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Title: **Auto-Antibodies to Post-Translationally Modified Proteins in Osteoarthritis**

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Abstract

Objective: Autoantibodies (AutoAbs) have been observed in osteoarthritis (OA) with broad antigenicity, although their prevalence and role remain unclear. Post-translational modification (PTMs) of proteins (oxidation, carbamylation, citrullination) is associated with synovitis and can lead to AutoAb development. Given the prevalence of synovitis, we explored whether AutoAbs to PTM-antigens are common in OA compared with rheumatoid arthritis (RA).

Methods: Serum (n=895) was obtained from healthy controls, OA and RA patients; and arthritic synovial fluid (SF, n=290). ELISAs were used to quantify anti-citrullinated peptide (ACPA), anti-carbamylated protein (anti-CarP), anti-oxidized collagen (anti-ROS-Ci/CII) antibodies.

Results: In sera, positivity for PTM-antigens AutoAbs was observed at a lower frequency in OA with 64.1% (95%CI: 57.2%-70.1%) more ACPA+ and 29.8% (21.0%-37.3%) more anti-CarP+ patients in RA (both $p<0.0001$). Levels of ACPA, anti-CarP were also lower in OA ($p<0.0001$). Anti-ROS-CII positivity was lower in OA compared to RA (16.6%, 4.8%-28.6%) less frequent, $p=0.033$) but not anti-native-CII. There was no impact of age/gender on AutoAbs associations with diseases either looking at positivity or levels. In SF, OA patients were often ACPA+ (45.9%) although less frequently than in RA ($p=0.004$). Anti-CarP were rarely observed (<5% all samples). All collagen AutoAbs were more frequent in RA compared to OA (all $p<0.010$) but only levels of anti-CII and anti-ROS-CII were significantly higher in they RA ($p<0.050$).

Conclusion: Although the frequency of AutoAbs for PTM proteins were lower in OA sera compared to RA, a higher proportion of OA SF were positive. The relative retention of AutoAbs in the OA joint requires further investigation.

Introduction

Osteoarthritis (OA) is considered an age-related disease. It affects millions of people around the world (1). However, many elements of the complex OA pathogenesis remain poorly understood. Many autoantibodies (AutoAbs) have been described in OA, although it is not commonly considered an autoimmune disease. These include autoimmune responses against proteins of the musculoskeletal system (aggrecan, cartilage link protein (LP), proteoglycan, fibronectin, cartilage oligomeric matrix protein (COMP) and the native form of type I and II collagen) (2). The prevalence of these AutoAbs in the blood has not been well described although some have been proposed as biomarkers (3, 4), and reports showed detectable to increased levels compared to healthy control (HC) sera. There are also many reports of the presence of AutoAbs in the OA synovial fluid (SF), adding another repertoire of AutoAbs, including to bone related proteins (osteocalcin, osteopontin, osteoprotegerin), hyaluronic acids, proteases and related enzymes (MMP9, TIMP1) (5-7). Again, these were mainly observations of raised levels, lacking details on prevalence and association with other features of OA. The development of AutoAbs is however, also a feature of ageing (8) leaving open the debate as to their relevance in an age-related disease such as OA (9). Ageing-associated AutoAbs detected in OA include rheumatoid factor (RF) and anti-nuclear antibody (ANA) (10, 11).

Numerous studies have provided evidences that local inflammation in OA joints (synovitis) makes a contribution to OA processes (12). OA joint structural deterioration (including changes in cartilage, menisci, and subchondral bone) and synovitis can be detected in many individuals over the age of 40, even if these become symptomatic in only 15% of people aged over 60 (13-15). A hallmark of tissue inflammation such as synovitis is cellular stress resulting in the expression of enzymes as well as the production of chemicals (reactive species, urea) responsible for the post translational modifications (PTM) of proteins by citrullination, oxidation, glycation or carbamylation (16-19). PTM are deleterious additions to native protein structures and are involved in diseases (20). They are also an important source of antigenicity for autoantibody development(21). Such enzymes and chemicals have been reported in OA synovitis (22-24) though the presence of synovitis-related AutoAbs in OA remains

poorly documented. Circulating anti-citrullinated peptide (ACPA) and anti-carbamylated protein (anti-CarP) AutoAbs were detected at low frequency in OA sera (25), while anti-oxidised-Collagen-II (anti-ROS-CII) AutoAbs were detected in sera and SF of OA patients with evidence of synovitis (26). In contrast, ACPA, anti-CarP, anti-ROS-CII Abs are well described in rheumatoid arthritis (RA) and the later also in Systemic Lupus Erythematous and diabetes (27). While PTM such as citrulination and carbamylation have not been particularly well studied with respect to ageing, it is well documented that oxidation processes are implicated in ageing (18, 20).

