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Urinary soluble CD163 is a putative non-invasive biomarker for primary sclerosing cholangitis

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ABSTRACT

Soluble CD163 (sCD163) is a selective marker of macrophages whose circulating levels have been found to be induced in patients with active inflammatory bowel disease (IBD). Urinary proteins are emerging as non-invasive diagnostic biomarkers, and here, sCD163 levels were measured in the urine of 18 controls and 63 patients with IBD by enzyme-linked immunosorbent assay. Urinary sCD163 levels did, however, not differentiate IBD patients from controls. Analysis of sCD163 in the serum of 51 of these patients did not show higher levels in IBD. Primary sclerosing cholangitis (PSC) is often associated with IBD, and sCD163 was higher in the urine of the 21 patients and in the serum of the 13 patients with PSC compared to patients with IBD. Of clinical relevance, urinary sCD163 levels were higher in PSC patients compared to those with other chronic liver diseases ($n = 16$), while serum sCD163 levels were comparable between the two groups. Serum sCD163 of IBD and PSC patients positively correlated with serum C-reactive protein. Serum creatinine and glomerular filtration rate, surrogate markers for renal function, did not significantly correlate with urinary or serum sCD163 levels in IBD or PSC patients. Moreover, urinary sCD163 was not related to fecal calprotectin levels whereas serum sCD163 of IBD patients showed a positive trend. PSC associated with IBD and PSC without underlying IBD had similar levels of urinary sCD163 while serum sCD163 tended to be higher in the latter group. In PSC patients, urinary sCD163 did not correlate with serum aminotransferase levels, gamma glutamyl transferase, alkaline phosphatase, bilirubin or the Model for End Stage Liver Disease score. Ursodeoxycholic acid was prescribed to our PSC patients and fecal levels of ursodeoxycholic acid and its conjugated forms were increased in PSC compared to IBD patients. Otherwise, fecal bile acid levels of IBD and PSC patients were almost identical, and were not correlated with urinary and serum sCD163 in PSC. In summary, our study identified urinary sCD163 as a potential biomarker for PSC.

1. Introduction

Activation of macrophages leads to the shedding of the macrophage-specific scavenger receptor CD163, and soluble CD163 (sCD163) is regarded as a circulating marker of activated monocytes and macrophages (Buechler et al., 2013; Moller, 2012).

CD163 is a haemoglobin–haptoglobin receptor and mediates the uptake of this complex by monocytes/macrophages (Buechler et al., 2013; Ryter, 2022). Cellular CD163 protein is induced by anti-inflammatory drugs such as glucocorticoids as well as the cytokines IL-10 and IL-6. Tumor necrosis factor (TNF) and lipopolysaccharide (LPS) cause downregulation of CD163 (Atri et al., 2018; Buechler et al., 2013; Moller, 2012).

The soluble form of CD163 also contributes to haemoglobin uptake (Moller, 2012). Inflammatory mediators such as LPS and oxidative stress stimulate the shedding of CD163, and levels of sCD163 were increased in response to LPS *in vivo* (Nielsen et al., 2019). Hence, systemic levels of sCD163 were found to be induced in various inflammatory diseases such

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as rheumatoid arthritis, psoriasis, sepsis (Buechler et al., 2013) and inflammatory bowel disease (IBD) (Dige et al., 2014; Moller, 2012).

Crohn's disease (CD) and ulcerative colitis (UC) are the main IBD entities (Brown and Mayer, 2007; Gajendran et al., 2018). Although many studies have described factors associated with the pathogenesis, the etiology of IBD remains elusive (Lee et al., 2018). Intestinal macrophages in patients with IBD are exposed to cytokines such as TNF and bacterial toxins. The number of CD163-expressing macrophages was increased in the colonic lamina propria of IBD patients (Demetter et al., 2005; Franze et al., 2013; Tsuda et al., 2019). It was also observed that the CD163 abundance of peripheral blood cells was higher in IBD (Dige et al., 2014; Franze et al., 2013). Plasma sCD163 levels were also found to be induced in IBD patients compared to healthy controls (Dige et al., 2014). Treatment of IBD patients with anti-TNF antibodies caused a rapid decrease in circulating sCD163 levels, which was not associated with the clinical response as assessed by fecal calprotectin levels and the Truelove and Witts criteria (Dige et al., 2014).

Primary sclerosing cholangitis (PSC) is a rare disease associated with IBD, and about 70% of PSC patients have IBD (PSC-IBD) (Mertz et al., 2019). The precise pathogenic mechanisms of PSC-IBD remain unknown but likely involve gut dysbiosis, disturbed bile acid homeostasis and aberrant activation of the immune system (Liang et al., 2017; Mertz et al., 2019). This progressive disease is characterized by destruction of the bile ducts, leading to cholestasis, liver fibrosis, and ultimately to liver cirrhosis. Diagnosis of PSC is challenging (Pria et al., 2022) and annual liver function screening for PSC in IBD patients, regardless of symptoms, is recommended (Mertz et al., 2019).

