Circulating Biomarkers of Macrophage Activation in Different Stages of Chronic Pancreatitis

A Pilot Study

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Objectives: Activation of type M2 macrophages has been implicated in the pathogenesis of chronic pancreatitis (CP). In a clinical pilot study, we investigated blood-based markers of macrophage activation at different stages of CP.

Materials and Methods: We performed a cross-sectional analysis of prospectively collected plasma samples from healthy controls and patients with suspected or definitive CP according to the M-ANNHEIM criteria. Plasma concentrations of soluble CD163 (sCD163), soluble CD206 (sCD206), and monocyte chemoattractant protein-1 (MCP-1) were analyzed using enzyme-linked immunosorbent assays. Group and pairwise comparisons of analytes were performed using regression models and area under the receiver operating curves (AUC-ROC).

Results: In total, 73 subjects with CP (28 suspected CP and 45 definitive CP) and 40 controls were included. Compared to controls, the median plasma concentrations of sCD163 ($P = 0.019$) and sCD206 ($P = 0.033$) were elevated in patients with definitive CP. sCD206 was also elevated in patients with definitive CP ($P = 0.042$) compared to suspected CP. ROC analysis revealed the optimal sCD163 cutpoint to distinguish definitive CP from controls was 1.84 mg/mL (AUC-ROC 0.65; 95% confidence interval [CI], 0.54–0.77). The optimal sCD206 cutpoint to distinguish definitive CP from controls was 0.24 mg/mL (AUC-ROC 0.66; 95% CI, 0.54–0.78). MCP-1 concentrations showed no differences across subgroups.

Conclusion: Our study demonstrates that subjects with definitive CP, sampled during a clinically quiescent phase, exhibited increased levels of sCD163 and sCD206. This indicates the presence of activated M2 macrophages in patients with CP at advanced, but not early, clinical stages.

Key Words: macrophages, biomarkers, chronic pancreatitis, immunology

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Chronic pancreatitis (CP) is a progressive, fibro-inflammatory disease that causes irreversible fibrosis and destruction of the pancreatic tissue.[1] Its pathogenesis involves a complex interplay of genetic, environmental, and immune factors.[2] Many patients develop CP following recurrent acute pancreatitis (RAP) attacks.[3–5] Thus, CP progression is linked to ongoing or recurrent inflammation and increasing fibrogenesis, supporting the concept of CP as a continuous disease often evolving from acute pancreatitis (AP) to RAP and ultimately to probable and definitive CP.[6,7]

Preclinical and clinical pilot studies highlight the crucial role of immunological mechanisms in the development and course of CP.[8–11] During acute pancreatic inflammation, innate immune cells release proinflammatory mediators in response to damage-associated molecular patterns (DAMPS).[1,9] These proinflammatory mediators, like monocyte chemoattractant protein-1 (MCP-1), recruit monocytes that differentiate into classical macrophages (M1 polarization) induced by various immunological signals.[1,9,12–14] Importantly, an imbalance in the communication between innate immune cells and macrophages can lead to alternative activation of macrophages (M2 polarization).[9] M2-polarized macrophages, in turn, stimulate pancreatic stellate cells (PSC), which are central to the fibrogenesis associated with CP.[1,13] Activated PSCs not only promote fibrosis but also further enhance the M2 polarization of macrophages, creating a self-perpetuating feedforward loop.[13] Upon polarization, M2 macrophages express specific membrane-bound proteins like CD163 and CD206 on their cell surface.[14–17] M2 macrophages are subdivided into 4 subsets: M2a, M2b, M2c, and M2d macrophages.[18,19] M2a macrophages, which are involved in fibrogenesis,

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express CD206. In contrast, M2c macrophages, which play a role in the phagocytosis of apoptotic cells, express CD163.[18,19]

In various inflammatory conditions, activation of macrophages leads to cleavage of CD163 and CD206, yielding soluble forms (sCD163 and sCD206) that serve as systemic indicators of macrophage activation.[15–17] Elevation of sCD163 and sCD206 is proposed to be a marker of M2 polarized macrophages and indicates a profibrogenic milieu as opposed to the proinflammatory milieu typically associated with M1 macrophages.[20,21] Previous studies have shown that elevated plasma levels of sCD163 and sCD206 are present across a variety of chronic fibro-inflammatory conditions, including rheumatoid arthritis,[22–24] spondyloarthropathy,[25,26] multiple sclerosis,[27] Crohn's disease,[20] and chronic liver disease,[28] where they have been used as diagnostic and prognostic biomarkers. However, to our best knowledge, the soluble forms of CD163 and CD206 have not yet been investigated in CP.

