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Urinary Hydroxyproline as an Inflammation-Independent Biomarker of Inflammatory Bowel Disease

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Abstract: Predicting responses and monitoring the severity of inflammatory bowel disease (IBD) is challenging due to a lack of specific biomarkers. This study identifies urinary hydroxyproline, a marker of collagen turnover elevated in experimental colitis, as independent of conventional biomarkers like creatinine, glomerular filtration rate, C-reactive protein, and fecal calprotectin. Among 71 IBD patients, urinary hydroxyproline levels were significantly higher compared with 36 controls, with an area under the receiver operating characteristic curve of 0.814, highlighting its potential as a diagnostic tool. No significant difference in hydroxyproline levels was observed between the 50 Crohn's disease and 21 ulcerative colitis patients, nor was there a correlation with kidney function markers, gastrointestinal symptom severity, or stool consistency. Disease localization was not associated with urinary hydroxyproline levels. Interestingly, 14 patients with primary sclerosing cholangitis and IBD also exhibited elevated urinary hydroxyproline levels, comparable to IBD patients but higher than healthy controls. This underscores the role of urinary hydroxyproline as an independent biomarker for IBD diagnosis, without association with disease severity or established markers like fecal calprotectin.

Keywords: urine; calprotectin; primary sclerosing cholangitis; C-reactive protein; IBD; hydroxyproline

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1. Introduction

Inflammatory bowel disease (IBD), comprising Crohn's disease (CD) and ulcerative colitis (UC), is a chronic inflammatory condition with increasing prevalence and incidence, especially in so-called developed countries [1–6].

Biomarkers in feces are useful for the diagnosis and assessment of the response to treatment of IBD. Fecal calprotectin is an established tool for measuring intestinal inflammation and predicting disease recurrence in clinical settings [7,8]. Granulocytes release this biomarker, which is induced in inflammatory diseases and thus is not specific to IBD [9,10].

Urinary metabolites have been used for clinical diagnosis for a long time [11–13]. Urinary chemerin levels, a protein initially described for its chemotactic activity, are strongly increased in active IBD [14]. Levels of 3-indoxyl-sulfate are higher in the urine of IBD patients compared with controls but are not related to disease severity [15]. Urinary formate and hippurate may serve as potential biomarkers for IBD diagnosis, while levoglucan and

leukotriene E4 may be useful for predicting clinical relapse and assessing disease activity, respectively [16].

Hydroxyproline is a major component of collagen and is mainly formed by hydroxylation of proline in procollagen. This irreversible modification is important for the correct assembly and stability of mature collagen. Collagens make up about a third of all proteins in the body, and because hydroxyproline cannot be used for protein synthesis, it is degraded in the liver and kidneys. Comparatively little hydroxyproline is excreted in the urine [17].

Urinary hydroxyproline is a marker of collagen turnover and has been used in the past as an indicator of bone disease [18]. Studies have shown that urinary hydroxyproline excretion is increased in several conditions, including Marfan syndrome [19], hyperthyroidism [20], Paget's disease [21], and acromegaly [22]. The urine of adult patients with coeliac disease and other malabsorption disorders also contained elevated levels of hydroxyproline [23]. An improvement in malabsorption was associated with the normalization of hydroxyproline excretion [23].

Trinitrobenzenesulphonic-acid-induced colitis in rats more than doubled urinary hydroxyproline excretion within 3 weeks. In these animals, colitis was linked to decreased bone density, and osteopenia was supposed to cause higher levels of hydroxyproline in the urine [24]. Patients with Crohn's disease nevertheless had reduced urinary hydroxyproline levels compared with controls. Dietary supplementation improved phosphorus, nitrogen, and calcium, which were low in these patients, and led to an increased level of urinary hydroxyproline [25].

Intestinal fibrosis is a frequent complication of IBD and is linked to the development of intestinal strictures and fistulas. Fibrosis is characterized by the deposition of extracellular matrix proteins such as collagens [26].

IBD is a complex disease involving the mucosal immune system, submucosa, and luminal components such as gut microbiota. Chronic inflammation drives fibrosis, a process of increased extracellular matrix formation and degradation. Biomarkers reflecting fibro-inflammatory processes in patients with IBD may be useful for monitoring of disease activity [27]. Serological biomarkers for collagen degradation were found to be elevated in IBD, distinguishing between moderate and severe disease stages with clinical relevance [28,29].