Urinary proteins and metabolites are emerging as biomarkers for various diseases (Tews et al., 2023). The potential of urinary sCD163 as a biomarker for renal function was described in lupus nephritis (Gupta et al., 2021) and IgA nephropathy (Gong et al., 2021). Renal manifestations occur in about 6% of patients with IBD (Ambruzs and Larsen, 2018; Dincer et al., 2022). IBD patients with renal involvement had lower glomerular filtration rate and higher serum creatinine in comparison to IBD patients without renal disease (Dincer et al., 2022). To our knowledge, associations between urinary sCD163 and laboratory measures of renal function have not been analyzed in IBD.

An established fecal biomarker for IBD is calprotectin, which is derived from neutrophil granulocytes, and correlates with disease activity assessed by endoscopy (Alghoul et al., 2022). This protein is an indicator of mucosal inflammation and is not a specific biomarker for IBD (Chassaing et al., 2012; Moein et al., 2017; Sands, 2015). Accordingly, there is a need to identify additional biomarkers in stool and/or urine for confirming the diagnosis, predicting the course and for monitoring therapeutic response of IBD patients.

Here, sCD163 was measured in urine and serum of patients with IBD and PSC. Urinary and serum sCD163 were similar in IBD patients and controls. Of clinical relevance, urinary and serum sCD163 were elevated in PSC, a disease associated with IBD (Lazaridis and LaRusso, 2016).

2. Materials and methods

2.1. Patients

Patients with IBD or PSC diagnosis from the out / inpatient clinic at the Department of Internal Medicine I (University Hospital of Regensburg) were recruited from December 6, 2021 to January 31, 2023. IBD and PSC were diagnosed based on histologic, endoscopic, and clinical criteria (EASL, C. P. G. o. s. c, 2022; Kucharzik et al., 2019; Sturm et al., 2019). The patients were actually treated with corticosteroids (14 patients), mesalazine (20 patients), anti-interleukin 12/23 antibodies (18 patients), anti-TNF antibodies (18 patients) and azathioprine (8 patients). The 21 PSC patients enrolled in our study were treated with ursodeoxycholic acid. Patients who had coagulopathy were not included in the study. Serum (10 mL) and spot-urine (10 mL) of patients as well as controls were collected at the same time, aliquoted and stored at −80 °C until use. Serum and urine samples for each patient and control were collected at different times between 8 am and 4 pm. Controls for this retrospective case-control study were students, hospital staff and partners of the patients. The controls were healthy and lived in the same area as the patients. The 16 patients with chronic liver diseases included 4 patients with primary biliary cholangitis, 8 patients with chronic hepatitis B virus infection, 1 patient with non-alcoholic steatohepatitis, 1 patient with IgG4 related cholangiopathy and 2 patients with autoimmune hepatitis. The study groups are described in Table 1.

2.2. Enzyme-linked immunosorbent assays (ELISAs)

The ELISA to measure urinary sCD163 was from EUROIMMUN (Cat. No.: EQ6851–9601-U; Lübeck, Germany), and urine was used undiluted. The coefficient of variation (provided by the manufacturer) for the intra-assay precision was 2.5, 2.5 and 4.0%, and for the inter-assay precision 3.9, 6.0 and 6.4%. This assay is an *in vitro* diagnostic tool used to assist in diagnosing acute renal diseases.

For analysis of serum sCD163, serum was diluted 1:10 fold in Reagent Diluent (1% BSA in PBS, pH 7.2–7.4, 0.2 μm filtered) as recommended by the provider of the ELISA (Cat. No.: DC1630; R&D Systems, Wiesbaden-Nordenstadt, Germany). The coefficient of variation

Table 1
Characteristics of the study groups. The PSC cohort includes 15 patients with PSC and IBD and 6 patients with PSC without underlying IBD. The IBD cohort does not include patients with PSC. Patients with chronic liver disease (CLD) do 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 and CLD patients. Statistical test used: Kruskal-Wallis Test (alanine aminotransferase (ALT), alkaline phosphatase (AP), aspartate aminotransferase (AST), gamma glutamyl transferase (gamma GT), glomerular filtration rate (GFR), not determined (n.d.)). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for the comparison of IBD and PSC patients, && $p < 0.01$ for the comparison of PSC and CLD patients.

Characteristics	IBD	PSC	Controls	CLD
Number (females / males)	63 (29 / 34)	21 (8 / 13)	18 (11 / 7)	16 (11 / 5)
Age (years)	44.3 (19.1–69.9)	51.1 (18.2–63.0)	45.0 (22.9–78.1)	59.5 (29.0–72.0)
BMI (kg/m^2)	24.3 (15.5–44.3)	24.9 (16.3–41.8)	n.d.	26.0 (20.0–34.0)
C-reactive protein (mg/L)	2 (0–144)	3 (0–26)	n.d.	2 (1–15)
Creatinine (mg/dL)	0.83 (0.51–1.14)	0.86 (0.60–1.43)	n.d.	0.86 (0.57–1.39)
GFR (mL/min)	100 (67–136)	96 (56–135)	n.d.	76 (49–121)
Fecal calprotectin (μg/g)	45 (0–3889)	35 (0–999)	n.d.	n.d.
AST (U/L)	25 (10–41) *	27 (15–161) *	n.d.	30 (14–45)
ALT (U/L)	20 (7–63)	25 (5–205)	n.d.	26 (11–51)
Gamma GT (U/L)	24 (8–100) *	46 (10–458) *	n.d.	41 (8–282)
AP (U/L)	64 (38–142) ***	107 (35–587) ***	n.d.	96 (54–123)
Bilirubin (mg/dL)	0.45 (0.10–1.90) **	0.60 (0.30–4.30) ** &&	n.d.	0.40 (0.30–0.70) &&
MELD Score	n.d.	6 (6–12)	n.d.	7 (5–10)

