

Cytokines in temporomandibular joint synovial fluid and tissue in relation to inflammation

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Funding information

This study was supported by grants from the Swedish Dental Society, University of Bergen and HelseVest funding, Haukeland University Hospital, Bergen, Norway.

Abstract

Background: Synovial tissue is known to be the origin of inflammation in joint disease. Despite this, synovial fluid is the main biological specimen of choice in temporomandibular joint (TMJ) inflammation and pathology biomarker research. No comparison of TMJ protein content between synovial fluid and synovial tissue has been made.

Objectives: The aim of this study was to investigate whether cytokine concentrations in synovial fluid can be related to cytokine concentrations in synovial tissue and to analyse correlation of clinical parameters reflecting local inflammation to cytokine concentrations.

Methods: Synovial tissue and fluid samples were obtained during the same surgical procedure from a cohort of 101 patients with TMJ disorders. Interleukin (IL) 1 β , IL-6, IL-8, IL-10 and tumour necrosis factor α (TNF- α) were analysed in the samples and an intraindividual correlation made. Various patient-specific factors related to TMJ inflammation were associated with the cytokine concentrations in synovial fluid and tissue.

Results: No correlation between cytokine concentration in synovial fluid and synovial tissue was found, except for IL-8 ($\rho = .284$, $p = .024$). Synovial tissue cytokines correlated strongly to inflammation-related factors: diagnosis (IL-1 β , $p = .001$; TNF- α , $p = .000$; IL-10, $p = .000$), TMJ palpation pain (IL-1 β , $p = .024$; TNF- α , $p = .025$), synovitis score (IL-1 β , $p = .015$) and subjective TMJ pain (TNF- α , $p = .016$). Synovial fluid cytokines showed no significant relations to inflammation.

Conclusions: The investigated cytokine concentrations showed weak correlations between synovial fluid and synovial tissue, besides IL-8. Synovial tissue appeared to reflect inflammation to a higher extent than synovial fluid. Thus, suggesting that synovial tissue research should complement synovial fluid in future explorations of TMJ pathology and inflammation.

KEYWORDS

biomarkers, cytokines, interleukins, synovial fluid, synovial membrane, temporomandibular joint

1 | INTRODUCTION

Chronic orofacial pain is a common symptom in patients with long-standing temporomandibular disorder (TMD).¹ Temporomandibular joint (TMJ) surgical interventions are most often performed after a substantial round of non-invasive treatment, which is why patients scheduled for surgery usually have a long anamnesis of pain, that is chronic pain from the TMJ area.^{2,3} Thus, the need for robust predictions of surgical or medical treatment outcomes is of great importance for caregivers, patients and health authorities, to offer the best possible treatment regimens with the most effective health economy.

In TMJ surgery, patient-specific clinical variables predicting outcome have been investigated with somewhat contradictory results.²⁻⁷ Monitoring biomarkers in synovial tissue or synovial fluid might be another way to not only gain knowledge about surgical prediction and treatment response, but also enhance diagnostic precision.^{8,9} Synovial fluid has gained the most interest in TMD research, possibly because it is easier to access and less invasive compared with sampling from the synovial tissue.⁹⁻¹¹ Synovial fluid and extracellular fluid in the synovial tissue are locally produced by immunocompetent cells in the synovium and derived from blood plasma by ultrafiltration.^{9,12} Since healthy synovium functions as a semi-permeable membrane, the proportion of dialysate in synovial tissue and fluid correlates to synovial tissue interstitial space and vascular permeability.^{13,14} The continuous turnover of synovial fluid, that is filtration into the joint cavity and simultaneous interstitial drainage, is in equilibrium during normal conditions but is altered during inflammation and the synovial fluid volume increases.^{13,14} Generally, joint inflammation leads to higher concentrations of plasma-derived proteins and lower concentrations of large lubricating molecules, such as hyaluronan and lubricin.^{13,14} Many studies have focused on inflammatory cytokine concentrations in TMJ synovial fluid during health and disease. The most frequently investigated cytokines include tumour necrosis factor α (TNF- α), interleukin (IL) 1 β , IL-6, IL-8 and IL-10.^{9-11,15} However, a draw-back to synovial fluid samples is that it only provides indirect information of the synovial tissue inflammatory process.^{8,16}

TMJ synovial tissue research has often been limited to immunohistochemical analyses of sampled tissues to localise cytokine presence, whilst analyses of protein concentrations in the tissue have rarely been performed.^{3,17-20} Synovial tissue research in rheumatology has mainly focused on other joints rather than the TMJ.⁸

