

Parotid saliva and blood biomarkers in juvenile idiopathic arthritis in relation to temporomandibular joint magnetic resonance imaging findings

Alexandra Dimitrijevic Carlsson^{1,2,3}  | Kerstin Wahlund⁴ |
Bijar Ghafouri⁵ | Erik Kindgren^{6,7,8} | Martina Frodlund⁹ | Hanna Salé¹⁰ |
Eva Klintström^{11,12} | Carin Starkhammar Johansson² | Per Alstergren^{1,3,13,14}

¹Orofacial Pain and Jaw Function, Malmö University, Malmö, Sweden

²Centre for Oral Rehabilitation, in Linköping, and Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden

³Scandinavian Center for Orofacial Neurosciences (SCON), Malmö, Sweden

⁴Department of Orofacial Pain and Jaw Function, Kalmar County Hospital, Kalmar, Sweden

⁵Rehabilitation Medicine, Department of Medicine and Health Sciences, Linköping University, Linköping, Sweden

⁶Department of Pediatrics, Västervik Hospital, Västervik, Sweden

⁷Division of Pediatrics, Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden

⁸Department of Pediatrics, Skövde Hospital, Sweden

⁹Rheumatology/Division of Inflammation and Infection, Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden

¹⁰Department of Neuroradiology, Center of Medical Imaging and Physiology, Skåne University Hospital, Lund, Sweden

¹¹Center for Medical Image Science and Visualization (CMIV), Linköping University, Linköping, Sweden

¹²Department of Radiology and Department of Health, Medicine and Caring Sciences, Linköping University, Linköping, Sweden

¹³Skåne University Hospital, Specialized Pain Rehabilitation, Lund, Sweden

¹⁴Orofacial Pain Unit, Malmö University, Malmö, Sweden

Correspondence

Alexandra Dimitrijevic Carlsson, Orofacial Pain and Jaw Function, Malmö University, Malmö, Sweden.

Email: alexandra.carlsson@regionostergotland.se

Abstract

Background: Juvenile idiopathic arthritis (JIA) often affects the temporomandibular joint (TMJ) caused by an abnormal immune system that includes overactive inflammatory processes. Salivary biomarkers may be a powerful tool that can help establishing diagnosis, prognosis and monitor disease progress.

Objective: The objective was to investigate biomarkers in parotid saliva and blood plasma in relation to temporomandibular joint (TMJ) magnetic resonance imaging (MRI) findings in patients with JIA and healthy individuals.

Methods: Forty-five children aged 6 to 16 years with JIA and 16 healthy age- and sex-matched controls were included. Unstimulated parotid saliva samples and venous blood were collected. Biochemical analyses were performed for the cytokine biomarkers. The participants underwent MR imaging of the TMJs, where changes in the inflammatory and the damage domains were assessed.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2024 The Author(s). *Journal of Oral Rehabilitation* published by John Wiley & Sons Ltd.

Results: In the JIA patients, lower concentrations of IL-6R and gp130 were found in parotid saliva than in plasma. Higher concentrations of IL-6 were found in parotid saliva than in plasma. IL-6, IL-6R and gp130 in parotid saliva explained the presence of bone marrow oedema and effusion in the JIA patients.

Conclusions: This study suggests that the IL-6 family in parotid saliva is associated with TMJ bone marrow oedema and effusion in patients with JIA, suggesting that IL-6 has promising properties as a parotid saliva biomarker for TMJ inflammatory activity.

KEYWORDS

arthritis, biomarkers, juvenile idiopathic arthritis, magnetic resonance imaging, parotid gland, temporomandibular joint

1 | INTRODUCTION

Juvenile idiopathic arthritis (JIA), a chronic rheumatic disease affecting children, is characterised by cartilage and bone tissue destruction in joints, including the temporomandibular joint (TMJ). These pathological changes are caused by an abnormal immune system that includes overactive inflammatory processes. Arthritis involving the TMJ in JIA sometimes also causes pain and growth disturbances.^{1,2}

Up to 70% of children with JIA develop temporomandibular joint (TMJ) arthritis.^{3,4} TMJ arthritis is often asymptomatic and therefore a particularly challenging joint to diagnose. Clinical assessment of the TMJ is hampered by the low sensitivity of joint pain and the absence of physical findings early in the disease process.⁵ More specific methods are therefore needed for diagnosing and predicting TMJ arthritis in JIA.

Advances in biotechnology have led to an interest in saliva diagnostics for monitoring disease.⁶ Saliva offers an attractive alternative to blood samples, particularly in children and when blood sample collection is painful and not repeatable. Salivary biomarkers have been found to be useful in diagnosing children with metabolic disorders such as obesity and diabetes.⁷ In our proof-of-concept study on biomarkers in parotid saliva in healthy controls, we detected the pro-inflammatory cytokines interleukin-6 (IL-6), IL-10 and IL-1 β , as well as tumour necrosis factor (TNF) and S100A8.⁸ Parotid saliva is uncontaminated until entering the oral cavity. Biomarkers can enter saliva by synthesis and secretion by the salivary gland or by passive diffusion.⁹ In contrast, whole saliva contains a mix of secretions from the salivary glands along with bacteria, gingival cervical fluids, epithelial cells, food debris and leucocytes.¹⁰

For example, IL-6 (together with IL-6R and gp130) is a strong pro-inflammatory cytokine associated with synovial inflammation and the destruction of cartilage and bone in joints, including the TMJ, in JIA.¹¹ IL-6 is involved in both local and systemic inflammation in JIA pathogenesis and is detectable in blood and tissue in all JIA subtypes.¹²⁻¹⁴

The aim of this study were to investigate biomarkers in parotid saliva and plasma in relation to temporomandibular joint MRI findings in the inflammatory and damage domains of JIA.

2 | METHODS

2.1 | Study population

Forty-five JIA patients (33 girls and 12 boys) aged 6 to 16 years and 16 healthy age- and sex-matched children were included. The JIA patients were consecutively referred from four paediatric departments in southeast Sweden (Linköping University Hospital, Vrinnevi Hospital/Norrköping, Motala Hospital and Västervik Hospital) to the Center of Oral Rehabilitation in Linköping (2015–2018). The healthy individuals were recruited among patients at public dental health clinics in Linköping (Table 1). All participants completed a questionnaire regarding general health before the clinical examination.

Clinical demographics for the study population (JIA and healthy individuals) are presented in Table 1. The same study population has participated in three previous studies by our research group.^{8,15,16}

Inclusion criteria were age between 6 and 16 years and a JIA diagnosis according to the 2004 International League of Associations for Rheumatology (ILAR) classification criteria.¹⁷ Exclusion criteria were diabetes mellitus, inflammatory bowel disease, chronic pain conditions other than JIA and psychiatric diseases, although depression and anxiety were allowed due to their frequency and importance among people with chronic pain (Table 2).