(provided by the manufacturer) for the intra-assay precision was 3.4, 3.5 and 3.8%, and for the inter-assay precision 4.1, 4.6 and 6.7%.

Urinary levels of sCD163 using the ELISA from EUROIMMUN were similar to the levels reported by Nielsen et al. (Nielsen et al., 2021) and Zhang et al. (Zhang et al., 2020) using different ELISAs. Our serum sCD163 levels are in the range of other studies (Feng et al., 2012; Sakumura et al., 2018). It was not possible to test whether the two ELISAs deliver identical values because these assays had been established for urine and serum, respectively.

Creatinine in urine was measured by the creatinine parameter assay kit (Cat. No.: KGE005; R&D Systems). For analysis, urine was diluted 1:20 fold. The coefficient of variation (provided by the manufacturer) for the intra-assay precision was 3.2, 3.2 and 3.5%, and for the inter-assay precision 4.0, 5.3 and 5.5%. All samples were measured in duplicate and the mean values were used for calculations.

2.3. Stool collection and fecal bile acid analysis

Feces were collected in 70% isopropanol and stored at −80 °C until use. Fecal samples were homogenized in a gentleMACS™ dissociator (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). The dry weight of the homogenate was determined by drying 1.0 ml of the mixture in a vacuum centrifuge. For further analysis, the raw fecal homogenates were diluted to a final concentration of 2.0 mg dry weight/mL. Fecal bile acids were quantified by LC-MS/MS using a modified serum stable isotope dilution analysis method (Krautbauer et al., 2016; Scherer et al., 2009). The primary bile acids cholic acid (CA), its glycine and taurine conjugates (GCA and TCA), and chenodeoxycholic acid (CDCA) and its conjugated forms GCDCA and TCDCA were measured. The secondary bile acids analyzed are deoxycholic acid (DCA), lithocholic acid (LCA), ursodeoxycholic acid (UDCA) and hyodeoxycholic acid (HDCA) and the corresponding glycine and taurine conjugates.

2.4. Statistical analysis

Data are shown as boxplots and outliers are marked as circles and asterisks. Mann Whitney *U* test, Kruskal-Wallis Test, receiver operating characteristic (ROC) curve and Spearman correlation were the statistical tests used (SPSS Statistics 26.0 program, IBM, Leibniz Rechenzentrum, München, Germany). A value of $p < 0.05$ was regarded as significant.

3. Results

3.1. Urinary sCD163 of patients with IBD and controls

Urinary sCD163 levels were measured in 18 controls, 63 patients with IBD (this cohort does not include PSC patients), 21 patients with PSC and 16 patients with chronic liver disease (CLD) other than PSC. Urinary sCD163 levels were normalized to urinary creatinine concentrations. Creatinine in the urine of PSC and IBD patients was similar ($p = 0.109$). The characteristics of the cohorts are summarized in Table 1. PSC patients had higher levels of aspartate aminotransferase (AST), gamma glutamyl transferase (gamma GT), alkaline phosphatase and bilirubin compared to IBD patients. Bilirubin was elevated in PSC patients compared to CLD patients with similar MELD scores (Table 1).

Females and males had similar levels of urinary sCD163 ($p = 0.741$ for IBD, $p = 0.186$ for PSC, and $p = 0.111$ for controls; Fig. 1A). Urinary sCD163 did not correlate with age in the control cohort ($r = 0.371$, $p = 0.129$), in the PSC cohort ($r = 0.123$, $p = 0.587$) and the IBD cohort ($r = 0.057$, $p = 0.656$). Body mass index (BMI) was only documented for the patients and was not related to urinary sCD163 of IBD patients ($r = 0.086$, $p = 0.523$) and PSC patients ($r = 0.161$, $p = 0.618$).

Urinary sCD163 of controls and patients with IBD was comparable and did not differ between CD and UC (Fig. 1B). Urinary levels of sCD163 of the 18 controls were 13.19 (0–48.74) ng/g, of the 42 patients with CD were 11.01 (0–92.59) ng/g and of the 21 UC patients were 12.33 (0–62.15) ng/g.

