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1 **Serum Tryptophan Metabolites are Associated with Erosive Hand Osteoarthritis**
2 **and Pain : Results from the DIGICOD Cohort**

3

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46

47

48 **Running Title: Tryptophan Metabolites in Hand Osteoarthritis**

49

50 **ABSTRACT**

51 **Objective:** To investigate host and gut-microbiota related Tryptophan metabolism in hand
52 osteoarthritis (HOA)

53 **Methods:** The baseline serum concentration of 20 Tryptophan metabolites was
54 measured in 416 HOA patients in a cross-sectional analysis of the DIGICOD cohort.
55 Tryptophan metabolites levels, metabolite-ratios and metabolism pathway activation were
56 compared between erosive (N=141) and non-erosive HOA (N=275) by multiple logistic
57 regressions adjusted on age, BMI and sex. The association between Tryptophan
58 metabolite levels and HOA symptoms was investigated by a Spearman's rank correlation
59 analysis.

60 **Results:** Four serum Tryptophan metabolites, eight metabolite ratios and one metabolism
61 pathway were associated with erosive HOA. Erosive HOA was negatively associated with
62 Tryptophan (odds ratio (OR)=0.41, 95% confidence interval [0.24-0.70]), indole-3-
63 aldehyde (OR=0.67 [0.51-0.90]) and 3-OH-anthranilic acid (OR=1.32 [1.13-1.54]) and
64 positively with 5-OH-Tryptophan levels (OR=1.41 [1.13-1.77]). The pro-inflammatory
65 kynurenine–indoleamine 2,3-dioxygenase pathway was upregulated in erosive HOA
66 (OR=1.60 [1.11-2.29]). Eleven metabolites were correlated with HOA symptoms and were
67 mostly pain-related. Serotonin and N-acetyl serotonin levels were negatively correlated
68 with number of tender joints. Indole-3-aldehyde level was negatively correlated and 3-
69 OH-anthranilic acid, 3-OH-kynurenine and 5-OH-Tryptophan levels were positively
70 correlated with number of patients-reported painful joints. Quinolinic acid and 3-OH-
71 kynurenine levels correlated positively with AUSCAN pain.

72 **Conclusions:** Tryptophan metabolites disturbance is associated with erosive HOA and
73 pain and emphasize the role of low-grade inflammation and gut dysbiosis in HOA.

74

75 **INTRODUCTION**

76 Osteoarthritis (OA) is the most common form of arthritis and affects more than 528 million
77 people worldwide¹. OA is heterogeneous in terms of clinical presentation, risk factors and
78 localization. It is no longer considered a “wear and tear” disorder but rather a complex
79 whole-joint disease involving cartilage degradation and synovial and subchondral bone
80 remodeling induced by local and systemic inflammation^{2,3}. Low-grade inflammation in OA
81 is driven in part by metabolic syndrome and obesity^{4,5}, and increasing evidence suggests
82 a potential role of gut microbiota^{6,7}.

83 Recent data on the “gut-joint axis” link gut dysbiosis and OA structural alteration⁸ but also
84 OA pain (the hallmark clinical symptom)^{9,10}. However, we do not know whether the gut
85 microbiota and related metabolites could act directly through OA-specific gut dysbiosis
86 and/or indirectly through gut dysbiosis induced by obesity and metabolic syndrome¹¹. The
87 role of gut microbiome in OA is complex since it cannot be easily dissociated of the
88 influence of overweight and obesity on weight-bearing joint, especially in knee OA. Hand
89 osteoarthritis (HOA) could be therefore a more appropriate OA localization to investigate
90 the potential role of gut dysbiosis^{12,13}. HOA, and especially its most severe form erosive
91 HOA has been associated with low-grade systemic inflammation. As compared with non-
92 erosive HOA, patients with erosive HOA present more severe clinical onset, more pain,
93 accelerated disease progression, joint destruction and reduced quality of life^{14,15}. We

94 could improve our understanding of disease mechanisms and management by studying
95 this specific HOA phenotype.

96 The effect of the gut microbiota on host physiology is notably mediated by metabolites
97 produced by gut microorganisms or by host cells under the influence of gut
98 microorganisms. Among the broad array of microbiota-dependent metabolites, those
99 derived from tryptophan (Trp) have emerged as crucial actors in host–microbiota
100 interactions. Trp is an essential amino acid that is a precursor for a large family of
101 metabolites in the indole, kynurenine, and serotonin pathways¹⁶. Indole pathway
102 metabolites are derived from the direct transformation of Trp by gut microbial species and
103 therefore their levels are directly altered in gut dysbiosis. Indole pathway metabolites
104 include ligands of the aryl-hydrocarbon receptor (AhR) and are involved in mucosal
105 immunity and intestinal permeability¹⁷. Indole pathway metabolites are derived from the
106 direct transformation of Trp by gut microbial species and therefore their levels are directly
107 altered in gut dysbiosis. Trp metabolism through kynurenine pathway (KP) is mediated by
108 intestinal immune and epithelial cells via indoleamine 2,3-dioxygenase (IDO) 1 enzyme
109 and is associated with inflammation and neurotransmission^{18,19}. Finally, serotonin
110 pathway metabolites derived from Trp transformation by enterochromaffin cells are
111 important precursors to neurotransmitters such as 5-hydroxytryptamine (5-HT) playing a
112 key role in the gut–brain signaling axis²⁰. Both indole-AhR, kynurenine-IDO and serotonin
113 pathways are under the direct and indirect influence of the gut microbiome¹⁶. Altered Trp
114 metabolism has been associated with several disorders such as inflammatory bowel
115 disease (IBD), colorectal cancer, obesity, depression, and rheumatoid arthritis^{21–25}. Trp

116 and its metabolites are promising therapeutic targets and have received growing attention
117 in drug discovery development²⁶.

