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Osteoarthritis and Cartilage

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SUMMARY

Objective: To investigate host and gut-microbiota related Tryptophan metabolism in hand osteoarthritis (HOA).
Methods: The baseline serum concentration of 20 Tryptophan metabolites was measured in 416 HOA patients in a cross-sectional analysis of the DIGICOD cohort. Tryptophan metabolites levels, metabolite-ratios and metabolism pathway activation were compared between erosive ($N = 141$) and non-erosive HOA ($N = 275$) by multiple logistic regressions adjusted on age, BMI and sex. The association between Tryptophan metabolite levels and HOA symptoms was investigated by a Spearman's rank correlation analysis.
Results: Four serum Tryptophan metabolites, eight metabolite ratios and one metabolism pathway were associated with erosive HOA. Erosive HOA was negatively associated with Tryptophan (odds ratio (OR) = 0.41, 95% confidence interval [0.24–0.70]), indole-3-aldehyde (OR = 0.67 [0.51–0.90]) and 3-OH-anthranilic acid (OR = 1.32 [1.13–1.54]) and positively with 5-OH-Tryptophan levels (OR = 1.41 [1.13–1.77]). The pro-inflammatory kynurenine–indoleamine 2,3-dioxygenase pathway was upregulated in erosive HOA (OR = 1.60 [1.11–2.29]). Eleven metabolites were correlated with HOA symptoms and were mostly pain-related. Serotonin and N-acetyl serotonin levels were negatively correlated with number of tender joints. Indole-3-aldehyde level was negatively correlated and 3-OH-anthranilic acid, 3-OH-kynurenine and 5-OH-Tryptophan levels were positively correlated with number of patients-reported painful joints. Quinolinic acid and 3-OH-kynurenine levels correlated positively with AUSCAN pain.
Conclusions: Tryptophan metabolites disturbance is associated with erosive HOA and pain and emphasize the role of low-grade inflammation and gut dysbiosis in HOA.

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Introduction

Osteoarthritis (OA) is the most common form of arthritis and affects more than 528 million people worldwide[1]. OA is heterogeneous in terms of clinical presentation, risk factors and localization. It is no longer considered a "wear and tear" disorder but rather a complex whole-joint disease involving cartilage degradation and synovial and subchondral bone remodeling induced by local and systemic inflammation[2,3]. Low-grade inflammation in OA is driven in part by metabolic syndrome and obesity[4,5], and increasing evidence suggests a potential role of gut microbiota[6,7].

Recent data on the "gut–joint axis" link gut dysbiosis and OA structural alteration[8] but also OA pain (the hallmark clinical symptom)[9,10]. However, we do not know whether the gut microbiota and related metabolites could act directly through OA-specific gut dysbiosis and/or indirectly through gut dysbiosis induced by obesity and metabolic syndrome[11]. The role of gut microbiome

		Total ($N = 416$)	Non Erosive HOA ($N = 275$)	Erosive HOA ($N = 141$)	*P*	Missing (%)
Demographics						
Sex (%)	Female	**348 (83.70)**	234 (85.10)	114 (80.90)	Ns	0
	Male	**68 (16.30)**	41 (14.90)	27 (19.10)		
Age (years), mean (SD)		**66.70 (7.21)**	66.01 (7.42)	68.05 (6.60)	0.006	0
Osteoarthritis duration (years), mean (SD)		12.95 (9.60)	11.40 (9.46)	15.96 (9.30)	<0.001	1
Metabolism						
BMI (kg/m^2), mean (SD)		**25.10 (4.30)**	25.11 (4.32)	25.12 (4.36)	Ns	1.70
Metabolic syndrome ATP III (%)		**148 (36.50)**	90 (33.50)	58 (42.60)	Ns	2.60 0
High-sensitivity CRP (mg/L), mean (SD)		**2.37 (4.60)**	2.55 (4.61)	2.01 (4.60)	Ns	
Patients-reported outcomes						
AUSCAN pain (0–100), mean (SD)		**25.83 (21.36)**	24.49 (20.98)	28.40 (21.92)	Ns	7.50
AUSCAN stiffness (0–100), mean (SD)		**32.68 (28.05)**	28.92 (26.95)	39.88 (28.80)	<0.001	6
AUSCAN function (0–100), mean (SD)		**36.58 (24.88)**	34.31 (24.53)	40.83 (25.08)	0.013	5.80
HADS score (0–21), mean (SD)		**12.56 (6.02)**	12.46 (5.94)	12.76 (6.19)	Ns	7.50
AIMS2-SF symptoms (0–100), mean (SD)		**33.17 (20.67)**	32.20 (19.53)	35.05 (22.69)	Ns	6.70
AIMS2-SF affect (0–100), mean (SD)		**28.67 (17.47)**	27.92 (17.06)	30.16 (18.21)	Ns	7
AIMS2-SF social (0–100), mean (SD)		**42.94 (16.55)**	43.89 (15.93)	41.08 (17.63)	Ns	
Clinical examination						
Number of tender joints (0–48), mean (SD)		**4.67 (4.67)**	4.18 (4.56)	5.61 (4.76)	0.003	0.20
Number of patient-reported painful joints (0–48), mean (SD)		**1.69 (3.28)**	1.44 (2.99)	2.18 (3.75)	0.029	0.20
Radiographic severity						
Number of joints with KL grade ≥2 (0–30), mean (SD)		**15.14 (6.29)**	113.48 (6.23)	18.61 (4.84)	<0.001	3.80
KL sum score (0–120), mean (SD)		**46.80 (18.02)**	40.72 (16.66)	59.57 (13.56)	<0.001	3.80
Verbruggen–Veys score (0–218), mean (SD)		**28.82 (21.28)**	17.95 (11.05)	51.47 (19.59)	<0.001	2.20
Treatments						
Acetaminophen (%)		**132 (32.50)**	85 (31.20)	47 (33.08)	Ns	1.20
NSAIDs (%)		**71 (17.30)**	45 (16.50)	26 (18.70)	Ns	1.20
Weak Opioids (%)		**34 (8.30)**	26 (9.60)	8 (5.80)	Ns	1.20
SYSADOAS (%)		**133 (32.40)**	80 (29.40)	53 (38.10)	Ns	1.20

AIMS2-SF: Arthritis Impact Measurement Scales 2 Short Format; BMI: body mass index; CRP: C-reactive protein; HADS: Hospital Anxiety Depression Scale; AUSCAN: Australian Canadian osteoarthritis hand index; KL: Kellgren–Lawrence; NSAIDs: non-steroidal anti-inflammatory drugs; ns: non-significant; SD: standard-deviation; SYSADOAS: symptomatic slow-acting drugs for osteoarthritis.

