 97 IU/L (normal range: 10-35 IU/L), LDH: 1059 IU/L and aldolase: 40 IU/L (normal range: 0-7,6 IU/L). An electromyogram showed significant features of IM while the muscle biopsy of the left quadriceps confirmed the diagnosis of PM (**Figure 2**). A computed tomography of the chest and abdomen was unremarkable. The patient was treated with methylprednisolone (48mg/day) and MTX (15mg/week) as a sparing agent of steroids. Six months later, the patient had significant clinical (muscle strength 5/5) and laboratory (AST: 16 IU/L, ALT: 97 IU/L, CK: 103 IU/L, LDH: 451 IU/L) improvement, while the dose of steroids was decreased to 4mg/day and the dose of MTX was stable.

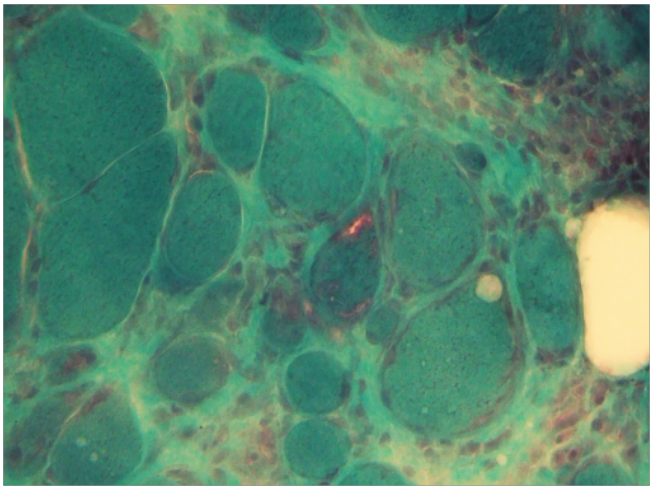

Figure 1. Muscle biopsy from a patient with SS and IBM (1st Case). Modified Gomori trichrome. The muscle biopsy shows: pronounced variation in fiber size, inflammatory response and a fiber with rimmed vacuole

DISCUSSION

According to the literature the percentage of patients with primary SS who manifested symptoms of IM, widely

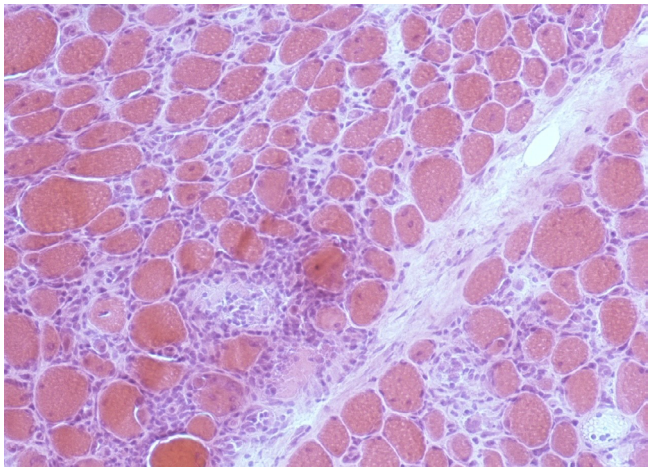

Figure 2. Muscle biopsy from a patient with SS and PM (2nd Case). Haematoxylin & Eosin The muscle biopsy shows: endomysial inflammatory infiltrates

vary between 2,5% - 10%.[16] Most studies showed significantly lower percentages of patients with primary SS who developed true IM (proven by biopsy). In the study of Colafrancesco et al. the percentage of the SS patients with manifestations of biopsy proven myositis was 0,75% (10 out of 1320).[17] Another recent study published by Kanellopoulos et al. demonstrated 3 out of 518 (0,6%) patients with primary SS who were diagnosed with myositis.[18] The study of Kraus et al raised this rate to 3%.[13] Both our cases had SS fulfilling the American European consensus group criteria[19] and both developed IM many years after the diagnosis of SS. In our first case the patient was diagnosed with IBM 13 years after the diagnosis of primary SS. Eight cases of SS patients, diagnosed according the performed biopsy, with IBM were reported (**Table 1**). The interval between the two diagnoses largely varied from 0 to 14 years. In the majority of the patients (7/8) ANA were positive. Anti Ro/SSA antibodies were positive in half of them (4/8). Anti La/SSB antibodies were positive only in one patient, while RF was positive in

Table 1: Published cases of primary Sjogren Syndrome associated with inflammatory myopathies (proven by biopsy).

Author (year)	Study	Sex	Age at onset of pSS	Age at onset of IM	Classification of IM (Biopsy)	Autoantibodies
Chad D. et al. (1982)	Case report	F	32	33	IBM	RF, ANA
Ringel SP. et al. (1982)	Case series (1/4)	F	64	64	DM	RF, ANA, Ro/SSA, La/SSB
Ringel SP. et al. (1982)	Case series (2/4)	F	42	42	PM	RF, ANA, Ro/SSA, La/SSB
Ringel SP. et al. (1982)	Case series (3/4)	F	55	55	PM	RF, ANA, Ro/SSA, La/SSB
Ringel SP. et al. (1982)	Case series (4/4)	F	29	29	PM	ANA, Ro/SSA, La/SSB
Gutmann L. et al (1985)	Case report	F	58	60	IBM	RF, ANA
Leroy JP. et al. (1990)	Case report	F	65	65	PM	RF, ANA, Ro/SSA, La/SSB
Khraishi MM. et al (1992)	Case report	F	-	69	IBM	RF, ANA, Ro/SSA, La/SSB
Imasaki T. et al. (1996)	Case report	F	53	53	PM	-
Kanellopoulos P. et al. (2002)	Case series (1/3)	F	33	47	IBM	ANA, AMA, Ro/SSA
Kanellopoulos P. et al. (2002)	Case series (2/3)	M	67	66	IBM	ANA, Ro/SSA
Kanellopoulos P. et al. (2002)	Case series (3/3)	F	52	45	IBM	ANA, Ro/SSA
Aoki A. et al. (2003)	Cohort study (1/5)	F	74	74	PM	ANA, Ro/SSA, La/SSB
Aoki A. et al. (2003)	Cohort study (2/5)	F	63	63	PM	ANA, Ro/SSA, RNP
Aoki A. et al. (2003)	Cohort study (3/5)	F	62	-	PM	RF, ANA, Ro/SSA, RNP
Aoki A. et al. (2003)	Cohort study (4/5)	F	57	57	PM	ANA, Ro/SSA, La/SSB
Aoki A. et al. (2003)	Cohort study (5/5)	F	50	50	PM	ANA
Misterska-Skora M et al. (2013)	Case report	F	47	47	IBM	RF, ANA
Colafrancesco S. et al. (2015)	Cohort study (1/6)	F	46	61	PM	RF, ANA, Ro/SSA, La/SSB
Colafrancesco S. et al. (2015)	Cohort study (2/6)	F	57	58	PM	-
Colafrancesco S. et al. (2015)	Cohort study (3/6)	F	68	69	IBM	RF
Colafrancesco S. et al. (2015)	Cohort study (4/6)	F	62	52	PM	RF, ANA, Ro/SSA,
Colafrancesco S. et al. (2015)	Cohort study (5/6)	F	37	42	PM	RF, ANA, Ro/SSA, La/SSB, Jo1, RNP
Colafrancesco S. et al. (2015)	Cohort study (6/6)	M	53	53	PM	ANA, Ro/SSA, Jo1
Migkos MP. et al. (Present study)	Case series (1/2)	F	57	70	IBM	ANA, Ro/SSA
Migkos MP. et al. (Present study)	Case series (2/2)	F	54	67	PM	ANA

pSS: primary Sjogren syndrome, IM: inflammatory myopathies, DM: dermatomyositis, PM: polymyositis, IBM: inclusion body myositis, ANA: antinuclear antibodies, RF: rheumatoid factor, M: male, F: female

5 out of 8 patients. In our case the patient was positive for ANA and anti-Ro/SSA antibodies.

IBM differentiates its clinical presentation from the other IM. IBM affects predominantly the distal muscles and more specific the foot extensors and finger flexors. IBM is usually refractory to treatment. Corticosteroids and immunosuppressive drugs (MTX, azathioprine, cyclosporine) have failed to show long term benefits.[12,20] Despite the transient effect of intravenous immune globulin, it remained ineffective in controlled trials.[21,22] IBM associated with SS responded better to treatment compared to patients diagnosed with IBM alone. In the study by Colafrancesco et al, one patient diagnosed with IBM was treated with intravenous immune globulin and achieved clinical and laboratory remission.[17] In a case report with biopsy proven IBM reported by Misterska-Skora et al., the patient was treated with steroids and MTX and achieved remission in 8 months.[23] Kanellopoulos et al published three cases with primary SS and IBM. Two of them were treated with HCQ and 1 received steroids and MTX. All three patients showed improvement in their muscle strength.[18] In our case the patient also received corticosteroids and MTX and had significant clinical and laboratory improvement 8 months after treatment.