118

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

122

123 **PATIENTS AND METHODS**

124 **Study design and patients**

125 The DIGItal COhort Design (DIGICOD) is a monocentric French university hospital-based
126 prospective cohort of patients with symptomatic HOA (ClinicalTrials.gov:
127 NCT01831570)²⁷. The DIGICOD study included patients aged ≥ 35 years old with a
128 diagnosis of symptomatic and radiographic HOA according to the American College of
129 Rheumatology criteria²⁸. Patients were included between April 2013 and June 2017 and
130 underwent clinical assessment of the hand, general examination, fasting blood sampling
131 and hands radiography scored by Kellgren-Lawrence (KL) grade and Verbruggen-Veys
132 score^{29,30} at the baseline visit. There was no pain threshold for the inclusion. Patients with
133 co-existing inflammatory, crystal induced arthropathies or secondary OA related to
134 traumatism or rare genetic disorders were excluded from the study. Characteristics of the
135 cohort were previously described²⁷. The present study is a cross-sectional analysis at the
136 time of inclusion. For the purpose of this work, we also excluded patients with co-existing
137 inflammatory bowel disease. All participants provided their written informed consent
138 before enrollment. The study obtained regulatory and ethics validation from the

139 institutional review board and ethics committee and was reported according to the
140 STROBE checklist for observational cohorts (<https://www.strobe-statement.org/>).
141 Patients and the public were involved by communications through patient's associations
142 and dedicated general articles to the public.

143

144 **Clinical and radiological assessments**

145 Erosive HOA was defined as the assessment of “E” (erosion) or “R” (remodeling) phases
146 for the Verbruggen-Veys score in ≥ 2 joints in anteroposterior radiographs³⁰.
147 Demographics and comorbidity data were collected: age, sex, body mass index (BMI),
148 disease duration, C-reactive protein (CRP) level, and metabolic syndrome as defined by
149 National Cholesterol Education Program - Adult Treatment Panel III (NCEP ATP III)
150 criteria³¹. Patient symptoms variables were recorded by clinical examination and patient-
151 reported outcomes (PRO) and included number of patient-reported painful joints, number
152 of tender joints at palpation based on the modified Doyle index (0-48)³²; Australian
153 Canadian osteoarthritis hand index (AUSCAN) subscores for pain, physical function and
154 stiffness normalized from 0 to 100³³; the Hospital Anxiety Depression Scale (HADS) (0-
155 21)³⁴ and the symptoms, affective and social components of the Arthritis Impact
156 Measurement Scales 2 short-form (AIMS2-SF)³⁵. The scores utilized in our study have
157 previously undergone validation in the French language^{33,35,36}. Our study incorporated
158 information related to oral treatments, specifically the use of acetaminophen, non-
159 steroidal anti-inflammatory drugs (NSAIDs), weak opioids (defined as the intake of
160 codeine, dihydrocodeine, or tramadol), and symptomatic slow-acting drugs for
161 osteoarthritis (SYSADOAs), including chondroitin sulfate, glucosamine sulfate, diacerein,

162 and avocado-soybean unsaponifiable. Radiographic severity was assessed with the
163 number of joints with a KL score ≥ 2 (0-30) , the sum score of KL in both hands (0-120)
164 ³⁶ and the Verbruggen-Veys score (0-218) ³⁰.

165

166 **Metabolites assessment**

167 Serum concentrations of 20 Trp metabolites were measured with a targeted metabolomic
168 approach from 500 μ L serum samples collected while patients were fasting. Levels of Trp
169 metabolites were measured by high-performance liquid chromatography (HPLC)
170 (Waters® (Millford, MA, USA)). Calibration curves and standards were assessed by using
171 100 μ L of serum samples and 100 μ L of a solution of internal standards and 300 μ L of
172 methanol. After stirring and incubation for 30 min at -20°C , each sample was centrifuged
173 (15,000 g for 15 min at 4°C) and the resulting supernatant (300 μ L) was transferred to 96-
174 well plates. After simultaneous evaporation, each well was resuspended in 100 μ L of a
175 methanol/water mixture (10/90). Finally, 5 μ L was injected into the HPLC including a
176 Kinetex C18 xb column (1.7 $\mu\text{m} \times 150 \text{ mm} \times 2.1 \text{ mm}$, temperature 55°C) associated with
177 a gradient of two mobile phases (Phase A: Water + 0.5% formic acid; Phase B: MeOH +
178 0.5% formic acid) at a flow rate of 0.4 mL/min. Trp metabolites included tryptophan,
179 metabolites from the kynurenine-IDO pathway (kynurenine, 3-OH kynurenine (3H-KYN),
180 kynurenic acid, xanthurenic acid, 3-OH anthranilic acid (3-HAA), picolinic acid, quinolinic
181 acid), serotonin pathway (5-hydroxytryptophan (5-HTP), serotonin, 5-hydroxyindole
182 acetic acid, N-acetyl-serotonin, melatonin), indole-ARH derivatives (indole-3-lactic acid,
183 indole-3-acetamide, indole-3-acetaldehyde (Iald), indole-3-acetic acid, and indoxyl
184 sulfate), tryptamine, and tryptophol (**Table S1**). . Metabolite ratios were calculated as the