Urinary sCD163 levels of PSC patients were 28.96 (3.34–489.51) ng/g and were increased in comparison to healthy controls and IBD patients (Fig. 1C). Urinary sCD163 of the patients with CLD was 0 (0–218.31) ng/g and was lower in comparison to PSC patients (Fig. 1C). The 2 PSC patients with very high urinary sCD163 levels (Fig. 1C) had very low creatinine in urine. PSC patients where disease etiology was not associated with IBD and PSC-IBD patients had similar levels of urinary

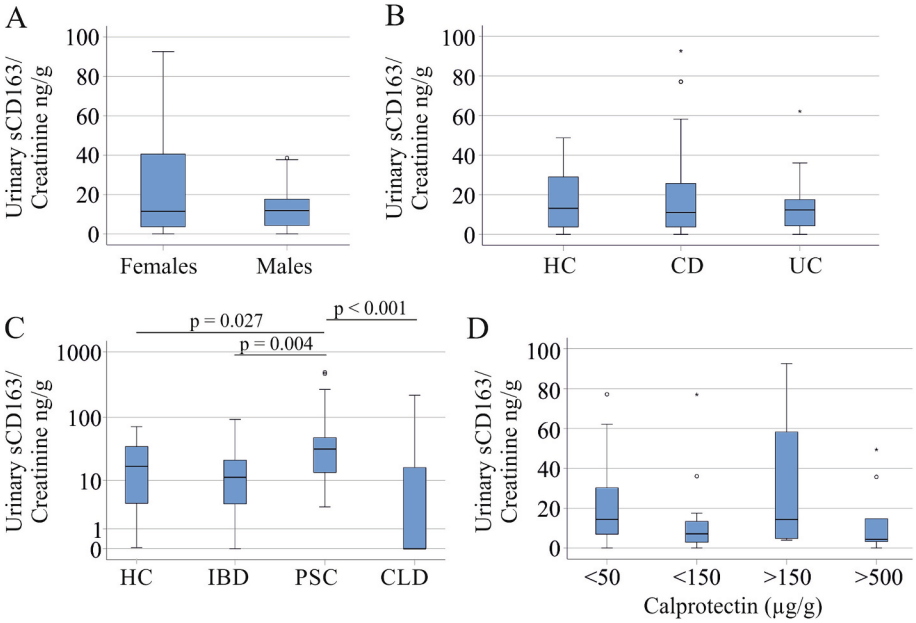

Fig. 1. Urinary sCD163 of inflammatory bowel disease (IBD) patients, primary sclerosing cholangitis (PSC) patients, chronic liver disease (CLD) patients and healthy controls (HC). A) Urinary sCD163 of female and male IBD patients. Statistical test used: Mann Whitney *U* test; B) Urinary sCD163 of healthy controls (HC), patients with Crohn's disease (CD) and patients with ulcerative colitis (UC). Statistical test used: Kruskal-Wallis Test; C) Urinary sCD163 levels of HC, IBD, PSC and CLD patients. Data are shown in a logarithmic scale. Statistical test used: Kruskal-Wallis Test; D) Urinary sCD163 of IBD patients stratified for fecal calprotectin. Statistical test used: Kruskal-Wallis Test. Small circles and asterisks in the figures indicate outliers.

sCD163 ($p = 0.606$).

3.2. Correlation of urinary sCD163 with markers of inflammation and renal function

Renal impairment is a relative common extra-intestinal manifestation of IBD (Dincer et al., 2022). In the IBD cohort, urinary sCD163 did not correlate with creatinine and glomerular filtration rate (GFR), which are clinical markers of renal function (Levey et al., 1988). C-reactive protein (CRP) levels in serum and fecal calprotectin levels were not related to urinary sCD163 (Table 2). Urinary sCD163 did not significantly correlate with creatinine, GFR, and CRP levels in serum or fecal calprotectin in PSC (Table 2).

Stratification of IBD patients (the cohort of PSC patients was too small for this analysis) for their fecal calprotectin levels revealed that urinary sCD163 did not vary between these groups ($p = 0.276$; Fig. 1D). Here, 31 patients had fecal calprotectin levels <50 μg/g, 14 patients levels <150 μg/g, 6 patients levels >150 μg/g and 9 patients levels >500 μg/g (data of 3 patients were not documented).

3.3. Serum sCD163 of patients with IBD and controls

A previous study has shown higher sCD163 in serum of patients with IBD (Dige et al., 2014), and thus serum sCD163 of our study cohort was also measured. Median serum sCD163 levels were about 450 ng/mL and 4500-fold higher in contrast to urinary levels with a median concentration of 0.1 ng/mL.

Serum of 51 IBD patients, of 13 patients with PSC, of 16 patients with CLD and 12 controls was available for this analysis. Serum sCD163 did not differ between IBD patients and controls ($p = 0.234$). The 34 CD and the 17 UC patients had similar serum sCD163 levels ($p = 0.484$) (Fig. 2A). Serum sCD163 was, however, increased in PSC as well as CLD patients compared to IBD patients (Fig. 2B). Controls, PSC patients and CLD patients had similar levels of serum sCD163 (Fig. 2B). PSC patients whose etiology was not associated with IBD had higher serum sCD163 levels than PSC IBD patients ($p = 0.056$).