To our knowledge, no attempt has been made to evaluate and compare with what extent TMJ synovial fluid and synovial tissue individually reflects ongoing disease processes. In the absence of such studies, solid conclusions concerning biomarkers of TMJ pathology might be difficult to draw. Thus, we hypothesised that cytokine concentrations in synovial tissue would differ from that of the synovial fluid regardless of diagnoses. The aim of the present study was to investigate whether the cytokine concentration in synovial tissue correlated to synovial fluid cytokine concentration. The secondary aim was to analyse any correlation between clinical

parameters, reflecting local TMJ inflammation and pain, to the cytokine concentration in synovial tissue and synovial fluid respectively.

2 | MATERIALS AND METHODS

2.1 | Study design and cohort

A prospective cohort study was performed at the Unit of Cranio- and Maxillofacial Surgery, Karolinska University Hospital, Stockholm, Sweden. Patients eligible for inclusion were those with disc displacement with reduction (DDwR), disc displacement without reduction (DDwoR) and degenerative joint disease (DJD) together with arthralgia according to the DC/TMD criteria and chronic inflammatory arthritis (CIA).²¹ CIA is a generic term for rheumatological diseases such as rheumatoid arthritis, psoriatic arthritis and spondylarthritis. Of those eligible for inclusion, additional criteria stated that non-invasive therapy should have been tried for at least three months, a visual analogue scale (VAS) value of ≥ 4 for TMJ pain on function and/or TMJ disability, and DDwoR patients had to have a maximum interincisal opening (MIO) of ≤ 35 mm. The pre-surgical treatment algorithm and the cut-off levels were according to the unit's treatment guidelines based on the Swedish National Board of Health and Welfare guidelines.²² In addition, patients also had to have synovial tissue and/or synovial fluid sampled during the operation to be eligible for inclusion. Exclusion criteria were < 18 years, patients unable to give informed consent, and a history of open TMJ surgery.

A written informed consent was required, and patients were included from December 2014 to January 2017. The Regional Ethics Review Board in Stockholm approved the study (registration number 2014/622-31/1).

2.2 | Clinical examination

Initially, all potential patients referred to the unit were examined by four calibrated assessors. Demographic information concerning age, sex and duration of TMJ disorder was collected. Patient-specific variables of TMJ palpation pain, TMJ diagnosis, TMJ pain on function, TMJ disability, synovitis score and degenerative score were also collected, possibly reflecting TMJ inflammation and/or pain. TMJ palpation pain and TMJ diagnosis, except CIA, were made according to DC/TMD instructions.²¹ All patients in the CIA group were diagnosed by a rheumatologist and had at least two affected joints, not including the TMJ, prior to inclusion in the current study. Patients with CIA also had to have a TMJ diagnosis in conformity with specific arthritis criteria.²³ Assessor calibration was held at the start of the clinical study and at approximately six-month intervals. Patient's self-assessed current TMJ pain on function, hereafter named TMJ pain, and TMJ disability on a 10-cm VAS. The endpoints were 0 ('No TMJ pain') and 10 ('Worst imaginable TMJ pain'). TMJ disability referred to the patient's experience of TMJ functional disability. Zero TMJ disability corresponded to normal range of motion, and ability

to eat and chew all kinds of food, whilst 10 described a situation with no ability to move the jaw and/or no possibility to chew any solid food.²⁴ TMJ palpation pain on the lateral aspect was registered as a positive or negative finding. During arthroscopy, synovitis and degenerative findings were graded 0–3 according to the Gynther scale.^{25,26}

2.3 | Collection of synovial fluid and tissue samples

Synovial fluid and synovial tissue were sampled during arthroscopy or discectomy by the same surgeon (M.U.). Unilateral cases were sampled from the affected TMJ (right TMJ, $n = 44$; left TMJ, $n = 46$), and bilateral cases were sampled from the TMJ most severely affected ($n = 11$; right TMJ, $n = 8$; left TMJ, $n = 3$), according to the patient's experience. All patients had general anaesthesia. The synovial fluid sample was taken before any penetration of the TMJ capsule, using a push-and-pull method.²⁷ A mixture of 3.5 ml saline solution (9 mg/ml) and 1.0 ml vitamin B12 (Behepan [hydroxocobalamin 1 mg/ml], Pfizer Inc.) was prepared. 4.0 ml of the mixture was used stepwise 1.0 ml at a time, to wash the intraarticular joint space using two 5-ml syringes connected with a stopcock valve (Figure 1). The resulting aspirate had to have a weight of ≥ 0.5 mg, no or hardly visible blood contamination, and a dilution factor (DF) ≤ 0.985 .²⁸ Possible blood contamination was assessed visually according to the stipulated 0–3 grading system. Samples with no visible blood contamination (score 0) and samples with hardly visible blood contamination (score 1) were considered acceptable for analysis. The absorption value of the aspirated solution (Abs_{Asp}) was determined using a spectrophotometer (Hitachi U-2000, Hitachi Ltd, Tokyo, Japan). 0.5 ml of undiluted washing solution remaining from the initial mixing was used as a reference and was also analysed in the spectrophotometer (Abs_{Wash}). The DF could then be calculated with the formula: $DF = Abs_{Asp} / Abs_{Wash}$.²⁸