185 ratio of downstream to upstream metabolites, with downstream metabolites being the end
186 products or the results of metabolic reactions and upstream metabolites being their
187 precursors (e.g. 5-HTP/tryptophan ratio). Metabolite pathway scores were defined by the
188 sum of metabolites of each pathway. Tryptamine, tryptophol, picolinic acid and melatonin
189 were not analyzed because their levels were below the detection threshold in large part
190 of the cohort (416, 416, 390 and 387 patients, respectively).

191

192 **Statistical analysis**

193 Data were described with descriptive statistics (mean (SD)), and Student *t* test and chi-
194 squared test were used to assess clinical differences between erosive and non-erosive
195 HOA. To investigate the association between metabolites and the presence of erosive
196 HOA, we applied a logistic regression for each metabolite, metabolite ratio and pathway
197 score. Logistic regression models were adjusted on age, BMI and sex. Metabolite
198 distributions were previously examined by a Shapiro-Wilk test and quantile-quantile
199 scatter plot. Trp metabolites, metabolite ratios and pathway scores were log₂-transformed
200 for the logistic regression analyses because the distribution of metabolites was skewed
201 (**Figure S1**). Odds ratios (OR) and 95% confidence intervals (CIs) were derived from the
202 logistic regression coefficients. Logistic regression was performed on complete cases.
203 Secondary analyses were performed to determine the potential association between Trp
204 metabolite levels and HOA symptoms (number of tender joints, patients reported painful
205 joints, AUSCAN subscore of function, pain and stiffness, AIMS2-SF symptoms, affect and
206 social, HADS) with a Spearman's correlation matrix and a correlation network on
207 complete cases. Symptom variables related to pain with statistically significant correlation

208 with at least one metabolite were also selected. Wilcoxon Mann-Whitney tests were used
209 to compare metabolite levels among patients with high and low pain-related symptom
210 intensity, grouped using the median values of symptom scores as the threshold. Two-
211 sided p-value <0.05 was considered statistically significant. In both primary and
212 secondary analyses, all p-values were corrected for multiple testing with a Benjamini-
213 Hochberg correction. Statistical analyses involved using R programming language (R
214 4.1.1 (2021-08-10)³⁷. Figures were designed with R and Affinity Designer Software.

215

216 **RESULTS**

217 **Patients characteristics**

218 Over the 426 patients included in the DIGICOD cohort, we excluded four patients with co-
219 existing inflammatory bowel disease and six patients with unavailable serum samples
220 **(Figure S2)**. We analyzed data for 416 patients; 141 (33.80%) had erosive HOA. The
221 final cohort consisted of 84% women, the mean age was 66.70 (7.20) years, mean BMI
222 25.10 (4.30) kg/m², and mean AUSCAN pain score 25.80 (21.40) **(Table 1)**. Erosive and
223 non-erosive HOA patients did not differ by sex or BMI, the mean age was 66 (7.40) and
224 68 (6.60) years in the non-erosive and erosive group (p = 0.006). Disease duration was
225 also higher in the erosive HOA group (p<0.001) There was no significant difference
226 regarding AUSCAN pain score between the groups and no difference for symptoms,
227 affective and social subscore of AIMS2-SF. Patients with erosive HOA reported a higher
228 number of painful joints and had a higher number of tender joints on palpation (2.18 (3.75)
229 and 5.61 (4.76), respectively) compared to non-erosive HOA (1.44 (2.98) and 4.17 (4.55),
230 respectively). Patients with erosive HOA presented significantly higher joint destruction

231 with higher KL sum score and higher number of joints with KL score ≥ 2 and higher
232 Verbruggen score ($p < 0.001$). There was no significant difference in treatment intakes
233 between erosive and non-erosive HOA for acetaminophen, weak opioids, NSAIDs and
234 SYSADOAs.

