

A seroimmunological profile of erosive hand osteoarthritis

Antonio Vannini¹, Marco Fusconi¹, Anna Chiara Dall'Aglio², Francesco Tovoli³, Magda Frisoni, Daniela Zauli³

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ABSTRACT

Objectives: Erosive hand osteoarthritis (EHOA) is an inflammatory disorder involving joints of the hands, which may be accompanied by acute phase reactants. The relationship between EHOA and classical osteoarthritis (OA) is still controversial, since some authors consider EHOA as a distinct disease, other as a subset of OA, and some as a border entity between OA and rheumatoid arthritis (RA). Scarce data are available about the seroimmunological profile of the disease, which could aid to identify a possible role of the immune system in EHOA pathogenesis, and could also allow to better differentiate EHOA both from OA and RA.

Material and methods: Blood was drawn from the following patients: 37 with EHOA, 35 with OA and 45 with RA. All sera were tested for rheumatoid factor, anti-cyclic citrullinated peptide antibodies (anti-CCP), antinuclear antibodies (ANA), anti-extractable nuclear antigens (anti-ENA) and anti-neutrophil cytoplasmic antibodies (ANCA).

Results: ANCA were never detected in OA, whereas they were found in 7 (19%) EHOA and 8 (18%) RA patients; the difference between EHOA and OA was statistically significant ($p < 0.01$). Anti-CCP antibodies, which were consistently negative in OA, were positive in 2 EHOA (5%) at a low titre and in 23 (51%) RA patients, usually at a very high titre. The difference between EHOA and OA was not statistically significant, while the number of RA positive patients was significantly higher ($p < 0.001$).

Conclusions: Our findings suggest that the seroimmunological profile of EHOA is different from that of OA. In EHOA patients ANCA and anti-CCP antibodies might be either markers of inflammation involving neutrophils and/or markers of an underlying autoimmune process.

Keywords: Anti-neutrophil Cytoplasmic Antibodies; Anti-cyclic Citrullinated Peptide Antibodies; Erosive Hand Osteoarthritis; Osteoarthritis; Rheumatoid Arthritis.

RESUMO

Objetivos: A osteoartrose nodal erosiva (OAE) é uma doença inflamatória que envolve as pequenas articulações das mãos que pode ser acompanhada de elevação dos marcadores de fase aguda. A relação entre a OAE e a osteoartrose (OA) clássica é ainda controversa, uma vez que alguns autores consideram que a OAE é uma doença completamente distinta, outros um subtipo de OA clássica e outros ainda uma entidade fronteira entre a OA e a artrite reumatoide (AR). Os dados existentes sobre o perfil seroimunológico da doença são escassos, podendo ser importante na identificação de um possível papel do sistema imunitário na fisiopatologia da OAE e poder ainda diferenciar a OAE da OA e da AR.

Material e Métodos: Foram colhidas amostras séricas de 37 doentes com OAE, 35 com OA e 45 com AR, sendo pesquisados o factor reumatoide, anticorpos anti-peptídeos cíclicos citrulinados (anti-CCP), anticorpos antinucleares (ANA), anticorpos anti-antigenios nucleares extraíveis (anti-ENA) e anticorpos anti-neutrófilos citoplasmáticos (ANCA).

Resultados: Foram detectados ANCA em 7 (19%), 8 (18%) e 0 doentes com OAE, AR e OA, respectivamente; a diferença entre grupos OAE e OA foi estatisticamente significativa ($p < 0.01$). Os anticorpos anti-CCP foram consistentemente negativos na OA, positivos em título baixo em 2 doentes (5%) com OAE e em 23 doentes (51%) com AR geralmente com título elevado; esta diferença foi estatisticamente significativa entre os grupos com OAE / OA e o grupo de doentes com AR ($p < 0.001$), mas não entre os dois primeiros.

Conclusões: Os resultados deste estudo sugerem que o perfil seroimunológico da OAE é diferente do da OA. Na OAE a presença de ANCA e anti-CCP poderão ser marcadores de inflamação envolvendo os neutrófilos

1. Azienda Ospedaliero-Universitaria di Bologna, Policlinico S. Orsola-Malpighi, Bologna, Italy

2. Azienda USL di Ravenna, Ospedale di Faenza, Ravenna, Italy

3. Dipartimento di Medicina Clinica, University of Bologna, Italy

e/ ou marcadores de um processo imuno-mediado subjacente.