PM is also associated with primary SS. In the literature review we identified 15 cases of primary SS and biopsy proven PM (**Table 1**). Thirteen out of 15 patients had positive ANA. Anti-Ro/SSA antibodies were present in 12 patients, while anti-La/SSB antibodies and rheumatoid factor was present in 8 and 7 out of 15 patients respectively. In our second case the patient had only positive ANA. Aoki et al presented 5 cases of PM associated with primary SS. The patients received prednisolone and they achieved clinical and laboratory remission. In three of them, relapse of the disease was mentioned with muscle weakness and elevation of CK. In those patients the dose of corticosteroids was increased, and remission was achieved without additional immunosuppressive therapy.[24] Colafrancesco et al diagnosed 5 patients with PM. All patients were treated with steroids and HCQ and according to their response pulses cyclophosphamide and rituximab were used for achieving remission.[17] Pulses of cyclophosphamide therapy were also used by Leroy et al in a patient with SS and PM.[15] Finally, Imasaki et al described a patient with bronchiolitis obliterans organizing pneumonia, primary SS and PM. The patient received prednisolone and two months later achieved remission.[25] In our second case, the patient was diagnosed with PM and received methylprednisolone and MTX successfully.

Only one case with biopsy-proven DM in a patient with SS was identified in the literature.[26]

Additionally, it should be mentioned that in rare cases of myositis associated with SS, with CK and electromyography abnormalities, muscle biopsy and histology examination were mandatory, in order not to overlook a real PM-overlap syndrome, misinterpreted as extraglandular involvement of SS.[27]

On the other hand, it is mandatory in patients with SS who have developed IM, to rule out other disorders known to cause myopathy. To this end, one must exclude lymphoma development, statin and HCQ induced myopathy. In the first case, a careful clinical examination with the appropriate imaging tests, tissue biopsy, as well as the detection for cryoglobulins, measurement of complement levels and serum electrophoresis will help the diagnosis of lymphoma.[28] In the cases of statin and HCQ-induced myopathy, a detail past medical history, the presence of antibodies against 3 –hydroxy-3 methylglutaril-coenzyme-A reductase (HMGCR), the enzyme target of statin therapy and muscle biopsy will differentiate these two entities from IM. It is reported that statin myopathy is associated with the presence of HMGCR antibodies and causes a necrotizing myopathy in the affected muscles.[29,30] In addition, HCQ-induced myopathy is a rare condition which may cause proximal muscle weakness and cardiomyopathy and the muscle biopsy shows curvilinear bodies and vacuolar changes.[31]

Finally, novel studies highlight that anti-Ku antibodies have been reported in a wide spectrum of autoimmune diseases, sometimes in association with IM. In the study of Rigolet et al, out of 34 anti-Ku positive patients, 11 had IM, 8 of them as part of an overlap syndrome defined as IM associated with connective autoimmune disease (systemic sclerosis, SS and systemic lupus erythematosus).[32] In a recent study published by Wielosz et al are reported 3 cases of anti-Ku positive patients: it concludes that the presence of anti-Ku antibodies is associated with a wide range of non-specific symptoms regarding muscle, joint and skin involvement (33). In another recent study published by Fiorentino et al, a new 60 kDa specificity was detected by immunoblotting HeLa cell lysates, the targeted autoantigen was identified as poly(U)-binding-splicing factor 60 kDa (PUF60). In this study it is reported that the new antibody anti-PUF60 it is present in SS patients with DM.[34]

CONCLUSION

Patients with primary SS and symptoms of muscle weakness should be investigated for associated IM. There is evidence of clinical association of primary SS and IM especially with IBM and PM. It seems that both entities respond better to treatment when they are associated with SS than as district entities.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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MEDITERRANEAN JOURNAL OF RHEUMATOLOGY | 29 2 2018

CLINICAL IMAGE

Calcified lymph nodes and systemic sclerosis

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Keywords: systemic sclerosis, scleroderma, lymphadenopathy.

CLINICAL IMAGE

Figure 1 depicts a chest X-ray of a 55-year-old female with a long history of diffuse systemic sclerosis. The patient first presented to the Rheumatology Clinic in 1984 with Raynaud's, sclerodactyly, cutaneous telangiectasias and recurrent ischemic digital ulcers with positive anti-Scl70 antibodies. Based on the clinical and laboratory findings a diagnosis of diffuse systemic sclerosis (SSc) was made.

Interestingly, in addition to all the above mentioned typical features of SSc, the patient also had a palpable, hard, supraclavicular lymph node from the beginning of her illness. An extensive work up was performed to rule out malignancy. Chest CT revealed extensive mediastinal lymphadenopathy. Notably, lymph nodes were calcified in an "eggshell" pattern. Supraclavicular lymph node biopsy ruled out malignancy; moreover, no evidence of granulomatous disease was found.

Throughout her 30 years of follow-up, the patient slowly but steadily deteriorated. She was treated with D-penicillamine, methotrexate and rituximab respectively; none of which had any effect on her radiographic findings. She never had SSc-related calcifications. She developed interstitial lung disease, gastrointestinal involvement with esophageal dysmotility and severe recurrent digital ulcers that lead to autoamputation of most of her fingers. At the later stages of the disease, she also developed heart failure and pulmonary arterial hypertension. Calcified mediastinal lymph nodes were always visible on plain x-rays during the course of her illness without any evidence of change over time.

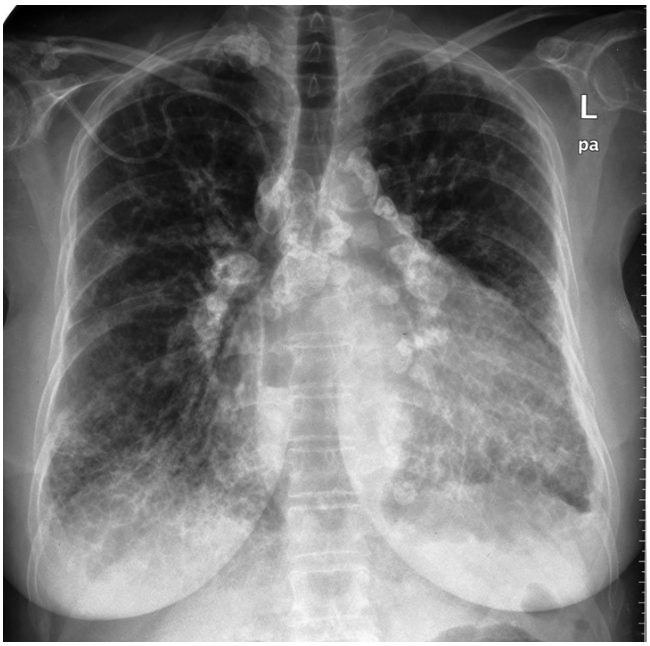

Figure 1.

Her chest x-ray presented herein, has many findings:

- A reticular pattern is noted in both lungs, more prominent in the lower lung zones. The appearance is consistent with interstitial lung disease in the context of SSc;
- The esophagus is dilated, with the presence of an air-fluid level, a common finding in SSc;

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- Abnormal cardiothoracic ratio, with a markedly enlarged heart due to heart failure;
- There is extensive mediastinal and hilar lymphadenopathy; the lymph nodes are calcified in an "eggshell" pattern. There are also similarly calcified supraclavicular lymph nodes on the right.