Primary sclerosing cholangitis (PSC) is a chronic liver disease characterized by progressive fibrosis of the biliary tree, leading to obstruction. PSC frequently co-occurs with IBD. The precise causes of PSC are largely unknown, with ongoing research aimed at elucidating the underlying pathophysiological mechanisms [30,31].

Carbon-tetrachloride causes liver fibrosis in rodents. Zinc treatment lowered hepatic collagen accumulation in these animals and this was accompanied by reduced urinary hydroxyproline levels [32]. Urinary levels of hydroxyproline in rats with dimethylnitrosamine-induced liver fibrosis increased in parallel with its serum levels [33].

Positive correlations of urine hydroxyproline with the Ishak fibrosis score were noted in patients with chronic hepatitis C infection [34]. Improvement of measures of liver injury of patients with chronic hepatitis B virus infection was, however, related to increased urine hydroxyproline levels [35].

Current evidence suggests that urinary hydroxyproline may serve as a noninvasive marker for diseases characterized by increased collagen turnover, such as intestinal and liver fibrosis. This study aimed to analyze urinary hydroxyproline to assess its suitability as a noninvasive biomarker for diagnosing IBD and PSC and evaluating disease severity.

2. Materials and Methods

2.1. Patient and Control Cohorts

From 6 December 2021 to 31 January 2023, patients diagnosed with IBD, PSC-IBD, or PSC without underlying IBD (PSCw/o) at the Department of Internal Medicine I (University Hospital of Regensburg) were recruited. Diagnosis of IBD and PSC was based on clinical, histologic, and endoscopic criteria [36–38]. In the IBD cohort, 19 patients were treated with corticosteroids, 19 with mesalazine, 17 with anti-interleukin 12/23 antibodies, 20 with

antitumor necrosis factor antibodies, and 8 with azathioprine. The PSC patients were all treated with ursodeoxycholic acid. Patients with coagulopathy were excluded from the study. The urine of patients, as well as controls, was collected and stored at −80 °C until analysis. For this retrospective study, the controls were partners of the patients, students, and hospital staff (from our Department and other Departments or Institutes at our hospital) who lived in the same area as the patients.

2.2. Enzyme-Linked Immunosorbent Assays (ELISAs)

The ELISA to measure urinary hydroxyproline was from Bio-Techne GmbH (Wiesbaden-Nordenstadt, Germany). Urine was used undiluted. Creatinine in urine was measured by the creatinine parameter assay kit (Bio-Techne GmbH) in 1:20 fold diluted urine. Urinary hydroxyproline levels were normalized to urinary creatinine concentrations.

2.3. Statistical Analysis

Data are presented in boxplots, with outliers indicated by circles and asterisks. The Kruskal–Wallis test, receiver operating characteristic (ROC) curve, Mann–Whitney U test, and Spearman correlation were utilized for statistical analysis using SPSS Statistics 26.0 (IBM, Leibniz Rechenzentrum, München, Germany). A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Urinary Hydroxyproline Levels of Patients and Controls

The cohort included 71 IBD patients, 14 patients with PSC and IBD (PSC-IBD), 5 patients with PSC without underlying IBD (PSCw/o), and 36 controls. Controls were older than patients with IBD and patients with PSC-IBD. PSCw/o patients were older than PSC-IBD patients. The cohorts had a comparable gender distribution (Table 1). PSC-IBD patients had higher aspartate aminotransferase (AST), alkaline phosphatase (AP), and bilirubin compared with IBD patients. PSCw/o patients had higher AP and bilirubin than IBD patients (Table 1).