Patients with JIA diagnoses included 19 (43%) with oligoarthritis, 15 (33%) with polyarthritis and 11 (24%) with other subtypes of JIA (systemic arthritis, psoriatic arthritis, enthesitis-related arthritis or undifferentiated arthritis). Sixteen (36%) of JIA patients were positive for antinuclear antibodies (ANA). The patient sample was representative of JIA patients in Scandinavia.^{18,19} Five patients were HLA-B27-positive. At inclusion, 41 patients had ongoing pharmacological therapy for their JIA, 27 of whom had treatment with disease-modifying anti-rheumatic drugs; 5 had methotrexate only, 7 methotrexate in combination with biologics (adalimumab or etanercept) and 3 biologics alone (adalimumab or etanercept); 31 patients had been prescribed non-steroidal anti-inflammatory drugs (NSAIDs), and six were on oral corticosteroid therapy (prednisolone).

Of the 16 healthy individuals, 11 were relatives of employees at the Oral Rehabilitation Clinic and one was a healthy sibling of a child with JIA. Exclusion criteria for the healthy individuals were diabetes

TABLE 1 Demographic data, disease activity and temporomandibular disorder diagnoses for 45 patients with juvenile idiopathic arthritis and 16 age- and sex-matched healthy individuals.

		PATIENTS					HEALTHY INDIVIDUALS				
		Median	Percentiles			n	Median	Percentiles			n
			25th	75th	% pos			25th	75th	% pos	
Individuals											
Age	Years	12	10	15		45	13	10	13		16
Sex	Boys/girls					12/33					5/11
Age at diagnosis	Years	9	5	12		45	n.a.				
Disease duration	Years	4	3	7		45	n.a.				
Disease activity											
JADAS71	0–101	6.0	2.8	9.8		45	n.a.				
Erythrocyte sedimentation rate ^a	mm/h	6	3	10		45	0	0	0		16
C-Reactive protein ^a	mg/L	0	0	0	9	45	0	0	0	0	16
Rheumatoid factor	U/mL	0	0	0	4	45	0	0	0	0	16
Anti-citrullinated antibodies	U/mL	0	0	0	4	43	0	0	0	0	16
DC/TMD diagnoses											
Myalgia	n					10					1
Myofascial pain with referral	n					2					0
Arthralgia	n (joints)					8					0
Headache attributed to TMD	n					3					0
Combinations from above											
Myalgia and arthralgia	n					2					0
Myalgia, arthralgia and headache	n					2					0

Abbreviations: DC/TMD, diagnostic criteria for temporomandibular disorders; JADAS71, 71-joint Juvenile Arthritis Disease Activity Score; n, number of observations; n.a., not applicable.

^aNormal values for ESR (<30 mm/h) and CRP (<5 mg/L) were counted as 0.

TABLE 2 Inclusion and exclusion criteria for the JIA patients.

Inclusion criteria	Exclusion criteria
● Age 6–16 years	● Diabetes
● JIA-diagnosis according to ILAR criteria	● Inflammatory-bowel disease
	● Other chronic pain condition than JIA
	● Psychiatric disease (depression and anxiety were allowed due to the frequent occurrence and important role in chronic pain)

Abbreviation: ILAR, International League of Associations for Rheumatology.

mellitus, inflammatory bowel disease, rheumatic disease, chronic pain conditions and psychiatric disease (other than depression and/or anxiety) (Table 3). A graphical representation of the study methods is shown in Figure 1.

2.2 | Clinical examination

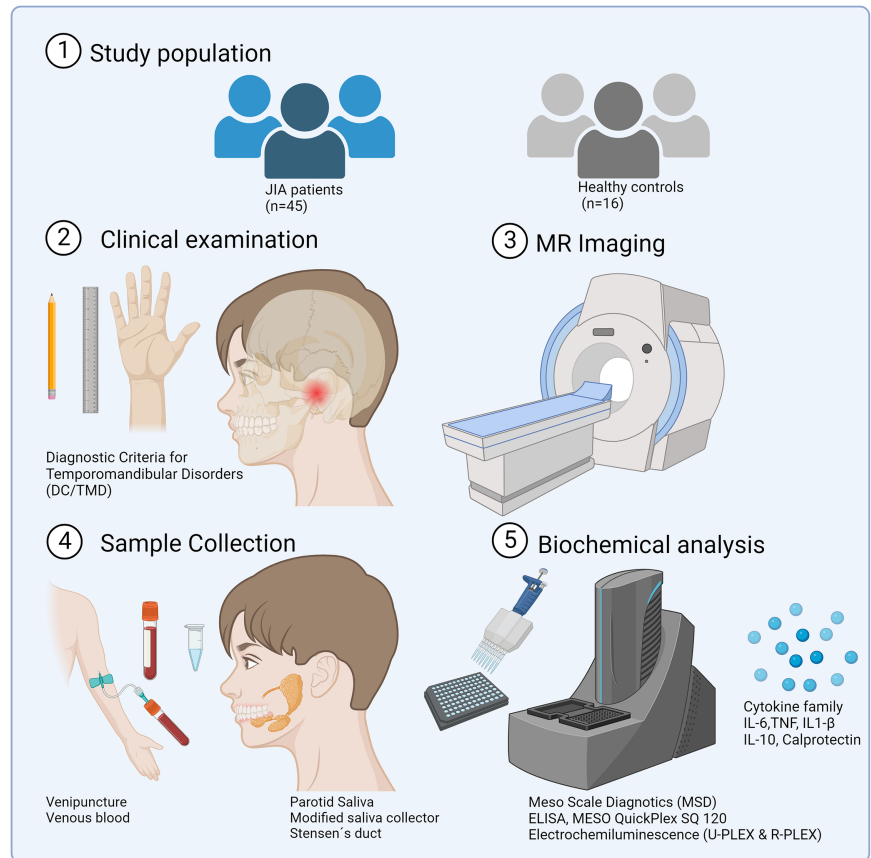
The diagnostic criteria (DC) for temporomandibular disorders (TMD) were used to diagnose the patients. The DC/TMD comprise two domains: Axis I for physical diagnoses and Axis II for several domains

TABLE 3 Inclusion and exclusion criteria for the healthy individuals.