The exact cause of calcific lymphadenopathy in this particular case was never proven. The differential diagnosis of calcific mediastinal lymphadenopathy is broad and includes tuberculosis, sarcoidosis, lymphoma, metastasis from thyroid cancer as well as osteogenic sarcoma, amyloidosis and Castleman disease. The extremely long history virtually rules out malignant or infectious causes. Sarcoidosis is included in the differential diagnosis, but the biopsy was not supportive; the patient also never had any typical features of this disease. Occupational exposure such as silicosis is one of the causes of "eggshell" calcific mediastinal lymphadenopathy. The patient was a dental technician, a profession known to associate with exposure to silica. Interestingly, silica is also a well known environmental trigger for SSc. We believe that the most likely scenario for our patient is that occupational exposure to silica triggered both SSc and lymphadenopathy. Of note, there is similar published case report in the literature.[1]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Stress perfusion Cardiac Magnetic Resonance in Patients with Antiphospholipid Syndrome

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ABSTRACT

Background: Antiphospholipid syndrome (APS) is characterized by the combination of recurrent arterial and venous thrombotic events and detection of persistently elevated antiphospholipid antibody titers in the serum or plasma. APS clinical manifestations also include non-thrombotic complications from various organ systems, mainly the CNS, kidneys, and heart. Cardiac manifestations of APS include valvulopathy, myocardial infarction and angina (stable, unstable, and Pritzmental angina). A previously published case series of cardiac magnetic resonance (CMR) in patients with APS has revealed a high rate of asymptomatic myocardial necrosis and scarring, but the prevalence of myocardial ischemia identified as CMR perfusion defects prior to development of necrosis is unknown. **Aims of the study:** To detect CMR imaging markers of myocardial ischemia in APS patients without symptoms of cardiovascular disease (CVD). **Methods:** We will scan fifty APS patients without symptoms of CVD stress-perfusion CMR in a 1.5 Tesla tomographer, after intravenous infusion of adenosine and gadolinium. In addition to markers of cardiac anatomy and function, we will record imaging markers of ischemia and scarring, namely perfusion defects (PDs), and late gadolinium enhancement (LGE). We will perform parametrics using dedicated software in order to derive each patient's myocardial perfusion reserve index (MPRI). Scans will be reviewed independently by two experienced reviewers, with evaluation of inter- and intra-observer reliability. Statistical hypotheses will be examined using Student's test and Pearson's correlation coefficient, or non-parametric equivalents (Kruskall-Wallis and Spearman) for continuous variables, and Fisher's exact test for binary variables. Linear or logistic regression analyses will be used to investigate APS-related determinants of subclinical myocardial ischemia. **Anticipated benefits:** We expect to identify CMR imaging patterns characteristic of APS, which will allow proactive therapeutic interventions for primary prevention of CVD and guide further research into the pathogenesis of APS cardiac manifestations.

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Abbreviations

APS:	Antiphospholipid syndrome
aPL:	Antiphospholipid antibodies
LAC:	Lupus Anticoagulant
aCL:	Anticardiolipin antibodies
PAPS:	Primary APS
SLE:	Systemic Lupus Erythematosus
HVD:	Heart valve disease

MI:	Myocardial infarction
EMF:	Endomyocardial fibrosis
CMR:	Cardiac magnetic resonance
Stress-CMR:	Stress perfusion-fibrosis CMR
SPECT:	Single photon emission computed tomography
PET:	Positron emission tomography
LGE:	Late gadolinium enhancement
ECG:	Electrocardiogram
TE:	Echo time
TR:	Repetition time
IR:	Inversion recovery
FA:	Fractional anisotropy
DTPA:	Diethylenetriamine pentaacetate
PD:	Perfusion defect
MPRI:	Myocardial perfusion reserve index
LV:	Left ventricle

INTRODUCTION

Antiphospholipid syndrome (APS) is an autoimmune disorder characterized by venous, arterial, and/or small vessel thrombosis, pregnancy morbidity, and elevated levels of antiphospholipid antibodies (aPL), namely lupus anticoagulant (LAC), anticardiolipin antibodies (aCL), and/or anti-beta2-glycoprotein I antibodies.[1] Any combination of thrombotic events is possible at variable intervals, and the syndrome can be either primary (PAPS) or associated with an underlying condition, most commonly systemic lupus erythematosus (SLE/APS). The 5-year mortality in APS is 5.3%, with up to 40% of deaths related to serious thromboembolic events such as stroke, pulmonary embolism and acute myocardial infarction.[2]

The most common type of cardiac involvement in APS is heart valve disease (HVD),[3,4] characterized by thickening and/or vegetation of the cardiac valves, as initially described by Libman and Sacks in patients with SLE.[5,6] The coexistence of antiphospholipid antibodies (aPL) with SLE is associated with a 3-fold greater risk of HVD. Valvulopathy tends to progress overtime, with a corresponding increase in the risk of stroke.

Non-valvular heart disease manifesting as myocardial infarction (MI) has been diagnosed in 5.5% of APS and was the presenting manifestation in 2.8% of APS.[2] Additionally, coronary vasospasm, has been described as a cause of myocardial ischemia without thrombosis (also known as Pritzmental's angina or cardiac syndrome X) in patients with APS.[7,8] Furthermore, myocardial ischemia can be caused by thrombotic cardiac microangiopathy, with the ischemic subendocardium acting as a triggering factor for clot formation, especially in the presence of left ventricular dysfunction.[9] Finally, endomyocardial fibrosis (EMF) has been reported as a cardiac complication of APS, possibly related to primary lesions of the coronary microcirculation.[10] Although the pathogenesis of EMF remains uncertain, the detection of antibodies in myocardial proteins of patients with this type of pathology suggests that the autoimmune response may be implicated in its etiology.

Cardiac magnetic resonance (CMR) is a reliable, non-invasive, radiation-free technique used in the evaluation of cardiac morphology, function, perfusion, and fibrosis. Contrast-enhanced stress perfusion-fibrosis CMR using adenosine (Stress-CMR) has demonstrated very high sensitivity in the detection on subendocardial perfusion defects over conventional imaging techniques in patients with coronary disease, cardiac syndrome X, and mincroangiopathy.[11-13] The CE-MARC study established the diagnostic accuracy of Stress-CMR for the detection of myocardial ischemia or scarring and confirmed its superiority over the single photon emission computed tomography (SPECT) and positron emission tomography (PET) scans.[14-16]

Stress-CMR is the technique of choice when quantification of scar or fibrotic tissue is needed (viability study).[17,18] The optimal time for scar detection is between 10 and 20 minutes of contrast administration, when characterization of scar, normal myocardium and blood pooling is most accurate, based on differences in contrast enhancement. This method is termed "late gadolinium enhancement" (LGE), and it is the gold standard for the evaluation of myocardial scarring in vivo.[19] One previous study of CMR in patients with APS has shown high rates of asymptomatic myocardial ischemia detection,[20] introducing CMR as an emerging technique for the evaluation of indolent cardiac disease in this population.

PATIENTS AND METHODS

Fifty patients meeting the updated Sapporo criteria for APS (2006) and followed in Rheumatology Unit of the First Propaedeutic Internal Medicine Department (Director: Professor P. Sfikakis) at Laikon Hospital, Athens, Greece, will be included in the study.[1] Patients will be considered to have the secondary form of APS, if they have concurrent SLE, as defined by the American College of Rheumatology classification criteria.[21] Patients with renal insufficiency, pregnancy and contraindications to CMR testing or to intravenous contrast administration, will be excluded. Informed consent will be obtained from all patients, following the protocol's institutional review board by the local committee. Demographic, clinical and laboratory characteristics of patients will be recorded. Patients will undergo CMR on a 1.5 Tesla scanner (Signa CV/i, GE Medical Systems) using ECG-triggered steady-state, free precession breath-hold cines (echo time (TE)/repetition time (TR) 1.6/3.2 ms, flip angle 60) in long-axis planes and sequential 8 mm short-axis slices (3 mm gap) from the atrioventricular ring to the apex. Stress perfusion CMR will be performed using 140 mg/kg/min adenosine for 4 minutes (12, 20) and 0.1 mmol/kg Gd-DTPA will be given during the first-pass perfusion sequence (IR bal-

anced Turbo Field Echo,TR 2.8 ms, TE 1.38 ms, FA 45, slice thickness 8 mm, preparation pulse delay 200 ms). A rest perfusion will be performed using the same protocol. Finally, late gadolinium enhancement (LGE) images will be acquired 10 min after intravenous gadolinium-DTPA (Schering; 0.2 mmol/kg) in identical short-axis planes using an inversion-recovery gradient echo sequence for fibrosis detection (3D-Turbo field echo sequence, TR 5.1 ms, TE 2.5 ms, FA 15, slice thickness 8 mm). Inversion times will be adjusted to null normal myocardium (typically 320–440 ms; pixel size 1.7x1.4 mm).

CMR scans will be analyzed independently by two experienced cardiologists at Onassis Cardiac Surgery Center, Athens, Greece, blinded to clinical data (S. Mavrogeni, G. Kolovou). A consensus will be used for discordant grades, and the intra and inter-observer variability will be calculated with a goal of 0.85. Ventricular volumes, anatomy, and function will be measured for both ventricles using standard techniques and analysed using specialized software. Perfusion defects (PDs) will be assessed by both visual and parametric analysis. Quantification will be performed using delineation of endo- and epicardial LV borders throughout first-pass perfusion (MEDIS system, Leiden, Netherlands). Stress and rest perfusion slopes will be derived using Fermi-fitting of signal intensity vs time and normalized to LV blood pool slope. A Myocardial Perfusion eserve Index (MPRI) will be calculated for each patient, defined as the ratio of stress to rest. Finally, LGE images will be assessed for midwall or subepicardial enhancement, compatible with myocarditis, with subendocardial or transmural enhancement in the distribution of a coronary artery, compatible with myocardial infarction, and for diffuse subendocardial fibrosis, compatible with vasculitis in this cohort.