The synovial tissue biopsy was taken from the posterior bilaminar zone in the superior joint compartment during either arthroscopy or discectomy. The triangulation technique was used

to collect biopsies under direct visualisation during arthroscopy (Figure 1).²⁹ The biopsy forceps (Karl Storz SE & Co) used resulted in approximately 4 mm² tissue samples. Synovial tissue samples were placed in RNeasy Lysis Buffer (Qiagen), refrigerated for 24 h after which they were stored at -80°C , prior to protein extraction.

2.4 | Analysis of synovial tissue and synovial fluid

The aspirated volumes of synovial fluid were small; therefore, cytokines with pro- or anti-inflammatory properties previously associated with the TMJ, were chosen for analyses.^{10,11} Synovial tissue was ground in liquid nitrogen to disrupt the piece of tissue. Proteins were extracted in ice-cold cell lysis buffer NP40 (Thermo Fisher Scientific) using 50 μl buffer per 10 mg of tissue.^{3,20,30} The mixtures were centrifuged at 20 000 g at 4°C for 10 min, and the supernatant retained. Total protein concentration in each tissue sample was determined using Qubit Protein Assay Kit and the Qubit Fluorometer (Thermo Fisher Scientific). The magnetic bead panel HCYTOMAG-60K (Merck Millipore) was used to determine the levels of IL-1 β , IL-6, IL-8, IL-10 and TNF- α with multi-analytic profiling using a Luminex 200 system (Luminex) and xMAP technology. Resulting data were analysed by xPONENT 3.1 software (Luminex).

Quantikine ELISA High Sensitivity Kits (R&D systems, Bio-Techne Corp.) were used to determine the concentration of IL-1 β (HSLB00D), IL-6 (HS600B), IL-8 (HS800), IL-10 (HS100C) and TNF- α (HSTA00D). The attained concentrations were corrected with the DF to obtain the synovial fluid cytokine concentrations.

2.5 | Statistical analyses

Stata version 15 SE (StataCorp) and IBM SPSS version 25.0 (IBM Corp) were used to analyse the data. The parameters were stratified by TMJ. Nota bene: only one TMJ per patient was sampled. Descriptive statistics regarding clinical characteristics were calculated as mean \pm standard deviation for all continuous data, and

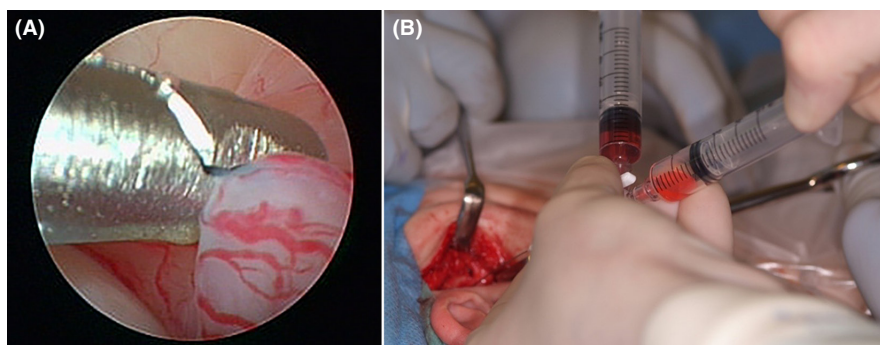


FIGURE 1 Illustrations of sampling from the temporomandibular joint (TMJ). (A) During arthroscopy, the triangulation technique was used to enable the surgeon to visualise the harvesting procedure. The picture shows biopsy forceps about to sample synovial tissue from the posterior bilaminar zone in the upper TMJ compartment. (B) Synovial fluid sampling with the push-and-pull method during a discectomy procedure before incision of the lateral joint capsule. A 27G needle is used to penetrate the superior joint compartment, and then, 1.0 ml at a time of the washing solution is flushed into the joint space and consecutively aspirated