Serum sCD163 positively correlated with BMI in the IBD cohort ($r = 0.368$, $p = 0.011$) but not in the PSC patients ($r = -0.291$, $p = 0.385$). Age was not related to serum sCD163 levels in IBD ($r = 0.148$, $p = 0.300$), PSC ($r = -0.005$, $p = 0.985$) and in controls ($r = 0.371$, $p = 0.129$). The 23 females and the 28 males with IBD had comparable sCD163 serum levels ($p = 0.297$). In IBD and PSC, serum sCD163 was positively related to serum CRP but did not correlate with serum creatinine and GFR (Table 3). Fecal calprotectin was positively associated with serum sCD163 in IBD (Table 3).

Stratification of IBD patients for their fecal calprotectin levels showed that serum sCD163 did not vary between these groups (Fig. 2C). Here, 25 patients had fecal calprotectin <50 μg/g, 13 patients levels <150 μg/g, 6 patients levels >150 μg/g and 7 patients levels >500 μg/g.

Serum and urinary sCD163 levels did not correlate with each other in the control cohort ($r = 0.437$, $p = 0.156$), the PSC patients ($r = 0.000$, $p = 1.000$), and the group of IBD patients ($r = -0.224$, $p = 0.126$).

3.4. Serum and urinary sCD163 and current medication

A recent study showed a decrease in serum sCD163 with anti-TNF therapy, while corticosteroids had no effect (Dige et al., 2014). In the IBD group there were 14 (13 for serum) patients treated with glucocorticoids. Serum sCD163 ($p = 0.598$) and urinary sCD163 ($p = 0.363$) did not differ among patients that were treated or not treated with corticosteroids. The 18 patients receiving anti-TNF antibodies had urinary sCD163 ($p = 0.125$) and serum levels (13 treated patients, $p = 0.294$) as patients without this therapy.

Furthermore, mesalazine treatment (20 patients) was not associated with altered sCD163 in urine ($p = 0.184$) or serum ($p = 0.499$, 14 patients treated). Anti-interleukin 12/23 antibody therapy (18 patients), and azathioprine (8 patients) treatment were not associated with

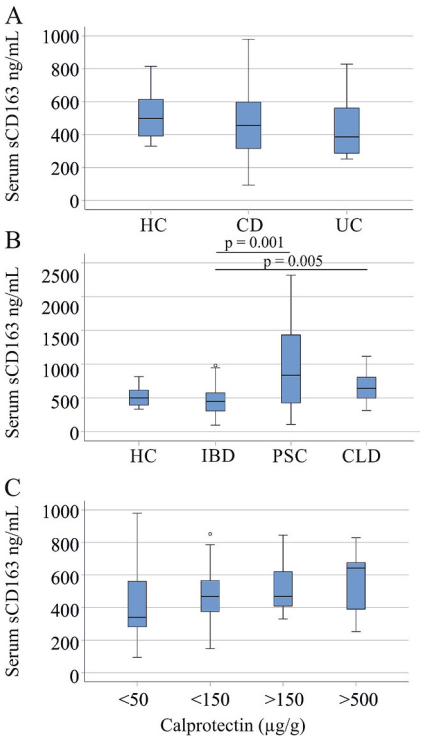

Fig. 2. Serum sCD163 levels of inflammatory bowel disease (IBD) patients, primary sclerosing cholangitis (PSC) patients, chronic liver disease (CLD) patients and healthy controls (HC). A) Serum sCD163 of healthy controls (HC), patients with Crohn's disease (CD) and patients with ulcerative colitis (UC). Statistical test used: Kruskal-Wallis Test; B) Urinary sCD163 levels of HC, IBD, PSC and CLD patients. Statistical test used: Kruskal-Wallis Test; C) Serum sCD163 of IBD patients stratified for fecal calprotectin levels. Statistical test used: Kruskal-Wallis Test. Small circles and asterisks in the figures indicate outliers.

Table 3
Spearman correlation of serum sCD163 with creatinine, glomerular filtration rate, C-reactive protein, and fecal calprotectin in IBD and PSC.

Correlation	Creatinine	Glomerular Filtration Rate	C-reactive Protein	Fecal Calprotectin
IBD	$r = 0.194$ $p = 0.173$	$r = -0.084$ $p = 0.559$	$r = 0.308$ $p = 0.031$	$r = 0.243$ $p = 0.085$
PSC	r = 0.064 $p = 0.853$	$r = -0.256$ $p = 0.422$	$r = 0.599$ $p = 0.040$	$r = 0.162$ $p = 0.615$

Table 2
Spearman correlation of urinary sCD163 with creatinine, glomerular filtration rate, C-reactive protein, and fecal calprotectin in IBD and PSC.