Studying the pathophysiological mechanisms of inflammatory pancreatic diseases in humans is challenging due to the inaccessibility and risks of pancreatic tissue samples. Recent studies have shown that pancreatic inflammatory responses and immunological mechanisms can be characterized by analyzing immune signatures in the systemic circulation.[8,29–31] We hypothesize that patients with CP at different clinical stages (suspected vs definitive CP according to the M-ANNHEIM criteria) have distinct concentrations of systemic macrophage-specific biomarkers. Our study aimed to explore serum concentrations of sCD163, sCD206, and MCP-1 at different stages of CP, using control subjects as a reference group.

MATERIALS AND METHODS

We conducted a phase 1 exploratory, cross-sectional study following the PROBE standards for the design of diagnostic and prognostic biomarker studies.[31,32] This study investigated plasma concentrations of sCD163, sCD206, and MCP-1 in subgroups of patients with CP and in control subjects. Human samples were obtained from the biorepository of the "Characterisation of the fibro-inflammatory process involved in progression from acute to chronic pancreatitis (PanFib)" study, which is a multicenter, prospective cohort study.[33] The study was approved by the Danish Data Protection Agency (VD-2018–298; I-suite no.: 6542) and the Regional Committee on Health Research Ethics (Journal-no.: H-18017705) and was conducted in compliance with the Declaration of Helsinki.

Study Population

The patient population included 2 CP subgroups classified according to the M-ANNHEIM system: suspected CP (n = 28) and definitive CP (n = 45).[7] The suspected CP group included patients with RAP, excluding cases induced by gallstones, and patients with probable CP as defined by the M-ANNHEIM criteria.[7] RAP was characterized by 2 or more episodes of AP, diagnosed according to the revised Atlanta Criteria.[34] Probable CP was defined as patients with a typical history of CP (persistent abdominal pain and/or a history of a single AP episode) along with any of the following: mild pancreatic duct changes (Cambridge I or II), recurrent or persistent pseudocysts, abnormal exocrine pancreatic function, or postpancreatitis diabetes mellitus (HbA1c > 48 mmol/L 3 months or later after pancreatitis diagnosis).[7] Definitive CP was also diagnosed using the M-ANNHEIM criteria, requiring patients to meet at least one of the following definitive CP criteria: pancreatic calcifications, moderate to severe pancreatic duct changes (Cambridge III or IV), or typical histological features of CP.[7,33]

To prevent any potential confounding of macrophage biomarker expression, subjects with either of the following medical conditions were excluded: chronic liver disease, renal failure, malignancy, chronic inflammatory bowel syndrome, chronic obstructive lung disease, or pulmonary fibrosis. Further, subjects treated with long-term anti-inflammatory medication, locally or systemically, at the time of inclusion were excluded. Additionally, pregnant women and patients for whom MRI was contraindicated were excluded.

Forty controls with no history of pancreatic or gastrointestinal diseases were included as a reference group. Supplementary Table 1, http://links.lww.com/MPA/B316, provides a detailed overview of the distribution of diagnostic criteria for the 3 study subgroups.

Demographics, Anthropometrics, and Clinical Parameters

Clinical and demographic variables were derived from an electronic study database (RedCAP version 13.7.14). Variables included gender, age, body mass index (BMI), smoking history, history of excessive alcohol consumption, etiological risk factors, pancreatic exocrine insufficiency (PEI), diabetes, glycated hemoglobin (HbA1c), and information on other comorbidities. Age was categorized into 3 groups: (i) < 40, (ii) 40–60, and (iii) > 60 years. BMI was categorized into 4 BMI groups: (i) < 18.5 kg/m^2, (ii) 18.5–24.9 kg/m^2, (iii) 25.0–29.9 kg/m^2, and (iv) > 30 kg/m^2. Smoking and excessive alcohol consumption were categorized as current, previous, and never.[35–38] Total smoking pack years were calculated by multiplying the average number of daily cigarette packs (assuming 20 cigarettes per package) by the duration of smoking. The total years with excessive alcohol consumption were defined as the number of years consuming more than 14 units per week for men and more than 7 units per week for women.

PEI was defined based on the presence of fecal elastase < 100 μg/g stool.[33] The presence of diabetes was determined by reviewing medical records and elevated HbA1c ≥ 6.5%. Comorbidities were quantified using the Charlson Comorbidity Index (CCI).[39] The CCI scores were categorized into 3 groups: "No comorbidities" (CCI = 0), "Mild comorbidity" (CCI = 1–2), and "Moderate to severe comorbidity" (CCI ≥ 3).