Inclusion criteria	Exclusion criteria
● Age 6–16 years	● Diabetes mellitus
● No subjective pain in the orofacial region	● Inflammatory-bowel disease
	● Rheumatic disease and other chronic pain condition
	● Psychiatric disease (other than depression and/or anxiety)

concerning pain and its consequences.²⁰ The applied clinical examination corresponds well to the consensus-based recommendations for clinical orofacial examination in JIA, except for examination of craniofacial deformations, which were not assessed in the present study.⁵ In this study, only DC/TMD Axis I was used. The clinical examination for Axis I diagnostics requires a pain history assessed on a questionnaire and a well defined and structured clinical examination. Clinical assessments evaluate familiar pain localizations, jaw movement capacity (lateral, protruding and mouth opening), familiar jaw movement pain, TMJ noises and familiar pain upon palpation of the masticatory muscles and TMJ. The criteria for DC/TMD Axis I diagnoses are validated for patients aged 18 years and older, and they

FIGURE 1 Graphical abstract of study methods. Created with [BioRender.com](https://www.biorender.com).



comprise TMJ arthralgia, masticatory muscle myalgia, headache attributed to TMD, degenerative joint disease and TMJ disk displacements. Multiple diagnoses are allowed in DC/TMD.²⁰

2.3 | Parotid saliva collection

Unstimulated parotid gland saliva was collected intraorally using a modified Carlson–Crittenden collector, described earlier by Dimitrijevic Carlsson and colleagues.⁸ In brief, the saliva was collected between 08:30 and 11:30 on the same day as the clinical examination. The children were instructed to sit upright with the jaw in a relaxed position and not to talk. The collector was placed over the papilla of Stensen's duct. Parotid saliva was collected during passive drooling via a 25-cm plastic tube into a 1.5-mL Eppendorf tube. The parotid saliva samples were stored at -80°C in a biobank facility at Linköping University.

2.4 | Venous blood collection

Venous blood samples were collected from each participant at hospitals and health centers in Linköping, Norrköping and Motala at a median of 13 days (25th/75th percentiles: 0/27, maximum value: 232 days) following the clinical examination. The two patients with the longest intervals forgot or cancelled their appointment for venous blood samples collection on several occasions.

Venous blood samples were collected in EDTA vacutainers and centrifuged at 18°C , 1800×10 min. The plasma collected was then aliquoted and stored at -80°C in a biobank facility at Linköping University. All samples were coded before analysis to ensure blinding of the operator.

Venous blood samples were also obtained to assess markers of disease activity (i.e. erythrocyte sedimentation rate, C-reactive protein, rheumatoid factor and anticyclic citrullinated peptide antibodies). The Juvenile Arthritis Disease Activity Score 71 (JADAS71) was calculated based on active joint count (out of 71), physician's global assessment, parental global evaluation and erythrocyte sedimentation rate.²¹

2.5 | Biochemical analysis

The concentration of 10 inflammatory cytokines and proteins was assayed in samples from parotid saliva samples and plasma. The biomarkers were chosen based on a literature review of JIA immunopathogenic mechanisms^{1,11,22,23} and outcomes from our proof-of-concept study.⁸ The biomarkers were the pro-inflammatory cytokines TNF, IL-6, Calprotectin, S100A8, IL-1 β and glycoprotein 130 (gp130), the anti-inflammatory TNF soluble receptor II (TNFSFRII), the IL-6 receptor (IL-6R) and the anti-inflammatory cytokines IL-10 and IL-1 receptor antagonist (IL-1ra). Two venous blood samples were missing from our original group of 45 patients. One of these was not delivered from the hospital to the biobank,

and the other was missing because the sample had not been obtained from the patient.

The samples were analysed using a commercially available R-PLEX (Calprotectin, S100A8, TNFsRII, IL-6R, gp130) and U-PLEX (TNF, IL-6, IL-10, IL-1 β , IL-1ra) human biomarkers singlet or multiplex assay Meso Scale Discovery (MSD; Rockville, MD, USA^{24,25}). The assays contained the 10 biomarkers mentioned above and was based on an electrochemiluminescent detection method and performed exactly according to the manufacturer's recommendations. Data were collected and analysed using the MESO QuickPlex SQ 120 instrument equipped with Discovery workbench data analysis software version 4.0.12 (Meso Scale Diagnostics; Rockville, MD, USA). The precision of the measurements, based on both intra- and inter-assay coefficients of variations, was <10% within the detection limits. The assay operators were blinded to whether samples were from patients or healthy controls.

2.6 | Magnetic resonance imaging evaluation of the temporomandibular joint

The patients and healthy controls underwent bilateral MR imaging of their TMJs at Linköping University Hospital at a median of 68 days (25th/75th percentiles: 46/106, maximum value: 266 days) following the clinical examination. The MR unit was an Achieva dStream 1.5 T (Philips Medical Systems; Best, the Netherlands) with flexible coils centred over the TMJ. An axial T1-weighted localizer was used to orient the long axis of the condyle in closed-mouth position. The sagittal images were perpendicular to the long axis, and the coronal images were parallel to the long axis. The sagittal images were proton- and T2-weighted images in closed-mouth position and proton-weighted images in the open-mouth position. The coronal images were proton-weighted, T2-weighted and short tau inversion recovery (STIR) sequences in closed-mouth position. The MR images were evaluated for findings within the inflammatory and damage domains according to Tolend and colleagues²⁶ modified to exclude the aspect 'joint enhancement'. Our modification of the Tolend's scoring system assesses an inflammatory domain and a damage domain. The inflammatory domain comprises bone marrow oedema, joint effusion and synovial thickening while the damage domain assesses condylar flattening, erosions and disc abnormalities. A detailed description of the MR imaging protocol for evaluation of TMJ changes on MR imaging in an observation by Dimitrijevic Carlsson et al.¹⁶

2.7 | Statistics

Non-parametric statistics were used due to the characteristics of the MR variables. For descriptive statistics median, and 25th and 75th percentiles were reported. For analytical statistics, the Mann-Whitney *U* test was used to calculate the significance of between-group differences. The Spearman-ranked correlation test

was used to calculate the significance of correlations between variables. Multiple logistic regression was used to determine the significance of relations between a dependent variable (dichotomized MR findings) and independent variables (clusters of biomarkers in parotid saliva and/or plasma). The area under the receiver operating characteristic (ROC) curve was calculated for each significant logistic regression and reported if the area under the curve was higher than 0.70. The following biomarker clusters were assessed in both parotid saliva and plasma in the logistic regression: (a) TNF and TNFsRII, (b) IL-6, IL-6R and gp130 and (c) IL-1 β and IL-1ra. All calculations were performed with Stata 15.1 Special Edition software (StataCorp; College Station, TX, USA). A probability level of $p < .05$ was considered significant.