Correlation	Creatinine	Glomerular Filtration Rate	C-reactive Protein	Fecal Calprotectin
IBD	$r = -0.098$ $p = 0.447$	$r = -0.024$ $p = 0.851$	$r = -0.175$ $p = 0.184$	$r = -0.173$ $p = 0.180$
PSC	$r = 0.073$ $p = 0.805$	$r = -0.368$ $p = 0.161$	$r = 0.207$ $p = 0.443$	$r = 0.064$ $p = 0.827$

Table 4
Bile acid (BA) species levels in stool of IBD and PSC patients. All bile acid concentrations are given in nmol/g dry weight. Primary bile acids: cholic acid (CA), its glycine and taurine conjugates (GCA and TCA), and chenodeoxycholic acid (CDCA) and its conjugated forms GCDCA and TCDCA were measured. Secondary bile acids: deoxycholic acid (DCA), lithocholic acid (LCA), ursodeoxycholic acid (UDCA) and hyodeoxycholic acid (HDCA) and the corresponding glycine and taurine conjugates. ** $p < 0.01$, *** $p < 0.001$.

	IBD			PSC			p
	Median	Min	Max	Median	Min	Max	
CA	616.96	0.00	73,855.29	429.50	0.00	10,144.54	
GCA	94.18	0.97	33,064.58	45.55	4.95	1977.18	
TCA	27.08	0.00	5398.21	6.84	0.00	1321.55	
CDCA	665.53	0.00	38,667.17	689.73	0.00	9513.81	
GCDCA	78.80	0.00	23,116.35	51.96	0.00	1672.43	
TCDCA	0.00	0.00	2838.87	0.00	0.00	1048.52	
DCA	3700.87	0.00	47,847.41	154.06	0.00	21,805.01	
GDCA	21.91	0.00	634.22	4.62	0.00	158.11	
TDCA	2.46	0.00	2802.98	0.00	0.00	50.21	**
LCA	4560.40	0.53	18,423.33	491.16	34.04	70,643.23	
GLCA	3.30	0.00	26.91	2.15	0.00	70.44	
TLCA	1.45	0.00	86.29	0.00	0.00	30.04	
UDCA	221.13	0.00	15,442.02	5713.92	5.99	41,507.24	***
GUDCA	0.00	0.00	510.89	118.96	0.00	6313.30	***
TUDCA	1.28	0.00	384.18	17.03	0.00	1750.98	***
HDCA	273.36	0.00	6008.50	246.69	0.00	1386.33	
GHDCA	0.54	0.00	47.15	0.00	0.00	3.49	
THDCA	0.47	0.00	45.20	0.23	0.00	12.49	
Total BA	18,379	173	116,771	25,754	299	96,588	

changes of urinary or serum sCD163 (data not shown).

3.5. Serum and urinary sCD163 and fecal bile acids

All 21 PSC patients were treated with ursodeoxycholic acid. Accordingly, PSC patients had higher UDCA, GUDCA and TUDCA in stool compared to IBD patients (Table 4, fecal bile acid levels of IBD patients have been published by our group before (Sommersberger et al., 2023)). Taurine conjugated deoxycholic acid levels of PSC patients were lower in comparison to IBD (Table 4). This difference was also observed when patients with IBD and PSC-IBD patients were compared ($p = 0.004$). Fecal bile acid levels in patients with PSC without underlying IBD and PSC-IBD were similar ($p > 0.05$ for all).

Urinary sCD163 negatively correlated with glycohyodeoxycholic acid in IBD ($r = -0.456$, $p = 0.010$). In PSC, sCD163 in urine and serum were not associated with fecal bile acid species levels ($p > 0.05$ for all).

3.6. Correlation of urinary and serum sCD163 and parameters of liver function of patients with PSC

AST, ALT, gamma GT, AP, and bilirubin did not correlate with urinary or serum sCD163 levels in the IBD cohort (data not shown). Of clinical importance, urinary sCD163 was not associated with any of these liver parameters in PSC (Table 5). Serum sCD163 of the PSC patients positively correlated with AST and bilirubin. Urinary and serum sCD163 were not related to the MELD score (Table 5).

Table 5
Spearman correlation of urinary and serum sCD163 with laboratory parameters of liver function in PSC patients. Alanine aminotransferase (ALT), alkaline phosphatase (AP), aspartate aminotransferase (AST), gamma glutamyl transferase (gamma GT), Model for End Stage Liver Disease (MELD).

Correlation	Urinary sCD163	Serum sCD163
AST (U/L)	$r = -0.118$, $p = 0.602$	$r = 0.571$, $p = 0.042$
ALT (U/L)	$r = -0.105$, $p = 0.643$	$r = 0.378$, $p = 0.203$
Gamma GT (U/L)	$r = 0.005$, $p = 0.982$	$r = 0.314$, $p = 0.296$
AP (U/L)	$r = -0.105$, $p = 0.642$	$r = 0.450$, $p = 0.123$
Bilirubin (mg/dL)	$r = 0.195$, $p = 0.383$	$r = 0.754$, $p = 0.003$
MELD Score	$r = -0.078$, $p = 0.743$	$r = 0.238$, $p = 0.456$

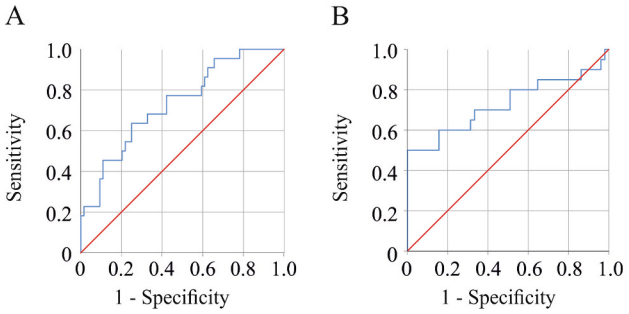

Fig. 3. Receiver operating characteristic (ROC) curve using data of IBD patients and PSC patients. A) Urinary sCD163; B) Serum sCD163.

