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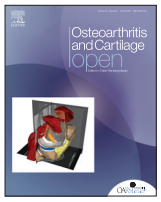

Brief Report

Association between type III collagen degradation and local tissue damage of a single joint

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ABSTRACT

Objective: The development of disease-modifying drugs is limited by OA's heterogeneity and the challenge of defining clinical endpoints. Serological biomarkers are considered potential surrogate endpoints, but their contribution from single joints to systemic levels in OA patients is unclear. In this exploratory study we longitudinally assessed systemic biomarker levels' response to tissue damage and healing before and after surgery in patients undergoing knee or hip joint replacement revision for aseptic failure. Patients with chronic pain associated with a prior hip or knee arthroplasty, but not receiving revision surgery were included as control.
Method: The serological biomarker of MMP mediated type III collagen degradation C3M, associated with synovial tissue degradation, was measured at baseline before revision surgery, after revision surgery and at a 6-month follow-up in 48 patients with aseptic loosening of a knee or hip prosthesis and in 18 patients with chronic pain from a hip or knee prosthesis. Longitudinal changes in biomarkers were modeled using linear mixed models.
Results: No differences between the aseptic loosening and chronic pain groups were observed at baseline. Revision surgery in the aseptic loosening group led to a swift increase in C3M, which normalized within 2–3 months. No changes in biomarker level were observed in chronic pain patients over three months.
Conclusion: These findings suggest that tissue damage in a single joint significantly impacts systemic biomarker levels and underscores the relevance of systemic biomarkers in assessing local tissue remodeling.

1. Introduction

The landscape of osteoarthritis (OA) research and therapeutic development has undergone significant transformation, shifting from a primarily structural focus to an emphasis on patient-reported outcomes (PROs) [1,2]. Central to understanding this emphasis on PROs is recognizing that pain is a crucial determinant. Pain, which partly originates from the soft tissue of the joint, is intricately linked with the structural integrity of joint tissues [3].

This transition in OA research aligns with the latest guidelines from the FDA on drug development for OA, which emphasize the importance of capturing how patients feel, function, and ultimately survive [4]. Historically, biomarkers predicting X-ray structural progression or MRI-detected cartilage loss have been the prime outcome focus of clinical trials [5]. Yet, as the dynamics of the field evolve, there's growing interest in identifying biomarkers from the entire joint, including not just the cartilage but also the innervated areas of the synovium and soft tissue. Such biomarkers may be more closely associated with pain and function and can potentially provide insights into disease progression and the efficacy of interventions on PROs. Recent findings, such as those from the FGF-18 (Sprifermin) FORWARD clinical study, underscore the importance of this shift. Despite demonstrating an effect on MRI cartilage gain, the studies did not show significant impacts on PROs of pain, highlighting the need to identify predictors for both total joint replacements (TJRs) and PRO worsening [5].

Serological biomarkers of tissue remodeling have been proposed as potential surrogate endpoints that could accelerate the development of novel OA treatment. If a treatment can effectively reduce pathological alterations to the soft tissue, it might directly influence OA pain, underscoring the importance of tissue-specific biomarkers [1].

However, the contribution of a disease that often only affects a limited number of joints to the systemic pool of a biomarker has been questioned, and therefore a significant knowledge gap persists: how does tissue damage in a single joint influence systemic biomarker levels?

Type III collagen is a major constituent of the interstitial membrane and importantly the synovial membrane and turnover is upregulated

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during inflammation, healing and repair, including synovitis [6,7]. C3M is a biomarker of type III collagen degradation carried out by matrix metalloproteinases and represents soft tissue turnover of the joint [7]. Inflammatory conditions such as Rheumatoid Arthritis (RA) present with increased C3M levels, and such levels have been associated with PROs [8]. In the context of OA changes in weight directed by diet and exercise interventions have been shown to reduce pain and improve function, and interestingly, here changes in C3M have been associated with weight loss and improvement in symptoms, suggesting that an intervention that alter type III collagen remodeling may affect patient reported outcome in OA [9]. However, whether C3M is released directly from tissues in and around the joint has not been shown clinically.