235

236 **Tryptophan metabolites and pathways are modulated in erosive HOA**

237 To compare the differences in tryptophan (Trp) metabolism variables between patients
238 with and without erosive hand osteoarthritis (HOA), multiple logistic regression models
239 were used. The models were adjusted for age, sex, and body mass index (BMI), and were
240 applied to a subset of 409 complete cases out of the 416 patients initially included in the
241 study. We identified four Trp metabolites, eight metabolite ratios and one metabolite
242 pathway score differentially associated with erosive HOA compared to non-erosive HOA
243 (**Figure 1, Table 2**). Higher Trp levels were associated with decreased odds of having
244 erosive HOA. For each twofold increase in concentration (pmol/l) we observed about 59
245 % decrease in the odds of having erosive HOA (OR 0.41, 95% CI [0.24-0.70], $p = 0.007$).
246 Kynurenine-IDO pathway upregulation was significantly associated with erosive HOA
247 (**Figure S3**) (OR 1.60 [1.11-2.29], $p = 0.04$). Similarly, higher levels of 3-HAA were
248 associated with increased odds of having erosive HOA (OR 1.32 [1.13-1.54] $p = 0.004$).
249 Higher 3H-KYN/kynurenine and 3-HAA/3H-KYN ratios were positively associated with
250 increased odds of having erosive HOA (OR 1.65 [1.17-2.33], $p = 0.02$, OR 1.43 [1.17-
251 1.74], $p = 0.007$), while inversely higher xanthurenic acid/3H-KYN, quinolinic acid/3-HAA
252 acid ratios were negatively associated with odds of having erosive HOA (OR 0.86 [0.76-
253 0.97], $p = 0.043$; OR 0.72 [0.62-0.85], $p < 0.001$). In the serotonin pathway, higher levels

254 of 5-HTP were associated with increased odds of having erosive HOA and for each
255 twofold increase in concentration (pmol/l) the odds by 41 % (OR 1.41 [1.13-1.77] p =
256 0.014). An increase of 5-HTP/tryptophan ratio was positively associated with odds of
257 erosive HOA ((OR 1.51 [1.23-1.84], p =0.002) and an increase of 5-hydroxyindole acetic
258 acid (5-HIAA) /5-HTP ratio was negatively associated with odds of having erosive HOA
259 (OR 0.70 [0.57-0.85] p = 0.004). Finally, in the indole–AhR pathway, increased of IAld
260 levels were associated with decreased odds of having erosive HOA, for each twofold
261 increase in concentration (pmol/l) the odds decreased by 33 % (OR 0.67 [0.51-0.59] p =
262 0.026). Increased indoxyl sulfate/tryptophan ratio was positively associated with odds of
263 having erosive HOA ((OR 1.28 [1.05-1.57] p = 0.043).

264

265 **Tryptophan metabolites are associated with pain in HOA**

266 We built a Spearman's correlation matrix for 371 patients with complete data for
267 symptoms (**Figure 2A, 2B; Table 3**) and identified 11 significant correlations between
268 HOA symptoms and Trp metabolite levels. Number of tender joints was negatively
269 correlated with N-acetyl-serotonin and serotonin levels (r = -0.20, p<0.001, r = -0.17,
270 p=0.002). Number of patient-reported painful joints was positively correlated with levels
271 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)
272 and negatively with lald level (r = -0.15, p=0.016) AUSCAN pain score was positively
273 correlated with quinolinic acid and 3H-KYN (r = 0.16, p=0.006, r = 0.14 p =0.02). AIMS2-
274 SF symptoms score was positively correlated with 5-HTP (r = 0.17 p = 0.003). AIMS2-SF
275 affect was negatively correlated with serotonin and tryptophan levels (r = -0.13 p = 0.032,
276 r = -0.13 p = 0.028). Among symptoms significantly correlated with metabolite levels the

277 majority were related to pain. Trp metabolite levels were not significantly correlated with
278 AUSCAN subscore of function and stiffness, AIMS2-SF social score and HADS score.
279 To further support our results, we performed a complementary analysis of significantly
280 correlated pain-related symptoms by using an additional Mann-Whitney Wilcoxon test
281 (**Figure 2C, Table S3**). Patients were divided into low and high pain intensity groups
282 based on the following median values: 3 for number of tender joints, 0 for number of
283 patient-reported painful joints, 20 for AUSCAN pain score, 33 for AIMS2-SF symptoms.
284 Patients with more than 3 tender joints had lower N-acetyl serotonin and serotonin levels
285 ($p < 0.001$ and 0.005). Patients with at least one reported painful joint had higher levels of
286 3-HAA, 3H-KYN, and 5-HTP ($p < 0.001$, $p = 0.005$, $p < 0.001$) and lower lald levels ($p = 0.006$).
287 Patients with AUSCAN pain score > 20 had higher quinolinic acid and 3H-KYN levels
288 ($p = 0.037$, $p = 0.02$) (**Figure 2C**) and patient with AIMS2-SF symptoms score > 33 had a
289 higher 5-HTP levels ($p = 0.020$)

290

291 **DISCUSSION**

292 In this study, we investigated the potential role of tryptophan (Trp) metabolism in
293 osteoarthritis (OA) by analyzing serum Trp metabolites in 416 patients from the DIGICOD
294 HOA cohort. Our finding revealed significant alterations in Trp metabolism in erosive
295 HOA, as well as a potential association with pain (**Figure 3**) These results are consistent
296 with a previous study by Rushing et al., who used an unsupervised fecal metabolomics
297 analysis to identify Trp metabolism as the second most significantly altered metabolic
298 pathway in OA compared to control³⁸.