Table 1. Characteristics of the study groups. The cohort includes 14 patients with PSC and IBD (PSC-IBD) and 5 patients with PSC without underlying IBD (PSCw/o). The IBD cohort does not include patients with PSC. Data are reported as median, minimum, and maximum values. The Model for End-Stage Liver Disease (MELD) score was documented for PSC patients. Statistical test used: Kruskal–Wallis Test (alanine aminotransferase (ALT), alkaline phosphatase (AP), aspartate aminotransferase (AST), body mass index (BMI), gamma-glutamyl transferase (gamma GT), glomerular filtration rate (GFR), not determined (n.d.)). For the comparison of IBD and PSC-IBD, a $p < 0.05$, aa $p < 0.01$, for the comparison of controls and IBD patients b $p < 0.05$, for the comparison of controls and PSC-IBD patients c $p < 0.05$, for the comparison of PSCw/o and PSC IBD patients d $p < 0.05$, and for the comparison of IBD and PSCw/o patients e $p < 0.05$.

Characteristics	IBD	PSC-IBD	PSCw/o	Controls
Number (females/males)	71 (36/35)	14 (5/9)	5 (2/3)	36 (18/18)
Age (years)	42 (19–70) b	41 (18–63) c d	57.0 (37.4–63.04) d	54 (23–78) b c
BMI (kg/m^2)	24.7 (15.5–44.3)	24.9 (16.3–41.8)	19.9 (18.0–21.8)	n.d.
C-reactive protein (mg/L)	2.0 (0–144)	2.4 (0–26)	0	n.d.
Creatinine (mg/dL)	0.80 (0.51–1.18)	0.83 (0.60–1.43)	2.52 (1.09–3.94)	n.d.
GFR (mL/min)	100 (67–136)	100 (56–135)	42 (12–72)	n.d.
Fecal calprotectin (µg/g)	55 (0–3402)	41 (0–999)	0	n.d.
AST (U/L)	25 (10–41) a	27 (17–161) a	31 (15–70)	n.d.
ALT (U/L)	19 (7–62)	28 (8–205)	27 (5–61)	n.d.
Gamma GT (U/L)	24 (8–71)	29 (10–458)	54 (11–234)	n.d.
AP (U/L)	63 (38–142) aa e	107 (35–587) aa	112 (70–426) e	n.d.
Bilirubin (mg/dL)	0.40 (0.15–1.90) aa e	0.60 (0.30–4.30) aa	0.80 (0.40–1.50) e	n.d.
MELD Score	n.d.	6 (6–12)	7 (6–20)	n.d.

Urinary hydroxyproline levels were measured in patients and controls. To normalize hydroxyproline levels, urinary creatinine was also analyzed. Patients with IBD (13.7 (1.8–113.8) ng/µg) and PSC (PSC-IBD and PSCw/o; 19.3 (4.6–326.9) ng/µg) exhibited similar urinary hydroxyproline levels, which were significantly higher than those of the controls with 4.0 (0.01–27.1) ng/µg (Figure 1A).

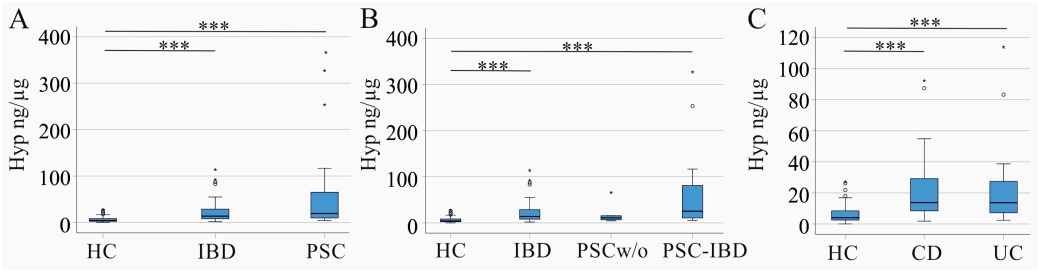

Figure 1. Urinary hydroxyproline (Hyp) of healthy controls (HC), patients with inflammatory bowel disease (IBD), and patients with primary sclerosing cholangitis (PSC). (**A**) Urinary hydroxyproline of HC, IBD, and PSC patients; (**B**) urinary hydroxyproline of HC, IBD, PSCw/o, and PSC-IBD patients); (**C**) urinary hydroxyproline of HC, patients with Crohn's disease (CD), and patients with ulcerative colitis (UC). Outliers are indicated by small circles and asterisks *** $p < 0.001$.