Magnetic Resonance Imaging

Upon inclusion in the PanFib study, all subjects (CP patients and controls) underwent magnetic resonance imaging (MRI) of the pancreas to determine the presence of inclusion criteria as outlined above. The modified Cambridge score was used to assess the degree of ductal pathology in compliance with the M-ANNHEIM criteria.[7,40]

Blood Samples and Analysis of Macrophage-Specific Biomarkers

Plasma samples were drawn during a clinically quiescent phase. Consequently, an AP flare or exacerbation in CP-related symptoms requiring hospital admission led to

TABLE 1. Demographic and Clinical Characteristics of Controls and Chronic Pancreatitis (CP) Subgroups

	Controls (n = 40)	Suspected CP (n = 28)	Definitive CP (n = 45)	*P*
Male sex, n (%)	23 (58)	19 (68)	32 (71)	0.399
Age, y (SD)	50.9 (15.6)	42.0 (15.6)*	54.2 (15.0)§	0.005
Age group, n (%)				
< 40 y	11 (28)	15 (54)	9 (20)	0.049
40–60 y	14 (35)	8 (29)	20 (44)	
> 60 y	15 (38)	5 (18)	16 (36)	
BMI, kg/m^2 (IQR)	24.7 (22.1–27.6)	26.1 (24.2–29.6)*	24.1 (22.0–26.5)¶	0.039
BMI category, n (%)#				
< 18.5	0	1 (4)	1 (2)	0.218
18.5–24.9	21 (58)	9 (32)	24 (56)	
25.0–29.9	11 (31)	12 (43)	15 (35)	
> 30	4 (11)	6 (21)	3 (7)	
Smoking history, n (%)#				
Current	2 (5)	6 (21)	19 (43)‡,‖	< 0.001
Previous	14 (35)	8 (29)	19 (43)	
Never	24 (60)	14 (50)	6 (14)	
Smoking pack years, y (IQR)#				
Current	14.6 (1.8–27.5)	15.0 (10.5–17.5)	34.5 (20.4–40.0)	0.069
Previous	7.5 (3.5–12.8)	23.5 (2.4–34.6)	30 (10.5–40.0)†	0.016
Excessive alcohol consumption, n (%)#				
Current	1 (3)	0†	2 (5)‡,§	< 0.001
Previous	1 (3)	3 (11)	15 (34)	
Never	38 (95)	25 (89)	27 (61)	
Excessive alcohol, y (IQR)#				
Current	0	0	19 (9–29)	
Previous	34 (34–34)	20 (5–20)	22 (9–29)	0.147
Etiological risk factor, n (%)**				
Toxic		8 (29)	35 (78)	< 0.001
Idiopathic or hereditary		12 (43)	13 (29)	0.311
Immunological		0	0	1.000
Other		14 (50)	18 (40)	0.471
Metabolic complications				
Diabetes, n (%)#	0	2 (7)	14 (31)‡,§	< 0.001
PEI, n (%)#	0	6 (21)†	33 (73)‡,¶	< 0.001
Charlson comorbidity category, n (%)				
0	37 (93)	23 (82)	27 (60)†,§	0.002
1–2	3 (8)	4 (14)	17 (38)	
> 2	0	1 (4)	1 (2)	
Blood samples				
CRP, mg/L (IQR)#	0.9 (0.5–4.1)	2.2 (0.7–3.8)	1.7 (0.9–2.5)	0.155
Pancreas-specific amylase, U/L (IQR)#	26.5 (22.5–33.5)	33.0 (20.0–53.0)	15.0 (9.0–27.0)	< 0.001
HbA1c, mmol/mol (IQR)				
Diabetes		65 (64–66)	62 (52–81)	0.937
No diabetes		38 (33–46)	39 (36–43)	0.546

Due to rounding, the total percentage may deviate slightly from 100%.
*Statistically significant difference between control subjects and CP subgroups: $P < 0.05$.
†Statistically significant difference between control subjects and CP subgroups: $P < 0.01$.
‡Statistically significant difference between control subjects and CP subgroups: $P < 0.001$.
§Statistically significant difference between suspected and definitive CP: $P < 0.05$.
‖Statistically significant difference between suspected and definitive CP: $P < 0.01$.
¶Statistically significant difference between suspected and definitive CP: $P < 0.001$.
#Missing data for the following variables: BMI (n = 6), smoking history (n = 1), excessive alcohol consumption (n = 1), excessive alcohol years (n = 1), diabetes (n = 2), EPI (n = 1), CRP (n = 13), amylase (n = 9), HbA1c (n = 9), and smoking pack years (n = 1).
**Subjects may have multiple etiological risk factors and use multiple analgesics.
BMI indicates body mass index; CP, Chronic pancreatitis; CRP, C-reactive protein; HbA1c, glycated hemoglobin; IQR, Interquartile range; PEI, Pancreatic exocrine insufficiency; SD, Standard deviation.

a quarantine period of 4 weeks before blood was drawn.[33] The resolution of pancreatitis was documented by the assessment of C-reactive protein (CRP) and plasma amylase,[33] analyzed using standard routine biochemical testing. Samples for macrophage biomarkers were stored at −80°C until analysis.