3.7. Receiver operating characteristic curve of sCD163 for PSC

To evaluate the diagnostic power of urinary and serum sCD163 for PSC, receiver operating characteristic (ROC) curve using data of IBD patients and PSC patients was analyzed (Fig. 3). The area under the curve (AUC) for urinary sCD163 was 0.733 ($p = 0.001$) and for serum sCD163 was 0.728 ($p = 0.003$).

4. Discussion

This study provides evidence for urinary sCD163 as a potential non-invasive biomarker for PSC. Monitoring of urinary sCD163 in IBD may be used to detect development of PSC in IBD.

Though macrophages are essential players in IBD (Franze et al., 2013; Vavricka and Rogler, 2009) there are to the best of our knowledge only two studies that have measured serum sCD163 in IBD. One of these studies described higher sCD163 in patients with IBD compared to patients with irritable bowel syndrome. However, levels of sCD163 did not differentiate patients with active disease from patients in clinical remission (Caviglia et al., 2020). Further, Dige et al. showed increased sCD163 in active IBD in comparison to healthy controls, and patients' sCD163 levels were approximately 33% higher (Dige et al., 2014). However, our analysis could not identify significant differences in

sCD163 levels among healthy controls and IBD patients. Of note, patients with high fecal calprotectin had about 29% more serum sCD163 in contrast to healthy controls. Because the number of patients with highly active disease was small in our cohort, this elevation of sCD163 was not significant. Current evidence thus suggests that sCD163 in the circulation is modestly increased in active IBD in comparison to healthy controls. There is also consent that serum sCD163 is not a marker of disease activity for IBD patients (Caviglia et al., 2020; Dige et al., 2014). Contrary to the observation of this previous study, which did not observe a correlation of serum sCD163 and CRP (Dige et al., 2014), serum sCD163 was positively associated with CRP in our study.

Corticosteroids have no effect on sCD163 abundance at the cellular level (Nielsen et al., 2019) and IBD patients treated or not treated with these drugs had comparable sCD163 levels. The fact that one week of prednisolone treatment did not reduce sCD163 levels (Dige et al., 2014) is in line with this observation. Patients receiving anti-TNF antibody therapy, mesalazine, azathioprine or anti-interleukin 12/23 antibody therapy had urinary and sCD163 levels comparable to patients on other therapies. It has to be noted that all of our patients were treated. Therefore, effects of individual medications but not the outcome in disease severity were analyzed.

Our analysis could not identify positive correlations between urinary and serum sCD163. This indicates that circulating sCD163 contributes little to urinary levels. Urinary sCD163 did not correlate with GFR and creatinine and is most likely not the result of impaired kidney function in IBD.

PSC is characterized by an early recruitment of macrophages to the biliary and peribiliary environment, which plays a pathogenic role in bile duct inflammation and liver fibrosis (Cadamuro et al., 2020). Our study showed that both serum as well as urinary sCD163 were elevated in patients with PSC compared to IBD patients. Levels also differed between PSC patients and controls, but this was only significant for urinary sCD163. Moreover, serum sCD163 levels of PSC patients tended to be higher than of PSC-IBD patients. Serum sCD163 did not differ between these two groups in the study published by Bossen et al. (Bossen et al., 2021). Therefore, further prospective analysis in larger cohorts are needed.

Serum sCD163 has been found to be induced in patients with chronic liver diseases (Gantzel et al., 2020). Accordingly, serum sCD163 of our patients with chronic liver disease other than PSC was increased. This suggests that higher serum sCD163 levels in PSC are associated with hepatic injury, and positive correlations with bilirubin and AST have been identified in our PSC cohort. Serum sCD163 has previously been reported to increase with disease severity in PSC, as assessed by liver enzyme levels and the enhanced liver fibrosis test (Bossen et al., 2021). Serum sCD163 correlated with CRP in PSC and IBD patients showing that it is also associated with systemic inflammation, which may limit its clinical utility.

Whether urinary sCD163 is a biomarker for progressive liver fibrosis has not been studied as far as we know. Our study showed that urinary sCD163 levels did not correlate with laboratory measures of liver function such as aminotransferases, bilirubin and the MELD score. This indicates that higher concentrations of sCD163 in urine of PSC patients are not related to these clinical measures of liver injury. Urinary sCD163 was not elevated in patients with chronic liver disease other than PSC, so urinary sCD163 seems to be specifically increased in PSC. In PSC, although not statistically significant, there was a moderate negative correlation between urinary sCD163 and GFR. The PSC patients with extremely high urinary sCD163 had low urinary creatinine. Thus, renal factors may play a role in high urinary sCD163. As our cohorts were small, these preliminary findings need to be confirmed in larger study groups.