PSC-IBD patients had higher urinary hydroxyproline levels than controls. The five PSCw/o patients had 11 (4.6–65.8) ng/µg, and the PSC-IBD patients had 25.4 (5.5–326.9) ng/µg urinary hydroxyproline, but this difference was not significant (Figure 1B). Urinary hydroxyproline of PSCw/o patients and controls was similar (Figure 1B).

The IBD cohort included 50 patients with CD and 21 patients with UC, and urinary hydroxyproline was comparable between these two groups. CD and UC patients had higher levels than the healthy controls (Figure 1C).

The area under the receiver operating characteristic curve (AUROC) for the discrimination of IBD from controls was 0.814 ± 0.044 ($p < 0.001$), for the discrimination of PSC from controls was 0.865 ± 0.048 ($p < 0.001$), and for the discrimination of PSC-IBD from controls was 0.895 ± 0.045 ($p < 0.001$) (Figure 2A–C). There were only five PSCw/o patients, and the AUROC was not determined.

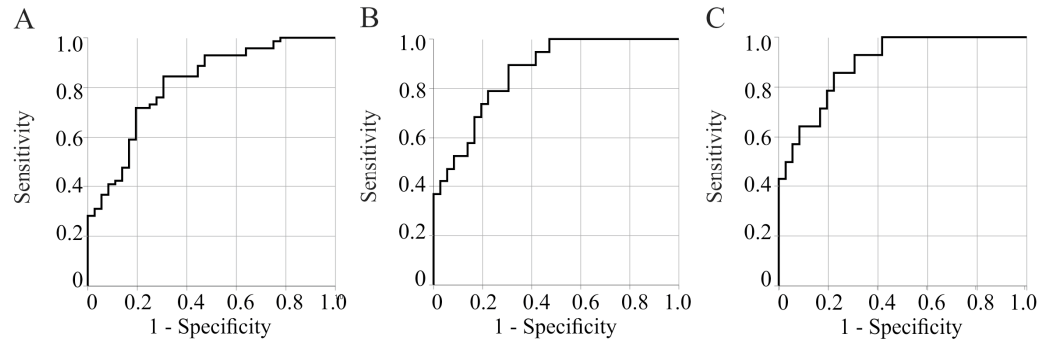

Figure 2. Receiver operating characteristic curve (ROC) for discrimination of patients and controls: (**A**) ROC for discrimination of inflammatory bowel disease (IBD) patients and controls; (**B**) ROC for discrimination of patients with primary sclerosing cholangitis (PSC) and controls; (**C**) ROC for discrimination of patients with PSC-IBD and controls.

3.2. Urinary Hydroxyproline in Relation to Age, BMI, and Gender

In the control cohort, urinary hydroxyproline was not correlated with age (r = −0.014, $p = 0.935$) and was similar in both sexes ($p = 0.293$).

Urinary hydroxyproline did not correlate with age in IBD (r = 0.001, $p = 0.994$), PSC (r = −0.002, $p = 0.994$), or PSC-IBD (r = 0.099, $p = 0.737$). BMI in IBD (r = −0.121, $p = 0.351$), PSC (r = 0.203, $p = 0.505$), and PSC-IBD (r = −0.009, $p = 0.979$) patients was not related

to the levels of this metabolite. Females had higher hydroxyproline levels than males in IBD and PSC ($p = 0.074$ for IBD and $p = 0.063$ for PSC). In PSC-IBD, this difference was not significant ($p = 0.147$).

3.3. Urinary Hydroxyproline in Relation to Measures of Inflammation and Kidney and Liver Function

Stratifying IBD patients by fecal calprotectin levels showed no significant variation in urinary hydroxyproline levels among the groups ($p = 0.118$; Figure 3A). A total of 34 patients had fecal calprotectin levels below 50 µg/g, 16 patients had levels between 50 and 150 µg/g, 10 patients between 150 and 500 µg/g, and 9 patients had levels above 500 µg/g. Data for two patients were not documented.