The role of AutoAbs in OA pathogenesis therefore remains unclear, with absence of data on prevalence. In this study, we explore whether systemic (sera) and local (SF) AutoAbs related to synovitis were more common in OA patients than in age-matched HC. We investigated mainly AutoAbs to PTM-proteins including ACPA, anti-CarP and anti-ROS-CI/II AutoAbs as well as RF as an age-related AutoAbs and compared OA with RA.

Methods

Subjects and samples

Serum and SF samples were obtained from patients attending outpatient clinics and surgical procedures at the Chapel Allerton Hospital, Leeds Teaching Hospitals National Health Service (NHS) Trust. All patients/controls provided written informed consent for their samples to be used in research. Samples were grouped as described in Table 1.

OA and RA patient serum (total n=718) were collected from several studies (28-31). Blood was allowed to clot for 1h, then spun for 10 minutes and aliquots were stored at -80^{o}C. Samples were grouped by anatomical sites: predominant hand OA, predominant knee OA (n=322) and multiple joint (MJ) OA (n=77, including at least 2 locations with a large joint (knee or hip) and others thumb, hand or ankle). 8 patients had hand OA only and 8 more had no detailed location precluding assignment to an anatomical group. Inflammatory arthritis (IA) patients were recruited from an early arthritis clinic (all rheumatic disease modifying drug naïve) and included in an early IA register. RA patients classified using the 2010 EULAR/ACR criteria were specifically selected for this study (n=303).

Health controls sera (HC, n=177) were also included and recruited as part of the early IA register. We included a question about any kind of joint pain (particular for the older individual), and excluded people diagnosed with any form of arthritis (notably OA which was allowed in the RA-register as disease control rather than heathy controls), any other disease, any current medication including analgesics for any kind of pain.

The OA studies were approved under ethical committee references REC 12/YH/0345, REC12/YH/0151, REC 07/Q1205/27, REC 13/YH/0279, and the RA/HC register under the reference REC-09/H1307/98.

Samples of SF (n=290, highly variable volumes) obtained during knee arthroscopy (NHS consent for the use of discarded tissue in research, between 1995 and 1999) or more recently during joint replacement surgery (REC 14/YH/0087). Fluids were spun and stored at -80^{o}C. Fluids were then

retrieved from our tissue bank (fully anonymised, except for diagnosis, OA versus RA). Before assays were performed, fluids were treated with a hyaluronidase solution (1 mg/ml in PBS, 1:10 volume to SF) incubated 30 minutes at 37°C and spun at high speed (in a micro-centrifuge 14000 rpm) for 15 minutes. SF matched with a serum samples were available as pairs from 10 OA patients only from recent joint replacement surgery.

Enzyme-linked immunosorbent assay (ELISA)

In the absence of absolute standards for these assays, arbitrary unit (AU) or optical density (OD) values were used to describe levels observed. ACPA levels were measured using Diagnostic grade tests according to manufacturer's instructions by local NHS services (BioRad tests). Cut-off for positivity were set according to manufacturers' instructions at 2 AU/mL and saturated values capped at 350 AU/mL. Data from patients recruited in the early arthritis register were retrieved from hospital records while data from OA patients (processed similarly by local NHS services) were retrieved from study records. ACPA levels, when negative, were not recorded while the lowest detectable levels were 0.1 AU/mL. Rheumatoid factor (RF) was measured by nephelometry and levels were obtained from NHS routine services (cut-off at 20 AU/mL) for all samples. Levels when negative, were also not recorded.

Anti-CarP Abs were measured in Leiden University (Leiden, The Netherlands) as previously described (11). Briefly, ELISA plates were coated with carbamylated foetal calf serum (10 μg/mL) to pull down autoantibodies from samples. Patient positive for Anti-CarP Abs were defined by values above the mean +2SD of the distribution of AU values for British HC (32). All samples were processed as a single batch.