In this exploratory hypothesis generating study we aim to explore the relationship between tissue damage and its subsequent impact on circulating biomarker levels of C3M. By analyzing serum samples from OA patients undergoing revision surgeries due to aseptic loosening and comparing them with patients experiencing chronic pain from an existing hip or knee joint prosthesis who are not receiving revision surgery, we aim to shed light on the systemic effects of localized joint damage. We aim to show the potential of systemic biomarkers in providing a molecular understanding of tissue remodeling of the joint arising from direct tissue damage.

2. Methods

2.1. Study population

The cohort was derived from the prospective PRIS study. The primary aim of the study was to improve outcomes following joint arthroplastic complications [10]. Approval of the PRIS project was obtained from the Research Ethics Committee for the North Denmark Region (N-20110022) and the Danish Data Protection Agency (2008-58-0028). Participants gave written and verbal consent to participate in the study in accordance to guidelines from the Research Ethics Committee for North Denmark Region.

Patients were included in PRIS from referrals to the Department of Orthopedic Surgery, Aalborg University Hospital from practitioners or other hospital departments. The main PRIS project inclusion criteria were all patients older than 18 years with a total knee or hip prosthesis experiencing prosthetic failure, categorized into either suspected infection, aseptic loosening or chronic pain associated with a knee or hip prosthesis. Patients <18 years of age we excluded from the study. Prosthetic failure was defined as unexplained pain and/or mechanical problem such as loosening or wear, based on history, clinical examination, microbiology, and x-rays. Chronic pain cases were suspected if none of the criteria for PJI or AF were met according to a standardized set of criteria as previously described [10].

In the current study, only aseptic patients were included in the analysis [10]. Since diagnosis could be revised during the PRIS study, cases were included in the current study based on the final diagnosis determined at the end of the PRIS study.

In total 47 patients with aseptic failure (hip: n = 24, knee: n = 23), and 18 patients with a chronic pain from either a hip (n = 8) or knee prosthesis (n = 10) were included in the current study. Patients with aseptic loosening underwent revision surgery according to the department routine standard of care. Serum samples from aseptic loosening patients were drawn at baseline prior to surgery, 1–7 days after surgery, and at 2 and 6 months post-operatively. In patients with chronic problems, serum was drawn at baseline, at follow-up after 1–2 months and after 6 months. No surgery was conducted on the chronic pain patient group.

2.2. Biomarkers

Non-fasted serum samples were analyzed in duplicates using competitive ELISA for the assessment of type III collagen degradation (C3M) (Nordic Bioscience, Denmark). All measurements were within measurement range of the assay (2.8-80-2 ng/mL). C3M detects an MMP-derived neo-epitope of type III collagen.

2.3. Statistics

This was an exploratory post-hoc study in a limited population of the PRIS study and no power calculation or sample size estimation was conducted. Demographic data are presented as mean ± SD when normally distributed or as median (IQR) when following a skewed distribution. Discrete variables are shown as counts (percentage, %). Biomarkers were log transformed to approximate normal distribution and longitudinal changes in average biomarker levels estimated using linear mixed models with visit as fixed and patient id as random effect. The difference between baseline and after surgery was assessed by 1-way ANOVA, and the difference between diagnosis was assessed by Mann-Whitney *U* Test.