Plasma sCD163 and sCD206 were analyzed in duplicate samples of plasma using in-house sandwich enzyme-linked immunosorbent assays (ELISAs) on a BEP2000 ELISA analyzer (Dade Behring, Marburg, Germany), essentially as described.[40,41] For sCD163, Rabbit anti-CD163 (1.5 mg/L) was coated onto microtiter wells, and diluted samples (serum, 1:201) were added in duplicates and incubated for 1.5 hours at 37°C. After washing, biotinylated monoclonal anti-CD163 (Clone Mac2-158, IQ-products, 0.100 µg/mL) was added and incubated for 1 hour at 37°C. After washing, plates were incubated with Avidin/HRP (Sigma cat. no. A7419) and Lysozyme (Sigma,

cat. no. L6876-5G) for 1 hour. Serum standards ranging from 6.25 to 200 µg/L, and human control samples were run on every plate. Human normal control samples were from the Danish Institute for External Quality Assurance for Laboratories in the Health Sector (DEKS) and run on every plate. For sCD206, Goat anti-CD206 (0.8 mg/L) was coated onto microtiter wells, and diluted samples (serum, 1:51) were added in duplicates and incubated for 1.5 hours at 37° C. After washing, biotinylated monoclonal anti-CD206 (Clone 7-450, Acris antibodies, 0.083 µg/mL) was added and incubated for 1 hour at 37°C. After washing, plates were incubated with Avidin/HRP (Sigma cat. no. A7419) and Lysozyme (Sigma, cat. no. L6876-5G) for 1 hour. Recombinant CD206 standards ranging from 0.94 to 120 µg/L and human control samples were run on every plate. Human normal control samples were from the Danish Institute for External Quality Assurance for Laboratories in the Health Sector (DEKS) and run on every plate. The coefficients of variation was 4.0% and 6.9% for sCD163 (3 runs, n = 6) and sCD206 (3 runs, n = 6), respectively. The sCD163 calibrator was traceable to purified CD163, and the sCD206 calibrator was traceable to recombinant protein. sCD163 and sCD206 are robust to thawing, and stability has been verified at −20° C for at least 9 months.[40,41] The concentration of MCP-1 was analyzed by a commercially available ELISA (Biolegend, ELISA MAX Deluxe Set Human MCP-1/CCL2 kit) according to the manufacturer's instructions.

Statistical Analysis

The sample size was based on sample availability from the PanFib study.[33] Demographic and clinical data were presented as means (standard deviations [SD]), medians (interquartile range [IQR]), or count (frequencies [%]). Analysis of variance (ANOVA), Kruskal-Wallis test, Student's *t* test, Wilcoxon rank sum tests, and Fisher's exact test were used to investigate differences in demographic and clinical characteristics between subgroups, as appropriate. Box plots were used to visually present the data distributions across subgroups. Uni- and multivariate regression models were applied to examine differences in plasma concentrations of macrophage-specific biomarkers between subgroups. Multivariate models were used to adjust for potential confounders, including age, excessive alcohol consumption, smoking, and BMI, as these variables may influence levels of sCD163 and sCD206.[15,42–44] Trend analysis was performed to assess the linear-by-linear trend in the mean response scores of macrophage-specific biomarker levels across subgroups. The diagnostic accuracy, sensitivity, specificity, and posttest probabilities (likelihood ratios) of macrophage-specific biomarker levels for discriminating subgroups were calculated using the area under the receiver operating characteristics curve (AUC-ROC). The Youden method was applied to assess the empirical optimal cutpoint for discriminating subgroups.[45] A P value < 0.05 was considered statistically significant. Due to the exploratory nature of the study, P values were not adjusted for multiple comparisons. The software packages R Studio, Version 2023.09.1 + 494 (RStudio PBC, Boston, MA) and STATA, Version 18.0 (StataCorp LLC, College Station, TX) were used for the statistical analyses.

RESULTS

This analysis included 73 patients with CP (28 suspected CP and 45 definitive CP) and 40 controls.

TABLE 2. Median Plasma Concentrations of sCD163, sCD206, and MCP-1 in Controls and Chronic Pancreatitis (CP) Subgroups

				Controls vs Suspected CP		Controls vs Definitive CP		Suspected vs Definitive CP	
	Controls (n = 40)	Suspected CP (n = 28)	Definitive CP (n = 45)	Unadjusted *P* Value	Adjusted *P* Value	Unadjusted *P* Value	Adjusted *P* Value	Unadjusted *P* Value	Adjusted *P* Value
sCD163, mg/L (IQR)	1.66 (1.38–1.94)	2.04 (1.38–2.25)	1.90 (1.56–2.73)	0.112	0.208	**0.007**	**0.019**	0.169	0.301
sCD206, mg/L (IQR)	0.21 (0.16–0.25)	0.20 (0.18–0.24)	0.26 (0.20–0.33)	0.384	0.857	**0.006**	**0.033**	**0.005**	**0.042**
MCP-1, pg/mL (IQR)	280 (198–385)	256 (214–374)	286 (217–394)	0.489	0.799	0.298	0.647	0.305	0.472

Bold typeface indicates statistical significance between groups. Groupwise comparisons were adjusted for age, excessive alcohol consumption, and smoking.
CP indicates chronic pancreatitis; IQR, interquartile range; MCP-1, monocyte chemoattractant protein-1; sCD163, soluble CD163; sCD206, soluble CD206.