Table I

Osteoarthritis and Cartilage

DIGICOD cohort patients characteristics according to the presence of erosive HOA

in OA is complex since it cannot be easily dissociated of the influence of overweight and obesity on weight-bearing joint, especially in knee OA. Hand osteoarthritis (HOA) could be therefore a more appropriate OA localization to investigate the potential role of gut dysbiosis[12,13]. HOA, and especially its most severe form erosive HOA has been associated with low-grade systemic inflammation. As compared with non-erosive HOA, patients with erosive HOA present more severe clinical onset, more pain, accelerated disease progression, joint destruction and reduced quality of life[14,15]. We could improve our understanding of disease mechanisms and management by studying this specific HOA phenotype.

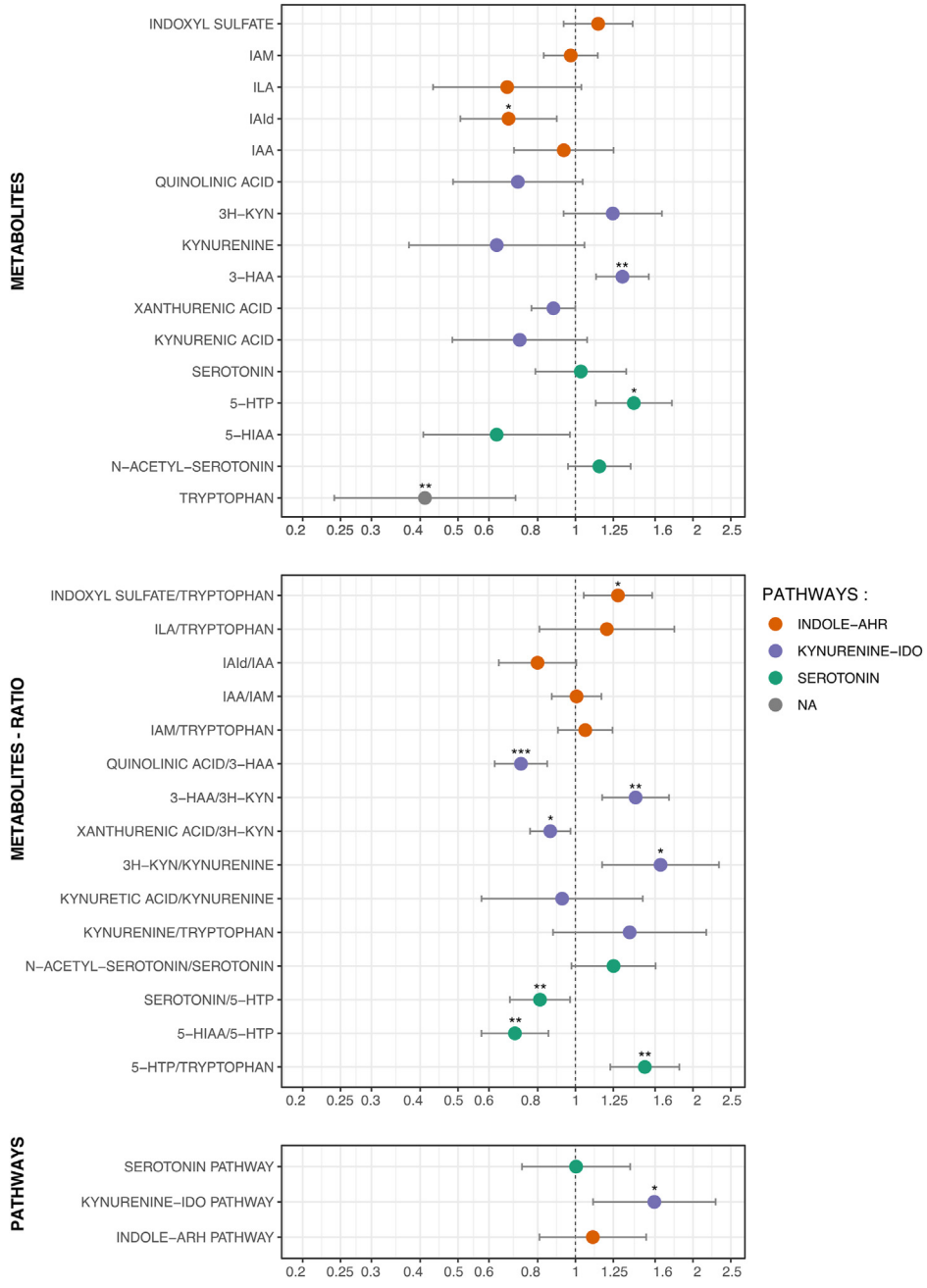

Fig. 1

Osteoarthritis and Cartilage

Multiple regression analysis of the association of Trp metabolites and metabolite ratios and pathways scores with erosive hand osteoarthritis (HOA). Odds ratios were adjusted on BMI, age, and sex. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, 3-HAA: 3-hydroxyanthranilic acid; 3H-KYN: 3-hydroxykynurenine; 5-HTP: 5-hydroxytryptophan; IIAA: indole acetic acid; IAld: indole-3-aldehyde; IAM: indole-3-acetamide; IDO: indoleamine 2,3-dioxygenase; ILA: indole-3-lactic acid; 5-HIAA: 5-hydroxyindoleacetic acid.

The effect of the gut microbiota on host physiology is notably mediated by metabolites produced by gut microorganisms or by host cells under the influence of gut microorganisms. Among the broad array of microbiota-dependent metabolites, those derived from tryptophan (Trp) have emerged as crucial actors in host–microbiota interactions. Trp is an essential amino acid that is a precursor for a large family of metabolites in the indole, kynurenine, and serotonin pathways[16]. Indole pathway metabolites are derived from the direct transformation of Trp by gut microbial species and therefore their levels are directly altered in gut dysbiosis. Indole pathway metabolites include ligands of the aryl-hydrocarbon receptor (AhR) and are involved in mucosal immunity and intestinal permeability[17]. Indole pathway metabolites are derived from the direct transformation of Trp by gut microbial species and therefore their levels are directly altered in gut dysbiosis. Trp metabolism through kynurenine pathway (KP) is mediated by intestinal immune and epithelial cells via indoleamine 2,3-dioxygenase (IDO) 1 enzyme and is associated with inflammation and neurotransmission[18,19]. Finally, serotonin pathway metabolites derived from Trp transformation by enterochromaffin cells are important precursors to neurotransmitters such as 5-hydroxytryptamine (5-HT) playing a key role in the gut–brain signaling axis[20]. Both indole-AhR, kynurenine-IDO and serotonin pathways are under the direct and indirect influence of the gut microbiome[16]. Altered Trp metabolism has been associated with several disorders such as inflammatory bowel disease (IBD), colorectal cancer, obesity, depression, and rheumatoid arthritis[21–25]. Trp and its metabolites are promising therapeutic targets and have received growing attention in drug discovery development[26].