Bovine CI (Cellsystems®) and CII (MD Biosciences, Inc.) were chemically modified (by glycation) to generate post-translationally modified CI/CII using ribose as previously described (33). Bovine serum albumin (BSA; Sigma) was similarly modified and used as normalising antigen. An ELISA was performed using ROS-CI/ROS-CII and native CI/CII as targets, as previously described (33). Briefly, ELISA plates were coated with 10μg/ml of ROS-CI/ROS-CII or CI/CII as bait for autoantibodies in serum or SF

samples. The ELISA OD values obtained for BSA and ROS-BSA were used as background controls to normalize the respective OD values for CI/CII and ROS-CI/ROS-CII. To control fluctuation in the antigen modification, all ELISA were performed using the same batch of modified proteins. Patients positive for anti-collagen autoantibodies were defined by values above the mean +2SD of the distribution of normalised OD values for HC (details in Figure 1). All samples were processed as a single batch for all 4 in-house ELISAs.

Statistical analysis

Possible association between AutoAbs levels (continuous data) and age/gender were analysed in healthy controls using Spearman's correlation/MWU tests and considered significant if coefficient rho was >0.600 for age and p-value <0.05 for gender.

The distributions of frequencies between the 2 disease groups were compared using Pearson's chi-square test. Numerical levels of AutoAbs were not normally distributed therefore, non-parametric tests were used (Mann - Whitney U) for comparisons of AutoAbs continuous levels between the 2 diseases. A value of $p < 0.050$ was considered statistically significant. A binary logistic regression comparing an OA outcome (1) versus RA (0), was used to adjust (for age and gender) the association between the disease and each of the AutoAbs individually looking at status (categorical) and levels (continuous), (reporting OR and 95% CI, p-value). Data for ACPA/RF negative AutoAbs levels are qualitatively present (negative status) but numerically absent. We used censored likelihood multiple imputation (34) to impute value for ACPA and RF in the regression analysis. The Likelihood multiple imputation estimates the conditional cumulative distribution function for censored ACPA and RF levels given the outcome and potential confounders and fit a logistic regression on both imputed dataset, the algorithm and code details can be found in *Lodi* library of R. The model for imputing ACPA/RF AutoAbs levels considered negative included OA outcome versus RA and two confounders: age and sex. The number of datasets to impute was 5. Data were presented using GraphPad Prism 8 and analysed using SPSS-v26 and *R-4.0.3*.

Results

Autoantibodies in healthy controls

ACPA were tested in 70 HC (only 1 positive sample) while 6 positive levels were recorded for RF (NHS records). To compare AutoAbs status (i.e. frequency of positivity) between patient groups for the in-house ELISAs, we first established cut-off values for the categorisation between positive and negative sera. For in-house ELISA, previous studies in Dutch, Swedish and American HC showed similar distributions of anti-CarP levels (35-40). Individual study cut-offs were derived for each population and were quite similar (ranging from 200-300 AU/mL). The range of values obtained for anti-CarP Abs in our British HC (Figure 1, n=174, removing 3 outliers, range 1-556 AU/mL) suggested a mean+2SD cut-off value at 235 AU/mL (SUP Table 1), slightly lower than previously reported using the top 95% CI of the distribution at 250 AU/mL (n=95) (32). There was no previously reported correlation between age and anti-CarP levels (35-40) and none was observed in this group (SUP Table 1, low correlation coefficient =0.122). No gender bias was observed either (SUP Table 1, p=0.215). Previously published data for anti-CII and anti-ROS-CII in HC from London, Leeds and Italy (26, 27) showed a similar distribution of ODs as the data obtained here in 98 new HC (Figure 1). These studies did not report AutoAbs levels relationships with age or gender. No correlation with age or gender were observed here either (SUP Table 1). 2 data points showed clear outlier features and were excluded to calculate cut-off based on a mean + 2SD for each of the ELISA individually (SUP Table 1).

Systemic (sera) and local (SF) AutoAbs levels were then explored in OA patients and compared with RA patients. Due to limitations in the volume of serum available, not all AutoAbs could be tested in all samples.

Autoantibodies in serum

ACPA, RF, anti-CarP and anti-CI/CII antibodies were measured in patients with OA and RA (Figure 2, bars represent the number of patients tested for each AutoAbs). Positivity for Abs differed significantly between disease groups for ACPA, anti-CarP and RF autoantibodies (all $p<0.0001$) which were

significantly more frequent in RA compared to patients with OA (64.1% (95%CI: 57.2%-70.1%) more frequent for ACPA, 29.8% (21.0%-37.3%) more for anti-CarP and 57.2% (45.2%-64.4%) more for RF). ELISAs for collagen-I were only performed in OA patients. In OA, there was an increase in frequencies of 8.8%% (95% CI 1.6%%- 15.9%) between positivity for anti-ROS-CI (27.2%) compared to anti-naïve CI (18.4%). For collagen-II, anti-native CII AutoAbs were equally represented in OA (17.3%) and RA (17.3%) but significantly less frequent in OA (29.8%) by 16.6% (4.8%-28.6%) for anti-ROS-CII compared to RA (44.4%, p=0.033). An increase in frequency from anti-native to anti-ROS-modified antigen was also observed for CII in both OA (17.3% to 29.8%, p=0.037) and RA (17.3% to 44.4%, p<0.0001).