Our main investigative hypotheses are the following:

1) There is a high prevalence of asymptomatic cardiac involvement in patients with APS, which can be readily detected using CMR. Specifically, we expect to find high rates of silent myocardial ischemia and fibrosis, manifested as subendocardial PDs, low MPRI, and abnormal LGE.

2) Distinct patterns of abnormal CMR findings will be found in PAPS and SLE/APS patients.

Additional hypotheses include:

3) Possible correlation of PDs, MPRI, and LGE abnormalities with double and triple antiphospholipid antibody positivity, a history of arterial, venous, or dual thrombosis, the number of prior thrombotic events, a history of a history of thrombotic versus obstetric-only APS, and exposure to corticosteroids, biologic agents, or other immunosuppressants.

4) Detection of Stress-CMR abnormalities even in APS patients without traditional cardiovascular risk factor comorbidities.

STATISTICAL ANALYSIS

Investigational hypotheses of correlation between measured variables will be carried out using Student's test or Pearson's correlation coefficient, or non-parametric alternatives as appropriate (Kruskall-Wallis test, Spearman's coefficient) for continuous variables. For binary categorical variables, Fisher's exact test will be used. Multiple linear or logistic regression analyses will be used to investigate associations of continuous or binary CMR outcome measures, respectively, with recorded demographic and clinical parameters. The pre-specified statistical significance level is set at a=0.05. STATA software version 12.0 (College Station™, Texas, USA) will be used for all analyses.

AIMS OF THE STUDY

The present study will be the first provide high-quality epidemiologic data on the prevalence of CMR-detected cardiac involvement in a representative sample of patients with APS. We will estimate the prevalence of significant lesions indicative of valvulopathy, myocardial dysfunction, microangiopathy, ischemia, or fibrosis in PAPS and SLE/APS patients, and determine the frequency of clinically silent cardiac disease in this population. We will provide detailed results on the prevalence of PDs, MPRI, and LGE abnormalities. We will perform statistical analyses to examine potential correlations of abnormal imaging findings with disease-related clinical characteristics and comorbidities. Based on the coexistence of different abnormalities in individual patients, we will describe CMR imaging patterns of cardiac involvement in APS, and detect pattern differences between PAPS and SLE/APS patients.

ANTICIPATED BENEFITS

The present study will contribute valuable epidemiologic data on the prevalence and the patterns of asymptomatic cardiac complications in APS. This will be the first description of CMR-detected myocardial ischemia in Greek APS patients, and is expected to further our knowledge on the rarer cardiac manifestations of APS, such as microangiopathy and endomyocardial fibrosis. Finally, this will be an ideal screening for indolent cardiovascular disease in our high-risk APS population, allowing appropriate treatment and prevention of future complications.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Five-year prospective multi-center cohort study of patients with giant cell arteritis in Greece

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ABSTRACT

Giant cell arteritis (GCA) is the most common systemic vasculitis in the aged population associated with significant morbidity due to the long term administration of corticosteroids and the presence of various comorbidities. Data regarding its current treatment modalities, comorbidities, morbidity and mortality in Greece are limited. In this multi-center, prospective study that begun at the end of 2015 patients with newly diagnosed GCA according to the modified 1990 ACR criteria, as well as individuals with established or relapsing disease have been included. During the 1st phase of the study that is still ongoing, data are being collected concerning demographic and clinical characteristics of the patients, treatment at the onset of the disease and at relapses, relapses, adverse events of therapy, comorbidities, hospitalizations and deaths. During the 2nd and 3rd phase of the study patients will be reevaluated 2 and 5 years after their 1st evaluation. The study is expected to provide valuable data regarding the current clinical characteristics, co-morbidities, therapeutic regimens used, relapse rate, morbidity and mortality of patients with GCA.

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INTRODUCTION

Giant cell arteritis (GCA) is the most common type of systemic vasculitis,[1] typically affecting individuals older than 50 years, with an estimated incidence ranging between 0.1 to 33 cases per 100,000.[2] Data regarding its prevalence and characteristics in the Greek population are limited. The latest available data indicate a prevalence of 0.08%[3] while there are no long-term data available regarding its course, current treatment modalities, comorbidities and mortality.

GCA is a heterogeneous disease often not conforming to a single clinical presentation. Ischemic tissue damage, which is the most serious complication, results in vision loss in 15-20% of cases and requires urgent treatment.[1] Glucocorticoids remain the mainstay of treatment for GCA and should be initiated promptly. Improvement of headaches, malaise, fever and polymyalgia rheumatic symptoms are often dramatic. While glucocorticoids are highly effectively at controlling systemic inflammation and preventing acute damage (i.e. vision loss), they fail to cure the disease or to induce long-term, treatment-free remission.

Data from epidemiological and retrospective studies indicate that 40-60% of GCA patients will relapse during follow-up, therefore requiring prolonged treatment courses.[4,5] Approximately 50% of the relapses will take place during the 1st year of follow-up, while 36% of patients will flare more than 2 times.[5] Most frequent symptoms at relapse are polymyalgia rheumatica, headaches, scalp tenderness, jaw claudication and vision disturbances.[5]

Due to the frequent relapses, approximately 40% of patients do not manage to discontinue corticosteroids,[4] resulting in long-term exposure and increased risk of adverse events. These include osteoporotic fractures, cataracts, diabetes and vulnerability to infections in an aged population.[6] Data from a large multi-centric study showed that the long-term use (>12 months) of high doses of corticosteroids (> 10 mg/day) was associated with an increased risk of deaths due to serious infections.[7]

Despite these findings, a number of unanswered questions regarding the current state of treatment, the rate of side effects, the rate of comorbidities and the long-term survival of these patients remain unanswered.

AIM OF THE STUDY

The aim of this multicenter, prospective cohort study is to evaluate the clinical characteristics, course, treatment efficacy, co-morbidities, morbidity and mortality in Greek patients with GCA.

METHODS

Since the end of 2015, a prospective epidemiological study supported by the Greek Rheumatology Society has begun, and so far, 84 patients have been enrolled. Participating centers include academic and non-academic rheumatology clinics, National Health System outpatient clinics and private offices. Ethical approval has been obtained by the local institutional boards of participating centers.

Patients newly diagnosed with GCA according to the modified 1990 American College of Rheumatology (ACR) classification criteria as well as patients with established or relapsing GCA have been included in the study.

The design of this prospective study includes 3 successive phases.

During phase 1 of the study, which is still ongoing, the following data are being collected:

- demographic and clinical patient characteristics
- current or previous treatment(s)/ dose (including corticosteroids, non-biologic and biologic immunosuppressives)
- adverse treatment events
- comorbidities
- relapses (relapse is defined as new disease activity after a period of remission, or worsening disease activity that occurred during follow up)
- hospitalizations and
- deaths

Data are entered either in a printed form, or electronically via a specially designed portal (rheumstudygrps.gr), where participants log in by using personal codes provided to them by website administrators. Patients are registered by their first name/surname initials and at the end of each registration, a unique verification code is provided. Only the study coordinator has full access to data entered by all participants. Each participant had access to the data entered by his/her own center and could edit them until the closing date of this phase.

During the second phase of the study, all patients from the initial cohort will be re-evaluated 24 months (2 years) after their 1st evaluation. Data collection will be performed by the same methods that were used in phase 1 (printed and web-based form). In this phase, patients are registered exclusively with the unique verification code provided during phase 1.

During this third phase, a 3rd evaluation of the same cohort (5 years after the 1st and 3 years after the 2nd evaluation) will be performed. Data collection will be done with the same methods that were used in phase 2 (printed and web-based form).

Statistical analysis

All analyses will be performed with the use of Microsoft Excel 2013 and IBM SPSS Statistics v.20 software. Data will be analyzed by descriptive statistics. Demographic and descriptive continuous variables will be expressed as mean (standard deviation, SD) and median values (interquartile range, IQR). Categorical variables will be expressed as percentages. Chi square or Fisher's exact test will be used for comparison of dichotomous and Mann-Whitney or t-test for continuous variables. Binary logistic regression analysis will be performed in order to identify variables associated with relapses, serious infections, hospitalizations, corticosteroid discontinuation and death.