Palavras-Chave: ANCA; Anti-CCP; Osteoartrose Nodal Erosiva; Osteoartrose; Artrite Reumatóide.

INTRODUCTION

Erosive hand osteoarthritis (EHOA) is a progressive inflammatory and destructive disorder involving both proximal (PIP) and distal (DIP) interphalangeal joints of the hands¹, which can affect up to 3.3% of men and 9.9% of women of the general population². Little is known about the pathogenetic mechanisms leading to the disease. Both genetic and environmental factors might play important roles in the disease pathogenesis. Stern *et al.* have reported an association between EHOA and a genomic region containing the interleukin-1 β (IL-1 β) 5810 single nucleotide polymorphism, thus suggesting a potential role for IL-1 in the pathogenesis of this condition, particularly in cartilage matrix degradation³. In a recent study by Ramonda *et al.*, the human leucocyte antigen (HLA) DRB1*07 allele was found to be associated with both the development and greater severity of the disease in North Italian EHOA patients⁴.

The relationship between EHOA and classical osteoarthritis (OA) is still controversial, as some authors view EHOA as a subset of OA, whereas some consider it as a distinct disease, and some regard it as a border entity between OA and rheumatoid arthritis (RA). According to the guidelines of the American College of Rheumatology (ACR) and of the European League against Rheumatism (EULAR), however, EHOA must be considered a more severe variant of OA^{5,6}. EHOA may be accompanied by acute phase reactants positivity, particularly erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) elevation, which, rather than for diagnostic purposes, may be useful for monitoring the disease activity⁷. Sometimes it may be difficult to differentiate EHOA from RA and psoriatic arthritis (PsA), with both of which it may share some clinicopathological features. Capillaroscopic modifications similar to those of PsA have been described in EHOA, suggesting some relationship between the two arthropathies⁸.

Several years ago, Utsinger *et al.*, comparing EHOA with RA, found that changes in levels of serum IgG,

lymphocytotoxic antibodies and cryoglobulins were found almost exclusively in RA⁹. The same authors observed that synovial fluid and synovial membrane from patients with EHOA, similarly to RA, had increased number of Ia+ (activated) T lymphocytes, as compared to those with OA¹⁰. Serum levels of soluble receptor of interleukin-2 (sIL-2R), a marker of lymphocyte activity, have been found increased in EHOA and higher than those in OA¹¹.

On the other hand, in a study by Morozzi *et al.*, no anti-cyclic citrullinated peptide antibodies (anti-CCP), specific serologic markers of RA, were detected in EHOA sera¹².

Recently, Bijsterbosch *et al.* showed that erosive evolution in EHOA is clustered in certain patients and in certain families, suggesting that underlying systemic processes are involved in EHOA pathogenesis¹³. Kortekaas *et al.* found that ultrasound inflammatory signs are more frequent in joints of EHOA patients than in OA cases, again suggesting an underlying, but not yet identified, systemic process leading to erosions¹⁴.

Although EHOA may be associated with autoimmune diseases, such as systemic sclerosis, Sjögren's syndrome and autoimmune thyroiditis^{15,16}, scarce data are available about the seroimmunological profile of EHOA, which could help to identify a possible role of the immune system in EHOA pathogenesis.

In the present study we aimed to verify the occurrence of a panel of rheumatic disease-associated serum autoantibodies in a small but well characterized cohort of EHOA patients. The results were compared to those obtained in a group of OA and RA patients. A seroimmunological profile closer to that of RA may suggest that EHOA is a border entity between OA and RA, whereas an OA-like profile could support the hypothesis that EHOA is a subset of OA.

MATERIAL AND METHODS

Blood was drawn from the following patients after obtaining informed consent: 37 consecutive patients with EHOA (F/M=35/2, median age 70, range 51-86), 35 with OA (F/M 31/4, median age 63, range 47-84) and 45 with RA (F/M 38/7, median age 66, range 30--88). Diagnosis of EHOA was made in presence of the ACR criteria of OA of the hand⁵ associated with radiological evidence of central erosions of at least 2 PIP or DIP joints (excluding the first finger), conferring the typical "gull wing" appearance¹⁷. OA patients were clas-