as number, and percentage for bivariate data. The concentration of specified proteins (pg/ml) was used in the statistical analyses. Histograms of cytokine concentration showed no normal distribution, and median and standard errors of the mean were used for descriptive statistics. Correlation between synovial fluid and synovial tissue cytokine concentrations was investigated with Spearman's rank correlation. To analyse clinical parameters in relation to cytokine concentrations, uni- and multivariate linear and logistic regression analyses were used, dependent on the clinical parameter. The reference in logistic regression for synovitis and degenerative score was those with scores 0 and 1 (no and mild synovitis/degenerative score), opposed to those with scores 2 and 3 (moderate and severe synovitis/degenerative score). Logistic regression reference regarding the parameter TMJ palpation pain was no pain on palpation, in contrast to pain on TMJ palpation. Lastly, the parameter TMJ diagnoses had the reference DDwR and DDwoR, opposed to DJD and CIA. Age, as a continuous variable, and gender were incorporated in the multivariate computations to adjust for possible confounding. A p -value of $\leq .05$ was regarded as significant.

3 | RESULTS

One hundred and twenty-eight patients were originally considered for inclusion in the study. Twenty-seven patients were excluded for various reasons; nine were not willing to participate, another nine were excluded due to exclusion criteria, seven improved spontaneously and therefore lacked indication for surgery, and two patients dropped out. These 27 patients had a mean age of 40.4 years (15.3), and 82% were women. Thirteen patients (48%) had DDwoR, three (11%) DDwR, eight (30%) DJD, one (4%) CIA and two patients (7%) with no diagnosis defined. However, none of these variables differed significantly from the final cohort. Thus, 101 patients met the inclusion criteria and remained within the study.

3.1 | Correlation between cytokine concentration in synovial tissue and synovial fluid

Seventy-one patients provided both analysable synovial fluid and synovial tissue and were available to test for correlations in cytokine concentrations between the two sample types (Figure 2). Included patients served as their own control. The measured concentrations of IL-1 β , IL-6, IL-8, IL-10 and TNF- α together with patient's characteristics are presented in Table 1. Some cytokine concentrations were identified to be below the lowest standard (<3.2 pg/ml) in the multiplex protein analyses of the tissue preparations. These were set at 3.2 pg/ml, whereas values above the highest standard (10 000 pg/ml) were set at 10 000 pg/ml. Samples with cytokine measurements outside of the precision and recovery of the assays had no values indicated and were consequently regarded as missing values. The number of tissue samples with detectable levels of the specific protein concentration in each group is indicated in Table 2. The

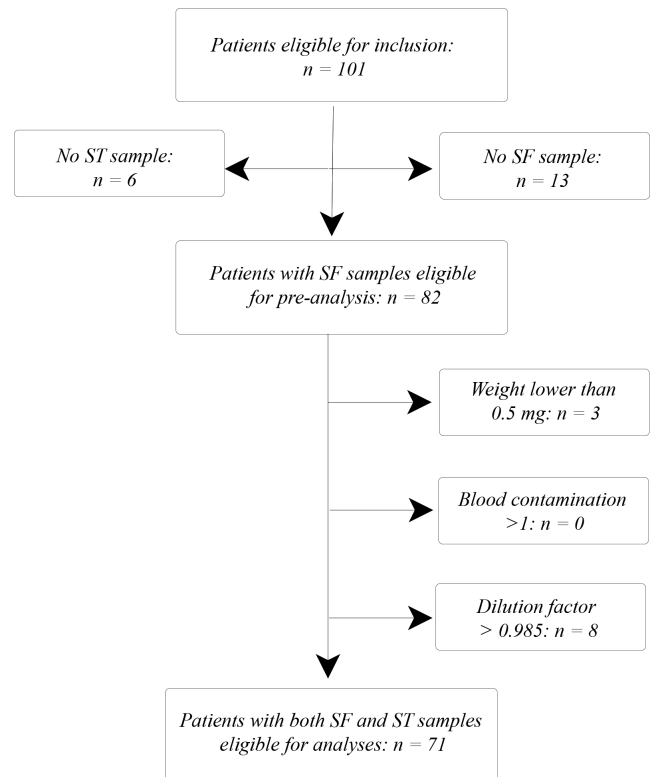


FIGURE 2 Flow chart illustrating the number of patients with both synovial fluid (SF) and synovial tissue (ST) samples fulfilling criteria for correlation analysis and the reasons for exclusion