ANTICIPATED BENEFITS

This is the first multi-center prospective study of such scale in Greece that is anticipated to provide valuable

data regarding the clinical characteristics, co-morbidities, therapeutic regimens used (including their efficacy, safety, rate of discontinuation), relapse rate, morbidity (hospitalizations, infections, malignancies) and mortality of patients with GCA.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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RESEARCH PROTOCOLS-PROPOSALS

Correlates of sedentary behaviour and light physical activity in people living with rheumatoid arthritis: protocol for a longitudinal study

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ABSTRACT

Background: Sedentary behaviour (SB) is associated with adverse health outcomes in the general population. Replacing sedentary time with light intensity physical activity (LPA) has been linked with improvements in all-cause and cardiovascular disease mortality in adults. People with Rheumatoid Arthritis (RA) typically spend long periods of time sedentary, but the health consequences of 'too much sitting', and possible benefits of LPA, have not been fully explored in this population. Moreover, little is known regarding the determinants of these behaviours among people living with RA, and such knowledge is required for the development of effective behavioural interventions. **Aims:** To examine longitudinal relationships between: 1) objectively-assessed SB/LPA with health outcomes in RA, 2) hypothesised determinants of SB/LPA with objectively-assessed SB/LPA in RA. **Methods:** This longitudinal study will secure assessments at baseline (Time 1) and 6-month follow-up (Time 2) from RA patients. At both time points, physical assessments will be undertaken, and questionnaires administered to measure physical (e.g., percentage body fat, disease activity, physical function, pain) and psychological (e.g., depression, anxiety, vitality) health outcomes. Additional questionnaires will be administered to establish hypothesised determinants (i.e., psychosocial, individual differences, and physical environmental). Participants will wear the ActiGraph GT3X accelerometer and activPAL3$^{\mu TM}$ for 7 days to objectively measure SB and LPA. **Discussion:** Findings will elucidate the health correlates of SB in RA, as well as the relevance of interventions targeting reductions in SB by promoting LPA. Results will also assist in identifying intervention targets (i.e., determinants), with the potential to encourage SB change in RA.

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INTRODUCTION

Rheumatoid Arthritis

Rheumatoid Arthritis (RA) is a chronic autoimmune disease that affects approximately 0.3-1% of people worldwide.[1] RA is characterised by high-grade local and systemic inflammation, which leads to severe joint pain, stiffness and swelling in synovial joints of the body.[2-4]

People living with RA are exposed to a 50% increase in cardiovascular risk,[5] with heightened universal inflammation implicated in the common development of cardiovascular disease (CVD) -related morbidity and mortality in this patient group.[6,7-9] In addition, this elevated inflammation contributes to an increased risk of poor mental health in RA.[10]

Sedentary Behaviour

Sedentary behaviour (SB) is defined as 'any waking behaviour characterised by an energy expenditure ≤1.5 metabolic equivalents (METS) while in a sitting, reclining, or lying posture'.[11,12] It is distinct from physical inactivity, which refers to lack of regular engagement in moderate-to-vigorous intensity physical activity (MVPA [≥3 METS]) in accordance with physical activity recommendations (i.e., 30 minutes x 5 days per week for adults). Examples of common SBs include sitting watching television, reading a book, working at a computer and travelling in a vehicle.[13] In the general population, SB has been consistently associated with increased inflammation, and it has been proposed that this may represent a mechanism through which SB leads to an increased risk of poor health.[14,15] For example, prospective studies demonstrate high levels of SB to associate with worsened cardiovascular and cardiometabolic health; both of which are linked to heightened systemic inflammation.[16-25] In addition, a recent systematic review and dose-response meta-analysis reported that total sitting time and television viewing time were associated with a greater risk of several major chronic disease outcomes, including all-cause, CVD, and cancer mortality, as well as incident diabetes.[26] Importantly, such adverse health outcomes associated with engagement in SB have been shown to occur, despite the level of MVPA an individual engages in.[27,28] That is, SB represents an independent risk factor for poor health.[26]

SEDENTARY BEHAVIOUR AND RHEUMATOID ARTHRITIS

It has been reported that people living with RA typically spend long periods of time sedentary,[3,29,30] and recent accelerometry studies suggest people with this condition can spend up to 9 waking hours sedentary per day.[31,32] On the basis of emerging evidence for the association between SB and inflammation, Fenton and Kitas[29] hypothesised that high levels of SB in RA may exacerbate already elevated systemic inflammation in these patients, and contribute to the progression of RA outcomes. Akin to this proposition, Fenton et al.[31] summarised results of non-RA studies, demonstrating the adverse links between SB with inflammation and chronic diseases with an inflammatory component (e.g., CVD, type 2 diabetes), underlining the relevance of these findings for RA. That is, as evidence for the associations between SB, inflammation and poor health continues to accumulate, it is important to evaluate the role of SB as an independent risk factor for disease outcomes in RA – a population experiencing compromised health and at high risk of comorbidity.

Whilst studies to date are yet to determine the role of SB for inflammation in RA, research has begun to examine the implications of SB for broader RA outcomes. Such investigations have employed either device-based, or self-report methods to quantify SB. For example, Khoja et al.[33] reported positive associations between accelerometer-assessed SB with disease activity, and Greene et al.[34] and Giles et al.[35] showed that high levels of self-reported SB was associated with poorer physical function in RA patients. In addition, Prioreschi et al.[3] used accelerometry to determine SB patterns in people living with RA and found an inverse association between SB and bone mass. More recently, research studies have indicated that higher levels of accelerometer-assessed SB are associated with more pain and fatigue,[36] and a higher risk of CVD.[37] Specifically, Fenton et al.[37] found that total sedentary time was adversely associated with 10-year CVD risk in a sample of RA patients. Interestingly, this study was the first to examine whether the manner in which sedentary time was accumulated held implications for health outcomes in RA. Findings revealed a positive relationship between sedentary time accumulated in bouts ≥20 minutes and 10-year CVD risk.

This finding[37] is aligned with novel prospective and experimental studies, which indicate that shorter sedentary bouts (i.e., the duration of uninterrupted sedentary periods) and more frequent sedentary breaks (i.e., interruptions in sedentary time)with light physical activity {LPA [1.6 – <3 METS]), are beneficially linked to health outcomes in non-clinical and clinical populations.[20,27,38-43] For example, regularly breaking up SB with LPA has been associated with better cardiometabolic and cardiovascular health in adults (≥20 years)[20,38] and older adults,[27] and has also been linked with a reduced risk of disability among older adults.[41] A recent systematic review and meta-analysis of experimental and observational studies also revealed that LPA (overall, and sedentary breaks) could play a role in improving adult cardiometabolic health and reducing mortality risk.[44] In RA, recent studies have reported beneficial associations between daily LPA with CVD risk,[37] depression and vitality.[45]

Such findings illustrating the potential health benefits of LPA are particularly relevant to people with RA, given the pain and physical dysfunction characteristic of this condition. Indeed, approaches to reduce SB by promoting LPA ('sedentary breaks'), may be better tolerated than those targeting MVPA, the traditional focus of physical activity behaviour change interventions in this patient group. Moreover, the strong inverse correlation between SB and LPA in studies with RA patients, certainly points

to the potential of behavioural interventions which aim to displace SB with LPA among this population.[33,45]
Whilst studies on this topic are beginning to emerge, a number of important limitations mark the extant literature in this field, which should be addressed in future research. First, a heavy reliance on self-report measures to assess SB and physical activity within this patient group brings issues regarding social desirability, errors in participant recall, and a tendency to underestimate levels of SB and overestimate levels of physical activity.[16,46] Second, whilst several studies have employed accelerometers in an attempt to address the limitations of self-report, sedentariness is often incorrectly defined as activity ≤1 MET[33] or <1 MET.[47] As a result, SBs that require between 1-1.5 METS (e.g., sitting whilst watching television or reading) may be misclassified as LPA,[48,49] meaning the true significance of SB for RA outcomes cannot be accurately determined. Similarly, SB is sometimes defined as 'lack of engagement in purposeful physical activity' (i.e., physical inactivity, not meeting MVPA guidelines), which is inconsistent with the definition used by the SB Research Network (i.e., waking behaviour ≤1.5 METS, whilst sitting/reclining/lying),[11,12] employed almost globally across the SB literature. Finally, the majority of studies that identify associations between SB and health outcomes in RA are cross-sectional, posing a challenge when inferring a causal direction of these associations.[31] As such, research is required to address these methodological limitations, and generate important knowledge regarding the consequences of SB in RA for inflammatory burden and related RA outcomes. It is also essential that high quality studies investigate the potential benefits of LPA participation for people living with RA, in order to elucidate the potential relevance of interventions that focus on displacing SB with LPA.

DETERMINANTS OF SEDENTARY BEHAVIOUR AND LIGHT PHYSICAL ACTIVITY

In order to prevent the potential adverse consequences of SB for health in RA, interventions promoting SB change should target factors that influence this behaviour (i.e., determinants). If interventions are to focus on displacing SB with engagement in LPA, they will also need to consider the determinants of LPA.

Psychosocial determinants and individual difference factors

Self-efficacy. Social Cognitive Theory (SCT)[50] regularly serves as the theoretical basis for health behaviour change interventions.[51] Self-efficacy (i.e., situational-specific confidence), an underlying composite of SCT, has been identified as a consistent predictor of physical activity engagement.[51] Indeed, it has been well documented that self-efficacy is a significant determinant for the adoption and adherence of physical activity in different populations.[52-54] Contrastingly, few studies have considered self-efficacy as a determinant for SB change, particularly in the RA population. One study by Huffman and colleagues[47] indicated that self-efficacy for exercise was negatively and positively associated with SB and physical activity respectively in this patient group. As highlighted above, a criticism of this study is that an incorrect definition of SB as <1 MET was employed.