The main objective of our study was to assess Trp metabolism alterations in patients with erosive HOA compared to non-erosive counterparts. Our secondary aim was to determine whether HOA symptoms were correlated with Trp serum metabolites.

Patients and methods

Study design and patients

The DIGItal COhort Design (DIGICOD) is a monocentric French university hospital-based prospective cohort of patients with symptomatic HOA (ClinicalTrials.gov: NCT01831570)[27]. The DIGICOD study included patients aged ≥35 years old with a diagnosis of symptomatic and radiographic HOA according to the American College of Rheumatology criteria[28]. Patients were included between April 2013 and June 2017 and underwent clinical assessment of the hand, general examination, fasting blood sampling and hands radiography scored by Kellgren–Lawrence (KL) grade and Verbruggen–Veys score[29,30] at the baseline visit. There was no pain threshold for the inclusion. Patients with co-existing inflammatory, crystal induced arthropathies or secondary OA related to traumatism or rare genetic disorders were excluded from the study. Characteristics of the cohort were previously described[27]. The present study is a cross-sectional analysis at the time of inclusion. For the purpose of this work, we also excluded patients with co-existing IBD. All participants provided their written informed consent before enrollment. The study obtained regulatory and ethics validation from the institutional review board and ethics committee and was reported according to the STROBE checklist for observational cohorts (https://www.strobe-statement.org/). Patients and the public were involved by communications through patient's associations and dedicated general articles to the public.

Clinical and radiological assessments

Erosive HOA was defined as the assessment of "E" (erosion) or "R" (remodeling) phases for the Verbruggen–Veys score in ≥2 joints in anteroposterior radiographs[30]. Demographics and comorbidity data were collected: age, sex, body mass index (BMI),

	OR	95 % CI	*P*-value	
Metabolites				
Indole/AhR pathway				
Indoxyl sulfate	1.14	0.93–1.40	Ns	
IAM	0.97	0.83–1.14	Ns	
ILA	0.67	0.43–1.04	Ns	
IAld	0.67	0.51–0.90	0.026	*
IAA	0.93	0.70–1.25	Ns	
Kynurenine/IDO pathway				
Quinolinic acid	0.71	0.49–1.04	Ns	
3H-KYN	1.25	0.93–1.67	Ns	
Kynurenine	0.63	0.37–1.05	Ns	
3-HAA	1.32	1.13–1.54	0.004	**
Xanthurenic acid	0.88	0.77–1.00	Ns	
Kynurenic acid	0.72	0.48–1.07	Ns	
Serotonin pathway				
Serotonin	1.03	0.79–1.35	Ns	
5-HTP	1.41	1.13–1.77	0.014	*
5-HIAA	0.63	0.41–0.97	Ns	
N-Acetyl-serotonin	1.15	0.96–1.38	Ns	
Tryptophan	0.41	0.24–0.70	0.007	**
Metabolites-ratio				
Indole/AhR pathway				
Indoxyl sulfate/tryptophan	1.28	1.05–1.57	0.043	*
ILA/tryptophan	1.20	0.81–1.79	Ns	
IAld/IAA	0.80	0.64–1.00	Ns	
IAA/IAM	1.01	0.87–1.17	Ns	
IAM/tryptophan	1.06	0.9–1.24	Ns	
Kynurenine/IDO pathway				
Quinolinic acid/3-HAA	0.72	0.62–0.85	<0.001	***
Xanthurenic acid/3H-KYN	0.86	0.76–0.97	0.043	*
3-HAA/3H-KYN	1.43	1.17–1.74	0.007	**
Kynurenic acid/kynurenine	0.92	0.57–1.49	Ns	
3H-KYN/kynurenine	1.65	1.17–2.33	0.02	*
Kynurenine/tryptophan	1.38	0.88–2.16	Ns	
Serotonin pathway				
N-Acetyl-serotonin/serotonin	1.25	0.98–1.60	Ns	
Serotonin/5-HTP	0.81	0.68–0.97	Ns	.
5-HIAA/5-HTP	0.70	0.57–0.85	0.004	**
5-HTP/tryptophan	1.51	1.23–1.84	0.002	**
Metabolites pathway				
Indole-AhR pathway	1.11	0.81–1.52	Ns	
Kynurenine-IDO pathway	1.60	1.11–2.29	0.04	*
Serotonin pathway	1.00	0.73–1.38	Ns	

Trp metabolite levels and metabolite ratios and pathways scores were log2-transformed, multiple regressions and odds ratio were adjusted on age, sex and BMI, *P*-values are adjusted with a Benjamini–Hochberg correction. Metabolite ratios represented the ratio of downstream metabolites to upstream metabolites * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. OR: odds ratio; 95% CI: 95% confidence interval; 3-HAA: 3-hydroxyanthranilic acid; 3H-KYN: 3-hydroxykynurenine; 5-HTTP: 5-hydroxytryptophan; AHR: Aryl Hydrocarbon Receptor, IIAA: indole acetic acid; Iald: indole-3-aldehyde; IAM: indole-3-acetamide; IDO: indoleamine 2,3-dioxygenase; ILA: indole-3-lactic acid; 5-HIAA: 5-hydroxyindoleacetic acid; ns: non-significant.

