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Systemic inflammation and antibodies to citrullinated peptides in hand osteoarthritis

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ABSTRACT

Objective. *To investigate systemic inflammation and autoimmune response to citrullinated peptides in patients with erosive and non erosive "lone" hand osteoarthritis (HOA) with no hip/knee involvement and their relationship with radiographic structural damage.*

Methods. *Sera were obtained from a total of 99 patients with HOA (52 patients with erosive HOA and 47 patients with non-erosive HOA) and from 50 control subjects (NC). Hand radiographs were obtained from all patients and scored for joint damage according to the Kellgren-Lawrence and the Kallman scores. Serum levels of high-sensitivity CRP (hsCRP), IL-6, pentraxin-3 (PTX-3), anti-CCP and anti-modified citrullinated vimentin (MCV) antibodies were evaluated by a sandwich ELISA.*

Results. *Circulating levels of inflammatory biomarkers hsCRP, IL-6 and PTX3 were not significantly different in the two groups of patients with erosive and non-erosive HOA compared to NC and no significant difference was seen between non-erosive and erosive HOA. Anti-CCP positivity was detected respectively in 1 patient (2.1%) with non-erosive HOA and 1 patient (1.9%) with erosive HOA. Anti-MCV antibodies were present in 4 patients (8.5%) with non-erosive HOA, and 4 patients (7.7%) with erosive HOA. In the control group, one subject (2%) was positive for anti-CCP and 2 subjects (4%) had anti-MCV antibodies.*

Significant correlation was obtained only between body mass index and hsCRP concentration ($r=0.4071$; $p<0.0001$). No correlation between inflammation markers/autoantibodies and disease duration and radiological scores was found.

Conclusions. *Our study underlines the lack of systemic inflammation and autoimmunity in "lone" HOA and confirms the association between BMI and CRP levels.*

Introduction

Local inflammation is increasingly recognised as a contributing factor to the symptoms and progression of osteoarthritis (OA) (1). Several studies have shown that the acute-phase response may take place in OA; indeed CRP may be slightly elevated in OA patients (1). Levels of high-sensitivity-C reactive protein have been reported to be associated with local inflammation in hip and knee OA patients (2) and high circulating levels of IL-6 are associated with the development of radiographic knee OA (3).

Inflammatory mediators, such as TNF-α and IL-1, have been shown to induce CRP (albeit to a lesser extent compared to IL-6), an acute-phase protein belonging to the pentraxin family, as well as pentraxin-3 (PTX3). Evidence suggests that PTX3 is a useful new serological marker, rapidly reflecting tissue inflammation and damage under different clinical conditions (4). OA synoviocytes produce PTX3 (5), but no data on PTX3 systemic expression in OA patients are yet available.

Inflammatory changes in joint tissue are triggered by innate and adaptive immunological mechanisms (1). Even though much remains to know about the trigger antigens in OA, autoantigens have been suggested as putative candidates.

In inflamed rheumatoid joints, autoimmune reactions lead to the production of antibodies against citrullinated proteins; recently antibodies against these proteins have also been found in the sera of other arthritides such as OA (6). In rheumatoid arthritis (RA), anti-CCP positivity showed the strongest association with erosive arthritis (7) and the combined detection of antibodies against mutated citrullinated vimentin (anti-MCV), another type of citrullinated protein, might improve the diagnostic and prognostic value of these tests in clinical practice (7).

A subset of HOA is characterised by an inflammatory and erosive pattern, more severe symptoms, perimenopausal onset and destructive changes involving the proximal and distal interphalangeal joints (8, 9). Therefore, erosive HOA is unique, sharing features typical of OA and inflammatory arthritis.

Most studies addressing circulating biomarkers of inflammatory and autoimmune response have focused on knee and hip OA, whereas data on HOA are scarce and the applicability of most results obtained in knee and hip OA is unknown.

Table I. Demographic, clinical and radiographic findings of HOA patients and controls.