3 | RESULTS

3.1 | Parotid saliva and plasma concentrations in JIA patients and healthy individuals

Table 4 shows the cytokine concentrations in parotid saliva and plasma. The JIA patients had significantly higher parotid saliva concentrations of TNFsRII ($p = .025$), IL-1 β ($p = .037$) and calprotectin ($p = .034$) than the healthy controls. In plasma, the JIA patients had significantly higher concentrations of IL-6 ($p = .001$), IL-1ra ($p = .042$) and S100A8 ($p = .040$) than the healthy controls.

In plasma, JIA patients with anti-rheumatic drugs had higher concentrations of TNF ($p = .005$), IL-1ra ($p = .037$), IL-10 ($p = .043$) and S100A8 ($p = .006$) than patients without such medication. There was no significant difference in parotid saliva concentrations between JIA patients with or without anti-rheumatic drugs.

The JIA patients had significantly lower salivary flow than the healthy controls ($p = .039$). Table 5 shows the significant relations between parotid saliva concentrations of biomarkers and parotid saliva flow.

3.2 | Cytokine concentrations in parotid saliva and plasma in JIA patients and in healthy individuals

In the JIA patients, significantly lower concentrations of TNF ($p < .001$), TNFsRII ($p < .001$), IL-6R ($p < .001$), gp130 ($p < .001$), S100A8 ($p < .001$) and IL-10 ($p < .001$) were found in parotid saliva than in plasma. Significantly higher concentrations of IL-1 β ($p < .001$), IL-1ra ($p < .001$) and IL-6 ($p < .001$) were found in parotid saliva than in plasma (Table 4).

In healthy individuals, TNF ($p < .001$), TNFsRII ($p < .001$), IL-6R ($p < .001$), gp130 ($p < .001$), S100A8 ($p < .001$) and IL-10 ($p < .001$) were found in significantly lower concentrations in parotid saliva than in plasma. IL-1 β ($p < .001$), IL-6 ($p < .001$) and IL-1ra ($p = .023$) were found in significantly higher concentrations in parotid saliva than plasma (Table 4).

There was no significant correlation between parotid saliva and plasma concentrations of any of the investigated cytokines.

TABLE 4 Cytokine concentrations in parotid saliva and blood plasma from 45 patients with juvenile idiopathic arthritis and 16 age- and sex-matched healthy individuals.

Cytokine		JIA patients					Healthy individuals					Patients – healthy
		Median	Percentiles			n	Median	Percentiles			n	
			25	75				25	75			
Parotid saliva												
TNF	pg/mL	0.6	***	0.4	0.9	44	0.4	***	0.2	0.7	16	.025
TNFsRII	pg/mL	58	***	42	146	41	39	***	25	58	16	
IL-6	pg/mL	1.9	***	0.9	4.4	44	2.2	***	1.0	3.2	16	
IL-6R	pg/mL	111	***	83	163	41	119	***	57	201	15	
gp130	pg/mL	52377	***	40594	83284	39	45754	***	32818	81829	16	
IL-1β	pg/mL	0.9	***	0.4	2.0	44	0.5	***	0.4	0.7	16	.037
IL-1ra	pg/mL	11165	***	11002	11831	41	11066	***	10972	11130	16	
Calprotectin	pg/mL	109162		46637	321898	38	42565		31438	75225	14	.034
S100A8	pg/mL	6799	***	4941	14441	37	6687	***	4281	10464	12	
IL-10	pg/mL	0.06	***	0.05	0.09	44	0.05	***	0.03	0.07	16	
Saliva flow rate	mL/min	0.016		0.007	0.020	45	0.020		0.014	0.036	16	.039
Plasma												
TNF	pg/mL	1.5		1.2	2.2	43	1.31		1.0	1.6	16	
TNFsRII	pg/mL	6020		4644	8993	43	5903		5433	7086	16	
IL-6	pg/mL	0.5		0.4	0.7	43	0.3		0.2	0.4	16	.001
IL-6R	pg/mL	48418		40166	57499	42	44503		37572	54019	15	
gp130	pg/mL	375352		338382	409978	43	340126		317239	380989	16	
IL-1β	pg/mL	0.0		0.0	0.1	31	0.0		0.0	0.1	11	.042
IL-1ra	pg/mL	155		129	206	43	138		105	155	16	
Calprotectin	pg/mL	101336		62374	120723	43	69351		57353	82776		.040
S100A8	pg/mL	16021		11493	18423	43	11371		8984	15532	16	
IL-10	pg/mL	0.3		0.2	0.4	43	0.3		0.2	0.3	16	

Abbreviations: ***, probability level ($p < .001$) for significant differences in cytokine/protein concentration in parotid saliva and plasma in JIA patients as well as in healthy individuals; gp130, glycoprotein 130; IL-10, interleukin-10; IL-1ra, interleukin 1 receptor antagonist; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-6R, interleukin 6 receptor; N, number of observations; S100A8, S100 calcium-binding protein A8; TNF, tumour necrosis factor; TNFsRII, tumour necrosis factor soluble Receptor 2.

3.3 | Relation between parotid saliva and plasma biomarkers versus markers of systemic inflammatory activity in JIA patients

TNFsRII in parotid saliva and IL-1ra in plasma were related to systemic inflammatory activity (Table 5).

3.3.1 | Magnetic resonance imaging findings in relation to parotid saliva and plasma cytokines

Table 6 shows the MRI findings in the inflammatory and damage domains.

3.4 | Bone marrow oedema

Logistic regression using presence of TMJ bone marrow oedema as the dependent variable and IL-6, IL-6R and gp130 in parotid saliva as

independent variables statistically explained the presence of bone marrow oedema ($p = .004$; Table 7 and Figure 2) in the JIA patients. The area under the ROC curve was 0.758. This relation was not possible to calculate in the healthy individuals because there were too few positive observations.

3.5 | Joint effusion

Logistic regression using presence of TMJ effusion as the dependent variable and IL-6, IL-6R and gp130 in parotid saliva as independent variables statistically explained the presence of TMJ effusion ($p = .012$; Table 7) in the JIA patients. The area under the ROC curve was 0.667. In addition, logistic regression using presence of TMJ effusion as the dependent variable and IL-1β and IL-1ra in plasma as independent variables statistically explained the presence of TMJ effusion ($p = .004$; Table 7) in the JIA patients. The area under the ROC curve was 0.808. These relations were not significant in the healthy individuals.

3.6 | Erosions

S100A8 in parotid saliva was negatively correlated to erosions ($r_s = -.41$, $n = 37$, $p = .011$) in the JIA patients.

4 | DISCUSSION

This study indicates that the IL-6 family in parotid saliva is associated with TMJ bone marrow oedema and effusion in patients with JIA, suggesting that IL-6 has promising properties as a parotid saliva biomarker for TMJ inflammatory activity. In addition, there seems to be local production or enrichment of the IL-6 family in the parotid

TABLE 5 Significant correlations between measures of systemic inflammatory activity and biomarkers in parotid saliva and blood plasma, as well as saliva flow rate, in 45 patients with juvenile idiopathic arthritis.