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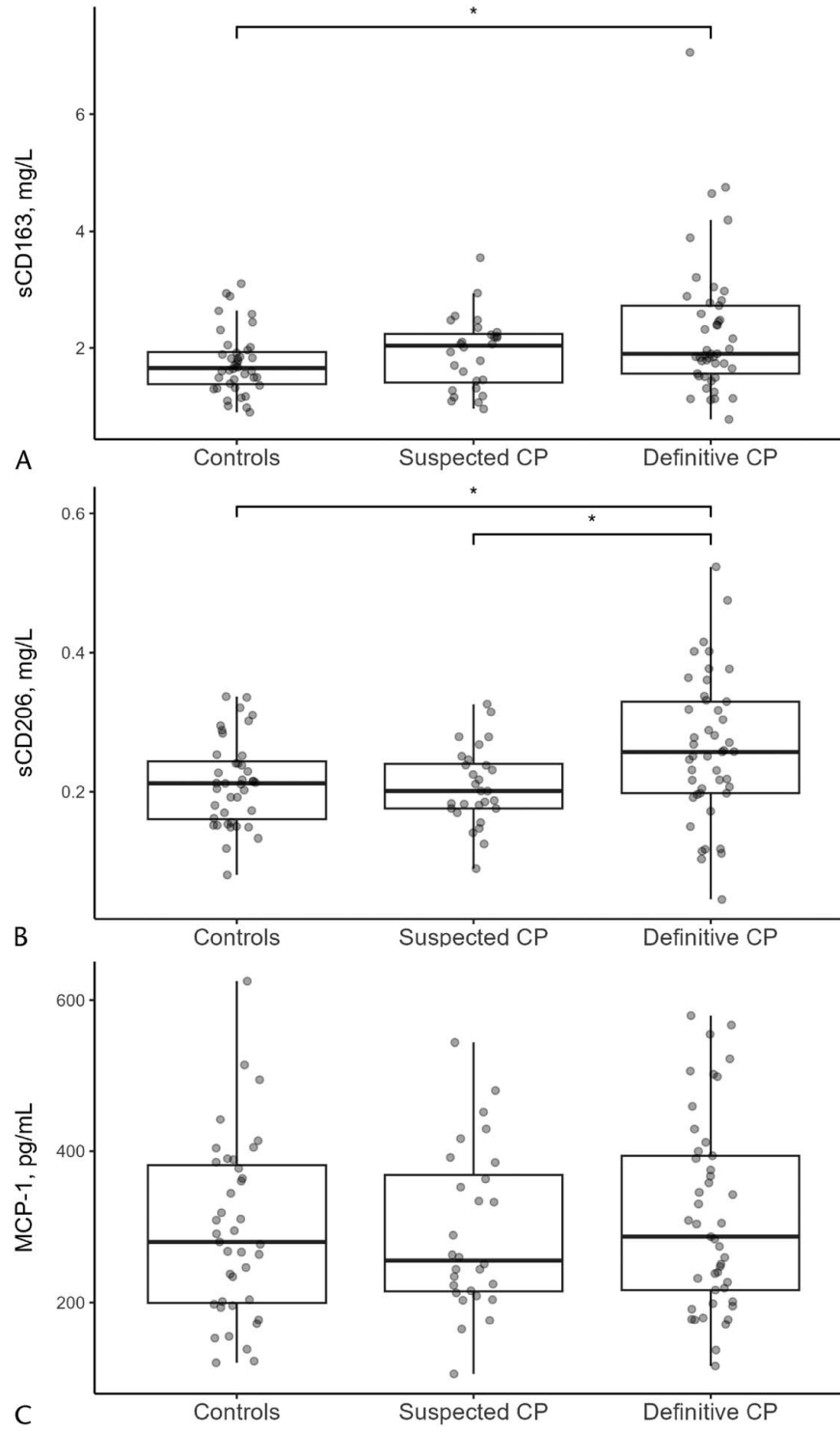

FIGURE 1. Box- and scatterplots of (A) soluble CD163 (sCD163), (B) soluble CD206 (sCD206), and (C) monocyte chemoattractant protein-1 (MCP-1) plasma levels in controls and chronic pancreatitis (CP) subgroups. *$P < 0.05$, adjusted for age, excessive alcohol consumption, smoking, and body mass index. One outlier for MCP-1 was removed because it deviated over 10-fold from the median in the control group.