Quality of motivation. Self-Determination Theory (SDT)[55] proposes that variability in the reasons 'why' a person chooses to engage (or not to engage) in a behaviour, holds important implications for levels of engagement. Specifically, SDT suggests that an individual's motivation may vary in its degree of relative autonomy, with more autonomous reasons for engagement (e.g., fun, enjoyment, personally important) linked to an increased likelihood of adopting and persisting with engagement in a behaviour (e.g., physical activity). In contrast, more controlled reasons for participation (e.g., other people's approval, feeling guilty) are linked to a lesser chance of sustaining behaviour. The implications of quality of motivation have been demonstrated in a considerable amount of physical activity research with different populations,[56] including RA.[57]
SDT also postulates that humans have three basic psychological needs; namely, autonomy, competence and social relatedness. Fulfilment of these needs leads to fostering more autonomous motivation towards a behaviour, as well as benefits in mental health (e.g., vitality and well-being).[58] SDT suggests the social environment is central to the satisfaction of these three basic needs, and holds implications for encouraging behaviour change through promoting more autonomous motivation. Specifically, the provision of autonomy support from an 'important other' (e.g., peer, parent, spouse, health professional) is reported to hold positive implications for need satisfaction, quality of motivation and behavioural engagement.[59-62] Fenton et al.[45] demonstrated perceptions of autonomy support from an 'important other' to be positively associated with LPA, in turn benefiting psychological health (i.e., depression and vitality), in RA patients. However, no studies have examined the role of the basic psychological needs and quality of motivation in this relationship, nor have the determinants of SB accumulation been explored in the RA population through a SDT lens. For example, it is possible that more autonomous motivation for reducing SB (e.g., identification with health benefits) is associated with lower levels of sedentariness.

Physical environmental determinants

The physical environment has been identified as a modifiable determinant of SB.[63] Furthermore, the Systems of SBs framework[64] emphasises the importance of prioritising investigation into physical environmental factors, and their relationship with SB accumulation in different populations. Distinguishing between specific factors within the

physical environment (e.g., home, workplace, neighbourhood) has been stressed as important when examining the determinants of SB, in order to ensure interventions can be properly targeted. At present, research studies examining the physical environmental determinants of SB often fail to be domain-specific, which can lead to contradicting evidence.[63]

The Systems of SBs framework also depicts that elements of the physical environment may influence SB engagement in different ways, among different populations. For example, examining the determinants of SB in the workplace might not be as relevant to an older adult population as the home or neighbourhood environment might be.[63] Indeed, aspects of the home environment, such as the number of televisions and motorised vehicles, have been positively correlated with levels of SB in adults (mean age = 57.5 years).[63] Furthermore, aesthetic features outside of the home environment (e.g., public parks, trees) have been inversely associated with leisure-time SB in adults (mean age = 52.2 years).[65]

To date, no studies have investigated the physical environmental correlates of SB and LPA in the RA population. This patient group is highly heterogeneous, representing different ages, variability in disease activity, physical function and employment status. This underlines the need to examine physical environmental determinants across multiple domains of SB and LPA engagement (i.e., in the home, workplace and neighbourhood).

AIMS OF THIS RESEARCH

This study will address the knowledge gaps and limitations of the existing literature, in order to build an evidence base regarding the health-related correlates of SB and LPA, and hypothesised determinants of these behaviours in RA.

The aims of this study are twofold:

1) To investigate the longitudinal relationships between objectively-assessed SB patterns (i.e., overall sedentary time, sedentary bouts and sedentary breaks) and LPA, with health outcomes in people living with RA. On the basis of evidence establishing an association between SB and inflammation in non-RA cohorts, we hypothesise that SB may contribute to disease outcomes in RA via perpetuating heightened systemic inflammation. As such, our primary health outcomes are: inflammatory biomarkers (e.g., Tumour Necrosis Factor-alpha [TNF-α], Interleukin 6 [IL-6], high-sensitivity C-Reactive Protein [CRP], Erythrocyte Sedimentation Rate [ESR]), which have previously been used as primary endpoints in non-RA studies examining the association between SB, inflammation and health.[14,15] Secondary health outcomes are: broader disease-related outcomes (e.g., disease activity, CVD risk, pain, fatigue, physical function), indices of psychological wellbeing (e.g., depression, anxiety, vitality, satisfaction with life, positive and negative affect), and quality of life.

2) To examine the longitudinal associations between hypothesised determinants (i.e., psychosocial [e.g., autonomy support], individual differences [e.g., self-efficacy] and physical environmental [e.g., home and neighbourhood environment]) of SB and LPA (i.e., for: 1) reducing overall SB, 2) regularly breaking up SB and 3) physical activity), with objectively-assessed SB patterns (i.e., overall sedentary time, sedentary bouts and sedentary breaks) and LPA, in people living with RA.

METHODOLOGY

Participants and Recruitment

Participants will be recruited from Rheumatology Outpatient Clinics in a hospital in Dudley, England. Inclusion criteria will be: a clinical diagnosis of RA according to the American College of Rheumatology-European League Against Rheumatism (ACR-EULAR) Criteria and aged ≥18 years old. Exclusion criteria will be: wheelchair users and those unable to ambulate independently with the use of an assistive device.

Eligible patients will be approached about the study during Rheumatology Outpatient Clinics. A member of the research team will provide patients with information about study procedures and patients will be given the opportunity to ask the researcher any questions. Willing patients will provide informed consent to participate in the study. This study has been approved by the local National Health Service Research Ethics Committee (West Midlands – Black Country Research Ethics Committee 16/WM/0371).

Protocol

This study will adopt a longitudinal design. Participants will be asked to visit the hospital at 2 time points; baseline (Time 1) and 6-month follow-up (Time 2). At each time point, participants will be asked to undertake 2 visits (i.e., visits 1 and 2) separated by a 7-day period. Specific protocols to be followed are described below.

Visit 1

Participants will visit the hospital to undertake physical assessments and complete questionnaires. At the end of Visit 1, participants will be fitted with the ActiGraph (GT3X) accelerometer and activPAL3$^{\mu TM}$ posture sensor to wear for the subsequent 7 days. The researcher will give verbal and written instructions, plus a demonstration, regarding how to wear each device. Participants will also be given the Bouchard Physical Activity Record (BAR)[66] to complete on 3 of the days during which they wear the GT3X and activPAL3$^{\mu TM}$.

Visit 2

After 7 days, participants will re-visit the hospital to provide a fasted blood sample, and return the GT3X accelerometer, activPAL3$^{\mu TM}$ posture sensor and BAR to the

researcher. During this visit, they will also be asked to complete questionnaires with specific reference to their experiences of pain and fatigue over the previous 7 days.

Measures

Visit 1

Participant characteristics. Information will be recorded pertaining to participants' age, gender, ethnicity, marital status, education, date of diagnosis, existing chronic conditions (e.g., heart disease, diabetes, depression), current medical treatment, smoking status and living arrangements.

Anthropometrics. Taken in duplicate, height, weight and body composition will be measured with participants bare-foot, whilst wearing light and loose-fitting clothing. Height will be measured to the nearest 0.1cm using a stadiometer (SECA, Leicester Height Measure). Weight will be measured to the nearest 0.1kg using the Tanita Body Composition Scales (Tanita BC-418 MA P). Body Mass Index (BMI) will be calculated as weight (kg)/height^2 (m^2). Percent body fat and muscle mass will be measured using the Tanita Scales via bioelectrical impedance analysis.

Resting blood pressure. Resting blood pressure (systolic and diastolic) will be taken in duplicate with an automatic blood pressure machine (Mindray Accutorr PLUS). The blood pressure cuff will be placed over the brachial artery as standard, after the participant has rested in a supine position for 5 minutes.[36,67]

Physical function. Gait speed will be assessed using the 20-Metre Timed Walk Test to provide an objective measure of physical function. Participants' time to walk from a 'start line' to a 'finish line', 20 metres apart, will be recorded using a stopwatch. The stopwatch will be started once the participant begins to walk from the 'start line', and will be stopped once the participant's heel has completely crossed the 'finish line'. Gait speed has been previously used in studies of RA to provide an objective measure of physical function (e.g., walking 15.24 metres).[68] Research has also shown that slower gait speed is associated with RA outcomes, such as pain, fatigue and joint deformation.[69] As such, it may also serve as a proxy for other important aspects of RA health, directly related to physical function.

Questionnaires. Validated questionnaires will be administered to the participant to assess self-reported RA outcomes and hypothesised determinants of SB and physical activity (see **Table 1**). Questionnaire scores will be calculated according to validated scoring instructions (e.g., mean scores will be calculated for the 'autonomous motivation for reducing SB' dimension in the Behavioural Regulation in Exercise Questionnaire-2).