Table II Osteoarthritis and Cartilage

Multiple regression analysis of the association of Trp metabolites and metabolite ratios and pathways with erosive HOA adjusted on age, sex and BMI

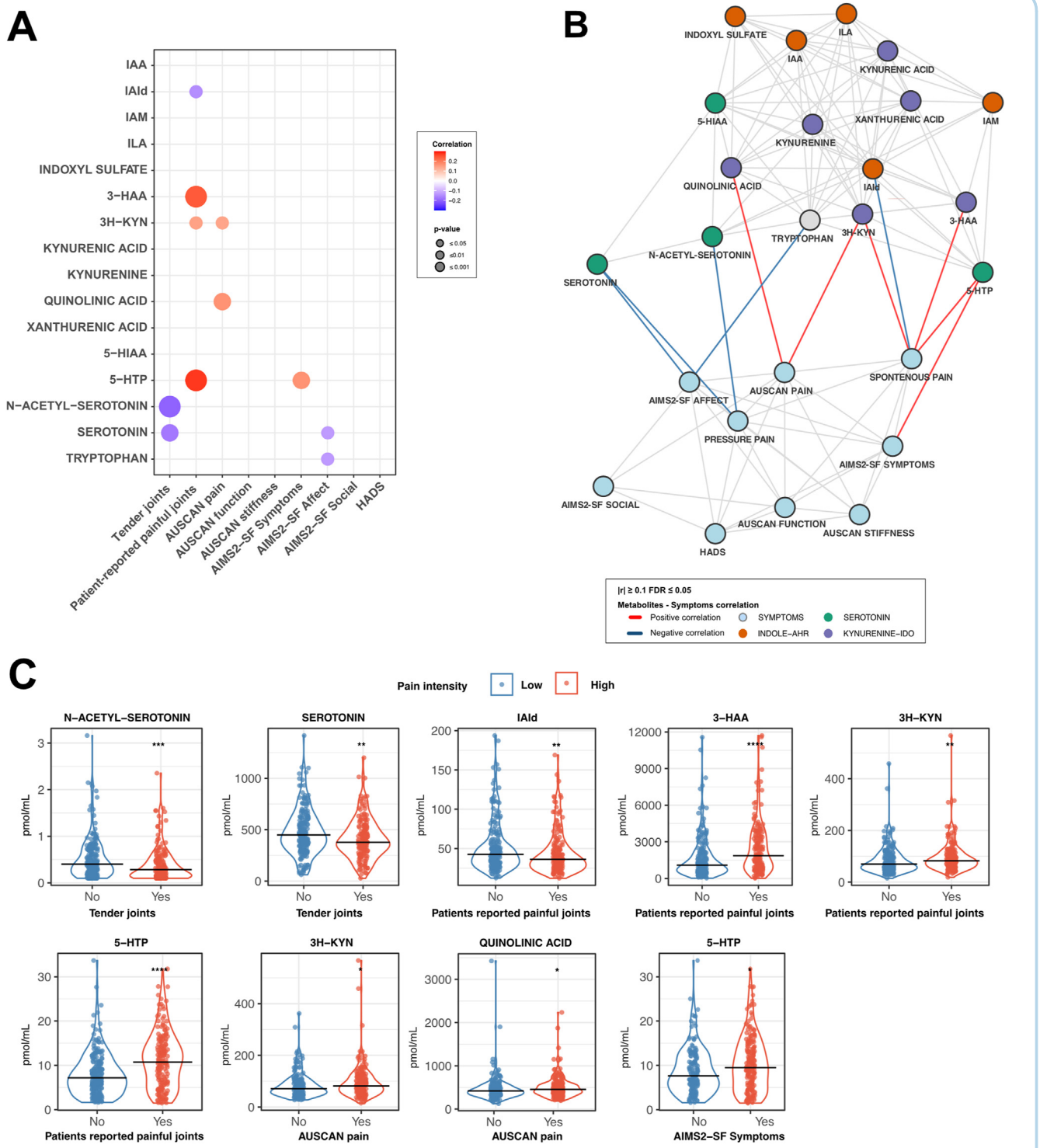
A
IAA
IAId
IAM
ILA
INDOXYL SULFATE
3−HAA
3H−KYN
KYNURENIC ACID
KYNURENINE
QUINOLINIC ACID
XANTHURENIC ACID
5−HIAA
5−HTP
N−ACETYL−SEROTONIN
SEROTONIN
TRYPTOPHAN
Tender joints
Patient−reported painful joints
AUSCAN pain
AUSCAN function
AUSCAN stiffness
AIMS2−SF Symptoms
AIMS2−SF Affect
AIMS2−SF Social
HADS
Correlation
p-value
B
INDOXYL SULFATE
ILA
IAA
KYNURENIC ACID
5-HIAA
XANTHURENIC ACID
IAM
KYNURENINE
QUINOLINIC ACID
IAId
3-HAA
TRYPTOPHAN
3H-KYN
N-ACETYL-SEROTONIN
SEROTONIN
5-HTP
AIMS2-SF AFFECT
AUSCAN PAIN
SPONTENOUS PAIN
PRESSURE PAIN
AIMS2-SF SYMPTOMS
AIMS2-SF SOCIAL
AUSCAN FUNCTION
AUSCAN STIFFNESS
HADS
|r| ≥ 0.1 FDR ≤ 0.05
Metabolites - Symptoms correlation
Positive correlation
Negative correlation
SYMPTOMS
INDOLE−AHR
SEROTONIN
KYNURENINE−IDO
C
Pain intensity
Low
High
N−ACETYL−SEROTONIN
SEROTONIN
IAId
3−HAA
3H−KYN
pmol/mL
No
Yes
Tender joints
Patients reported painful joints
5−HTP
QUINOLINIC ACID
AUSCAN pain
AIMS2−SF Symptoms

Fig. 2

Symptoms	Metabolites	Correlation coefficient	*P*-value	
Number of tender joints	N-Acetyl-serotonin	−0.20	<0.001	***
Number of tender joints	Serotonin	−0.17	0.002	**
Number of patient-reported painful joints	3-HAA	0.24	<0.001	***
Number of patient-reported painful joints	3H-KYN	0.13	0.03	*
Number of patient-reported painful joints	5-HTP	0.27	<0.001	***
Number of patient-reported painful joints	IAld	−0.15	0.01	*
AUSCAN pain	Quinolinic acid	0.16	0.006	**
AUSCAN pain	3H-KYN	0.14	0.02	*
AIMS2-SF symptoms	5-HTP	0.17	0.003	**
AIMS2-SF Affect	Serotonin	−0.13	0.032	*
AIMS2-SF Affect	Tryptophan	−0.13	0.028	*

Correlations were selected with an absolute coefficient value $|r| \geq 0.1$ and adjusted *P*-value ≤0.05 after Benjamini–Hochberg correction. 3-hydroxyanthranilic Acid; 3H-KYN: 3-hydroxykynurenine, 5-HTP: 5 hydroxytryptophan; AIMS2-SF: Arthritis Impact Measurement Scales 2 Short Format; AUSCAN: Australian Canadian osteoarthritis hand index; IAld: indole-3-Aldehyde. * $0.01 < P \leq 0.05$; ** $0.001 < P \leq 0.001$; *** $P < 0.001$.