299

300 We observed a significant decrease in serum tryptophan levels among patients with
301 erosive hand osteoarthritis (HOA) compared to those with non-erosive HOA. Decreased
302 serum Trp level has been previously described in patients with rheumatoid arthritis and
303 was correlated with radiographic destruction and joint space narrowing reflecting cartilage
304 loss³⁹. We also found an overactivation of the kynurenine-IDO pathway in erosive HOA,
305 along with significant alterations in kynurenine-IDO related metabolites in both pain and
306 erosive HOA. Kynurenine pathway is highly involved in inflammatory response, and in
307 neurotransmission^{18,19}. More specifically we observed that 3-HAA levels were increased
308 in erosive HOA additionally, we also found a positive correlation between 3-HAA, 3H-
309 KYN, quinolinic acid and pain. These findings are of significant interest as oxidative
310 kynurenine metabolites such (3-HKYN, 3-HAA, and quinolinic acid) have been associated
311 with neurotoxicity and alteration of nerves ending of afferent sensory neurons⁴⁰. Similarly
312 we found an increase of 3H-KYN/kynurenine, 3-HAA/3H-KYN ratios suggesting an
313 overactivation of kynurenine 3-monooxygenase (KMO) and kynureninase, inhibiting KMO
314 has shown therapeutic potential in preclinical models of neuropathic pain⁴¹. Several
315 studies highlighted the role of kynureninase activation in inflammatory and cardiovascular
316 diseases⁴². In contrast, we found negative associations between the ratios of quinolinic
317 acid/3-HAA and xanthurenic acid/3H-KYN with erosive HOA. These findings are
318 noteworthy as they suggest a potential decrease in kynurenine aminotransferase or
319 aminoadipate aminotransferase (AADAT) activity as observed in inflammatory bowel
320 diseases in which AADAT low levels were associated with disease severity and flares⁴³.
321 Altogether, these results reinforce the potential role of kynurenine metabolites in HOA
322 inflammation and pain

323

324 Our study reveals significant alterations in metabolites of the indole-AhR pathway, which
325 are directly produced by the gut microbiome, indicating a potential involvement of gut
326 dysbiosis and intestinal permeability in hand osteoarthritis (HOA) pathogenesis.
327 Specifically, we found a significant decrease in lald levels in both pain and erosive HOA.
328 Previous research linked decreases of serum lald levels to gut dysbiosis and impaired
329 intestinal barrier function, and animal models have provided evidence that lald
330 supplementation can restore intestinal barrier integrity^{44,45}. Several studies have
331 implicated gut dysbiosis and intestinal permeability in OA-related pain. For instance,
332 serum CD14 levels have been positively associated with self-reported knee pain, while
333 independent cohorts have linked an abundance of Streptococcus species to increased
334 knee pain^{9,46}. Furthermore, additional research has suggested a potential connection
335 between intestinal permeability and osteoarthritis, with Loeser et al. reporting an
336 association between serum lipopolysaccharide and obesity-related osteoarthritis¹¹. These
337 findings support the notion that the gut microbiome and intestinal barrier function may
338 play a crucial role in the development and progression of osteoarthritis.
339 Finally, in the serotonin pathway we observed that 5-HTP levels were increased in both
340 pain and erosive OA patients. Additionally, we observed an increase in the 5-
341 HTP/Tryptophan ratio specifically in erosive OA patients, which suggests an
342 overactivation of tryptophan hydroxylase (Tph). Tph is mostly produced by mast cells and
343 has been implicated in immune tolerance regulation and inflammation⁴⁷. These findings
344 highlight the potential role of Tph in the pathogenesis of erosive OA and pain. N-acetyl
345 serotonin and serotonin levels were also lower in patients with pain. Serotonin plays a

346 major role in pain perception modulation and low levels of serotonin has been associated
347 with chronic pain⁴⁸. The particular association of reported pain and decreased serotonin
348 level suggests a specific role in OA pain beyond inflammation and nociception.

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

369 interesting therapeutic target and a promising treatment for OA. Trp metabolites
370 modulation could have potential therapeutic properties in OA⁵⁰. Further studies are
371 needed to investigate the pathophysiological role of Trp metabolites in OA and OA-related
372 pain.

373

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

379

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390 **Contributions**

391 All authors included met the authorship criteria according to the ICJME recommendations
392 (<https://www.icmje.org>) . JS, HS, FB conceived and designed the study. PE and AC
393 contributed to the acquisition of the data. MB, BM, EMF and JS analyzed the data. MB,
394 AC, MK, PR, EMF, FB, HS and JS contributed to the interpretation of the data. MB, EMF
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397 All the authors approved the final version of the manuscript. MB, and JS
398 (marie.binvignat@sorbonne-universite.fr, jeremie.sellam@aphp.fr) take responsibility for
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408 **Ethics**

409 The DIGICOD study complies with the Declaration of Helsinki and obtained all the
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412

413 **Data availability**

414 Data sharing will be subject to the terms of DIGICOD cohort and the AP-HP data-sharing
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417 **Competing interest statement**

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617 **TABLES LEGEND:**

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619 **Table 1: DIGICOD cohort patients characteristics according to the presence of**

620 **erosive HOA.** AIMS2-SF: Arthritis Impact Measurement Scales 2 Short Format; BMI:

621 body mass index; CRP: C-reactive protein; HADS: Hospital Anxiety Depression Scale;

622 AUSCAN: Australian Canadian osteoarthritis hand index; KL: Kellgren-Lawrence;

623 NSAIDs: Non-steroidal anti-inflammatory drugs; ns: non-significant; SD: standard-

624 deviation; SYSADOAS: symptomatic slow-acting drugs for osteoarthritis

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626

627 **Table 2. Multiple regression analysis of the association of Trp metabolites and**