TABLE 3. Trend Analysis of sCD163, sCD206, and MCP-1 Mean Plasma Concentrations Across Controls and Chronic Pancreatitis (CP) Subgroups

	Controls (n = 40)	Suspected CP (n = 28)	Definitive CP (n = 45)	Trend Coefficient (SE)	*P* for Linear Trend
sCD163, mg/L (SD)	1.74 (0.54)	1.91 (0.62)	2.28 (1.16)	0.20 (0.07)	**0.005**
sCD206, mg/L (SD)	0.21 (0.06)	0.21 (0.06)	0.26 (0.10)	0.02 (0.01)	**0.005**
MCP-1, pg/mL (SD)	376 (116)	293 (107)	317 (126)	7.13 (9.60)	0.457

The bold typeface indicates a statistically significant linear trend.
MCP-1 indicates monocyte chemoattractant protein-1; sCD163, soluble CD163; sCD206, soluble CD206; SD, standard deviation; SE, standard error.

Demographic and clinical characteristics are summarized in Table 1. Age differed across subgroups ($P = 0.005$); subjects with suspected CP were younger than those with definitive CP ($P < 0.05$) and controls ($P < 0.05$). Subjects with definitive CP were more frequently smokers ($P < 0.01$). Additionally, subjects with definitive CP were more likely to have a history of excessive alcohol consumption ($P < 0.05$). Pancreas-specific amylase and CRP were within the normal reference range for all CP patients and controls. Compared to controls and the suspected CP groups, patients with definitive CP demonstrated a higher incidence of diabetes ($P < 0.001$), PEI ($P < 0.001$), and comorbidities indicated by the CCI score ($P = 0.002$).

Soluble Biomarkers of Macrophage Activation in Definitive CP Versus Controls

In the univariate analyses, the median plasma concentrations of sCD163 ($P = 0.007$) and sCD206 ($P = 0.006$) were significantly elevated in individuals with definitive CP compared to controls (Table 2 and Fig. 1). These differences remained significant for both sCD163 ($P = 0.019$) and sCD206 ($P = 0.033$) in multivariate analyses. MCP-1 concentrations did not differ between the 2 groups in either uni- or multivariate analyses (Fig. 1 and Table 2).

Soluble Biomarkers of Macrophage Activation in Suspected CP Versus Controls

The median plasma concentrations of sCD163, sCD206, and MCP-1 did not differ between suspected CP and controls in either uni- or multivariate analyses (Fig. 1 and Table 2).

Soluble Biomarkers of Macrophage Activation in Definitive CP Versus Suspected CP

In the univariate analysis, the median plasma concentration of sCD206 ($P = 0.005$) was significantly elevated in definitive CP compared to suspected CP (Table 2 and Fig. 1). The differences remained significant in the multivariate analysis ($P = 0.042$). Concentrations of sCD163 and MCP-1 did not differ between the 2 groups in either uni- or multivariate analyses (Fig. 1 and Table 2).

Soluble Biomarkers of Macrophage Activation in Definitive CP With and Without Diabetes

Due to the strong correlation between diabetes and the stage of CP, particularly with a higher prevalence of diabetes in the definitive CP group (Table 1), diabetes could not be included as a covariate in the main analysis because of collinearity issues. To explore the potential impact of diabetes (and glucose-lowering therapies) on macrophage biomarkers, we stratified the definitive CP group into diabetics and nondiabetics. No significant differences were observed in sCD163 ($P = 0.687$), sCD206 ($P = 0.096$), or MCP-1 ($P = 0.724$) levels between these subgroups (Supplementary Table 2, http://links.lww.com/MPA/B317).

Trend Analyses of Macrophage Activation Across Controls and CP Subgroups

Trend analyses indicated an increasing trend of sCD163 plasma levels ($P = 0.005$) and sCD206 plasma levels ($P = 0.005$) across the control versus suspected CP versus definitive CP subgroups (Table 3).

TABLE 4. Diagnostic Performance of sCD163, sCD206, and MCP-1 Plasma Levels to Distinguish Controls and Chronic Pancreatitis (CP) Subgroups