	Erosive HOA (n=52)	Non-Erosive HOA (n=47)	Controls (n=50)	*p*-value
Men/Women	2 / 50	1 / 46	2 / 48	
Age*, years	66.4 (7.2)	66.3 (7.4)	66.4 (7.5)	NS§
Body Mass Index*, kg/m^2	24.8 (2.9)	24.3 (3.5)	24.4 (3.6)	NS§
Disease duration**, years	11 (6–15)	7 (5–13)	–	0.0275#
Radiological score*				
Kallman,	92.6 (22.1)	74.8 (22.6)	–	<0.001§
Kellgren-Lawrence	31.4 (11.6)	20.2 (12.7)	–	<0.001§
Number of IP joint nodes**	10 (7–12)	7 (6–11)	–	0.0252#
Number of central erosion**	3 (2–4)	0 (0–0)	–	<0.001#

Results are reported as * mean (SD) or ** median (Interquartile Range) according to distribution of data; *p*-values were obtained by applying § One-way ANOVA with Bonferroni's correction for multiple comparison or unpaired *t*-test (two independent groups) under the hypothesis of normality and variance equality of data sets, if the assumptions were not fulfilled, # non-parametric analysis was performed by Mann-Whitney U-test (two independent groups).
NS: not significant.

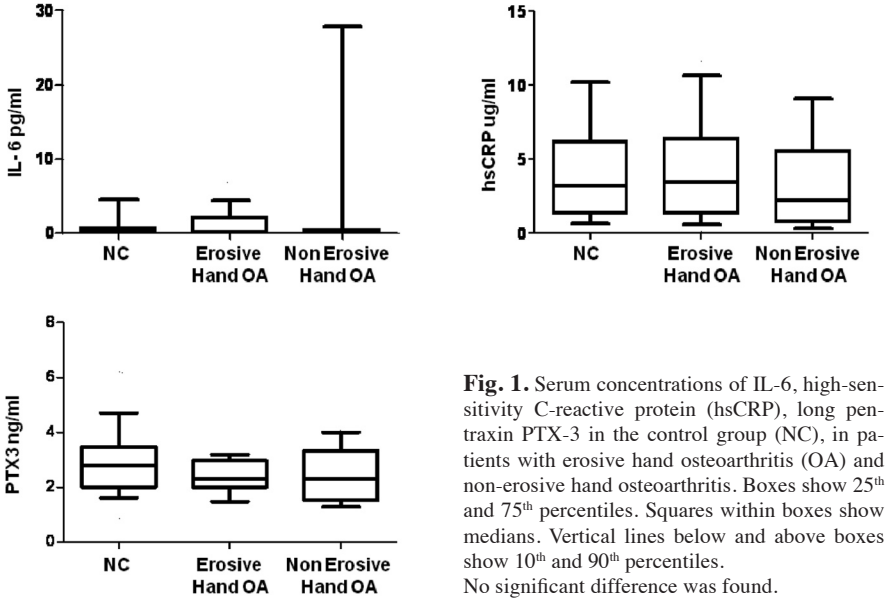

Fig. 1. Serum concentrations of IL-6, high-sensitivity C-reactive protein (hsCRP), long pentraxin PTX-3 in the control group (NC), in patients with erosive hand osteoarthritis (OA) and non-erosive hand osteoarthritis. Boxes show 25th and 75th percentiles. Squares within boxes show medians. Vertical lines below and above boxes show 10th and 90th percentiles.
No significant difference was found.

Furthermore, since HOA is frequently accompanied by OA in larger joints, the biomarker specificity occurrence should be evaluated in patients with OA confined only to the hands to obtain real hand-related results.

In the present study we investigated systemic inflammation (by evaluating CRP, IL-6, PTX3 circulating levels) and autoimmune response to citrullinated peptides (anti-CCP and anti-MCV) in patients with erosive and non-erosive "lone" HOA (with no hip/knee joint clinical involvement) and their relationship with radiographic structural damage.

Materials and methods

Patients

A total of 99 patients (3 men and 96 women, age range 50–88 years, mean 65 years) with HOA (52 patients with erosive HOA and 47 patients with non-erosive HOA) were included in this study.