TABLE I. SEROIMMUNOLOGICAL PROFILE OF THE PATIENTS UNDER STUDY

Cases	F/M	Age median (range)	Disease duration (years) mean \pm SD	ESR (mm/h) median (range)	CRP (mg/dl) median (range)	RF positivity	ANCA positivity	Anti-ENA positivity	Anti-CCP positivity	ANA positivity
EOA (37)	35/2	70 (51-86)	9.8 \pm 6.7	15 (3-57)	<0.2 (<0.2-2.0)	2 (5%)*	7 (19)%**	0	2 (5%)***	11 (30%)****
OA (35)	31/4	63 (47-84)	5.1 \pm 3.3	13 (3-38)	0.21 (<0.2-3)	4 (11%)	0	0	0	13 (37%)
RA (45)	38/7	66 (30-88)	11.9 \pm 7.8	34 (3-115)	1.5 (<0.2-9.3)	39 (87%)	8 (18)%	0	23 (51%)	20 (44%)

EOA: hand erosive osteoarthritis; OA: osteoarthritis; RA: rheumatoid arthritis; SD: standard deviation; ESR: erythrocytes sedimentation rate; CRP: C-reactive protein; RF: rheumatoid factor; ANCA: anti-neutrophil cytoplasmic antibodies; anti-ENA: anti-extractable nuclear antigen; anti-CCP: anti-cyclic citrullinated peptide antibodies; ANA: antinuclear antibodies

*p not significant vs OA; p <0.001 vs RA; **p <0.01 vs OA; p not significant vs RA; ***p not significant vs OA; p <0.001 vs RA;

****p not significant vs both OA and RA

sified according to the ACR criteria^{5,18,19}. Hand involvement was present in 23 OA cases (66%). RA patients were classified according to the ACR revised criteria²⁰. As to current therapy, in the RA group 8 (18%) patients were receiving DMARDs, 7 (16%) steroid therapy (Prednisone 5 to 25 mg daily, or Methylprednisolone 4 to 16 mg daily), 13 (29%) both DMARDs and steroids. In the EHOA group, 4 (11%) patients were undergoing DMARD therapy, 1 (3%) was receiving steroid therapy (Prednisone 10 mg q.d.). In OA group, 1 (3%) patient was undergoing steroid therapy (Prednisone 5 mg q.d.), the remaining were taking NSAIDs as needed. Patients affected by rheumatologic or autoimmune comorbidities and infectious diseases were not included in the present study.

All sera were tested for: (1) rheumatoid factor (RF), by a routine turbidimetric assay; (2) anti-cyclic citrullinated peptide antibodies (anti-CCP), by a second generation commercial ELISA kit (Axis-Shield, Dundee, Scotland, UK), with sera diluted 1:100, according to the manufacturer's instructions; (3) antinuclear antibodies (ANA), by indirect immunofluorescence (IIF) on HEP-2 cells, with serum diluted 1:40 and fluorescent polyvalent anti-human immunoglobulins as secondary antibody; (4) anti-extractable nuclear antigens (anti-ENA), by counter immuno-electrophoresis with an aqueous extract of acetone powder of rabbit thymus (Peel Freeze, Rogers, USA); (5) anti-neutrophil cytoplasmic antibodies (ANCA), by IIF on alcohol-fixed human neutrophils, with a serum dilution of 1:20 and fluorescent polyvalent anti-human immunoglo-

bulins as secondary antibody, as previously described²¹; positive sera were further titrated.

Acute phase reactants (ESR, CRP) were determined by routine methods in the Hospital central laboratory.

Statistical analysis were performed by the Fisher's exact test when applicable; p value <0.05 was considered significant.

RESULTS

Results are summarized in Table I. In EHOA patients both ESR and CRP levels were similar to OA and lower than RA patients levels. RF was positive in 39 (87%) RA, 2 (5%) EHOA and 4 (11%) OA patients; there was no statistically significant difference between EHOA and OA, while there was a significant difference (p<0.001) between EHOA and RA. ANA were found more frequently in RA (20 cases, 44%) as compared to OA (13 cases, 37%) and EHOA (11 cases, 30%), although the difference was not statistically significant. ANCA were never detected in OA, whereas were found in 7 (19%) EHOA and 8 (18%) RA patients; the difference between EHOA and OA resulted statistically significant (p<0.01). In our cases ANCA were most often of the "perinuclear" type (6 out of the 7 EHOA positive and 6 out of the 8 RA positive patients) and were not associated with clinical evidence of small-vessel vasculitis. ANCA titres were similar in EHOA and RA patients, resulting between 1:40 and 1:160 in the two groups. Anti-CCP antibodies, which were consistently

negative in OA, were positive in 2 EHOA (5%) at a low titre and in 23 (51%) RA patients, usually at a very high titre. The difference between EHOA and OA was not statistically significant, while the number of RA positive patients was significantly higher ($p < 0.001$). The two anti-CCP positive EHOA subjects were in no way different from the remaining 35 negative ones. Anti-ENA antibodies were negative in all the studied patients.