	Correlation		
	r_s	n	p
Parotid saliva			
JADAS71			
TNF-sRII	.31	42	.048
Plasma			
JADAS71			
IL-1ra	.33	43	.030
Salivary flow rate (mL/min)			
Parotid saliva			
IL-1 β	-.45	44	.002
S100A8	-.39	37	.016
gp130	-.54	39	<.001
TNF-sRII	-.41	41	.008
IL-6R	-.55	39	<.001

r_s = Spearman's ranked correlation coefficient; n = number of observations; p = probability level, JADAS71 = 71-joint Juvenile Arthritis Disease Activity Score. *Normal values for ESR (<30 mm/h) and CRP (<5 mg/L) were counted as 0.

gland. TNFsRII concentrations in parotid saliva seem to be associated with systemic inflammatory activity. However, parotid saliva does not seem to reflect the content of the investigated biomarkers in plasma in JIA patients.

In our study, the IL-6 family in parotid saliva was associated with bone marrow oedema and effusion as assessed by MRI. The MRI signs of TMJ bone marrow oedema and joint effusion points to ongoing inflammatory activity. TMJ bone tissue erosion implies ongoing structural joint damage, and the presence of bone marrow oedema may be predictive of later bone tissue erosions.²⁷ Bone marrow oedema is associated with increased levels of erythrocyte sedimentation rate, IL-6 and C-reactive protein in patients with early-stage rheumatoid arthritis.²⁸ IL-1 β , IL-1ra, TNF and TNFsRII in TMJ synovial fluid have found to be associated with inflammatory activity in TMJs, which may result in pain as well as TMJ cartilage and bone tissue destruction rheumatoid arthritis.^{29,30} IL-6 (together with IL-6R and gp130) is a strong pro-inflammatory cytokine associated with both synovial inflammation and cartilage and bone destruction in joints, including the TMJ, in patients with JIA.¹¹ IL-6 is involved in both local and systemic inflammation in JIA pathogenesis and is detectable in blood and tissues in all JIA subtypes.¹²⁻¹⁴ Parotid salivary levels of IL-6 have been found to be higher in patients with RA than in healthy controls.³¹ Higher parotid saliva IL-6 concentrations have been observed in adult patients with Sjögren's syndrome than in healthy subjects, and IL-6 has been suggested as a potential biomarker of disease activity in Sjögren's syndrome.³² Given these observations, IL-6 in parotid saliva seems to be a strong candidate as a potential biomarker of disease activity or prognosis for the TMJ in patients with JIA.

In the present study, the JIA patient has lower parotid salivary flow than the healthy individuals. The decreased salivary flow rate in the JIA patients may be explained by the autoimmune disease causing inflammation in the salivary gland, influencing salivary flow by local effects in the gland, changes in sympathetic/parasympathetic control of the gland, or medication. These factors may very well affect the function of the salivary gland and the composition of saliva.^{8,33,34} For example, Sjögren's syndrome has been shown to cause chronic inflammation and damage to the salivary glands, hyposalivation and increased pro-inflammatory cytokine content in

TABLE 6 Distribution (number and percentage) of temporomandibular joint (TMJ) magnetic resonance imaging findings (MRI), assessed according to Tolend et al.²⁶ in 90 TMJs in 45 patients with juvenile idiopathic arthritis (JIA) and 32 TMJs in 16 healthy individuals.

MRI findings		JIA patients			Healthy individuals		
		0	1	2	0	1	2
Inflammatory domain							
Bone marrow oedema	0-1: Absent, present	83 (92%)	7 (8%)	n.a.	31 (97%)	1 (3%)	n.a.
Joint effusion	0-2: Absent, small, large	64 (71%)	22 (24%)	4 (4%)	24 (75%)	8 (25%)	0 (0%)
Synovial thickening	0-2: Absent, mild, moderate/severe	80 (89%)	9 (10%)	1 (1%)	31 (97%)	1 (3%)	0 (0%)
Damage domain							
Condylar flattening	0-2: Absent, mild, moderate/severe	48 (53%)	28 (31%)	14 (16%)	30 (94%)	2 (6%)	0 (0%)
Erosions	0-2: Absent, mild, moderate/severe	83 (92%)	6 (7%)	1 (1%)	32 (100%)	0 (0%)	0 (0%)
Disc abnormalities	0-1: Absent, present	39 (43%)	51 (57%)	n.a.	17 (53%)	15 (47%)	n.a.

TABLE 7 Significant results from logistic regression using magnetic resonance imaging signs of temporomandibular joint arthritis (TMJ; erosion, effusion and bone marrow oedema) as dependent variables and parotid saliva and plasma concentrations of clusters of cytokine biomarkers as independent variables.

	Odds ratio	Confidence interval (95%)		
		Low	High	
TMJ effusion				
Cytokine family: IL-6 ($n=39$; $p=.012$)				
Parotid saliva	IL-6	1.932	1.146	3.258
	IL-6R	1.001	0.996	1.006
	gp130	0.999	0.999	1.000
Cytokine family: IL-1 ($n=31$; $p=.004$)				
Plasma	IL-1 β	1.20×10^8	8.386	1.70×10^{15}
	IL1-ra	0.998	0.075	2.235
TMJ bone marrow oedema				
Cytokine family: IL-6 ($n=39$; $p=.004$)				
Parotid saliva	IL-6	1.831	0.920	3.645
	IL-6R	1.008	0.995	1.020
	gp130	0.999	0.998	0.999

Parotid saliva and plasma clusters of cytokine biomarkers: 1) TNF, TNFR2, 2) IL-6, IL-6R and gp130, 3) IL-1 β and IL-1ra. No significant findings were found for TMJ erosion.