Table III

Osteoarthritis and Cartilage

Spearman's correlation coefficient between Trp metabolites and HOA symptoms

disease duration, C-reactive protein (CRP) level, and metabolic syndrome as defined by National Cholesterol Education Program – Adult Treatment Panel III (NCEP ATP III) criteria[31]. Patient symptoms variables were recorded by clinical examination and patient-reported outcomes (PRO) and included number of patient-reported painful joints, number of tender joints at palpation based on the modified Doyle index (0–48)[32]; Australian Canadian osteoarthritis hand index (AUSCAN) subscores for pain, physical function and stiffness normalized from 0 to 100[33]; the Hospital Anxiety Depression Scale (HADS) (0–21)[34] and the symptoms, affective and social components of the Arthritis Impact Measurement Scales 2 short-form (AIMS2-SF)[35]. The scores utilized in our study have previously undergone validation in the French language[33,35,36]. Our study incorporated information related to oral treatments, specifically the use of acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs), weak opioids (defined as the intake of codeine, dihydrocodeine, or tramadol), and symptomatic slow-acting drugs for osteoarthritis (SYSADOAs), including chondroitin sulfate, glucosamine sulfate, diacerein, and avocado-soybean unsaponifiable. Radiographic severity was assessed with the number of joints with a KL score ≥2 (0–30), the sum score of KL in both hands (0–120)[36] and the Verbruggen–Veys score (0–218)[30].

Metabolites assessment

Serum concentrations of 20 Trp metabolites were measured with a targeted metabolomic approach from 500 μL serum samples collected while patients were fasting. Levels of Trp metabolites were measured by high-performance liquid chromatography (HPLC) (Waters® (Millford, MA, USA)). Calibration curves and standards were assessed by using 100 μL of serum samples and 100 μL of a solution of internal standards and 300 μL of methanol. After stirring and incubation for 30 min at −20°C, each sample was centrifuged (15,000 g for 15 min at 4°C) and the resulting supernatant (300 μL) was transferred to 96-well plates. After simultaneous evaporation, each well was resuspended in 100 μL of a methanol/water mixture (10/90). Finally, 5 μL was injected into the HPLC including a Kinetex C18 xb column (1.7 μm × 150 mm × 2.1 mm, temperature 55°C) associated with a gradient of two mobile phases (Phase A: Water + 0.5% formic acid; Phase B: MeOH + 0.5% formic acid) at a flow rate of 0.4 mL/min. Trp metabolites included tryptophan, metabolites from the kynurenine-IDO pathway (kynurenine, 3-OH kynurenine (3H-KYN), kynurenic acid, xanthurenic acid, 3-OH anthranilic acid (3-HAA), picolinic acid, quinolinic acid), serotonin pathway (5-hydroxytryptophan (5-HTP), serotonin, 5-hydroxyindole acetic acid, N-acetyl-serotonin, melatonin), indole-ARH

A. Dot heatmap of significant Spearman's correlation between Trp metabolite and HOA symptoms. The significant correlations between Trp metabolites and symptoms are visually represented using dots. Red dots indicate positive correlations, while blue dots indicate negative correlations. The size of each dot reflects the inverse of the corresponding ***P***-value. B. Spearman's Correlation network of Trp metabolites and HOA symptoms. Symptoms and metabolites are depicted as nodes and significant correlations are represented as edges. Only correlations with an absolute coefficient above 0.1 and an adjusted ***P***-value of 0.05 or less are displayed. Positive and negative correlations between symptoms and metabolites are denoted by red and blue, respectively. The size of the node corresponds to its significance. C. Violin plot analysis between Trp and pain related symptoms (low and high intensity). Differences were tested by a Mann–Whitney–Wilcoxon test. For each symptom, patients were classified as symptomatic based on the median value for the 376 patients. This median was 0 for patient-reported painful joints, three tender joints, 20 for AUSCAN pain, 33 for AIMS2-SF symptoms. *$\boldsymbol{P} \leq 0.05$, **$\boldsymbol{P} \leq 0.01$, ***$\boldsymbol{P} \leq 0.001$. 3-Hydroxyanthranilic acid; 3H-KYN: 3-hydroxykynurenine; 5-HTP: 5-hydroxytryptophan; AIMS2-SF: Arthritis Impact Measurement Scales 2 Short Format; AUSCAN: Australian Canadian osteoarthritis hand index; IAld: indole-3-aldehyde.

derivatives (indole-3-lactic acid, indole-3-acetamide, indole-3-acetaldehyde (Iald), indole-3-acetic acid, and indoxyl sulfate), tryptamine, and tryptophol (Table S1). Metabolite ratios were calculated as the ratio of downstream to upstream metabolites, with downstream metabolites being the end products or the results of metabolic reactions and upstream metabolites being their precursors (e.g., 5-HTP/tryptophan ratio). Metabolite pathway scores were defined by the sum of metabolites of each pathway. Tryptamine, tryptophol, picolinic acid and melatonin were not analyzed because their levels were below the detection threshold in large part of the cohort (416, 416, 390 and 387 patients, respectively).

Statistical analysis

Data were described with descriptive statistics (mean (SD)), and Student t test and chi-squared test were used to assess clinical differences between erosive and non-erosive HOA. To investigate the association between metabolites and the presence of erosive HOA, we applied a logistic regression for each metabolite, metabolite ratio and pathway score. Logistic regression models were adjusted on age, BMI and sex. Metabolite distributions were previously examined by a Shapiro–Wilk test and quantile–quantile scatter plot. Trp metabolites, metabolite ratios and pathway scores were log 2-transformed for the logistic regression analyses because the distribution of metabolites was skewed (Fig. S1). Odds ratios (OR) and 95% confidence intervals (CIs) were derived from the logistic regression coefficients. Logistic regression was performed on complete cases. Secondary analyses were performed to determine the potential association between Trp metabolite levels and HOA symptoms (number of tender joints, patients reported painful joints, AUSCAN subscore of function, pain and stiffness, AIMS2-SF symptoms, affect and social, HADS) with a Spearman's correlation matrix and a correlation network on complete cases. Symptom variables related to pain with statistically significant correlation with at least one metabolite were also selected. Wilcoxon Mann–Whitney tests were used to compare metabolite levels among patients with high and low pain-related symptom intensity, grouped using the median values of symptom scores as the threshold. Two-sided *P*-value <0.05 was considered statistically significant. In both primary and secondary analyses, all *P*-values were corrected for multiple testing with a Benjamini–Hochberg correction. Statistical analyses involved using R programming language (R 4.1.1 (2021-08-10))[37]. Figures were designed with R and Affinity Designer Software.