3. Results

3.1. Study population

A total of 65 patients were included in the analysis. Of these 47 patients had aseptic loosening in either a knee (n = 24) or hip (n = 23) prosthesis and underwent revision surgery. One patient was excluded from the analysis due to initially being diagnosed with prosthetic joint infecting and therefore receiving surgery for infection instead of aseptic loosening. The median follow time after baseline for patients with aseptic loosening were 1 day for visit 2, 67 days for visit 3, and 188 days for visit 4. 18 patients were included with chronic pain in a hip (n = 8) or knee (n = 10) prosthesis. The median follow-up time was 38 days and 185 days. Patients were slightly overweight in both diagnosis groups while patients with aseptic loosening generally had a slightly older prosthesis than patients with chronic pain (Table 1). There was an equal distribution of hip and knee revisions between the aseptic loosening and chronic pain groups. Both patient subsets exhibited similar symptoms, clinical presentations and biomarker level at baseline.

Table 1
Baseline characteristics of study participants.

	Aseptic loosening (N = 47)	Chronic problem (N = 18)	p value
Age (years)			0.129[a]
Median (Q1, Q3)	72.0 (67.0, 78.5)	68.0 (66.0, 72.2)	
Sex			0.1752[b]
Female	22 (46.8%)	12 (66.7%)	
Male	25 (53.2%)	6 (33.3%)	
BMI (kg/m^2)			0.917[a]
Median (Q1, Q3)	29.0 (27.1, 33.1)	29.8 (26.0, 34.0)	
Joint			0.778[b]
Hip	24 (51.1%)	8 (44.4%)	
Knee	23 (48.9%)	10 (55.6%)	
Age of prosthesis (years)			0.298[a]
Median (Q1, Q3)	8.3 (3.8, 18.6)	6.8 (2.2, 14.1)	
Treatment			<0.001[b]
None	0 (0.0%)	18 (100.0%)	
Surgery for aseptic failure	47 (100.0%)	0 (0.0%)	
Redness			0.273[b]
No	47 (100.0%)	17 (94.4%)	
Yes	0 (0.0%)	1 (5.6%)	
Warmth			
No	47 (100.0%)	18 (100.0%)	
Yes	0 (0.0%)	0 (0.0%)	
Swelling			1.000[b]
No	38 (80.9%)	14 (77.8%)	
Yes	9 (19.1%)	4 (22.2%)	
Pain			1.000[b]
No	1 (2.1%)	0 (0.0%)	
Yes	46 (97.9%)	18 (100.0%)	
Fever			0.281[b]
No	47 (100.0%)	17 (94.4%)	
Yes	0 (0.0%)	1 (5.6%)	

[a] Pearson's Chi-squared test with simulated p-value (based on 2000 replicates).
[b] Kruskal-Wallis rank sum test.