concentration of IL-8 in synovial fluid and synovial tissue showed a significant correlation (number of pairwise observations [obs.] = 63, $\rho = .284$, $p = .024$), whereas IL-1 β (obs. = 27, $\rho = .059$, $p = .771$), IL-6 (obs. = 33, $\rho = -.158$, $p = .381$), IL-10 (obs. = 53, $\rho = .233$, $p = .093$) and TNF- α (obs. = 43, $\rho = .171$, $p = .272$) showed weaker and non-significant correlation. Scatter plots are presented to show the distribution of sample concentrations (Figure 3).

Subgroup analyses were also performed, stratifying the patient cohort according to diagnoses. Spearman's rank correlation was used and correlation between synovial fluid and tissue cytokine concentration were tested. In the diagnosis group, DDwR and CIA weak and non-significant correlations were found. TNF- α concentrations in synovial fluid and tissue showed strong correlations in DJD (obs. = 8, $\rho = .738$, $p = .037$) and IL-10 in DDwoR (obs. = 24, $\rho = .576$, $p = .003$). Applying Bonferroni correction resulted in weak and non-significant correlations in all computations.

3.2 | Correlation of clinical parameters to the cytokine concentration in synovial tissue or fluid

Objective and subjective parameters, possibly reflecting local TMJ inflammation, were related to cytokine concentrations in both synovial fluid and synovial tissue. Patients with solely synovial fluid or synovial tissue samples were also included in this statistical computation since paired samples were not needed. The whole cohort of 101

TABLE 1 Demographic data, clinical variables and cytokine concentrations in patients (n=71) that provided both synovial fluid and synovial tissue

	Diagnoses				
	DDwR	DDwoR	DJD	CIA	Total
Number of patients, n (%)	17 (24)	32 (45)	10 (14)	12 (17)	71 (100)
Age (years), mean (SD)	38.6 (11.9)	40.0 (15.7)	43.7 (20.6)	42.5 (12.1)	40.6 (14.9)
Sex, W/M	12/5	28/4	10/0	11/1	61/10
Duration of symptoms (months), mean (SD)	40.0 (37.5)	23.3 (28.0)	38.7 (52.2)	29.8 (20.9)	30.4 (33.7)
Objective and subjective parameters indicating local inflammation, mean (SD) unless where indicated					
TMJ pain (VAS 0–10)	3.9 (2.5)	5.6 (2.4)	7.1 (1.8)	5.7 (2.1)	5.4 (2.4)
TMJ palpation pain, Y/N	5/12	24/8	7/3	11/1	47/24
Synovitis score (0–3)	na	1.7 (0.5)	1.9 (0.9)	2.1 (0.6)	1.9 (0.6)
Degenerative score (0–3)	na	1.5 (0.9)	1.7 (1.2)	2.1 (0.8)	1.6 (1.0)
Cytokine concentration (pg/ml) in synovial fluid, mean (SEM); samples with detectable concentration, n					
IL-1 β	2.9 (1.0); 8	8.6 (8.4); 15	260.7 (254.7); 2	9.2 (7.1); 6	6.7 (16.7); 31
IL-6	13.3 (3.8); 14	10.7 (77.3); 31	5.5 (183.8); 9	6.8 (23.7); 10	9.4 (45.5); 64
IL-8	4.1 (2.5); 14	13.4 (4.2); 32	13.1 (8.2); 10	23.5 (12.5); 8	11.2 (3.1); 64
IL-10	14.8 (29.2); 12	9.6 (7.1); 25	21.0 (12.7); 8	17.6 (20.0); 9	14.2 (8.2); 54
TNF- α	15.9 (4.8); 10	8.3 (0.9); 20	3.6 (4.5); 8	7.4 (17.5); 6	8.3 (2.8); 44
Cytokine concentration (pg/ml) in synovial tissue, mean (SEM); samples with detectable concentration, n					
IL-1 β	0.5 (0.2); 16	1.8 (0.9); 29	6.0 (4.6); 10	9.8 (4.5); 10	1.7 (1.3); 65
IL-6	4.5 (1.0); 7	10.0 (8.1); 10	22.2 (13.8); 9	38.4 (25.1); 12	13.4 (9.4); 38
IL-8	5.9 (1.8); 17	27.1 (3.4); 32	11.3 (58.3); 10	7.4 (2.2); 11	15.1 (8.5); 70
IL-10	2.5 (0.6); 17	7.2 (1.6); 31	15.4 (20.6); 10	28.7 (3.9); 12	6.1 (3.5); 70
TNF- α	2.3 (0.4); 17	4.6 (0.9); 32	7.7 (5.2); 10	12.2 (3.8); 11	4.5 (1.2); 70

Note: The data are presented according to TMJ diagnoses.