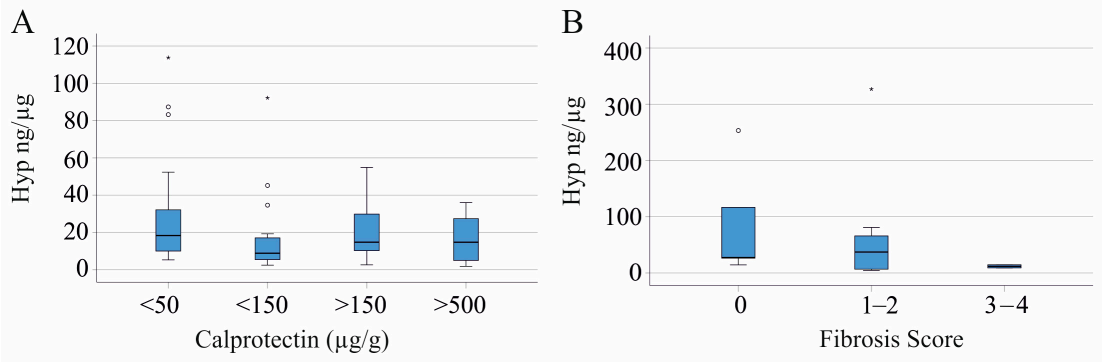

Figure 3. Urinary hydroxyproline in relation to fecal calprotectin and liver fibrosis: (**A**) urinary hydroxyproline of IBD patients in relation to fecal calprotectin; (**B**) urinary hydroxyproline of PSC patients in relation to liver fibrosis grades. Outliers are indicated by small circles and asterisks.

Serum creatinine ($p = 0.719$) was not changed with increasing levels of fecal calprotectin. GFR was increased with higher fecal calprotectin levels ($p = 0.030$).

In IBD, PSC, and PSC-IBD, urinary hydroxyproline did not correlate with creatinine, GFR, C-reactive protein, or fecal calprotectin (Table 2).

Table 2. Spearman correlation of urinary hydroxyproline with creatinine, glomerular filtration rate, C-reactive protein, and fecal calprotectin in IBD, PSC (PSC-IBD and PSCw/o), and PSC-IBD patients.

Correlation	Creatinine	Glomerular Filtration Rate	C-reactive Protein	Fecal Calprotectin
IBD	r = −0.247 $p = 0.051$	r = 0.108 $p = 0.399$	r = 0.069 $p = 0.595$	r = −0.203 $p = 0.095$
PSC	r = −0.279 $p = 0.334$	r = 0.029 $p = 0.923$	r = 0.145 $p = 0.622$	r = 0.058 $p = 0.837$
PSC-IBD	r = −0.210 $p = 0.513$	r = −0.154 $p = 0.632$	r = 0.042 $p = 0.897$	r = −0.028 $p = 0.929$

ALT, AST, gamma GT, and AP did not correlate with urinary hydroxyproline of IBD, PSC, or PSC-IBD patients ($p > 0.05$ for all).

The fibrosis grade of 17 patients was determined using FibroScan: 5 PSC patients exhibited no fibrosis, 10 patients had grade 1 or 2 fibrosis, and 2 patients had grade 3 or 4 fibrosis. Urinary hydroxyproline levels were similar across these groups (Figure 3B).

3.4. Urinary Hydroxyproline in Relation to Time since First Diagnosis, Disease Localization, Ileocecal Surgery, and Fistula

Time since the first diagnosis of IBD was documented for 63 patients and was 13 (1–42) years. Urinary hydroxyproline did not correlate with disease duration (r = 0.115,

p = 0.368). The disease localization of 10 CD patients was ileocecal and that of 37 CD patients was ileocecal, with further parts of the gastrointestinal tract affected, and 3 patients had inflammation in the colon. Urinary hydroxyproline did not significantly differ between these groups (Figure 4A). Nineteen patients with CD had fistulas, but this was not related to increased urinary hydroxyproline (p = 0.429).