The selection criteria have been reported elsewhere (10). In addition, patients with clinically demonstrated involvement of hip/knee were excluded.

Plain hand radiographs were obtained from all patients. All radiographs were assessed for joint damage using the Kellgren-Lawrence and Kallman scores, as described elsewhere (10).

The number of joints with central subchondral erosions were identified radiologically by the presence of the classic erosion patterns: gull wing or saw-tooth appearance. The number of Heberden's and Bouchard's nodes was evaluated clinically according to the presence of hard (bony) enlargement respectively in distal interphalangeal and proximal interphalangeal joints.

In addition, 50 control subjects (NC) (2 men, 48 women, age range 51–86 years, mean 65 years) were selected among individuals showing no clinical signs of osteoarticular disorders who attended the orthopaedic or rheumatology outpatient clinics of the Rizzoli Orthopaedic Institute (Bologna) for minor non-specific complaints.

Demographic, clinical and radiographic findings of HOA patients and controls are reported in Table I.

Ethical approval for the study was obtained from the ethics committee and informed written consent was obtained from all subjects.

Serum biomarker analyses

Serum levels of high-sensitivity CRP (hsCRP), IL-6, anti-CCP and anti-MCV antibodies were evaluated by commercial sandwich enzyme immunoassay (ELISA) kits (respectively HYPHEN BioMed, Neuville-sur-Oise, France; Bender MedSystems GmbH, Vienna, Austria; Euro-Diagnostica, Malmö, Sweden; ORGENTEC Diagnostika GmbH, Mainz, Germany) following the manufacturer's instructions. The cut-off point levels for the anti-CCP and anti-MCV antibody tests were respectively 5 U/ml and 20 U/ml, as indicated by the manufacturers.

PTX3 was evaluated with a sandwich ELISA based on monoclonal antibody (mAb) MNB4 (ascites diluted 1:5000 in coating buffer) and rabbit antiserum as previously described (11).

Statistical analysis

Results are reported as mean and standard deviation or median and interquartile range according to distribution of data.

One-way ANOVA with Bonferroni's correction for multiple comparison or unpaired *t*-test (two independent

groups) were applied under the hypothesis of normality and variance equality of data sets. If the assumptions were not fulfilled, non-parametric analysis was performed by the Kruskal-Wallis test with Dunn's multiple comparison post-hoc test or Mann-Whitney U-test (two independent groups).
Spearman's correlation analysis was used to assess relationships between variables.
The statistical analysis was carried out using GraphPad Prism for Windows (CA, USA).

Results

Circulating levels of inflammatory biomarkers hsCRP, IL-6 and PTX3 were not significantly different in the groups of patients with erosive and non-erosive HOA compared to NC, and no significant difference was seen between non-erosive and erosive OA (Fig. 1).
To check whether a subgroup of erosive HOA patients with relatively elevated inflammatory markers was identified on the basis of clinical/radiological features, we analysed the distribution of hsCRP, IL-6 and PTX3 serum concentration values in this group of patients. Concerning CRP levels, we considered 10 μg/ml as the cut-off level (the normal range being 0.2–10 μg/ml); on this basis 8 patients out of 52 with erosive HOA showed CRP serum levels above the cut-off value. The two HOA patient groups, defined according to the CRP serum value, were compared for radiological scores, node and erosion number and no significant difference was found (data not shown).
On the other hand, the distribution analysis of PTX3 and IL-6 serum concentration values in erosive HOA patient did not identify a subset of patients characterised by a systemic elevation of these inflammatory markers (data not shown).
Anti-CCP positivity was detected respectively in 1 patient out of 47 (2.1%) with non-erosive HOA and in 1 patient out of 52 (1.9%) with erosive HOA. Anti-MCV antibodies were present in 4 patients out of 47 (8.5%) with non-erosive HOA, and 4 patients out of 52 (7.7%) with erosive HOA. In the control group, 1 subject out of 50 (2%) was positive for anti-CCP and 2 out of 50 (4%) had anti MCV antibodies.
Positive correlation between BMI and circulating CRP values was found (taking into account the total study population: patients and controls) ($r=0.4071$; $p<0.0001$). No correlation between inflammatory and autoimmune markers and disease duration or radiological score was found.