Actigraph GT3X. The GT3X triaxial accelerometer (27g; 3.8cm x 3.7cm x 1.8cm) will be attached to an adjustable elastic belt and worn on the right hip in a vertical position.[70-74] Participants will be asked to remove the device only for sleeping and water-based activities (e.g., swimming, bathing), and to record all replacement/removal in wear time logbooks provided. The GT3X will record accelerations in 1-second epochs, which will be converted into activity counts. These counts will then be interpreted to determine the frequency, intensity and duration of SB and physical activity.
Data collected by the GT3X will be cleaned and analysed using Actilife Software (version 6). For inclusion in analyses, participants will be required to have worn the GT3X for ≥4 days, for ≥10 hours per day, including a weekend day.[25,75-78] Non-wear (e.g., 60 minutes of 'zero counts') will be defined in accordance with previous research among older adults[75,78] and people living with RA.[45] Time spent in SB, LPA and MVPA will be determined using cut-points that have been validated in previous research with adults (e.g., Troiano et al.),[74] and used in studies investigating SB and physical activity in the general population,[79] in people with rheumatic diseases[80] and in osteoarthritis.[81]

ActivPAL3$^{\mu TM}$. The activPAL3$^{\mu TM}$ posture sensor (9g; 2.35cm x 4.3cm x 0.5cm) will be initialised using activPAL3$^{\mu TM}$ software and attached by the researcher to the front of the right thigh, in a mid-anterior position, with a waterproof, adhesive Tegaderm dressing. Participants will be asked to wear the activPAL3$^{\mu TM}$ for 24 hours a day to enable assessment of time spent sitting/lying, standing and stepping, as well as sit-to-stand transitions.[82-85] activPAL3$^{\mu TM}$ software will be used to download the activPAL3$^{\mu TM}$ data. Sleep time will be determined using self-reported information from the wear time logbooks and BAR, in conjunction with non-wear periods identified via algorithms applied to GT3X data. Specifically, the GT3X output generated by Actilife details at which time the participant removes (e.g., at bedtime) and replaces (e.g., at waking) the accelerometer throughout the 7-day period. This will be checked against the BAR, which details (over 3 days) when the participant woke up and went to bed.
Participants will be required to have worn the activPAL3$^{\mu TM}$ for ≥4 days, for ≥10 hours per day, including a weekend day, to be included in analyses.[76,86-88] Variables derived for analyses will include: time spent sitting/lying, standing and stepping (i.e., hours per day), as well as the number of sit-to-stand transitions (i.e., sedentary breaks per day).

Bouchard Physical Activity Record. Participants will be asked to self-report the dominant activity undertaken every 15 minutes, over 3 days of the study week, including a weekend day (e.g., Thursday, Friday and Saturday).[66,89,90] They will be asked to report this information in real-time, in

Table 1. Questionnaires administered at baseline (Time 1) and 6-month follow-up (Time 2), specifically on visit 1 to the hospital

Outcome	Questionnaire	Description	Example
Pain	McGill Pain Questionnaire	Questions pertaining to the past 2 weeks • 17 items - Sensory descriptors - Affective descriptors - Present pain - Average pain	• For each of these words, please place a tick in one column: - E.g., Throbbing - (0 = none, to 3 = severe)
Fatigue	Multidimensional Assessment of Fatigue Scale	Questions pertaining to the past 2 weeks • 16 items • 4 dimensions: - E.g., Degree and severity • Global fatigue index	• Please complete the following items based on the past 2 weeks: - E.g., To what degree have you experienced fatigue? - (1 = not at all, to 10 = a great deal)
Fatigue	Multidimensional Fatigue Inventory	Questions pertaining to the past 2 weeks • 20 items • 5 dimensions: - E.g., Physical fatigue	• Over the past 2 weeks: - E.g., I feel fit - (1 = yes that is true, to 5 = no that is not true)
Physical function	Health Assessment Questionnaire	Questions pertaining to the past 2 weeks • 8 categories: • Self-reported ability to complete activities of daily living - E.g., Dressing and Grooming	• Are you able to: - E.g., Dress yourself, including tying shoelaces and doing buttons? - (0 = without any difficulty, to 3 = unable to do)
Physical function	Dartmouth Coop Functional Assessment Charts	Questions pertaining to the past 2 weeks • 6 items • 6 dimensions: - E.g., Physical fitness	• During the past 2 weeks: - E.g., what was the hardest physical activity you could do for at least 2 minutes? - (1 = very heavy, to 5 = very light)
Sleep	Pittsburgh Sleep Quality Index	Questions pertaining to the past 2 weeks • 18 items • Aspects of sleep - E.g., Sleep disturbances • Global sleep index	• In the past 2 weeks: - E.g., How often have you had trouble sleeping because you have to get up to use the bathroom? - (0 = not during the past 2 weeks, to 3 = three or more times a week)
Satisfaction with life	Satisfaction With Life Scale	Questions pertaining to the present time • 5 items • Satisfaction with life	• I currently feel: - E.g., In most ways, my life is close to my ideal - (1 = strongly disagree, to 7 = agree)
Vitality	Subjective Vitality Scale	Questions pertaining to the past 2 weeks • 6 items • Vitality	• Over the past 2 weeks, generally: - E.g., I have been feeling alive and vital - (1 = not at all true, to 7 = very true)
Anxiety and depression	Hospital Anxiety and Depression Scale	Questions pertaining to the past 2 weeks • 14 items • Depression (7 items) • Anxiety (7 items)	• Over the past 2 weeks: - E.g., I still enjoy the things I used to enjoy - (0 = definitely as much, to 3 = not at all)
Positive and negative affect	Positive and Negative Affect Schedule	Questions pertaining to the past 2 weeks • 20 items • Words describing different feelings/emotions	• Over the past 2 weeks: - E.g., Interested - (1 = very slightly or not at all, to 5 = extremely)
Quality of life	World Health Organisation Quality Of Life Scale	Questions pertaining to the past 2 weeks • 26 items • 4 domains: - E.g., Physical health	• Thinking about the past 2 weeks: - E.g., How would you rate your quality of life? - (1 = very poor, to 5 = very good)

Determinant	Questionnaire	Description	Example
Support from participant-identified other for physical activity, reducing sedentary behaviour and breaking up sitting time	Important Other Climate Questionnaire	• 6 items - Perceived autonomy support from important other regarding physical activity, reducing sedentary behaviour and breaking up sitting time	• With regards to being physically active/reducing my sedentary behaviour/breaking up my sitting time: - E.g., I feel that my important other provides me with choices and options about reducing my sedentary behaviour - (1 = strongly disagree, to 7 = strongly agree)
Support from consultant for physical activity, reducing sedentary behaviour and breaking up sitting time	Health Care Climate Questionnaire	• 6 items - Perceived autonomy support from consultant regarding physical activity, reducing sedentary behaviour and breaking up sitting time	• With regards to being physically active/reducing my sedentary behaviour/breaking up my sitting time: - E.g., I feel that my consultant understands how I see things with respect to reducing my sedentary behaviour - (1 = strongly disagree, to 7 = strongly agree)
Need satisfaction for physical activity	Psychological Need Satisfaction For Exercise Scale	Questions pertaining to the past 4 weeks • 18 items - Personal experiences of physical activity	• With regards to my experiences of physical activity: - E.g., I feel free to do physical activity in my own way - (1 = false, to 6 = true)
Self-efficacy for physical activity and breaking up sitting time	Self-Efficacy for Exercise Scale	• 9 items - Extent of self-efficacy to take part in physical activity in different situations - Extent of self-efficacy to break up sitting time in different situations	• How confident would you feel taking part in physical activity (e.g., walking) 3 times per week for 20 minutes if: - E.g., You felt pain when being physically active - (1 = not confident, to 10 = very confident) • How confident would you feel breaking up your sitting time every 20 minutes if: - E.g., You did not enjoy breaking up your sitting time - (1 = not confident, to 10 = very confident)
Motivation for physical activity, reducing sedentary behaviour and breaking up sitting time	Behavioural Regulation in Exercise Questionnaire-2	Questions pertaining to the past 4 weeks • 19 items - Reasons for taking part in physical activity, reducing sedentary behaviour and breaking up sitting time • 5 dimensions: - Intrinsic regulation (autonomous motivation) - Identified regulation (autonomous motivation) - Introjected regulation (controlled motivation) - External regulation (controlled motivation) - Amotivation	• I take part in physical activity/reduce my sedentary behaviour/break up my sitting time: - E.g., Because it is fun - E.g., Because I value the benefits of doing this - E.g., Because I feel guilty when I am not doing this - E.g., Because other people say I should - E.g., But I don't see why I should
Motivation to limit screen time	Motivation to Limit Screen Time Questionnaire	Questions pertaining to the past 4 weeks • 9 items - Feelings/beliefs regarding screen-time behaviour in leisure time	• I try to limit my screen-time because: - E.g., I believe too much screen time is bad for my health - (1 = not true at all, to 7 = very true)
Environmental perceptions – active travel and physical activity	Assessing Levels of Physical Activity Scale	• 3 sections - Home and neighbourhood environment - Walkability	• About how long would it take to get from your home to the nearest businesses or facilities listed below if you walked to them? - E.g., Supermarket - (1 = 1-5 min, to 5 = more than 30 min)

order provide information regarding the context in which SB and physical activity were undertaken. For this study, the BAR has been adapted to include visual analogue scales, in which participants will be asked to report their average, minimum and maximum pain and fatigue on each of the 3 days. Specifically, at the end of each day, participants will be asked to mark a vertical line along a 100mm continuum from 'no pain/fatigue' to 'extreme pain/fatigue'.