When looking at the levels of AutoAbs in sera (SUP Figure 1), wide ranges of levels were observed in OA and RA samples for ACPA, anti-CarP and RF (RA only) as well as for the collagen AutoAbs. There were significant differences in levels of AutoAbs considered positive (due to the absence of reported negative levels) for ACPA (p<0.0001) between OA and RA. The RF levels observed in OA (n=3) were low and not sufficient to allow comparison. For anti-CarP and the collagen AutoAbs, continuous levels (combining levels considered positive and negative), were significantly higher in RA for anti-CarP (p<0.0001) and anti-ROS-CII (p<0.0001) while not for anti-native CII (no data available in RA for CI).

We performed binary logistic regressions comparing an OA outcome (1) versus RA (0), both for AutoAbs status and then levels (Table 2), first unadjusted and then, adjusting for age and gender. There was an absence of any noticeable change between unadjusted and adjusted OR (95% CI) and p-values of being OA if positive for an AutoAb or for having high levels, suggesting a limited effect of age and gender on the difference in AutoAbs status or levels observed between OA and RA patients. On the other hands, this analysis suggested that being positive for any of these AutoAbs (ACPA, RF, anti-CarP and anti-ROS-CII) was significantly less likely to be associated with OA than with RA (OR being below 1), although more for the PTM antigen while less so for naïve CII.

Autoantibodies in multiple joint OA sera

We further analysed samples based on the anatomical sites affected with OA (Figure 3) separating samples with predominantly knee OA (1 joint, n=303) from those with multiple joints involved (MJ OA, n=74, some including a knee). Positivity for ACPA tended to be mainly restricted to knee OA (22/303, 7.3%) compared to MJ OA (1/74 positive samples, p=0.041). Anti-CarP Abs were present at similar frequency between both anatomical sites (18.0% and 20.4%), however with higher levels in knee OA (SUP Figure 3). For RF (data not displayed), only 1 positivity was observed (other positive samples being in hand/missing anatomical data). AutoAbs to native-CI showed a site pattern with slightly increased frequency in MJ OA (23.7%) compared to knee OA (17.3%, p=0.262). This pattern was repeated for ROS-CI anti-Abs, with higher frequency in MJ OA (35.6%) than knee (25.0%; p=0.103). This was not observed for CII AutoAbs, with similar frequencies between knee (18.4%) and MJ OA (15.3%) as well as for ROS-CII knee (26.0%) and MJ OA (22.0%). Levels of collagen related AutoAbs were similar between knee and MJ OA (SUP Figure 2).

Autoantibodies in synovial fluid

We then investigated the presence of AutoAbs in SF from knee joints of patients with OA and RA (Figure 4a). The presence of ACPA was detected more frequently by 28.2% (13.4%-36.8%) in RA fluid (n=85/119, 71.4%) compared to OA (n=56/122, 45.9%, p=0.004). ACPA levels showed a similar range in RA as in OA (SUP Figure 3, up to 350 AU/mL) while altogether significantly higher in RA (p<0.0001).

Anti-CarP were only detected in very few fluid samples (n=6/73 tested, 8.2%), but showed more positivity in RA samples (n=5/21, 23.8%), than in OA (n=1/52, 1.9%). Comparison of continuous levels of anti-CarP in SFs suggested higher levels in RA (p<0.0001) although this remains hypothetical due to small number of positive samples.

The overall range of values detected for the AutoAbs to collagen in SF were different from levels observed in serum with a shift towards lower values. Results from AutoAbs status showed similar trends (Figure 4a) with increased positivity by 14.3% (95% CI: 6.2%-34.5%) of anti-native CI in RA

241 (23.8%) compared to OA (9.5%, p=0.007) as well as anti-ROS-CI with 28.6% (39.7%-79.3%) more in RA
242 (38.1%) versus OA (9.5%, p<0.0001). For anti-native-CII the differences were similar with an increase
243 of 22.5 % (9.4%-35.1%) in RA (35.8%) compared to OA (13.3%) also observed for anti-ROS-CII with
244 39.2% (24.2%-52.1%) more in RA (59.7%) than in OA (37.8%, both p<0.010). In contrast, levels of anti-
245 collagen AutoAbs were not significantly different (SUP figure 3) with the exception of anti-ROS-CII
246 which were higher in RA (p=0.001).