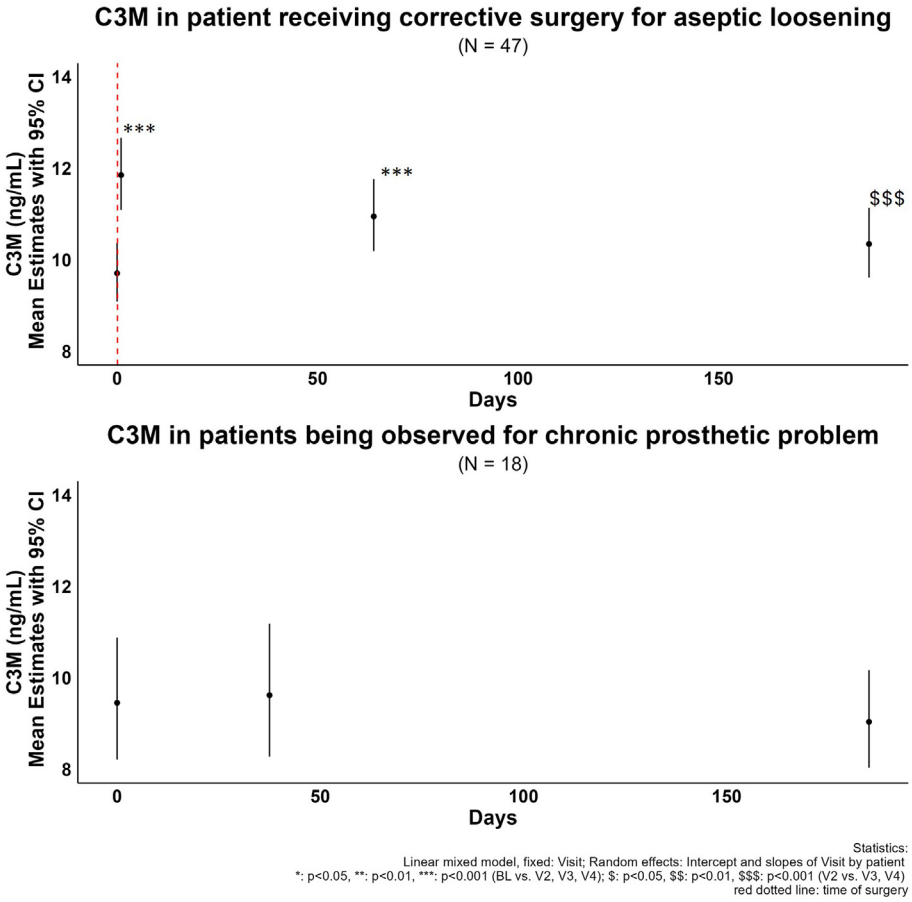

Fig. 1. Effect of revision surgery on biochemical marker of synovial tissue degradation C3M in patients with aseptic loosning versus patients with chronic problem not undergoing surgery. Serum samples from baseline, Visit 2 (median follow-up: Aseptic loosening 1 day; Chronic problem 38 days), Visit 3 (median follow-up: Aseptic loosening 67 days; Chronic problem 185 days), and Visit 4 (median follow-up: Aseptic loosening – 188 days) blood collection was assayed for C3M. Data are shown as ng/ml. *t*-test was used to compared baseline to follow-up differences within diagnosis group.

3.2. Systemic levels of type III collagen metabolites are associated with local joint tissue damage

The C3M biomarker recognize a neoepitope, that is generated by MMP cleavage of type III collagen in the soft connective tissues including synovium, tendons and ligaments but not in bone and the articular cartilage.

C3M levels were similar between aseptic loosening and chronic pain groups at baseline. C3M levels increased in patients with aseptic loosening from baseline to post-surgery by 22% ($p < 0.0001$). Biomarker levels normalized after 2 months almost to the level pre-surgery. Levels of C3M did not change from baseline to follow-up visits in patients with chronic pain, that did not undergo surgery (Fig. 1).

To assess whether these findings were affected by the prosthesis site, either hip or knee, data were also modeled separately for knee and hip. In the aseptic loosening group the changes following surgery were similar between the two joints (Supplementary figure 1). Due to the limited number of patients in the chronic pain group, subgroup analysis was not feasible.

4. Discussion

OA is often affecting only single joints, and only target joints are being clinically evaluated in clinical trials. Considering the emerging intra-articular DMOADS in clinical trials, understanding the contribution to biomarkers in circulation from a single joint is critical [11]. In this study, we describe the contribution of tissue damage caused by arthroplasty revision surgery to soft-tissue degradation biomarker levels in circulation.

In this study, we find a rapid increase in levels of degradation biomarker C3M post-surgery, which then normalizes after 1–3 months. These findings suggest that tissue degradation arising from a significant insult to the joint tissue exemplified by an arthroplasty revision surgery results in the release of type III collagen degradation products into circulation, that can be quantified by the C3M biomarker. The increase in C3M after joint replacement revision surgery, and the normalization at consecutive time points indicates that C3M is derived from soft and synovial tissue degradation localized in the joint. However, the degree of C3M deriving from the synovial membrane compared to the wound healing process associated with the penetration of the overlying tissue cannot be established in the current study, and future studies should seek to investigate C3M origin using either immunohistochemical approaches or assessing systemic levels following other surgical procedures to establish the wound healing contribution. In the light of these findings, it is reasonable to assume that trauma to tissue may result in acute increases in biomarkers of interstitial tissue remodeling such as C3M and this should be taken into account when interpreting biomarker data in the context of clinical studies involving surgery and acute biomarker measurements.