Abbreviations: CIA, chronic inflammatory arthritis; DDwoR, disc displacement without reduction; DDwR, disc displacement with reduction; DJD, degenerative joint disease; IL, interleukin; M, men; ml, millilitre; N, no; n, number; na, not applicable; pg, picogram; SD, standard deviation; SEM, standard error of means; TNF, tumour necrosis factor; VAS, visual analogue scale; W, women; Y, yes.

patients and their samples were consequently analysed, 77 patients had analysable synovial fluid, and 95 had synovial tissue (Figure 2). The demographic and clinical characteristics of this patient cohort have been reported on elsewhere.³ Unadjusted logistic regression analyses (synovitis score, degenerative score, TMJ diagnoses and TMJ palpation pain) and linear regression analyses (patient assessed pre-operative TMJ pain) were performed (Table 2). Only synovial tissue cytokine concentrations were significantly correlated to the different parameters (Table 2). Multivariate analyses were also made, but no relevant changes in either significant or non-significant findings of correlations compared with the univariate analyses were found.

4 | DISCUSSION

To our knowledge, cytokine concentrations in synovial fluid have not earlier been compared with synovial tissue cytokine concentrations. Synovial fluid originates from immunocompetent cells in the synovium and from exudation, which might suggest a similarity

in protein concentrations between synovial tissue and fluid.^{13,14} To test this assumption, we investigated the concentrations of five different cytokines detected earlier in several studies on healthy, as well as diseased TMJ's.^{3,6,15,17,18,20,27,28,31–38} The concentrations of IL-8 were found to have a strong correlation between synovium and synovial fluid, but weak non-significant correlations were found with respect to IL-1 β , IL-6, IL-10 and TNF- α . In the subgroup analyses stratified on diagnoses, TNF- α in DJD and IL-10 in DDwoR showed strong correlation between synovial tissue and fluid. Although, when applying Bonferroni correction, only weak correlations were found in the subgroup analyses. This suggests that synovial tissue and synovial fluid may mirror TMJ disease activity in different ways.

Diagnoses and clinical factors possibly reflecting local inflammation were compared with synovial fluid and synovial tissue cytokine concentrations. Synovial tissue cytokine concentrations showed several correlations with clinical factors, whilst this study failed to identify corresponding associations with synovial fluid cytokines. Of the parameters selected for their probable ability to mirror local

TABLE 2 Analysis of the correlation between inflammatory cytokines and patient-specific objective, and subjective parameters reflecting local TMJ inflammation

No. of obs.	ST/SF (max. 95/77)	Synovitis score ^a		Degenerative score ^a		TMJ palp. pain ^a		Diagnosis ^a		TMJ pain (VAS) ^b	
		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	Coeff	95% CI
IL-1 β	86	1.10 [†]	1.02-1.18	1.02	0.98-1.08	1.24 [†]	1.03-1.51	1.19 ^{**}	1.07-1.32	0.04	-0.01-0.09
	34	1.02	0.98-1.06	1.04	0.98-1.06	0.99	0.97-1.01	1.01	0.99-1.02	-0.00	-0.01-0.01
IL-6	47	1.00	0.99-1.01	1.00	0.99-1.02	1.00	0.99-1.01	1.03	1.00-1.06	-0.01	-0.02-0.01
	70	1.00	1.00-1.00	1.00	1.00-1.00	1.00	1.00-1.00	1.00	1.00-1.00	0.00	-0.00-0.00
IL-8	94	1.00	0.99-1.01	0.98	0.96-1.01	1.00	0.99-1.02	1.00	0.99-1.01	0.01	-0.00-0.01
	70	1.01	0.99-1.03	1.01	0.99-1.02	1.02	0.99-1.06	1.02	0.99-1.04	-0.00	-0.01-0.01
IL-10	93	1.04	1.00-1.08	0.99	0.98-1.01	1.04	1.00-1.09	1.10 ^{**}	1.04-1.16	0.02	-0.00-0.04
	58	1.00	0.98-1.01	1.00	0.99-1.02	1.00	0.99-1.01	1.00	0.99-1.01	-0.01	-0.02-0.01
TNF- α	92	1.03	0.98-1.09	1.03	0.98-1.09	1.09 [†]	1.01-1.17	1.18 ^{**}	1.09-1.28	0.06 [†]	0.01-0.11
	47	1.10	0.96-1.26	1.02	0.97-1.07	1.00	0.96-1.03	1.01	0.97-1.04	-0.02	-0.06-0.01

Abbreviations: CI, confidence interval; coeff, coefficient; IL, interleukin; max., maximum number of observations; No., number; obs., observations; OR, odds ratio; palp., palpation; SF, synovial fluid; ST, synovial tissue; TNF, tumor necrosis factor; VAS, visual analogue scale.