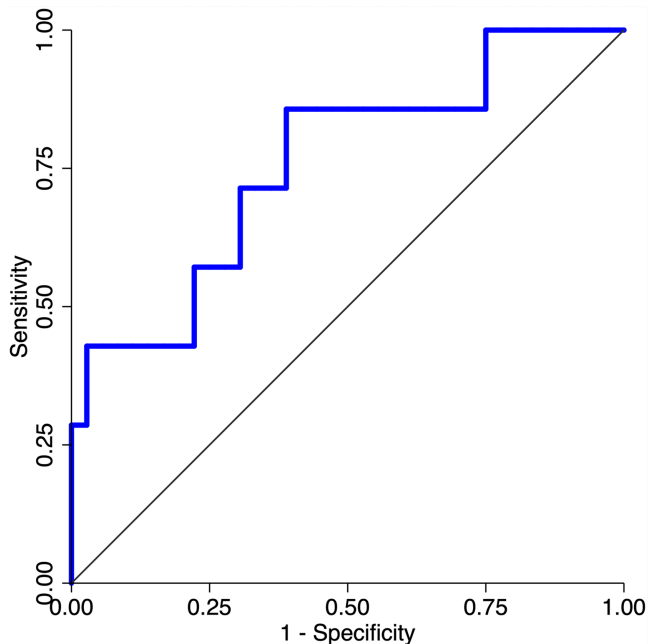


FIGURE 2 Receiver operating curve (ROC) showing the relation between sensitivity and specificity regarding presence of TMJ bone marrow oedema using the combination of IL-6, IL-6R and gp130 in parotid saliva as predictors ($p=.004$). The area under the ROC was 0.76.

whole saliva in patients with juvenile Sjögren's syndrome.³⁵⁻³⁷ In addition, RA patients have a lower parotid salivary flow than healthy controls.³¹ The reduced parotid salivary flow may also explain the

higher concentrations of certain mediators found in parotid saliva in the JIA patients than in the healthy individuals. It may also explain the higher parotid saliva concentrations of some of the investigated cytokines compared to plasma.

In this study, we analysed inflammatory biomarkers in JIA patients and healthy individuals. All investigated cytokines and receptors were detectable in our parotid saliva and blood samples. The JIA patients had higher levels of IL-1 β , Calprotectin and TNFR2 in parotid saliva and higher IL-1ra, IL-6 and S100A8 in plasma compared to their healthy peers. This suggests a role of these cytokines/receptors in JIA. In addition, IL-6 was found in higher concentration in parotid saliva than in plasma in both the JIA group and the healthy individuals. These results show that IL-6 is locally produced and released or enriched in the parotid gland in JIA and points to a possible potential of IL-6 as a JIA biomarker. In plasma, JIA patients on anti-rheumatic drugs had higher concentrations of TNF, IL-1ra, IL-10 and S100A8 than patients without such medication. One probable explanation is that these medications are mainly reserved for patients with higher systemic inflammatory activity, that is, higher plasma levels of these mediators. Taken together, this suggests a role of these cytokines/receptors in JIA. However, this must be further elucidated in future studies.

Wozniak and colleagues analysed whole and parotid saliva from healthy individuals for certain cytokines and chemokines.³⁸ That study indicated similar concentrations of chemokines and cytokines in whole and parotid saliva. However, whole saliva contains a mix of secretions from the salivary glands along with bacteria, gingival cervical fluids, epithelial cells, food debris and leucocytes. Parotid saliva, sampled using the methodology used in the present study, is uncontaminated until entering the oral cavity. We consider the use of parotid saliva, as used in the present study, is a major advantage in investigating potential biomarkers.

This study found no association between parotid saliva and plasma concentrations for any of the investigated mediators. This means that parotid saliva does not reflect plasma levels of these mediators and must therefore be regarded as a separate tissue. Indeed, there is limited evidence supporting any association between cytokine concentrations in saliva and blood.³⁹⁻⁴²

Children with JIA are at risk of developing pain and irreversible orofacial changes due to inflammation and degradation of cartilage and bone. By the time destruction is detectable with MR imaging, TMJ damage may already have caused lifelong joint destruction. The potential of biomarkers in saliva to function as a useful clinical tool to assess and monitor disease activity is therefore worthy of further investigation. Certainly, the specificity of IL-6 concentration in parotid saliva for the TMJ (or any other joint) is low, but it may be regarded as a piece of information that should be interpreted in the context of the medical history and clinical examination.

4.1 | Methodological considerations

The included patients received three MRI examinations over 2 years during the large research project of which this study was one part. For

several reasons, including ethical concerns such as risks of claustrophobia, anxiety and stress, reluctance to perform invasive procedures, the need to sedate healthy individuals without clinical indications for MRI, and the toxic contrasting agents used, we did not use gadolinium-enhanced MR in the present study. Clinical practice at the Center for Oral Rehabilitation avoids using intravenous contrast agents for MR imaging due to their potential toxicity. This limits our possibilities to detect joint enhancement, why this aspect was excluded from this study by the modified Tolend's score. All other aspects of the inflammatory and damage domains were possible to assess.

This is the first study to investigate the relation between parotid saliva and plasma cytokines and MR findings of the TMJ in JIA. The study is also unique to include healthy individuals as well as the focus on uncontaminated parotid saliva. In addition, this study is a part of a larger project that will longitudinally investigate JIA patients over a two-year period regarding clinical, psychosocial, biomarkers and MR findings.

The interval between the clinical examination and saliva sampling, on the one hand, and blood sampling as well as MRI examinations, on the other hand, was for some patients longer than expected. However, we consider the interval for the vast majority of both groups to be acceptable in relation to the aim of the study. The included patients had low systemic inflammatory activity, as assessed by CRP, indicating adequate medication. This is likely to ensure low and stable pro-inflammatory cytokine levels as well.⁴³ Unfortunately, there are no studies on JIA patients that reports cytokine levels in blood over time to compare with. Koelman et al.⁴⁴ showed that single plasma measurements of IL-6, IL-8, TNF- α , IL-10, IL-13 and IFN- γ in adult individuals accurately represents the average plasma concentrations over 4-month period. In healthy adolescent girls, neither the plasma nor saliva content of for example IL-6, TNF, IL-1 β and IL-10 varied substantially over a three-year period.⁴² Previous longitudinal studies with MR imaging have shown that inflammatory TMJ signs generally persist over a longer period of time in JIA⁴⁵⁻⁴⁷ as well as in adults with temporomandibular joint disorders^{48,49} and rheumatoid arthritis.⁵⁰

5 | CONCLUSIONS

This study indicates that the IL-6 family in parotid saliva is associated with TMJ bone marrow oedema and effusion in patients with JIA, suggesting that IL-6 has promising properties as a parotid saliva biomarker for TMJ inflammatory activity. In addition, there seems to be a local production in the parotid saliva gland in JIA and healthy regarding the IL-6 family. However, parotid saliva does not seem to reflect the plasma content regarding the investigated biomarkers in JIA.

AUTHOR CONTRIBUTIONS

ADC participated in planning the study, collecting the data, analysing the data, interpreting the results and writing the manuscript. KW participated in planning the study, analysing the data, interpreting the results and writing the manuscript. BG participated in planning

the study, analysing the data and interpreting the results and writing the manuscript. EK and MF participated in planning the study and writing the manuscript. HS and EK participated in planning the study, analysing the data and writing the manuscript. CSJ participated in planning the study and writing the manuscript. PA was the senior researcher responsible for planning the study, designing study, analysing the data, interpreting the results and writing the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

The authors thank all the children and their parents for participating in the study.