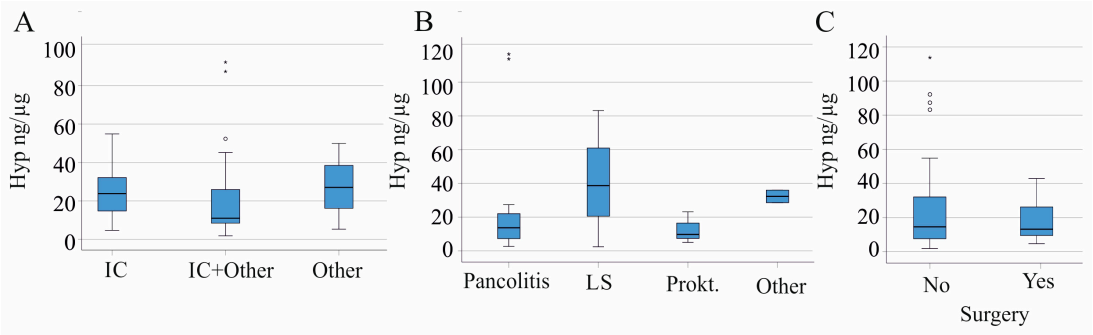

Figure 4. Urinary hydroxyproline levels in relation to disease localization and surgeries in the ileocecal region: (**A**) Urinary hydroxyproline levels of patients with Crohn's disease with ileocecal inflammation (IC), inflammation in the ileocecal region and further parts of the gastrointestinal tract (IC + other) and patients where inflammation was not located in the ileocecal region (other); (**B**) urinary hydroxyproline levels of patients with ulcerative colitis and pancolitis, left-sided colitis (LS), proctosigmoidosis (Prokt.) and patients with other localizations (other); (**C**) urinary hydroxyproline of IBD patients without (No) and with (Yes) surgery in the ileocecal region. Outliers are indicated by small circles and asterisks.

In the UC group, 13 patients had pancolitis, 3 patients had left-sided colitis, 3 patients had proctosigmoidosis, and 2 patients had proctitis. Urinary hydroxyproline was similar between these groups (Figure 4B).

Fifteen IBD patients had surgery in the ileocecal region and 56 patients did not (this was not documented for 8 patients). The urinary hydroxyproline levels of these two groups were similar (Figure 4C).

3.5. Urinary Hydroxyproline in Relation to the Gastrointestinal Symptom Rating Scale and Bristol Stool Score

The Gastrointestinal Symptom Rating Scale (GSRS) for assessment of disease symptoms such as abdominal pain and the number of stools per day was not associated with urinary hydroxyproline levels (Figure 5A). There were 2 IBD patients with no complaints, 43 patients with minor complaints, 21 patients with moderate complaints, and 3 patients with strong complaints (data of 2 patients were not documented).

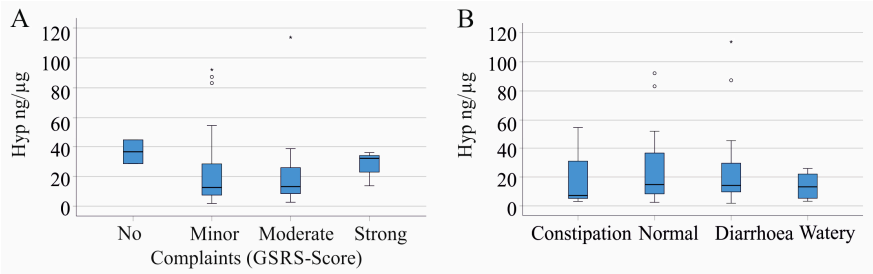

Figure 5. Urinary hydroxyproline levels in relation to the Gastrointestinal Symptom Rating Scale (GSRS) and the Bristol stool score: (**A**) urinary hydroxyproline levels in relation to the Gastrointestinal Symptom Rating Scale; (**B**) urinary hydroxyproline levels in relation to the Bristol stool score. Outliers are indicated by small circles and asterisks.

According to the Bristol stool chart, which was known for 59 patients, in the IBD cohort, 3 patients had constipation, 19 had normal stool, 32 had diarrhea, and 5 had watery stool. The urinary hydroxyproline of these patients was similar (Figure 5B).

3.6. Urinary Hydroxyproline in Relation to Current Medication

In the IBD cohort, the 19 patients currently treated with corticosteroids ($p = 0.959$), the 19 patients with mesalazine therapy ($p = 0.449$), the 17 patients with anti-interleukin 12/23 antibody therapy ($p = 0.227$), the 20 patients treated with antitumor necrosis factor antibodies ($p = 0.729$), and the 8 patients with azathioprine ($p = 0.670$) had the same urinary hydroxyproline levels as the patients not treated with these drugs.