^aLogistic regression.

^bLinear regression.

[†] $p < .05$; ^{**} $p < .005$.

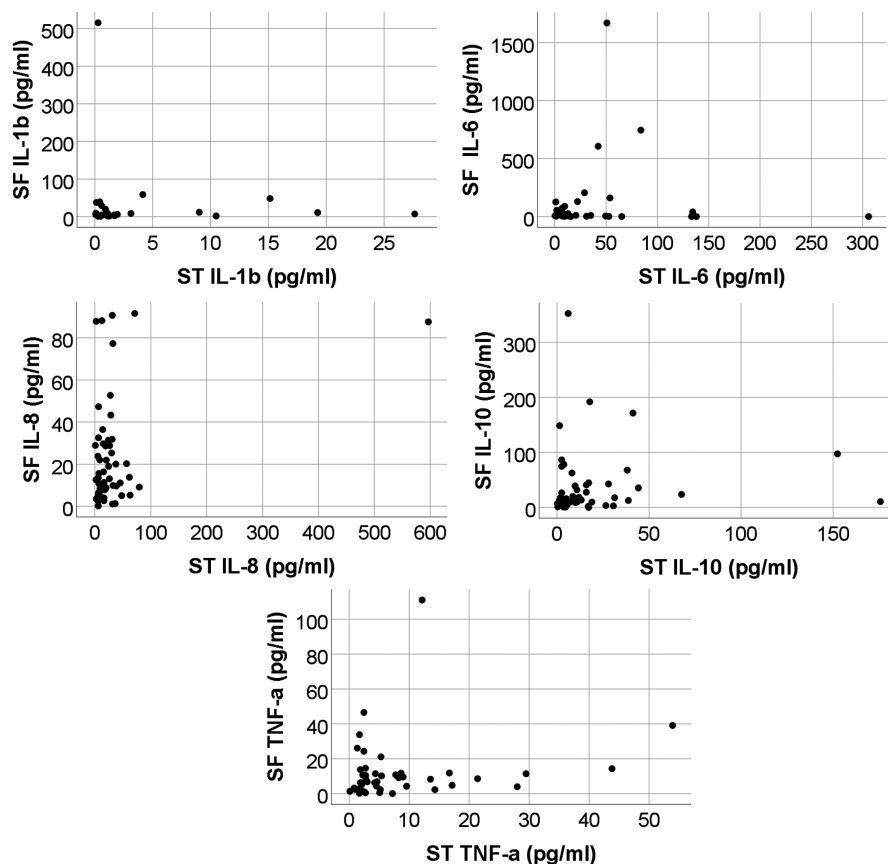
inflammation, TMJ diagnosis seem to best correlate with cytokine concentration. Higher concentrations of IL-1 β , IL-10 and TNF- α in synovial tissue correlated to diagnoses DJD/CIA. The lack of diagnosis-dependent correlation of synovial fluid cytokine concentration has earlier been reported.^{34,38} Positive findings of palpatory pain lateral to the TMJ also correlated with higher concentrations of IL-1 β and TNF- α in synovial tissue. IL-1 β and TNF- α in synovial fluid have been reported to be associated with TMJ palpation pain, which this study could not confirm.^{31,38} Patient assessed TMJ pain intensity was associated with TNF- α in synovial tissue but not in synovial fluid in our patient sample. Synovial fluid concentration of TNF- α in rheumatoid arthritis has been found to be related to TMJ pain, but in DDwR, DDwoR and DJD patients, no such relation has been found.^{32,33,35,37} In our study, the synovitis score was associated with IL-1 β concentrations in synovial tissue. Synovial fluid concentration of IL-6 and synovitis score has earlier been found to correlate, whilst IL-1 β did not.³⁶ Degenerative score was not correlated to any of the cytokine concentrations in our mixed patient sample, which might implicate that degenerative changes were merely a remnant sign of previous inflammation and degradation, thereby not reflecting present inflammation. According to our results, it may be questioned whether synovial fluid has limitations as

a marker for local TMJ inflammation. Although, the present investigation was not designed to answer that question. It seems reasonable to further investigate the correlation between synovial fluid and tissue biomarker concentrations, and potential relationships with clinical factors to improve the understanding of inflammation and degeneration of the TMJ.