	Optimal Cutpoint	AUC-ROC (95% CI)	Sensitivity, %	Specificity, %	Accuracy, %	LR+	LR−
Controls vs suspected CP							
sCD163, mg/mL	2.06	0.59 (0.44–0.73)	50	83	69	2.9	0.6
sCD206, mg/mL	0.18	0.48 (0.34–0.62)	79	33	51	1.2	0.7
MCP-1, pg/mL	202	0.50 (0.36–0.64)	89	28	54	1.2	0.4
Controls vs definitive CP							
sCD163, mg/mL	1.84	**0.65 (0.54–0.77)**	60	68	64	1.8	0.6
sCD206, mg/mL	0.24	**0.66 (0.54–0.78)**	58	75	66	2.3	0.6
MCP-1, pg/mL	390	0.53 (0.41–0.66)	29	82	54	1.6	0.9
Suspected CP vs definitive CP							
sCD163, mg/mL	2.57	0.57 (0.43–0.70)	29	93	53	4.0	0.8
sCD206, mg/mL	0.25	**0.68 (0.56–0.80)**	56	79	64	2.6	0.6
MCP-1, pg/mL	268	0.54 (0.40–0.67)	56	57	56	1.2	0.8

The bold typeface indicates statistically significant AUC-ROC.
AUC-ROC indicates area under receiver operating characteristics curve; CI, confidence interval; LR+, positive likelihood ratio; LR−, negative likelihood ratio; MCP-1, monocyte chemoattractant protein-1; sCD163, soluble CD163; sCD206, soluble CD206.

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Diagnostic Performance of Biomarkers for Discriminating CP Subgroups

The diagnostic performance of macrophage-specific biomarkers for discriminating subgroups is reported in Table 4 and visualized in Figure 2. The optimal sCD163 cutpoint for distinguishing definitive CP from controls was 1.84 mg/mL, with a corresponding AUC-ROC of 0.65 (95% CI, 0.54–0.77), an accuracy of 64%, a sensitivity of 60%, and a specificity of 68%. The optimal sCD206 cutpoint for distinguishing definitive CP from controls was 0.24 mg/mL, with a corresponding AUC-ROC of 0.66 (95% CI, 0.54–0.78), an accuracy of 66%, a sensitivity of 58%, and a specificity of 75%. The analytes did not significantly discriminate patients with suspected CP from controls.

DISCUSSION

We performed immunoassays to quantify plasma concentrations of soluble biomarkers of macrophage activation in patients at different clinical stages of CP and control subjects. All patients were carefully selected based on the absence of other fibro-inflammatory conditions and the use of anti-inflammatory treatments. Further, they were sampled during a clinically quiescent phase to avoid confounding from acute flares of pancreatitis. Upon inclusion, pancreatic morphology was characterized by MRI to ensure accurate group assignment. Patients with definitive CP (characterized by morphological features of advanced CP) exhibited elevated plasma concentrations of sCD163 and sCD206 compared to controls and patients with suspected CP (characterized by morphological features indicative of CP at an early stage). This indicates activation and M2 polarization of macrophages in advanced CP, suggesting a profibrogenic milieu in the pancreas.

Macrophage Expression in Chronic Pancreatitis

Elevated concentrations of sCD163 and sCD206 have been observed during both acute and chronic inflammation across multiple fibro-inflammatory diseases, including rheumatoid arthritis,[22–24] spondyloarthropathy,[25,26] multiple sclerosis,[27] Crohn's disease,[20] and chronic liver disease.[28] Acute and chronic inflammation involves macrophage activation, leading to the shedding of membrane-bound CD163 and CD206 and the release of their soluble forms, sCD163 and sCD206.[15–17] The physiological role of sCD163 and sCD206 is unknown.[21] Although increased expression of CD206 in human pancreatic tissue from CP patients was previously reported,[13] no studies have, to our knowledge, investigated the tissue expression of CD163 in CP or investigated the soluble forms of either CD163 or CD206.

Macrophages are versatile cells capable of swiftly changing their functions in response to local signals in the microenvironment.[13] They can take on either proinflammatory (M1) or profibrogenic (M2) characteristics, with these phenotypes fluctuating over time.[46] An imbalance in macrophage signaling can lead to the dominance of specific phenotypes.[13] Preclinical studies have shown different macrophage responses in AP and CP.[8–11] In AP, M1 macrophages are more abundant, whereas in CP, M2 macrophages infiltrate pancreatic tissue.[47] Interestingly, M1- and M2-polarized macrophages activate PSCs, which drive fibrosis in CP.[9,13] This PSC activation sets off a cycle where PSCs further promote M2 macrophage polarization, creating a feedforward loop.[13] This process involves cytokines like interleukin (IL)-4, IL-13, transforming growth factor beta (TGF-β), and platelet-derived growth factor (PDGF).[9,13] As M2 macrophages become active, specific markers like CD163 and CD206 are released into soluble forms (sCD163 and sCD206).[15–17] Although these markers are not exclusive to pancreatic macrophages, the meticulous patient selection process in our study excluded those with other fibro-inflammatory conditions. Consequently, the observed increase in biomarkers of macrophage activation as CP progresses strongly suggests that pancreatic macrophages are the primary source of these biomarkers, with a prevailing M2 macrophage polarization profile in advanced CP patients.