FUNDING INFORMATION

This research was funded by the Research Council of Southeast Sweden, grant number FORSS-748481; Public Dental Health Scientific Funds in Östergötland, grant number FOU 2-15-14, Sweden; and Swedish Dental Society's Scientific Fund.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

PEER REVIEW

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/joor.13806>.

DATA AVAILABILITY STATEMENT

Correspondence and request for data and materials should be addressed to the corresponding author (ADC).

ETHICS STATEMENT

The Regional Ethical Review Board in Linköping, Sweden, approved the study (Dnr 2014/461-31 and 2017/135-32). All participants and their parents received a written information about the study and signed an informed consent form before enrollment. The procedure followed the requirements of Declaration of Helsinki (1964).

ORCID

Alexandra Dimitrijevic Carlsson  <https://orcid.org/0000-0002-2260-8442>

REFERENCES

1. Prakken B, Albani S, Martini A. Juvenile idiopathic arthritis. *Lancet (London, England)*. 2011;377(9783):2138-2149.
2. Stoustrup P, Glerup M, Bilgrau AE, et al. Cumulative incidence of orofacial manifestations in early juvenile idiopathic arthritis: a regional, three year cohort study. *Arthritis Care Res*. 2019;72:907-916.
3. Stoll ML, Kau CH, Waite PD, Cron RQ. Temporomandibular joint arthritis in juvenile idiopathic arthritis, now what? *Pediatr Rheumatol Online J*. 2018;16(1):32.
4. Rongo R, Alstergren P, Ammendola L, et al. Temporomandibular joint damage in juvenile idiopathic arthritis: diagnostic validity of diagnostic criteria for temporomandibular disorders. *J Oral Rehabil*. 2019;46(5):450-459.

5. Stoustrup P, Twilt M, Spiegel L, et al. Clinical orofacial examination in juvenile idiopathic arthritis: international consensus-based recommendations for monitoring patients in clinical practice and research studies. *J Rheumatol*. 2017;44(3):326-333.
6. Pappa E, Kousvelari E, Vastardis H. Saliva in the "omics" era: a promising tool in paediatrics. *Oral Dis*. 2018;25:16-25.
7. Desai GS, Mathews ST. Saliva as a non-invasive diagnostic tool for inflammation and insulin-resistance. *World J Diabetes*. 2014;5(6):730-738.
8. Dimitrijevic Carlsson A, Ghafouri B, Starkhammar Johansson C, Alstergren P. Unstimulated parotid saliva sampling in juvenile idiopathic arthritis and healthy controls: a proof-of-concept study on biomarkers. *Diagnostics (Basel)*. 2020;10(4):251.
9. Kaufman E, Lamster IB. The diagnostic applications of saliva—a review. *Crit Rev Oral Biol Med*. 2002;13(2):197-212.
10. Edgar WM. Saliva: its secretion, composition and functions. *Br Dent J*. 1992;172:305-312.
11. Yilmaz M, Kendirli SG, Altintas D, Bingol G, Antmen B. Cytokine levels in serum of patients with juvenile rheumatoid arthritis. *Clin Rheumatol*. 2001;20(1):30-35.
12. Akioka S. Interleukin-6 in juvenile idiopathic arthritis. *Mod Rheumatol*. 2019;29(2):275-286.
13. Mangge H, Kenzian H, Gallistl S, et al. Serum cytokines in juvenile rheumatoid arthritis. Correlation with conventional inflammation parameters and clinical subtypes. *Arthritis Rheum*. 1995;38(2):211-220.
14. Eberhard BA, Laxer RM, Andersson U, Silverman ED. Local synthesis of both macrophage and T cell cytokines by synovial fluid cells from children with juvenile rheumatoid arthritis. *Clin Exp Immunol*. 1994;96(2):260-266.
15. Dimitrijevic Carlsson A, Wahlund K, Kindgren E, Skogh T, Starkhammar Johansson C, Alstergren P. Orofacial pain in juvenile idiopathic arthritis is associated with stress as well as psychosocial and functional limitations. *Pediatr Rheumatol Online J*. 2019;17(1):83.
16. Dimitrijevic Carlsson A, Wahlund K, Klintstrom E, et al. Juvenile idiopathic arthritis and the temporomandibular joint: a case-control study of magnetic resonance imaging findings in relation to clinical and psychosocial factors. *Eur J Paediatr Dent*. 2023;24(1):69-76.
17. Petty RE, Southwood TR, Manners P, et al. International league of Associations for Rheumatology Classification of Juvenile Idiopathic Arthritis: second revision, Edmonton, 2001. *J Rheumatol*. 2004;31(2):390-392.
18. Berntson L, Andersson Gare B, Fasth A, et al. Incidence of juvenile idiopathic arthritis in the Nordic countries. A population based study with special reference to the validity of the ILAR and EULAR criteria. *J Rheumatol*. 2003;30(10):2275-2282.
19. Berthold E, Mansson B, Kahn R. Outcome in juvenile idiopathic arthritis: a population-based study from Sweden. *Arthritis Res Ther*. 2019;21(1):218.
20. Schiffman E, Ohrbach R, Truelove E, et al. Diagnostic criteria for temporomandibular disorders (DC/TMD) for clinical and research applications: recommendations of the international RDC/TMD consortium network* and orofacial pain special interest Groupdagger. *J Oral Facial Pain Headache*. 2014;28(1):6-27.
21. Consolaro A, Ruperto N, Bazso A, et al. Development and validation of a composite disease activity score for juvenile idiopathic arthritis. *Arthritis Rheum*. 2009;61(5):658-666.
22. Consolaro A, Varnier GC, Martini A, Ravelli A. Advances in biomarkers for paediatric rheumatic diseases. *Nat Rev Rheumatol*. 2015;11(5):265-275.
23. Zaripova LN, Midgley A, Christmas SE, Beresford MW, Baildam EM, Oldershaw RA. Juvenile idiopathic arthritis: from aetiopathogenesis to therapeutic approaches. *Pediatr Rheumatol Online J*. 2021;19(1):135.
24. Dabito D, Margolick JB, Lopez J, Bream JH. Multiplex measurement of proinflammatory cytokines in human serum: comparison of the Meso scale discovery electrochemiluminescence assay and the Cytometric bead Array. *J Immunol Methods*. 2011;372(1-2):71-77.
25. Gerdle B, Bäckryd E, Falkenberg T, Lundström E, Ghafouri B. Changes in inflammatory plasma proteins from patients with chronic pain associated with treatment in an interdisciplinary multimodal rehabilitation program—an explorative multivariate pilot study. *Scand J Pain*. 2019;20(1):125-138.
26. Tolend MA, Twilt M, Cron RQ, et al. Toward establishing a standardized magnetic resonance imaging scoring system for temporomandibular joints in juvenile idiopathic arthritis. *Arthritis Care Res*. 2018;70(5):758-767.
27. Kellenberger CJ, Junhasavasdikul T, Tolend M, Doria AS. Temporomandibular joint atlas for detection and grading of juvenile idiopathic arthritis involvement by magnetic resonance imaging. *Pediatr Radiol*. 2018;48(3):411-426.
28. Tamai M, Kawakami A, Uetani M, et al. Bone edema determined by magnetic resonance imaging reflects severe disease status in patients with early-stage rheumatoid arthritis. *J Rheumatol*. 2007;34(11):2154-2157.
29. Alstergren P, Benavente C, Kopp S. Interleukin-1beta, interleukin-1 receptor antagonist, and interleukin-1 soluble receptor II in temporomandibular joint synovial fluid from patients with chronic polyarthritides. *J Oral Maxillofac Surg*. 2003;61(10):1171-1178.
30. Ahmed N, Petersson A, Catrina AI, Mustafa H, Alstergren P. Tumor necrosis factor mediates temporomandibular joint bone tissue resorption in rheumatoid arthritis. *Acta Odontol Scand*. 2015;73(3):232-240.
31. Silvestre-Rangil J, Bagan L, Silvestre FJ, Martinez-Herrera M, Bagan J. Periodontal, salivary and IL-6 status in rheumatoid arthritis patients. A cross-sectional study. *Med Oral Patol Oral Cir Bucal*. 2017;22(5):e595-e600.
32. Grisius MM, Bermudez DK, Fox PC. Salivary and serum interleukin 6 in primary Sjögren's syndrome. *J Rheumatol*. 1997;24(6):1089-1091.
33. Moen K, Bertelsen LT, Hellem S, Jonsson R, Brun JG. Salivary gland and temporomandibular joint involvement in rheumatoid arthritis: relation to disease activity. *Oral Dis*. 2005;11(1):27-34.
34. Helenius LM, Meurman JH, Helenius I, et al. Oral and salivary parameters in patients with rheumatic diseases. *Acta Odontol Scand*. 2005;63(5):284-293.
35. Verstappen GM, Pringle S, Bootsma H, Kroese FGM. Epithelial-immune cell interplay in primary Sjögren syndrome salivary gland pathogenesis. *Nat Rev Rheumatol*. 2021;17(6):333-348.
36. Hammenfors DS, Valim V, Bica B, et al. Juvenile Sjögren's syndrome: clinical characteristics with focus on salivary gland ultrasonography. *Arthritis Care Res*. 2020;72(1):78-87.
37. Gomez Hernandez MP, Starman EE, Davis AB, et al. A distinguishing profile of chemokines, cytokines, and biomarkers in the saliva of children with Sjögren's syndrome. *Rheumatology*. 2021;60:4765-4777.
38. Wozniak KL, Arribas A, Leigh JE, Fidel PL Jr. Inhibitory effects of whole and parotid saliva on immunomodulators. *Oral Microbiol Immunol*. 2002;17(2):100-107.
39. Williamson S, Munro C, Pickler R, Grap MJ, Elswick RK Jr. Comparison of biomarkers in blood and saliva in healthy adults. *Nurs Res Pract*. 2012;2012:246178.
40. Byrne ML, O'Brien-Simpson NM, Reynolds EC, et al. Acute phase protein and cytokine levels in serum and saliva: a comparison of detectable levels and correlations in a depressed and healthy adolescent sample. *Brain Behav Immun*. 2013;34:164-175.
41. Nam Y, Kim YY, Chang JY, Kho HS. Salivary biomarkers of inflammation and oxidative stress in healthy adults. *Arch Oral Biol*. 2019;97:215-222.
42. Riis JL, Out D, Dorn LD, et al. Salivary cytokines in healthy adolescent girls: Intercorrelations, stability, and associations with serum cytokines, age, and pubertal stage. *Dev Psychobiol*. 2014;56(4):797-811.
43. Walters HM, Pan N, Lehman TJ, et al. The impact of disease activity and tumour necrosis factor- α inhibitor therapy on cytokine levels in juvenile idiopathic arthritis. *Clin Exp Immunol*. 2016;184(3):308-317.
44. Koelman L, Pivovarova-Ramich O, Pfeiffer AFH, Grune T, Aleksandrova K. Cytokines for evaluation of chronic inflammatory