628 **metabolite ratios and pathways with erosive HOA adjusted on age, sex and BMI.**

629 Trp metabolite levels and metabolite ratios and pathways scores were log2-transformed,

630 multiple regressions and odds ratio were adjusted on age, sex and BMI, p-values are

631 adjusted with a Benjamini-Hochberg correction. Metabolite ratios represented the ratio of

632 downstream metabolites to upstream metabolites * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

633 OR: odds ratio; 95% CI: 95% confidence interval;3-HAA: 3-hydroxyanthranilic acid; 3H-

634 KYN: 3-hydroxykynurenine; 5-HTTP: 5-hydroxytryptophan; AHR: Aryl Hydrocarbon

635 Receptor, IIAA: indole acetic acid; lald: indole-3-aldehyde; IAM: indole-3-acetamide; IDO:

636 indoleamine 2,3-dioxygenase; ILA: indole-3-lactic acid; 5-HIAA: 5-hydroxyindoleacetic

637 acid; ns: non-significant

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640 **Table 3 Spearman's correlation coefficient between Trp metabolites and HOA**
641 **symptoms.**

642 Correlations were selected with an absolute coefficient value $|r| \geq 0.1$ and adjusted p-
643 value ≤ 0.05 after Benjamini-Hochberg correction. 3-Hydroxyanthranilic Acid; 3H-KYN: 3-
644 Hydroxykynurenine, 5-HTP: 5 Hydroxytryptophan; AIMS2-SF: Arthritis Impact
645 Measurement Scales 2 Short Format; AUSCAN: Australian Canadian osteoarthritis hand
646 index; IAld: Indole-3-Aldehyde.

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Table 1: DIGICOD cohort patients characteristics according to the presence of erosive HOA.

		Total	Non Erosive HOA	Erosive HOA	p	missing
		(N=416)	(N = 275)	(N = 141)		(%)
Demographics						
Sex (%)	Female	348 (83.7)	234 (85.1)	114 (80.9)	ns	0
	Male	68 (16.3)	41 (14.9)	27 (19.1)		
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 (9.6)	11.40 (9.46)	15.96 (9.30)	<0.001	1
Metabolism						
BMI (kg/m ²), mean (SD)		25.1 (4.3)	25.11 (4.32)	25.12 (4.36)	ns	1.7
Metabolic syndrome ATP III (%)		148 (36.5)	90 (33.5)	58 (42.6)	ns	2.6
High sensitive CRP (mg/L), mean (SD)		2.37 (4.60)	2.55 (4.61)	2.01 (4.60)	ns	0
Patients-reported outcomes						
AUSCAN pain (0-100), mean (SD)		25.83 (21.36)	24.49 (20.98)	28.40 (21.92)	ns	7.2
AUSCAN stiffness (0-100), mean (SD)		32.68 (28.05)	28.92 (26.95)	39.88 (28.80)	<0.001	7.5
AUSCAN function (0-100), mean (SD)		36.58 (24.88)	34.31 (24.53)	40.83 (25.08)	0.013	6
HADS score (0-21), mean (SD)		12.56 (6.02)	12.46 (5.94)	12.76 (6.19)	ns	5.8
AIMS2-SF symptoms (0-100), mean (SD)		33.17 (20.67)	32.20 (19.53)	35.05 (22.69)	ns	7.5
AIMS2-SF affect (0-100), mean (SD)		28.67 (17.47)	27.92 (17.06)	30.16 (18.21)	Ns	6.7
AIMS2-SF social (0-100), mean (SD)		42.94 (16.55)	43.89 (15.93)	41.08 (17.63)	ns	7.0
Clinical examination						
Number of tender joints (0-48), mean (SD)		4.67 (4.67)	4.18 (4.56)	5.61 (4.76)	0.003	0.2
Number of patient-reported painful joints (0-48), mean (SD)		1.69 (3.28)	1.44 (2.99)	2.18 (3.75)	0.029	0.2
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	3.8

KL sum score (0-120), mean (SD)	46.80 (18.02)	40.72 (16.66)	59.57 (13.56)	<0.001	3.8
Verbruggen-Veys score (0-218), mean (SD)	28.82 (21.28)	17.95 (11.05)	51.47 (19.59)	<0.001	2.2

Treatments

Acetaminophen (%)	132 (32.5)	85 (31.2)	47 (33.8)	ns	1.2
NSAIDs (%)	71 (17.3)	45 (16.5)	26 (18.7)	ns	1.2
Weak Opioids (%)	34 (8.3)	26 (9.6)	8 (5.8)	ns	1.2
SYSADOAS (%)	133 (32.4)	80 (29.4)	53 (38.1)	ns	1.2

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

METABOLITES	OR	95 % CI	p-value
INDOLE / AHR PATHWAY			
INDOXYL SULFATE	1.14	0.93-1.4	ns
IAM	0.97	0.83-1.14	ns
ILA	0.67	0.43-1.04	ns
IAId	0.67	0.51-0.9	0.026 *
IAA	0.93	0.7-1.25	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	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	0.24-0.7	0.007 **
METABOLITES-RATIO	OR	95 % CI	p-value
INDOLE / AHR PATHWAY			
INDOXYL SULFATE/TRYPTOPHAN	1.28	1.05-1.57	0.043 *
ILA/TRYPTOPHAN	1.2	0.81-1.79	ns
IAId/IAA	0.8	0.64-1	ns
IAA/IAM	1.01	0.87-1.17	ns