A control group consisting of TMJ healthy volunteers would have added base-line characteristics of the reported cytokines and validity to this report. We deliberately chose not to include healthy volunteers, foremost because the surgical procedures under which harvesting synovial tissue was done, might induce TMJ problems of uncertain magnitude. To perform these surgeries in healthy volunteers would be an ethical concern and not in line with the Helsinki declaration. Furthermore, the main objective was to compare synovial fluid to synovial tissue, which was why each unique patient served as their own control. From this perspective, a healthy control group was not needed.

The CIA patient group were heterogenous in this material and the results correlating synovial tissue and fluid in this subgroup might not be representative. Future studies on fluid/tissue correlations might focus on specific rheumatologic diagnoses, for instance rheumatoid arthritis or psoriatic arthritis.

FIGURE 3 Scatter plots demonstrating the intraindividual concentration of the cytokines IL-1 β , IL-6, IL-8, IL-10 and TNF- α . Cytokine concentrations in synovial fluid (SF) are seen on the y-axis and synovial tissue (ST) concentrations on the x-axis



The cytokine assays used were not the same due to considerable deviance in dilution between synovium samples and synovial fluid. This might of course influence the outcome, but since the same type of assay's were used for all synovial tissue samples or for all synovial fluid samples, the intraindividual differences should be proportional between patients. When comparing either synovial fluid or tissue separately to clinical variables, interindividual differences should be due to a real difference since the same type of assay was used. In all, this shortcoming should not bias the results in this study.

The size of the population was not determined by a prior power analysis. Earlier studies of this kind have not been performed; therefore, a power analysis only would be based on bold assumptions. In this material, 71 patients served as their own controls as both fluid and tissue samples were harvested and analysed. The highest number of pairwise observations (63) was made for IL-8, which was the only cytokine where a correlation was found. The other cytokines had pairwise observations that ranged from 23 to 53 cases. IL-10 had the largest number of 53 cases and were close to a significant result ($p = .093$). Thus, it might be inferred that higher numbers of observations could lead to more significant correlations for the other cytokines.

5 | CONCLUSIONS

In conclusion, this study suggests an important role for the synovial tissue cytokines IL-1 β , IL-10 and TNF- α in local TMJ inflammation

in patients with either DDwR, DDwoR, DJD or CIA. Synovial tissue and synovial fluid concentrations of IL-8 correlated, whilst the other investigated cytokines seem not to be related in these patients. For future research on TMJ inflammatory pathology, we propose additional analyses of synovial tissues and synovial fluid for a more comprehensive understanding of pathological mechanisms and possible interactions between intraarticular tissues.

ACKNOWLEDGEMENTS

The authors wish to thank the technical support of Nikolce Tudzarovski at the Department of Dental Medicine, Karolinska Institutet, Stockholm and Janne Elin Reseland, Safiyye Suslu, and AinaMari Lian at Oral Research Laboratory, Institute of Clinical Dentistry, University of Oslo. Carina Kruger-Weiner (C.K-W.), Head of Department of Oral- and Maxillofacial Surgery, Eastman Institute, Stockholm, for help examining included patients.

CONFLICT OF INTEREST

All the authors have declared no conflict of interest.

AUTHOR CONTRIBUTIONS

M. Ulmner contributed to conception, design, data acquisition, analysis and interpretation, drafted and critically revised the manuscript. R. Sugars contributed to conception, design, data acquisition, analysis and interpretation, and critically revised the manuscript. A. Naimi-Akbar contributed to data analysis and interpretation, and critically revised the manuscript. P. Alstergren contributed to data

analysis and interpretation, and critically revised the manuscript. B. Lund contributed to conception, design, data analysis and interpretation, and critically revised the manuscript. All authors gave their final approval and agreed to be accountable for all aspects of the work.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/joor.13321>.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Ulmner M, Sugars R, Naimi-Akbar A, Alstergren P, Lund B. Cytokines in temporomandibular joint synovial fluid and tissue in relation to inflammation. *J Oral Rehabil.* 2022;49:599-607. doi:[10.1111/joor.13321](https://doi.org/10.1111/joor.13321)