4. Discussion

Urinary hydroxyproline levels are elevated in patients with IBD and PSC-IBD, irrespective of disease severity as determined by laboratory markers and clinical scores.

This finding contrasts with a previous study that reported lower urinary hydroxyproline levels in Crohn's disease patients. The earlier study focused on adolescent IBD patients experiencing growth failure and compared them with healthy controls [25]. Thus, the lower urinary hydroxyproline levels might be attributed to growth retardation rather than to IBD itself.

Rats with experimental colitis had more than twofold elevated urinary hydroxyproline levels compared with the respective control animals [24]. The IBD patients of our cohort had 2.4-fold higher median urinary hydroxyproline levels compared with healthy controls. The median urinary hydroxyproline of patients with PSC-IBD was 6.3 times higher, and for patients with PSCw/o, it was 2.7 times higher than that of the control group. Urinary levels of hydroxyproline were, however, similar between IBD, PSC-IBD, and PSCw/o patients, and all of these disease entities appear to be related to elevated renal hydroxyproline excretion. Urinary hydroxyproline demonstrated an AUROC of 0.814 for IBD, of 0.865 for PSC (PSC-IBD and PSCw/o), and of 0.895 for PSC-IBD diagnosis, indicating an excellent discriminatory power between patients and controls [39].

Most of our CD patients had ileocecal disease localization and urinary hydroxyproline levels of patients with only this region affected, and patients in whom further regions of the gastrointestinal tract were involved were similar. Disease localization in UC was also not related to altered urinary hydroxyproline levels.

PSC is a chronic liver disease, and elevated urinary hydroxyproline levels have been observed in animal models of liver fibrosis [32,33] and in patients with chronic viral hepatitis C infections where positive correlations of urinary hydroxyproline with the Ishak fibrosis score were noted [34]. Measurement of hepatic hydroxyproline concentrations in liver tissue is commonly used to quantify fibrosis in experimental models [40]. Plasma levels of hydroxyproline are elevated in patients with liver fibrosis of different etiologies and were interrelated with hepatic levels and fibrosis scores [41]. It is therefore reasonable to assume that hydroxyproline in urine is related to liver fibrosis.

On the other hand, it has been shown that improved liver function of patients with chronic hepatitis B virus infection was associated with an increase in urine hydroxyproline levels [35]. Whether urinary hydroxyproline is related to liver disease etiology thus needs further study.

Our study indicates that both PSC and IBD are associated with elevated urinary hydroxyproline levels, suggesting that this biomarker is not specific to liver fibrosis. PSC-IBD patients had higher levels in comparison with PSCw/o patients, indicating that bowel diseases have a strong effect on urinary hydroxyproline levels. The number of PSCw/o patients was small, so this suggestion needs confirmation. Consistent with this hypothesis, patients with celiac disease and those with other absorption defects, such as enteritis, also exhibit increased urinary hydroxyproline [23].

Corticosteroids are anti-inflammatory drugs but did not alter urinary hydroxyproline levels in adult rats [42,43]. In our IBD patients, corticosteroid therapy was not associated

with altered urinary hydroxyproline levels. Other drugs, such as mesalazine or biologics, did not change urinary hydroxyproline levels. This analysis rules out the possibility that specific therapies are associated with urinary hydroxyproline levels.

Osteomalacia is a bone disease characterized by impaired mineralization of the bone matrix. Vitamin D and calcium deficiency due to malabsorption syndromes, corticosteroid, and cyclosporine therapy, as well as disease activity, is supposed to contribute to bone diseases in IBD [44,45]. Low bone mineral density in IBD has been associated with higher age, use of glucocorticoids, and elevated fecal calprotectin [46], and it may be prevalent in up to 50% of patients with IBD [45].

Osteomalacia is rare in patients with chronic liver disease, although low serum vitamin D levels are common in patients with liver cirrhosis. These patients more often suffer from osteoporosis [47]. Bone disorders are associated with higher urinary hydroxyproline levels [23] and may contribute to the elevated levels in the urine of IBD and PSC patients, as osteomalacia and osteopenia are both associated with inflammatory conditions [44,45,47].