Results

Patients characteristics

Over the 426 patients included in the DIGICOD cohort, we excluded four patients with co-existing IBD and six patients with unavailable serum samples (Fig. S2). We analyzed data for 416 patients; 141 (33.80%) had erosive HOA. The final cohort consisted of 84% women, the mean age was 66.70 (7.20) years, mean BMI 25.10 (4.30) kg/m2, and mean AUSCAN pain score 25.80 (21.40) (Table I). Erosive and non-erosive HOA patients did not differ by sex or BMI, the mean age was 66 (7.40) and 68 (6.60) years in the non-erosive and erosive group ($P = 0.006$). Disease duration was

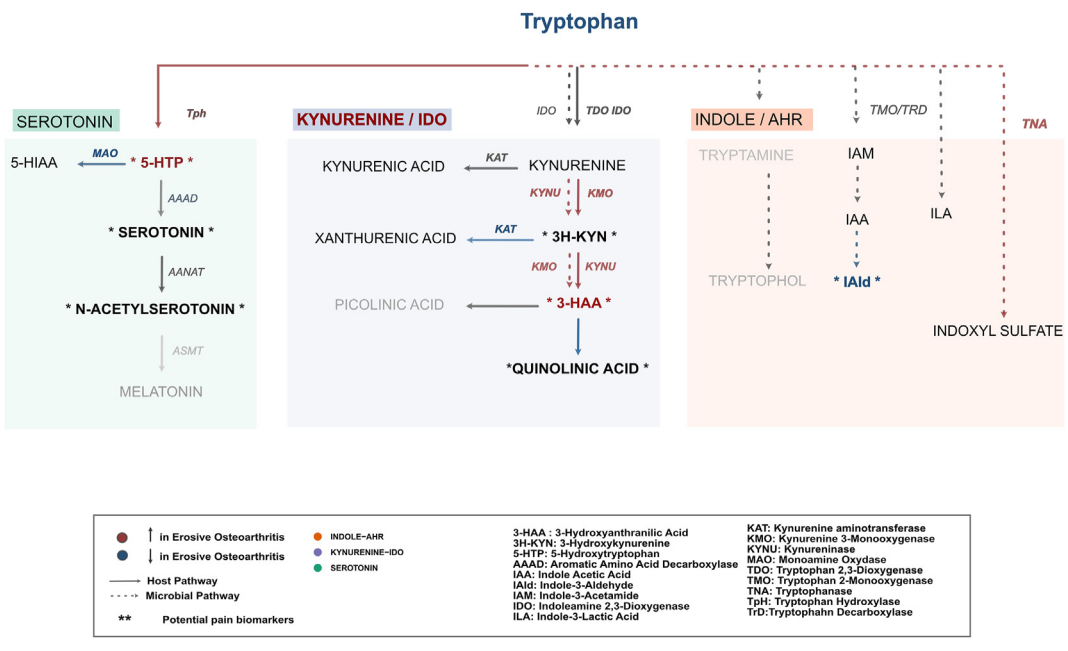

Fig. 3 Osteoarthritis and Cartilage

Overview of upregulated and downregulated Trp-metabolites in erosive versus HOA. Metabolites that were correlated with pain are labeled with an asterix. 3-HAA: 3-hydroxyanthranilic acid; 3H-KYN: 3-hydroxykynurenine; 5-HTP: 5-hydroxytryptophan; IIAA: indole acetic acid; IAld: indole-3-aldehyde; IAM: indole-3-acetamide; IDO: indoleamine 2,3-dioxygenase; ILA: indole-3-lactic acid; 5 HIAA: 5-hydroxyindoleacetic acid. Upregulated metabolites are in red, downregulated in blue, and metabolite ratio/enzymatic activity in arrows.

also higher in the erosive HOA group ($P < 0.001$) There was no significant difference regarding AUSCAN pain score between the groups and no difference for symptoms, affective and social subscore of AIMS2-SF. Patients with erosive HOA reported a higher number of painful joints and had a higher number of tender joints on palpation (2.18 (3.75) and 5.61 (4.76), respectively) compared to non-erosive HOA (1.44 (2.98) and 4.17 (4.55), respectively). Patients with erosive HOA presented significantly higher joint destruction with higher KL sum score and higher number of joints with KL score $\geq$2 and higher Verbruggen score ($P < 0.001$). There was no significant difference in treatment intakes between erosive and non-erosive HOA for acetaminophen, weak opioids, NSAIDs and SYSADOAs.

Tryptophan metabolites and pathways are modulated in erosive HOA

To compare the differences in tryptophan (Trp) metabolism variables between patients with and without erosive HOA, multiple logistic regression models were used. The models were adjusted for age, sex, and BMI, and were applied to a subset of 409 complete cases out of the 416 patients initially included in the study. We identified four Trp metabolites, eight metabolite ratios and one metabolite pathway score differentially associated with erosive HOA compared to non-erosive HOA (Fig. 1, Table II). Higher Trp levels were associated with decreased odds of having erosive HOA. For each twofold increase in concentration (pmol/l) we observed about 59 % decrease in the odds of having erosive HOA (OR 0.41, 95% CI [0.24–0.70], $P = 0.007$). Kynurenine-IDO pathway upregulation was significantly associated with erosive HOA (Fig. S3) (OR 1.60 [1.11–2.29], $P = 0.04$). Similarly, higher levels of 3-HAA were associated with increased odds of having erosive HOA (OR 1.32 [1.13–1.54] $P = 0.004$). Higher 3H-KYN/kynurenine and 3-HAA/3H-KYN ratios were positively associated with increased odds of having erosive HOA (OR 1.65 [1.17–2.33], $P = 0.02$, odds ratio (OR) 1.43 [1.17–1.74], $P = 0.007$), while inversely higher xanthurenic acid/3H-KYN, quinolinic acid/3-HAA acid ratios were negatively associated with odds of having erosive HOA (OR 0.86 [0.76–0.97], $P = 0.043$; OR 0.72 [0.62–0.85], $P < 0.001$). In the serotonin pathway, higher levels of 5-HTP were associated with increased odds of having erosive HOA and for each twofold increase in concentration (pmol/l) the odds by 41 % (OR 1.41 [1.13–1.77] $P = 0.014$). An increase of 5-HTP/tryptophan ratio was positively associated with odds of erosive HOA (OR 1.51 [1.23–1.84], $P = 0.002$) and an increase of 5-hydroxyindole acetic acid (5-HIAA)/5-HTP ratio was negatively associated with odds of having erosive HOA (OR 0.70 [0.57–0.85] $P = 0.004$). Finally, in the indole–AhR pathway, increased of IAId levels were associated with decreased odds of having erosive HOA, for each twofold increase in concentration (pmol/l) the odds decreased by 33 % (OR 0.67 [0.51–0.59] $P = 0.026$). Increased indoxyl sulfate/tryptophan ratio was positively associated with odds of having erosive HOA ((OR 1.28 [1.05–1.57] $P = 0.043$).