Prior studies have also linked C3M to synovial tissue degradation and synovitis. C3M is increased in conditioned media of synovial explants from late-stage OA patients following treatment with TNFα or IL-1β, showing a contribution from the synovium to C3M biomarker levels [7]. Rheumatoid arthritis patients have increased systemic C3M levels and levels are decreased in response to treatment with anti-IL-6 receptor therapy, which indicates a link to synovial inflammation in RA [12,13]. Interestingly, levels of C3M in RA patients have also been shown to

correlate with patient-reported outcomes [8]. In the IDEA study investigating the long-term benefits of weight loss, diet or a combination of both in overweight knee OA patients, C3M levels were positively correlated to IL-6 levels, suggesting an inflammatory link between weight loss, inflammation, and tissue destruction [9]. The impact of weight on OA pain and its subsequent influence on C3M levels provides another dimension to understanding the disease and may suggest that alterations in meta-inflammation and downstream tissue destruction associated with weight gain or loss may be reflected in the levels of type III collagen degradation quantified by C3M.

An increasing amount of attention has been directed to the clinical effect of weight loss therapies on OA pain. Overweight is associated with low-grade inflammation. In the IDEA trial, C3M was shown to decrease by a diet and exercise intervention after 18 months, while dietary intervention alone was unaffected, compared to an exercise control group. The effect was highly correlated to weight loss over 18 months, but interestingly also correlated to a reduction in WOMAC function [9]. The finding of a weak, but significant association between change in C3M in response to diet and exercise and WOMAC function, along with our findings that C3M is increased with tissue insult, could suggest that soft tissue destruction may indeed be interlinked with weight-related inflammation and symptoms, but this requires further studies.

There are several limitations to the current study. This study was post-hoc exploratory study utilizing already existing samples and therefore no power analysis or sample size estimation was conducted and these findings should therefore be further validated in other studies using predefined hypotheses. A major limitation of the study is the variability in the time between visits among patients and between different diagnoses. The main reason for this is the difference in diagnostic algorithm and treatment between patients suspected of aseptic loosening and more undefined chronic problems causing pain. Furthermore, in the current study we did not distinguish between knee and hip revision in the primary analysis. Joint distribution within each diagnosis was similar. The overall finding did not differ between joints in the aseptic loosening group when looking at the individual joint, while this analysis was not possible in the chronic pain group due to low number of patients. Furthermore, the fact that patients undergoing this type of treatment struggle with mobility may affect visit adherence, and we therefore cannot rule out a certain bias in patient adherence. This analysis was a secondary analysis and therefore no power calculation was possible.

In summary, in a longitudinal cohort of patients being treated for arthroplastic joint complications, type III collagen degradation was found to increase in response to tissue insult to the joint from revision surgery, while no change was observed in a non-surgical group with chronic pain of the joint over 6 months. The increase and gradual decrease throughout the study indicate a relationship between systemic levels of type III collagen degradation fragments and soft-tissue destruction and inflammation of the joint.

Credit author statement

CST: Idea and conception of biomarker study, Acquisition of biomarker data. Data analysis. Preparation of the manuscript. Revision of the manuscript and final approval. SR: Conception and design of clinical study. Revision of manuscript and final approval. MK: Conception of biomarker study. Revision of manuscript and final approval. ACBJ: Conception of biomarker study, revision of manuscript and final approval.

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Conflicts of interest

CST, ACBJ, and MK are employees of and owns stocks in Nordic Bioscience. The remaining authors declare no conflicts of interest. The author Sten Rasmussen has a potential relevant financial interest as a partner in ReGold ApS.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ocarto.2024.100527.

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