Discussion

Evidence of local inflammatory features in HOA is mainly detected clinically or, more recently, by ultrasound (US) or magnetic resonance imaging (MRI) analysis (12).
By US with Power Doppler signal, at least three groups showed active synovitis in a number of finger joints in patients with HOA (13-15).
Nonetheless, our findings showed normal levels of systemic inflammatory markers (hsCRP, IL-6) in both erosive and non-erosive HOA.
Our results on CRP circulating levels in HOA are in agreement with previous reports (16-18). Conversely, Olejárová *et al*. found increased CRP and erythrocyte sedimentation rate in non-erosive HOA (19), whereas Punzi *et al*. had opposite results (20). This discrepancy may be explained by the different rates of coexisting knee/hip OA in the HOA patient series evaluated in these studies. Indeed, increased hsCRP levels have been reported in knee and/or hip OA patients (2). That is why we selected HOA patients without knee/hip involvement.
A further explanation might be the widely reported positive correlation between CRP and BMI; indeed, the HOA patients included in our study did not show increased BMI compared to that of normal subjects.
In our series the finding of normal hsCRP levels are strengthened by evidence of normal levels of IL-6, the main inducer of CRP. This result is in agreement with findings reported by Pantsulaia I. and co-workers (21).
Increased levels of synovial fluid and serum IL-6 have been found in patients with large-joint OA (3). In the knee joint, the infra-patellar fat pad has been shown to be an important source of adypokines and cytokines, such as IL-6 (22), whilst in small joints, the adipocyte contribution to inflammatory soluble factor secretion is not known, but adipose tissue is certainly scarce in small joints.
An indirect relationship between inflammation and adipose tissue is supported by the widely documented positive correlation between BMI and circulating CRP values. This was also confirmed by our results.
A positive correlation between BMI and the risk of developing OA not only in weight-bearing joints but also in hand joints has been reported, although the level of evidence was moderate (23). Although the potential association between obesity and OA is still under debate, growing evidence supports the role of soluble factors released by fat tissue, such as adipokines, in inflammation and OA pathopysiology.
In particular, the involvement of adiponectin in HOA has been recently reported. Increased circulating levels of adiponectin, but not resistin or leptin, in erosive HOA patients has been showed by Filková M. and co-workers (18). Conversely, a recent study highlighted an inverse association between levels of adiponectin and HOA progression (24).
In the present study, in addition to hsCRP and IL-6, we investigated systemic inflammation also by evaluating PTX3 circulating levels.
Luchetti *et al*. (5) showed that in synovial biopsies obtained from OA patients PTX3 was barely expressed. Up to now, no data on PTX3 systemic levels in OA patients have been available. In the present study we found normal PTX3 circulating levels in HOA patients.
In general, we think that our findings might be related to a local low-grade release of proinflammatory soluble molecules, which is hardly detected at systemic level.
Inflammatory changes in joint tissue are triggered by immunological mechanisms (1), but the role of autoimmunity in OA is still under debate.
Du H. and co-workers (6) showed the presence of circulating anti-CCP antibodies in a subset of patients with early-stage but not late-stage knee OA; conversely Morozzi G. and co-workers (25) reported the absence of circulating

anti-CCP antibodies in patients with erosive OA.

Although no knowledge on anti-CCP local production in HOA joint is available, we may hypothesize that, even if occurring, no evidence is detectable at systemic level.

Circulating anti-MCV antibodies were found in a minority of HOA patients. This finding is in line with the previously reported observation of a higher prevalence of anti-MCV antibodies compared to anti-CCP antibodies in non-RA arthritis and in healthy subjects (26).

In conclusion, our study underlines the lack of systemic inflammation and autoimmunity in "lone" HOA and confirms the association between BMI and CRP levels.

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