- status in ageing research: reliability and phenotypic characterisation. *Immun Ageing*. 2019;16:11.
45. Weiss PF, Arabshahi B, Johnson A, et al. High prevalence of temporomandibular joint arthritis at disease onset in children with juvenile idiopathic arthritis, as detected by magnetic resonance imaging but not by ultrasound. *Arthritis Rheum*. 2008;58(4):1189-1196.
 46. Kuseler A, Pedersen TK, Gelineck J, Herlin T. A 2 year followup study of enhanced magnetic resonance imaging and clinical examination of the temporomandibular joint in children with juvenile idiopathic arthritis. *J Rheumatol*. 2005;32(1):162-169.
 47. Zwir LM, Terreri MT, Sousa SA, Fernandes AR, Guimaraes AS, Hilario MO. Are temporomandibular joint signs and symptoms associated with magnetic resonance imaging findings in juvenile idiopathic arthritis patients? A Longitudinal Study. *Clin Rheumatol*. 2015;34(12):2057-2063.
 48. Chiba M, Kumagai M, Fukui N, Echigo S. The relationship of bone marrow edema pattern in the mandibular condyle with joint pain in patients with temporomandibular joint disorders: longitudinal study with MR imaging. *Int J Oral Maxillofac Surg*. 2006;35(1):55-59.
 49. Higuchi K, Chiba M, Kondo T, Echigo S. The relationship between bone marrow edema and bone changes in the mandibular condyle: a longitudinal study with MR imaging. *Oral Sci Int*. 2013;10(1):33-39.
 50. Nieuwenhuis WP, van Steenberg HW, Stomp W, et al. The course of bone marrow edema in early undifferentiated arthritis and rheumatoid arthritis: a longitudinal magnetic resonance imaging study at bone level. *Arthritis Rheumatol (Hoboken, NJ)*. 2016;68(5):1080-1088.

How to cite this article: Carlsson AD, Wahlund K, Ghafouri B, et al. Parotid saliva and blood biomarkers in juvenile idiopathic arthritis in relation to temporomandibular joint magnetic resonance imaging findings. *J Oral Rehabil*. 2024;51:2082-2092. doi:[10.1111/joor.13806](https://doi.org/10.1111/joor.13806)