In our IBD cohort, we found no associations between urinary hydroxyproline and fecal calprotectin levels or serum CRP, both of which are markers of disease severity [48–50]. The GSRS and the Bristol stool score were evaluated for most of our patients but were not related to urinary hydroxyproline levels, further emphasizing that urinary levels of this metabolite are neither related to disease severity nor symptoms.

Fistulas are common in CD and are associated with intestinal fibrosis [26]. The CD patients with fistulas had urinary hydroxyproline levels comparable to patients without this complication. This suggests that urinary hydroxyproline levels are not related to intestinal fibrosis or the formation of a fistula.

Importantly, urinary hydroxyproline levels did not correlate with creatinine or GFR and were therefore not related to renal dysfunction.

There was also no association with patient age, in accordance with previous observations in healthy controls [51]. Positive associations with age in healthy females but not males were reported by another study [52]. In agreement with our results, urinary hydroxyproline did not correlate with BMI [52].

In our IBD and PSC cohort, women tended to have higher urinary hydroxyproline than men. Urinary hydroxyproline levels of the control group did not differ by sex in our study or the study by Onwuka et al. [51]. Female patients with primary aldosteronism had strongly increased urinary hydroxyproline levels compared with male patients, and a similar trend appeared in the normal controls [53]. Collagen degradation is thought to be the main pathway related to urinary hydroxyproline levels [54], and sex-related differences in the development of organ fibrosis are well described [55]. Evaluating the associations of gender and sex hormones with urinary hydroxyproline levels in healthy controls and patients with fibrotic diseases may clarify this issue.

Elevated urinary hydroxyproline is suggested to result from increased collagen breakdown [54]. In humans, 4-hydroxyproline accounts for about 99% of hydroxyproline in the body. It is converted through several chemical reactions to glyoxylate and glycolate in the mitochondria and into glycine in peroxisomes [17]. Glyoxylate reductase/hydroxypyruvate reductase (GRHPR) is an enzyme involved in this process and was found to be increased in the intestine of a murine IBD model and in patients with Crohn's disease [56]. Hydroxyproline degradation mainly takes place in the liver [17], and future research is needed to evaluate whether hydroxyproline degradation is also impaired in patients with increased urinary hydroxyproline levels such as IBD and PSC.

In IBD and PSC patients, urinary hydroxyproline did not correlate with markers of liver disease severity. Preliminary data also exclude a correlation with liver fibrosis in PSC. The origin of the higher hydroxyproline levels in the urine of patients with IBD and PSC is still unclear.

Notably, dietary hydroxyproline was shown to attenuate the severity of experimental colitis [57]. The conversion of hydroxyproline to glycine and the subsequent increased synthesis of glutathione is one of the beneficial effects of this imino acid [58]. The role

of dietary hydroxyproline is ambiguous, and a positive association with coronary heart disease risk was described [59]. This highlights the importance of exploring the metabolic role of this imino acid in different diseases.

This study has limitations. We did not determine the bone mineral density of our patient cohort. The intake of vitamin D and calcium was not documented. Urine samples were collected spontaneously and at only one time point, and patients were not required to follow a specific diet. However, it has been shown that the daily excretion of hydroxyproline is largely independent of diet and does not show diurnal variations [54]. A further limitation of our study is that the number of patients with PSC was low. For controls, only age and sex were recorded, and laboratory measurements and BMI were not documented. All of our controls had normal body weight and were healthy, indicating that their laboratory values were normal.

5. Conclusions

This study shows that patients with IBD and PSC-IBD exhibit elevated urinary hydroxyproline levels, independent of intestinal inflammation markers or liver disease severity. The diagnosis of IBD can be challenging [60], and urinary hydroxyproline was shown in this study to discriminate between IBD patients and healthy controls. It remains to be seen whether urinary hydroxyproline is similarly elevated in patients with gastrointestinal diseases other than IBD. The potential of urinary hydroxyproline as a standalone biomarker for diagnosing IBD and PSC-IBD, independent from disease severity and established markers such as fecal calprotectin, needs further study. Hydroxyproline analysis in spot urine can be used to screen patients with a high risk, such as a family history of IBD [61], to identify and treat patients with IBD at an earlier stage.

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