Serum CRP and fecal calprotectin are non-invasive biomarkers of inflammation in IBD and are being used in clinical practice. These markers have proved useful in grading inflammation and in monitoring the response to therapy (Sands, 2015). CRP and fecal calprotectin are not specific for IBD and are also increased in infectious diseases and other inflammatory processes (Sands, 2015). Elevated serum sCD163 levels have been already described in various inflammatory diseases (Buechler et al., 2013; Moller, 2012), and further studies need to show whether this is also true for urinary sCD163. Current recommendation is to annually screen for PSC in IBD patients (Mertz et al., 2019). Continuous monitoring of urinary sCD163 may be used as an additional marker for non-invasive exclusion of PSC development. This has to be tested in well-designed prospective studies.

UDCA is prescribed for PSC patients but its role in treatment is still controversial (Vesterhus and Karlsen, 2020). Higher stool levels of UDCA and its conjugated derivatives in PSC compared to IBD patients are caused by UDCA treatment. Our study showed that the secondary bile acid TDCA was low in the stool of PSC patients compared to IBD patients. Otherwise, PSC and IBD patients had similar levels of primary and secondary bile acids in feces in accordance with previous studies (Torres et al., 2018; Vaughn et al., 2019). Although we have no explanation for the decrease in TDCA in PSC, our results rule out the use of fecal bile acids as a diagnostic tool for PSC.

Secondary bile acids have been described to modulate programming of pro- and anti-inflammatory macrophages (Wammers et al., 2018; Wang et al., 2020). In our PSC cohort, sCD163 in urine and serum did not correlate with fecal bile acid species levels. Urinary sCD163 negatively correlated with fecal GHDCA in IBD, consistent with anti-inflammatory effects of HDCA (Zhu et al., 2022), but this needs further study.

Age may be a confounding factor for the use of sCD163 as a biomarker. Positive correlations of serum sCD163 with age have been described (Sporrer et al., 2009). Serum as well as urinary sCD163 did not correlate with age in controls, IBD and PSC patients. Serum sCD163 was also found to be increased in overweight / obesity (Fjeldborg et al., 2013; Sporrer et al., 2009; Zanni et al., 2012) and serum sCD163 positively correlated with BMI in the IBD cohort but not the PSC cohort. It has to be noted that serum and urinary sCD163 did not differ between sexes in the current cohorts in accordance with previous findings (Moller et al., 2003).

This study has several limitations. The PSC and CLD cohorts were relatively small and analysis of sCD163 in urine and serum of larger cohorts is required. This was a retrospective study and the diagnostic values of serum as well as urinary sCD163 have to be validated in prospective studies. This was, moreover, a monocentric study and multicenter studies are needed.

5. Conclusion

In conclusion, our analysis showed higher urinary and serum sCD163 levels in PSC compared with IBD patients without PSC. Serum sCD163 appears to be a marker of chronic liver disease, while urinary sCD163 is specifically elevated in PSC. The mechanisms are so far unexplained but warrants further studies.

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Ethics statement and consent to participate

The study was approved by the Ethics Committee of the University Hospital of Regensburg (Protocol No. 19–1309-101, Approval date: 20.02.2019 and Protocol No. 21–2390-101, Approval date: 19.05.2021) and all participants gave written informed consent to the study. The study was performed according to the updated guidelines of good clinical practice and updated Declaration of Helsinki.

CRediT authorship contribution statement

Tanja Elger: Writing – review & editing, Investigation. **Tanja Ferberger:** Writing – review & editing, Investigation. **Muriel Huss:** Writing – review & editing, Investigation. **Stefanie Sommersberger:** Writing – review & editing, Investigation. **Patricia Mester:** Writing – review & editing, Resources. **Petra Stoeckert:** Writing – review & editing, Resources. **Stefan Gunawan:** Writing – review & editing, Investigation. **Gerhard Liebisch:** Writing – review & editing, Formal analysis. **Johanna Loibl:** Writing – review & editing, Investigation. **Arne Kandulski:** Writing – review & editing, Conceptualization. **Martina Müller:** Writing – review & editing. **Christa Buechler:** Writing – review & editing, Writing – original draft, Conceptualization. **Hauke Christian Tews:** Writing – review & editing, Conceptualization.

Declaration of competing interest

Arne Kandulski (scientific presentations and scientific advisory activities): Roche Pharma AG, Eisai GmbH, Abbvie Germany AG, Janssen-Cilag GmbH, MSD Sharp & Dohme GmbH, Boston Scientific Corp., Fujifilm Germany, Micro-Tech Germany, Bayer Pharma AG Germany.

Hauke Christian Tews (scientific presentations and scientific advisory activities): Abbvie Germany AG, Janssen-Ciag GmbH, Celltrion, Bristol Myers Squibb, Pfizer Pharma GmbH.

Martina Müller (travel grants, scientific presentations): United European Gastroenterology, Abbvie Germany, Falk foundation, Germany.

Data availability

Data will be made available on request.

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