Visit 2

Fasted blood sample. Blood will be taken from the inside of the arm and collected in appropriate vacutainers. Standard laboratory procedures and Enzyme-Linked Immunosorbent Assays (ELISAs) will be used to measure: serum biomarkers of inflammation (i.e., TNF-α, IL-6, high-sensitivity CRP, ESR), plasma lipids (i.e., total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides), plasma glucose and insulin.

Table 2. Questionnaires administered at baseline (Time 1) and 6-month follow-up (Time 2), specifically on visit 2 to the hospital

Outcome	Questionnaire	Description	Example
Pain	McGill Pain Questionnaire	Questions pertaining to the past week • 17 items - Sensory descriptors - Affective descriptors - Present pain - Average pain	• For each of these words, please place a tick in one column: - E.g., Throbbing - (0 = none, to 3 = severe)
Fatigue	Multidimensional Assessment of Fatigue Scale	Questions pertaining to the past week • 16 items • 4 dimensions: - E.g., Degree and severity • Global fatigue index	• Please complete the following items based on the past week: - E.g., To what degree have you experienced fatigue? - (1 = not at all, to 10 = a great deal)
Fatigue	Multidimensional Fatigue Inventory	Questions pertaining to the past week • 20 items • 5 dimensions: - E.g., Physical fatigue	• Over the past week: - E.g., I feel fit - (1 = yes that is true, to 5 = no that is not true)

Disease activity score-28. The number of swollen and tender joints in 28 joints of the body (i.e., hands, wrists, elbows, shoulders and knees) will be examined. Tenderness will be assessed via participants' self-report when light pressure is applied to the joint by the researcher. The degree of swelling will be visually assessed and self-reported by the researcher. The number of swollen and tender joints will be used in conjunction with patients' ESR and a self-reported degree of overall health ranging from 0 (very good) to 100 (very poor), to determine patients' disease activity score-28 (DAS-28).[91]

Questionnaires. Validated questionnaires will be administered to the participant on visit 2 to assess RA-related pain and fatigue during the study week (see **Table 2**). Questionnaire scores will be calculated according to validated scoring instructions (e.g., pain scores from the McGill Pain Questionnaire will be calculated by summing the intensity rank values for descriptors of sensory, affective and total pain).

Power Calculation

Power calculations were conducted with G*Power (version 3.1.9.3) using data collected from the Physical Activity in Rheumatoid Arthritis (PARA) randomised controlled trial (Trial Number: ISRCTN04121489). In the PARA study, accelerometers were utilised to measure SB, LPA and MVPA in a subsample of RA participants, and high-sensitivity CRP was measured as a biomarker of systemic inflammation. Cross-sectional accelerometer data were available for n = 61 participants. A priori power calculation from this data indicated that a sample size of n = 125 would be sufficient to detect statistically significant relationships (power = 0.80, α error of probability = .05), between daily SB and LPA with high-sensitivity CRP (a key inflammatory biomarker in RA).

To ensure the robustness of our calculations for detecting significant changes in broader RA outcomes, we also conducted power calculations for physical function, overall cardiovascular risk score and vitality. For this, cross-sectional accelerometer data, Health Assessment Questionnaire scores (physical function), QRisk-2 scores (cardiovascular risk), and vitality scores (vitality) were available for n = 61, n = 61 and n = 59 participants respectively. A priori power calculation confirmed minimum sample sizes of n = 82 (physical function), n = 14 (QRisk-2) and n = 114 (vitality), would ensure adequate statistical power (power = 0.80, α error of probability = .05) to detect the hypothesised associations.

Statistical Analyses

SPSS (version 24) will be used to compute descriptive statistics for all measured variables. These will include information regarding participant characteristics (e.g., gender, mean age, ethnicity), health outcomes (e.g., DAS-28, BMI), determinants (e.g., quality of motivation, self-efficacy), as well as levels of SB and LPA among the RA sample. Missing value analyses will be conducted via multiple imputation[92] or expectation maximization[93] methods in SPSS, where missing data does not exceed 5%.

Cross-sectional associations between RA participants' objectively-assessed SB patterns and LPA, with health outcomes (Aim 1) and proposed determinants (Aim 2) will be examined using correlation and regression analyses. Longitudinal associations from baseline (Time 1) to 6-month follow-up (Time 2) will be analysed using regression models.

Aim 1. For cross-sectional analyses, objectively-assessed SB patterns and LPA will be independent variables. Dependent variables will include biomarkers of inflammation, disease activity, CVD risk, pain, fatigue, physical function, depression, anxiety, vitality, satisfaction with life, positive and negative affect, and quality of life. For longitudinal analyses, regression models will examine if changes in objectively-assessed SB patterns significantly predict change in health outcomes from baseline (Time 1) to 6-month follow-up (Time 2). Health outcomes will be examined in separate regression models, but analyses will be adjusted for other factors which may influence these associations, as appropriate (e.g., disease duration, age, gender, current medication, and GT3X and activPAL3$^{\mu TM}$ wear time).

Aim 2. For cross-sectional analyses, the determinants of SB in RA (e.g., autonomy support and self-efficacy for physical activity, reducing SB and breaking up SB) will be independent variables, and objectively-assessed SB patterns and LPA in RA will be dependent variables. For longitudinal analyses, regression models will examine if changes in hypothesised determinants of SB and LPA (e.g., autonomy support for reducing SB) significantly predicts change in objectively-assessed SB patterns and LPA from baseline (Time 1) to 6-month follow-up (Time 2). As above, all analyses will be adjusted for potential confounders (e.g., 7-day pain and fatigue [to consider the possibility of bi-directional relationships], GT3X and activPAL3$^{\mu TM}$ wear time).

Subsequent analyses will use AMOS (version 24) to conduct path analyses and structural equation modelling, in order to examine multivariate relationships and hypothesised process models. For example, psychological processes (e.g., autonomy support for reducing SB) proposed to underlie objectively-assessed accumulated sedentary time and LPA engagement, in turn influencing RA disease outcomes (e.g., DAS-28) will be explored.[37,45,94,95]

DISCUSSION

To date, there is a paucity of research on SB conducted in the RA population. The current study – investigating potential physical and psychological health consequences, as well as potential determinants, of SB and LPA in RA patients – is taking steps to address the limitations of previous studies, whilst simultaneously addressing important knowledge gaps in the field. Firstly, this study is employing two novel devices to measure SB, according to the SB Research Network definition,[11,12] and LPA in RA patients. The present study will also provide the first longitudinal evidence regarding possible changes in health consequences associated with changes in sedentariness and LPA in this patient group. Furthermore, this longitudinal study is the first to comprehensively explore the determinants (i.e., psychosocial, individual differences and physical environmental) of SB and LPA in RA. Indeed, this study will identify modifiable factors that might influence SB and LPA in this patient group above and beyond, for example, RA-related disease activity and physical function, which may demonstrate bi-directional associations with SB and LPA. These data will elucidate targets for intervention that have the potential to support people living with RA, to reduce their time spent sedentary (e.g., by regularly breaking up their sedentary time and displacing it with LPA).

Future Research Directions

Building on findings from this study, future research that aims to ascertain the health outcomes and determinants of SB in the RA population should be consistent in their approach. Specifically, studies should accurately define and conceptualise SB, and follow recommended protocols that utilise validated measures (subjective and objective) to measure sedentariness and its correlates in RA.[31] Additionally, future research studies should seek to further explore the 'sedentary-inflammation hypothesis',[31] which postulates cyclical relationships between SB, inflammation and adverse health outcomes in RA. Finally, interventions assessed by Randomised Controlled Trials must be developed, implemented and evaluated, to definitively test the relationships in this study in order to infer causality. Data from the present study will generate an evidence base which will inform the development of such interventions, and optimise their potential to encourage SB change and improve health outcomes among people living with RA.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest.

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