Tryptophan metabolites are associated with pain in HOA

We built a Spearman's correlation matrix for 371 patients with complete data for symptoms [Fig. 2(A) and (B); Table III] and identified 11 significant correlations between HOA symptoms and Trp metabolite levels. Number of tender joints was negatively correlated with N-acetyl-serotonin and serotonin levels ($r = -0.20$, $P < 0.001$, $r = -0.17$, $P = 0.002$). Number of patient-reported painful joints was positively correlated with levels of 3-HAA ($r = 0.24$, $P < 0.001$), 3H-KYN ($r = 0.13$, $P = 0.03$) and 5-HTP ($r = 0.26$, $P < 0.001$) and negatively with Iald level ($r = -0.15$, $P = 0.016$) AUSCAN pain score was positively correlated with quinolinic acid and 3H-KYN ($r = 0.16$, $P = 0.006$, $r = 0.14$ $P = 0.02$). AIMS2-SF symptoms score was positively correlated with 5-HTP ($r = 0.17$ $P = 0.003$). AIMS2-SF affect was negatively correlated with serotonin and tryptophan levels ($r = -0.13$ $P = 0.032$, $r = -0.13$ $P = 0.028$). Among symptoms significantly correlated with metabolite levels the majority were related to pain. Trp metabolite levels were not significantly correlated with AUSCAN subscore of function and stiffness, AIMS2-SF social score and HADS score.

To further support our results, we performed a complementary analysis of significantly correlated pain-related symptoms by using an additional Mann–Whitney Wilcoxon test [Fig. 2(C), Table S3]. Patients were divided into low and high pain intensity groups based on the following median values: 3 for number of tender joints, 0 for number of patient-reported painful joints, 20 for AUSCAN pain score, 33 for AIMS2-SF symptoms. Patients with more than 3 tender joints had lower N-acetyl serotonin and serotonin levels ($P < 0.001$ and 0.005). Patients with at least one reported painful joint had higher levels of 3-HAA, 3H-KYN, and 5-HTP ($P < 0.001$, $P = 0.005$, $P < 0.001$) and lower Iald levels ($P = 0.006$). Patients with AUSCAN pain score >20 had higher quinolinic acid and 3H-KYN levels ($P = 0.037$, $P = 0.02$) [Fig. 2(C)] and patient with AIMS2-SF symptoms score >33 had a higher 5-HTP levels ($P = 0.020$).

Discussion

In this study, we investigated the potential role of tryptophan (Trp) metabolism in osteoarthritis (OA) by analyzing serum Trp metabolites in 416 patients from the DIGICOD HOA cohort. Our finding revealed significant alterations in Trp metabolism in erosive HOA, as well as a potential association with pain (Fig. 3). These results are consistent with a previous study by Rushing *et al.*, who used an unsupervised fecal metabolomics analysis to identify Trp metabolism as the second most significantly altered metabolic pathway in OA compared to control[38].

We observed a significant decrease in serum tryptophan levels among patients with erosive HOA compared to those with non-erosive HOA. Decreased serum Trp level has been previously described in patients with rheumatoid arthritis and was correlated with radiographic destruction and joint space narrowing reflecting cartilage loss[39]. We also found an overactivation of the kynurenine-IDO pathway in erosive HOA, along with significant alterations in kynurenine-IDO related metabolites in both pain and erosive HOA. Kynurenine pathway is highly involved in inflammatory response, and in neurotransmission[18,19]. More specifically we observed that 3-HAA levels were increased in erosive HOA additionally, we also found a positive correlation between 3-HAA, 3H-KYN, quinolinic acid and pain. These findings are of significant interest as oxidative kynurenine metabolites such (3-HKYN, 3-HAA, and quinolinic acid) have been associated with neurotoxicity and alteration of nerves ending of afferent sensory neurons[40]. Similarly we found an increase of 3H-KYN/kynurenine, 3-HAA/3H-KYN ratios suggesting an overactivation of kynurenine 3-monooxygenase (KMO) and kynureninase, inhibiting KMO has shown therapeutic potential in preclinical models of neuropathic pain[41]. Several studies highlighted the role of kynureninase activation in inflammatory and cardiovascular diseases[42]. In contrast, we found negative associations between the ratios of quinolinic acid/3-HAA and xanthurenic acid/3H-KYN with erosive HOA. These findings are noteworthy as they suggest a potential decrease in kynurenine aminotransferase or aminoadipate aminotransferase (AADAT) activity as observed in IBDs in which AADAT low levels were associated with disease severity and flares[43]. Altogether, these results reinforce the potential role of kynurenine metabolites in HOA inflammation and pain.

Our study reveals significant alterations in metabolites of the indole-AhR pathway, which are directly produced by the gut microbiome, indicating a potential involvement of gut dysbiosis and intestinal permeability in HOA pathogenesis. Specifically, we found a significant decrease in Iald levels in both pain and erosive HOA. Previous research linked decreases of serum Iald levels to gut dysbiosis and impaired intestinal barrier function, and animal models have provided evidence that Iald supplementation can restore intestinal barrier integrity[44,45]. Several studies have implicated gut dysbiosis and intestinal permeability in OA-related pain. For instance, serum CD14 levels have been positively associated with self-reported knee pain, while independent cohorts have linked an abundance of Streptococcus species to increased knee pain[9,46]. Furthermore, additional research has suggested a potential connection between intestinal permeability and osteoarthritis, with Loeser *et al.* reporting an association between serum lipopolysaccharide and obesity-related osteoarthritis[11]. These findings support the notion that the gut microbiome and intestinal barrier function may play a crucial role in the development and progression of osteoarthritis.Finally, in the serotonin pathway we observed that 5-HTP levels were increased in both pain and erosive OA patients. Additionally, we observed an increase in the 5-HTP/Tryptophan ratio specifically in erosive OA patients, which suggests an overactivation of tryptophan hydroxylase (Tph). Tph is mostly produced by mast cells and has been implicated in immune tolerance regulation and inflammation[47]. These findings highlight the potential role of Tph in the pathogenesis of erosive OA and pain. N-acetyl serotonin and serotonin levels were also lower in patients with pain. Serotonin plays a major role in pain perception modulation and low levels of serotonin has been associated with chronic pain[48]. The particular association of reported pain and decreased serotonin level suggests a specific role in OA pain beyond inflammation and nociception.