247 We further examined 10 knee OA paired serum and SF for anti-Collagen AutoAbs (Figure 4b). In serum
248 samples, only 2 patients were positive for anti-native CI and 1 for native CII. SF samples were positive
249 for native CII (n=7) and showed 2- to 3-fold higher levels than the matched serum (data not shown).
250 For anti ROS-CII, despite no positivity in serum, n=6 patients were positive in SF. Therefore, SF
251 positivity can be observed in OA in the absence of detectable AutoAbs in the serum.

252

Discussion

Our data demonstrate that while autoantibodies to PTM-antigen such as ACPA and anti-CarP are more specifically associated with RA in the serum, AutoAbs to collagen (anti CI/CII/ROS-CI/ROS-CII) are present at frequencies ranging from 17 to 30% in patients with OA. In contrast, at the site of disease (reflected in SF), ACPA and collagen AutoAbs are present in both types of arthritis, although anti-CarP Abs are very infrequent. In addition to these differences in frequencies, levels of AutoAbs in the serum were higher in RA for ACPA, RF and anti-CarP compared to OA, while the collagen related AutoAbs showed similar levels, except for anti-ROS-CII. In the synovial fluids, levels of AutoAbs appeared at have similar ranges while biased toward higher levels in RA compared to OA for ACPA and anti-CII AutoAbs.

It is well accepted that ACPA positivity is detected in 50%-70% of patients with RA (41, 42), while only in ~2% of HC (43), independently of age (42). 6% of OA patients were reported to be ACPA+ in a previous study (44), as well as in ours, which suggests a 3-fold increase compared to HC. Levels of ACPA in OA were significantly lower than in RA and looking closely at the distribution of levels, it may be that over a 1/3 of these values (below 20 AU/mL) may represent false ACPA positivity. This may also be due to the use of 2nd generation CCP-tests while more recent 3rd generation tests use a higher cut-off and are more specific (45). However, similarly low levels in RA patients, in the context of other symptoms such as CRP and swelling of joints are more likely to be relevant. The OA patients were chosen from several studies in which study eligibility criteria included confirmation of OA diagnosis according to the American College of Rheumatology clinical criteria for OA (hand, knee or hip as relevant) (29-31). This, however, does not fully exclude that some of these OA patients are at-risk of developing RA, notably highlighted by ACPA positivity. Our data on the other hand indicate that ACPA are also present at a high frequency at the site of disease in OA (46% in SF), while no difference was observed between serum and SF in RA (both highly positive 70-71%). ACPA positivity was notably observed more frequently in knee of OA patients (7%) potentially with larger volume of synovitis than in MJ patients (1.4%) which include other (smaller) joints. Accordingly, high levels of citrullinated

peptides have been detected in knee SF of patients with RA and OA and have been correlated with the arthroscopic early detection of OA and the early stages of cartilage degradation in OA (22, 44).

Anti-CarP AutoAbs are also present at higher frequency in RA (46%) than observed in OA (18%) sera, at a slightly higher frequency than previously reported (11%) (11). Therefore, serum anti-CarP in OA are more frequent than in a smaller aged-match group of HC (n=98 (age 50-83) showing only 2 positive). Higher levels were however observed in RA sera. Carbamylation is irreversible and therefore likely to affect long-lived proteins such as in cartilage and bone (17, 35). Anti-CarP were rarely detected in SF (n=6/73 samples tested) and levels of AutoAbs were furthermore, much lower in SF (median 8, range 1-1240 AU/mL) than in sera (median 110, 1-2600 AU/mL). While we have previsouly showed that the biochemical nature of fluid does not block detection in ELISA, anti-CarP AutoAbs levels observed in SF are lower and present with a reduced avidity compared to serum AutoAbs (46). No impact of age was observed for anti-CarP AutoAbs so far and we did not observe increase reactivity in our oldest HC. Alternatively, our data may suggest that auto-reactivity to modified epitopes through carbamylation is not associated with synovitis (barely detectable in RA) and that it is possible that circulating anti-CarP AutoAbs are generated independently of synovitis. An ELISA using a musculoskeletal antigen may be required to fully address anti-CarP AutoAbs relationship with synovitis and prevalence in rheumatic diseases.