As previously mentioned, M2a macrophages involved in fibrogenesis express CD206, whereas M2c macrophages responsible for phagocytosing apoptotic cells express CD163.[18,19] Interestingly, we observed higher concentrations of sCD206 in definitive CP compared to suspected CP and controls, suggesting increased fibrogenesis in definitive CP. Additionally, we observed a gradually increasing trend of sCD163 concentrations, indicating progressive activation of M2c macrophages across the CP spectrum.

Despite the upregulation of sCD163 and sCD206 in definitive CP, the concentrations of acute phase reactants (CRP and MCP-1), as well as pancreas-specific amylase, remained within the normal clinical reference range. This

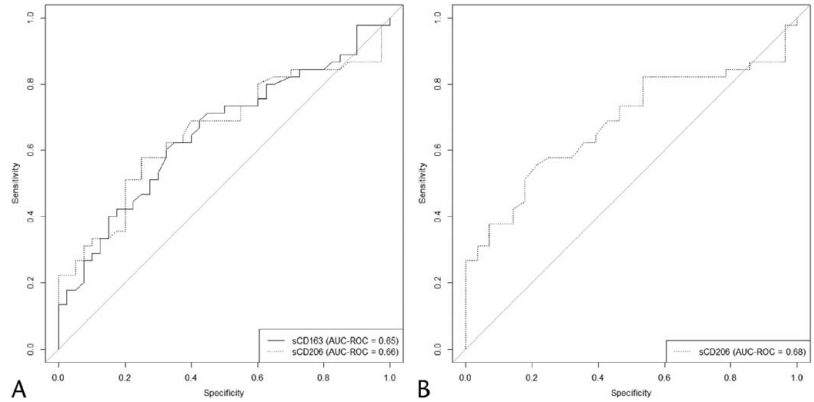

FIGURE 2. ROC analysis and the discriminatory performance of soluble CD163 and CD206 (sCD163 and sCD206) in definitive chronic pancreatitis (CP) versus controls (A) and definitive CP versus suspected CP (B). AUC-ROC, area under the receiver operating characteristics curve.

underscores that the subjects included in our study were all in a clinically quiescent phase, and as such, confounding from acute inflammation is unlikely to explain our observations. Rather, our observations are indicative of a chronic inflammatory milieu and fibrogenesis in patients at an advanced stage of CP and support the self-perpetuating feedforward loop between M2 macrophages and PSCs described above.[13,16,17,21,48,49]

Clinical Implications

In various inflammatory diseases, sCD163 and sCD206 have proven to be useful as diagnostic and prognostic biomarkers. However, in our study, their diagnostic performance for distinguishing between controls and definitive CP was only moderate. Additionally, there were no significant differences between controls and patients with suspected CP, the key subgroup for diagnostic biomarker development. This indicates that, although these macrophage activity markers can provide insights into the mechanisms underlying CP (ie, mechanistic biomarkers), their practical utility in clinical diagnosis may be limited. This limitation is further exacerbated by the selective nature of our study cohort, as many CP patients also have other fibro-inflammatory conditions, such as chronic obstructive pulmonary disease and chronic liver disease, which could potentially affect the measurement of pancreas-derived macrophage markers.

Strengths and Limitations

The study's primary strength lies in its comprehensive definition of CP subgroups achieved through MRI assessment for all subjects. Additionally, the methodological rigor employed across both study sites ensured the prospective collection of blood samples during clinically silent phases of CP, thereby avoiding potential confounding from AP flares. This was further supported by the absence of elevated acute-phase reactants, including CRP and MCP-1. Moreover, potential confounding factors from other fibro-inflammatory comorbidities were meticulously addressed by excluding patients with other chronic fibro-inflammatory conditions. However, this approach also restricts the generalizability of our findings and limits their applicability to a broader CP population with fibro-inflammatory comorbidities. Furthermore, the limited sample size of our study restricts our ability to adjust for all relevant confounders, including detailed data on smoking and alcohol intake. Despite this, our rigorous inclusion and exclusion criteria, designed to exclude potential confounding comorbidities, should help mitigate these issues. Additionally, the absence of an external validation cohort is another limitation of our study.

CONCLUSION

In our exploratory study, we found higher concentrations of sCD163 and sCD206 in individuals with definitive CP compared to controls and those with suspected CP. This suggests heightened M2 macrophage activity and a profibrogenic environment in the pancreatic tissue of CP patients at an advanced disease stage. Although these biomarkers show promise in shedding light on CP pathogenesis (ie, mechanistic biomarkers), they lack the necessary diagnostic sensitivity and specificity for clinical use as diagnostic biomarkers. These findings corroborate earlier observations from preclinical studies and highlight the importance of macrophage activation and polarization in CP pathogenesis, offering insights into its underlying pathophysiological mechanisms and potential targets for disease-modifying therapies.

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