DISCUSSION

The present data show that EHOA and OA are characterized by a similar seroinflammatory profile (ESR, CRP), but do not completely share the autoantibody profile: RF show similar prevalence in EHOA and OA, but ANCA and anti-CCP, always negative in OA, are positive in a proportion of EHOA patients. For ANCA the difference is statistically significant. ANA and anti-ENA autoantibodies show similar prevalences in the three studied groups. As above mentioned, patients affected by comorbidities potentially associated with autoantibodies production were not included in the present study.

The relatively high frequency of ANA and RF both in EHOA and OA can be, at least in part, explained by the age range of these patients. It is well established that ageing is characterized by an increased autoantibody production (particularly ANA and RF) without any associated autoimmune disease²². This is not the only condition in which ANA, even at high titres, are not harmful. The experience with anti-tumour necrosis factor alpha therapies has shown that, though they induce ANA production in most cases, a lupus-like syndrome is very rare, even when the lupus-specific anti-dsDNA antibodies are produced²³.

On the contrary, ANCA and anti-CCP have been rarely detected in healthy subjects. So far, ANCA have been detected in 18% apparently healthy centenarians²², in whom they are thought to reflect active neutrophil involvement to compensate the immunodeficiency associated with the ageing process (immunosenescence). However, in younger healthy people their prevalence does not exceed 2%²⁴.

No data are available in literature about the prevalence of ANCA in EHOA sera patients. In our cases, the finding of ANCA in EHOA and not in OA suggests that they are unrelated to age, but probably to an inflammatory pathway, involving neutrophils and resulting in the liberation of neutrophil constituents, which indu-

ce anti neutrophil immune response. Though originally described in small vessel vasculitides, where they mostly target myeloperoxidase (MPO) and proteinase 3 (PR3), ANCA have also been detected in other chronic inflammatory/autoimmune diseases, such as RA, inflammatory bowel disease and autoimmune liver diseases^{21,25}. In these conditions they may target different neutrophil proteins.

The finding of ANCA in our RA cases is line with other reported studies. ANCA, mostly of the perinuclear fluorescent pattern, have been reported in 18% to 33% RA patients, where they do not univocally correlate with a more severe disease pattern and extra-articular manifestations²⁵. In our experience, ANCA seropositivity could not be associated with any of these features either in RA or in EHOA.

As to anti-CCP, they have been originally described as highly specific for RA, where they correlate with the severity of the disease²⁶. Afterwards they have been detected in other arthritides and in autoimmune liver diseases²⁷, though with lower percentages. Even if they were detected in only 5% of our EHOA cases and not at high titres, their presence could suggest that the pathological mechanism underlying EHOA might share, at least in part and in certain patients, some features of RA.

The present study has limitations, the small number of patients being the most important one. Furthermore, a health population control group was not included in the study; however, prevalences of the tested autoantibodies in the health population are well known, as previously discussed.

CONCLUSIONS

Though obtained in a small cohort of patients, our data show that the seroimmunological profile of EHOA is partially different from that of OA. The finding of ANCA and anti-CCP in a proportion of EHOA and not in OA cases might imply that these antibodies are markers of an inflammatory process involving neutrophils and their by-products and/or markers of an underlying, yet undefined, autoimmune process.

Further studies on the autoantibody profile in larger series of EHOA patients could aid in a better understanding of the pathophysiological mechanisms of EHOA and, eventually, lead to properly allocate the disease, either as a subset of OA or as a distinct nosological entity.

CORRESPONDENCE TO

Antonio Vannini
 Azienda Ospedaliero-Universitaria di Bologna,
 Policlinico S. Orsola-Malpighi
 Medicina d'Urgenza e Pronto Soccorso
 Via Massarenti 9
 40138 Bologna, Italy
 E-mail: antoniiovannini@tiscali.it

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