IAM/TRYPHTOPHAN	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/TRYPHTOPHAN	1.38	0.88-2.16	ns
SEROTONIN PATHWAY			
N-ACETYL-SEROTONIN/SEROTONIN	1.25	0.98-1.6	ns
SEROTONIN/5-HTP	0.81	0.68-0.97	ns .
5-HIAA/5-HTP	0.7	0.57-0.85	0.004 **
5-HTP/TRYPHTOPHAN	1.51	1.23-1.84	0.002 **
METABOLITES PATHWAY	OR	95 % CI	p-value
INDOLE-AHR PATHWAY	1.11	0.81-1.52	ns
KYNURENINE-IDO PATHWAY	1.6	1.11-2.29	0.04 *
SEROTONIN PATHWAY	1	0.73 -1.38	ns

Trp metabolite levels and metabolite ratios and pathways scores were log2-transformed for logistic regression. Multiple regressions and odds ratio are adjusted on age, sex and BMI, p-values are adjusted with a Benjamini-Hochberg correction.

Metabolite ratios represent 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-HTP: 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 3 Significant Spearman's correlation coefficients between Trp metabolites and HOA symptoms.

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	*

Correlation 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.

FIGURE LEGEND:

Figure 1: Multiple regression analyses 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.

Figure 2: 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) s). 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, 3 tender joints, 20 for

AUSCAN pain, 33 for AIMS2-SF symptoms. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.
3-hydroxyanthranilic acid; 3H-KYN: 3-hydroxykynurenine; 5-HTP: 5-hydroxytryptophan;
AIMS2-SF: Arthritis Impact Measurement Scales 2 Short Form
AUSCAN: Australian Canadian osteoarthritis hand index; IAld: indole-3-aldehyde

Figure 3: 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.

Figure 1: Multiple regression analyses 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-values were adjusted with a Benjamini-Hochberg correction. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, 3-HAA: 3-hydroxyanthranilic acid; 3H-KYN: 3-hydroxykynurenine; 5-HTP: 5-hydroxytryptophan; IAA: 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.