AutoAbs to native collagen proteins were detected in both OA and RA at similar frequencies (~17%), approximately 3.5-fold more frequently than in age-matched controls. This is also observed in the SF although with higher frequencies in RA than in OA (23.8% and 9.5% for CI, 35.8% and 13.3% for CII), suggesting that these Abs to native proteins occur independently of the type of disease (RA or OA) and may be more associated with damage to joint structure. Frequencies and levels of anti-CII were higher than for CI, suggesting that CII epitopes may be more exposed than CI, or that they present a higher antigenicity. Anti-ROS-CI was observed with increased frequency in both RA SF (38%) compared to native protein (23%) suggesting an effect of the PTM on the antigenicity of CI. AutoAbs to ROS-CII were also observed at a high frequency in sera from OA and RA patients (30% and 44%). At the diseases

sites (SF), anti-ROS-CII were present at higher frequencies in RA (59%), while lower in OA (38%) although still higher than for the native-CII. Synovitis is more prevalent in RA and local inflammation is more pronounced (as exemplified by higher ar-Vas scores, Table 1) (47). We observed that positive levels of anti-native-CII and anti-ROS-CII levels correlated with ar-VA (Rho=0.627 and Rho=0.469 respectively, $p<0.03$) while this was not observed for native-CI, ROS-CI and ACPA (all Rho<0.275).

We observed more ACPA in the SF than in the blood of OA patients while such differences were not seen for RA. This observation suggests a segregation between the 2 compartments (synovium/SF and circulation) in OA but not in RA. The reason for this discrepancy in frequencies is unclear. Abs detected in the circulation are produced by plasma cells usually residing in the bone marrow (48, 49). Abs in the SF are more likely to derive from local B-cell activation/maturation. If synovitis related ACPA can both be generated in the RA and OA joint (50, 51), it may be that ACPA-secreting B-cells in OA are not able to live outside of the joint niche environment (i.e. migrate to the bone marrow). In contrast in RA, differentiating B-cells may be able to survive/migrate outside the joint and establish themselves in a bone marrow niche. Furthermore, circulating ACPA may also originated from alternative tissue niches in RA, where B-cells can establish themselves (for example lungs (52)) resulting in more sources of circulating ACPA, while being restricted to tissue where citrullination events occur in OA (i.e. synovitis/joints). On the other hand, rheological analysis of the interaction between hyaluronic acid (HA) and other proteins in the SF of OA patients has suggested a 3D physical network based on electrostatic interactions and dependent on HA content, binding proteins to the lattice (53). PTM antigens as well as auto-Ab may remained trapped in OA joints the same way these proteins are, while in RA, lower HA content may no longer be able to retain AutoAbs locally in the joint.

There are limitations to our study. First, the difficulty in recruiting elderly heathy blood donors should be noted. Despite an age range up to 90, we only had 20/175 samples from participants over the age of 65 and we may have underestimated any age effect. Furthermore, despite asking about musculoskeletal symptoms, asymptomatic OA structural damage could not be addressed as no X-rays were performed on HC not allowing us to fully excluded OA from that group. On the other hand, we

chose an early, drug naïve RA cohort (median symptom duration 6 months, IQR 9 month) to reduce the chances of including patients with secondary OA. Similarly, we cannot fully exclude some pre-RA patients in the OA group (notably those ACPA+).

Overall, our observations suggest that auto-reactivity to citrullination and oxidation occur at a high frequency in RA and OA synovitis (SF), and that B-cell tolerance for citrullinated and oxidised antigens may be altered in both diseases. Carbamylation may follow a similar pattern but an ELISA for a synovial antigen must be developed to fully answer this question. Importantly, there appear to be differences between the systemic and local AutoAbs frequencies in OA, while this is not observed in RA. This study therefore clearly opens the question of a potential role for AutoAbs in local pathogenesis in OA that remains to be further investigated.

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Author Contributions

XX, FP: Conception and design, collection and assembly of data, analysis and interpretation of data, statistical analysis.

MvD, LAT: collection and assembly of data, analysis and interpretation of data

FS: statistical analysis.

SRK, PGC: Provision of study materials, funding supporting the clinical studies providing the samples used.

XX, FP, SK, PGC, GMD: Drafting of the article.

XX, FP, PGC, LAT: obtained funding related to this work.

All authors critically contributed and approved the Final version of the manuscript.

Conflict of interest

LAT is listed as an inventor on a patent describing the detection of anti-CarP antibodies.

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