Our study has several limitations. We performed a descriptive and cross-sectional study without a non-HOA group; therefore, we can only describe an association between Trp metabolism and erosive HOA or OA symptoms, and we cannot infer any causality. We also used an indirect measurement of gut dysbiosis because we did not have stool samples. We did not include complementary measurements of intestinal permeability biomarkers, such as LPS or LPS-binding protein. Further investigations are warranted to explore these biomarkers and their potential implications in our findings. It is also important to acknowledge potential confounding factors, such as SYSADOAs intake, have been previously associated with gut microbiome alterations[49]. However, our analysis did not reveal any significant difference in treatment intake between patients with erosive and non-erosive HOA. It is also to note that our study did not include measurements for different types of pain. Finally, we did not perform an external validation because of no other existing data on Trp metabolites in HOA. Nevertheless, our study is the first to investigate Trp metabolites in HOA, a non-weight bearing joint, which thus limits the main confounding factors of overload and obesity in OA. We performed multiple adjustments and sensitivity analyses to limit potential biases of interpretation and to ensure the validity of our results. The DIGICOD study is a large cohort of accurately phenotyped HOA patients and a prospective study with a 6-year follow up. It will allow us to investigate patients' radiographic and clinical progression according to their Trp metabolite profile. Finally, altered Trp metabolite levels could be an interesting therapeutic target and a promising treatment for OA. Trp metabolites modulation could have potential therapeutic properties in OA[50]. Further studies are needed to investigate the pathophysiological role of Trp metabolites in OA and OA-related pain.

Altogether those results highlight the role of Trp metabolites in erosive HOA and HOA-related pain and thus provide new hypotheses for the HOA pathophysiology and potential new biomarkers. Our study reinforces the potential role of systemic inflammation and gut dysbiosis in OA and encourages the pursuit of explorations regarding gut dysbiosis and its related metabolites in OA and OA-related pain.

Contributions

All authors included met the authorship criteria according to the ICJME recommendations (https://www.icmje.org). JS, HS, FB conceived and designed the study. PE AC and DC, contributed to the acquisition of the data. MB, FM, BM, EMF and JS analyzed the data. MB, FM, AC, MK, PR, EMF, FB, HS and JS contributed to the interpretation of the data. MB, EMF and JS drafted the article, PE, BM, FM, AC, EM, DC, MK, PR AB, EMF, FB, HS and JS revised it critically for important intellectual content. JS obtained the financial support of the study. All the authors approved the final version of the manuscript. MB, and JS (marie.binvignat@sorbonne-universite.fr, jeremie.sellam@aphp.fr) take responsibility for the integrity of the work as a whole, from inception to finished article.

Competing interest statement

AC received fees from Novartis, Pfizer and BMS. PR reports fees from Pfzier and Pierre Fabre. AB is a co-founder and consultant to Personalis and NuMedii; consultant to Samsung, Mango Tree Corporation, and in the recent past, 10x Genomics, Helix, Pathway Genomics, and Verinata (Illumina); has served on paid advisory panels or boards for Geisinger Health, Regenstrief Institute, Gerson Lehman Group, AlphaSights, Covance, Novartis, Genentech, and Merck, and Roche; is a shareholder in Personalis and NuMedii; is a minor shareholder in Apple, Facebook, Alphabet (Google), Microsoft, Amazon, Snap, 10x Genomics, Illumina, CVS, Nuna Health, Assay Depot, Vet24seven, Regeneron, Sanofi, Royalty Pharma, AstraZeneca, Moderna, Biogen, Paraxel, and Sutro, and several other non-health related companies and mutual funds; and has received honoraria and travel reimbursement for invited talks from Johnson and Johnson, Roche, Genentech, Pfizer, Merck, Lilly, Takeda, Varian, Mars, Siemens, Optum, Abbott, Celgene, AstraZeneca, AbbVie, Westat, and many academic institutions, medical or disease specific foundations and associations, and health systems. AJB receives royalty payments through Stanford University, for several patents and other disclosures licensed to NuMedii and Personalis. AB's research has been funded by NIH, Northrup Grumman (as the prime on an NIH contract), Genentech, Johnson and Johnson, FDA, Robert Wood Johnson Foundation, Leon Lowenstein Foundation, Intervalien Foundation, Priscilla Chan and Mark Zuckerberg, the Barbara and Gerson Bakar Foundation, and in the recent past, the March of Dimes, Juvenile Diabetes Research Foundation, California Governor's Office of Planning and Research, California Institute for Regenerative Medicine, L'Oreal, and Progenity. EM reports personal fees for lecture, board membership, clinical investigation or invitation to congresses from Celgene, Expanscience, Fidia, Labrha, Meda-Mylan, Pierre Fabre, Sublimed, TRB Chemedica. FB received an institutional grant from TRB Chemedica and Pfizer and consulting fees from AstraZeneca, Boehringer Ingelheim, Bone Therapeutics, Cellprothera, Galapagos, Gilead, Grunenthal, GSK, Lilly, Merck-Serono, MSD, Nordic Bioscience, Novartis, Pfizer, Roche, Sandoz, Sanofi, Servier, UCB, Peptinov, 4P Pharma, and 4Moving Biotech. HS report lecture fee, board membership, or consultancy fees from Carenity, AbbVie, Astellas, Danone, Ferring, Mayoly Spindler, MSD, Novartis, Roche, Tillots, Enterome, BiomX, Biose, Novartis, Takeda,

Biocodex and is co-founder of Exeliom Biosciences. JS reports personal fees from MSD, Pfizer, Abbvie, Fresenius Kabi, BMS, Roche, Chugai, Sandoz, Lilly, Novartis, Galapagos, AstraZeneca, UCB and Janssen and research grants from Pfizer, MSD, Schwa Medico, and BMS.

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Ethics
The DIGICOD study complies with the Declaration of Helsinki and obtained all the regulatory and ethics validation from the local regulatory committee (Comité de Protection des Personnes, Paris Île-de-France IV).

Data availability
Data sharing will be subject to the terms of DIGICOD cohort and the AP-HP data-sharing agreement to ensure all users of the data adhere to the legal requirements of using personal data.

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Supplementary data

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