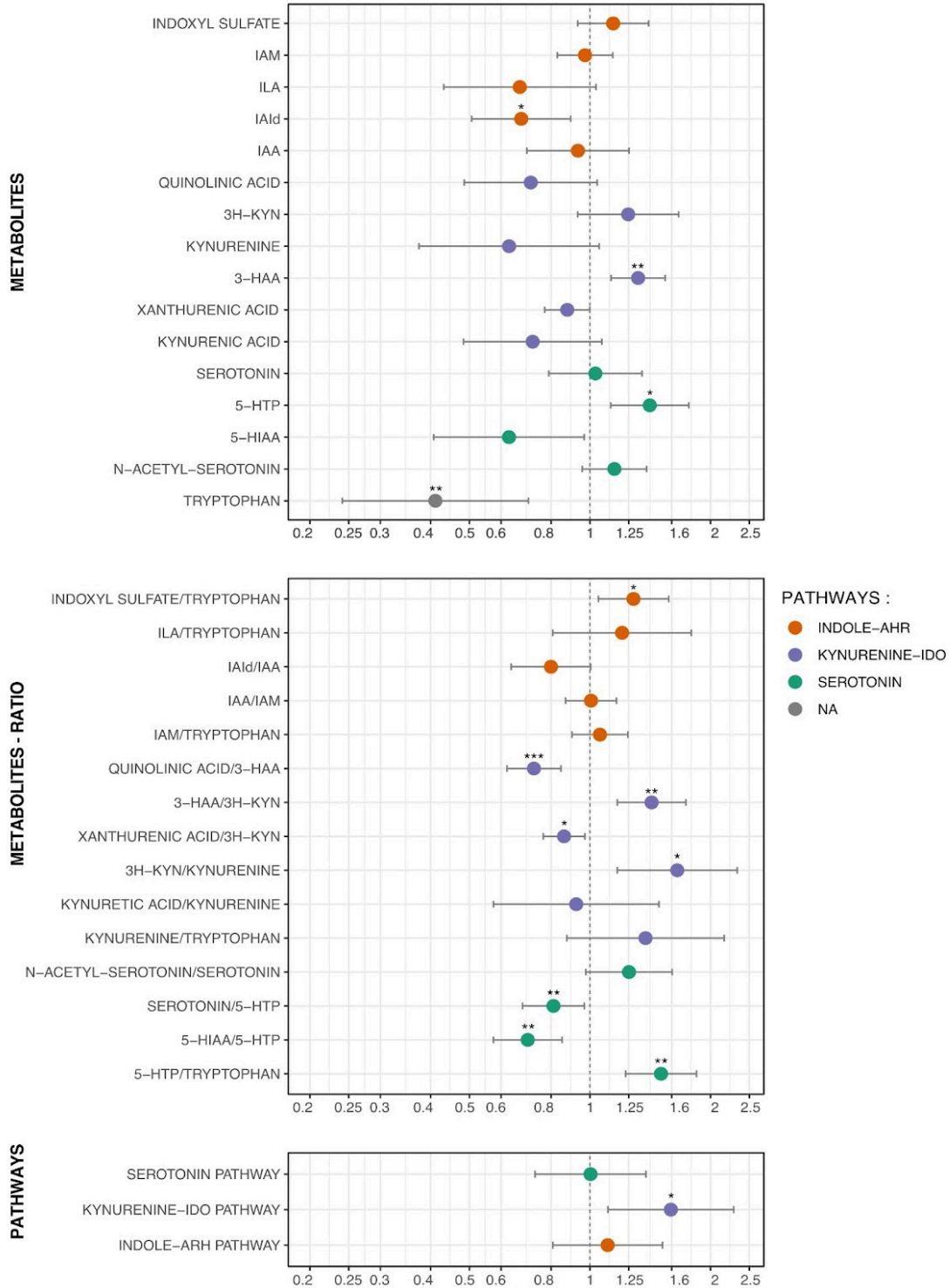


Figure 2: A. Dot heatmap of significant Spearman's correlation between Trp metabolite and HOA symptoms. The significant correlations between Trp metabolites and symptoms are visually represented using dots. Red dots indicate positive correlations, while blue dots indicate negative correlations. The size 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)s.** 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, 3 tender joints, 20 for AUSCAN pain, 33 for AIMS2-SF symptoms. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. AUSCAN: Australian Canadian osteoarthritis hand index; 3-hydroxyanthranilic acid; 3H-KYN: 3-hydroxykynurenine; 5-HTP: 5-hydroxytryptophan; IAld: indole-3-aldehyde

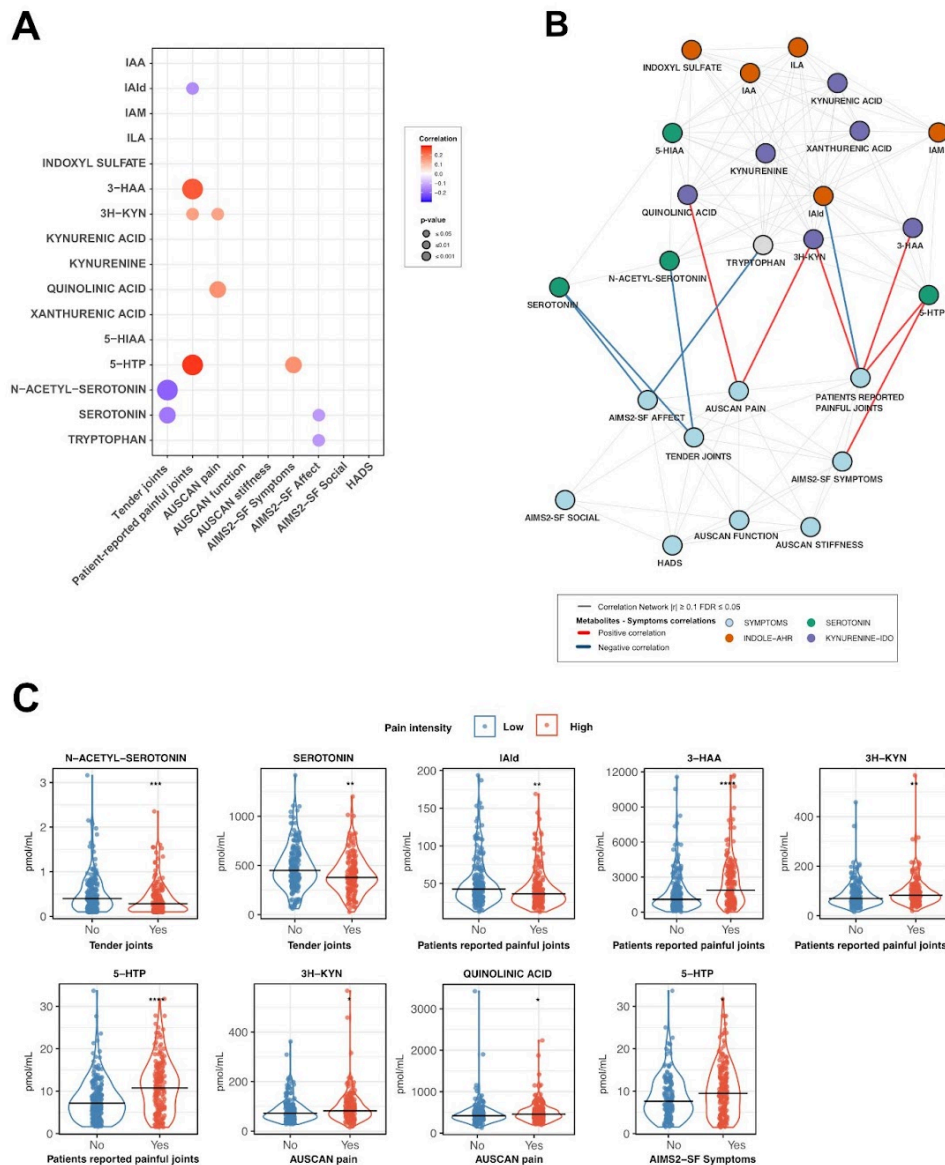


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