# Serum biomarker CD163 predicts overall survival in patients with pancreatic ductal adenocarcinoma

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# Abstract

Background The serum soluble CD163 (sCD163) is elevated in patients with infection disease and several types of cancer. However, the prognostic value of serum sCD163 in pancreatic ductal adenocarcinoma (PDAC) has not yet been investigated. Methods Serum level of sCD163 was measured by using the peripheral blood of 54 patients with PDAC, 20 patients with benign tumor of pancreas, and 30 healthy volunteers (healthy controls). The association between serum sCD163 level and overall survival was analyzed. Receiver operating characteristics (ROCs) curves were generated, and areas under the curve (AUC) were compared to evaluate the diagnostic accuracy, including CA 19-9, CEA, CA 125, CA 153, serum sCD163 level and combination of sCD163 and CA19-9. Results Serum sCD163 level of patients with PDAC was significant higher than patients with benign tumor ($p = 0.002$) and health controls ($p < 0.001$). Using ROCs curves, we found that the AUC values of serum sCD163 were higher than those of CA 125 and CA 153, but lower than those of CA 19-9 and CEA. The combination of sCD163 and CA19-9 had higher diagnostic accuracy than CA19-9 or sCD163 alone. In addition, the prognosis of PDAC patients with sCD163 ≥ median was worse than sCD163 < median by using univariate analysis ($p = 0.027$). Further, multivariate analysis showed that higher level of serum sCD163 was still associated with poorer overall survival ($p = 0.030$). Serum sCD163 was not associated with tumoral CD163 expression, whereas negatively correlated with lymphocyte to monocyte ratio ($r = -0.428$, $p = 0.001$). Conclusion The serum sCD163 has the potential as a new promising parameter to predict the prognosis in PDAC patients.


# Background

Pancreatic ductal adenocarcinoma (PDAC) is currently the fourth leading cause of cancer related mortality. The five-year survival is only 9% even surgical resection or surgical resection plus chemotherapy/radiotherapy was performed [1]. The previous studies revealed that tumor-associated macrophages (TAMs) play a key role in tumor progression and associate with a worse prognosis in several malignancies [2, 3]. TAMs deriving from peripheral blood monocytes and recruited into the tumor mass [4], which were educated by the tumor microenvironment [2], could promote tumor growth and metastasis. The hemoglobin/haptoglobin complex scavenger receptor, CD163, expressed almost exclusively on circulating monocytes and on tissue macrophages, has been recognized as a valuable specific macrophage marker [5]. Tumoral CD163 is a valuable biomarker of macrophage for predicting the poor overall survival (OS) in several types of cancer [6-8]. Upon acute and chronic inflammations, CD163 is shed from the surface of activated macrophages and released into peripheral blood in the soluble form [9, 10]. This serum biomarker might represent CD163 level on activated monocytes. Serum soluble CD163 (sCD163) has been highlighted as a representative of macrophage activation and as a disease prognostic biomarker in cutaneous T cell lymphoma, multiple myeloma, melanoma, hepatocellular carcinoma, and Hodgkin lymphoma [5, 11-14]. To our knowledge, serum sCD163 has not been previously analyzed in patients with PDAC. The aim of this study was to evaluate the diagnostic and prognostic potential of serum sCD163 in PDAC patients.

# Patients And Methods

## Patients

Peripheral blood was obtained from 54 patients with PDAC and 20 patients with benign tumor of pancreas at Fujian Medical University Union Hospital, Fuzhou, China, from December 2016 to August 2018. The benign tumor included Solitary fibrous tumor (n = 1), Solid pseudopapillary neoplasm (n =4), Serous cystadenoma (n = 10), Serous microcystic adenoma (n = 3), Mucinous cyst (n = 1) and cyst (n = 1) of pancreas. All patients with PDAC and benign tumor were confirmed by histology. None of the patients received neoadjuvant radiation or chemotherapy. Patients suffering from acute infection disease, such as pancreatitis and pneumonia, were excluded. Blood samples were collected from each subject during routing venipuncture before treatment. The stage of each malignant patient was assessed based on the American Joint Committee on Cancer version 8 (AJCC 8). All patients were followed up for survival status until January 2019. This study was approved by the Committee for the Ethical Review of Research, Fujian Medical University Union Hospital (No. 2016-ZQN-34) and all patients gave written informed consent for the collection of blood and data analysis.

## Blood samples

Thirty normal blood samples were obtained from volunteers (healthy controls). To remove blood cells, serum tubes were centrifuged at 3,000 rounds per minute for 15 min at 4 ℃. Serum samples were aliquoted and stored at -80 ℃ subsequently. Complete blood count, carbohydrate antigen 19-9 (CA 19-9), carcinoembryonic antigen (CEA), CA 125 and CA 153 were measured at the central laboratory of Fujian Medical University Union Hospital.

## Serum sCD163 ELISA

Serum sCD163 was quantified by enzyme-linked immunosorbent assays (ELISA) by using the human CD163 ELISA kit (RayBiotech catalog number ELH-CD163). Serum concentrations of sCD163 were measured in duplicate and analyzed in accordance with the manufacturers' recommendations. A 1:50 dilution was used for all the samples. The nonlinear standard curve was constructed according to polynomial regression (degree = 2).

## Immunohistochemistry

Tumor tissues of 35 patients in the PDAC cohort were collected for immunohistochemistry (IHC) staining. The corresponding formalin-fixed paraffin-embedded tumor tissues were cut into 4-μm-thick sections. The slides were then stained with anti-human CD163 antibody (EPR19518). The IHC protocols were described

in our previous studies [15]. The density of the CD163-positive cells was calculated with 400× magnification in five random fields, and the average was calculated. Each section was examined independently by two investigators in a blinded manner. The positive infiltration of $CD163^+$ macrophages was defined as more than 90 $CD163^+$ cells from an average of five 400× high-power fields (HPF), as described by Coati Sakakura et al. [16].

### Statistical analysis

Differences between groups were tested using $c^2$ test, Kruskal-Wallis H test for the different types of variables. The optimal cut-off value for the neutrophil to lymphocyte ratio (NLR) and lymphocyte to monocyte ratio (LMR) was determined by applying receiver operating characteristics (ROCs) curves analysis to differentiate PDAC patients, benign tumor patients and healthy controls. OS was measured from the date of diagnosis to the day of death from any cause or the last censored follow-up. The univariate and multivariate analysis were evaluated by the log-rank test and the cox proportional hazard model. The correlation between serum sCD163 and tumoral CD163 expression or LMR was evaluated by the Spearman's rank correlation coefficients. To evaluate the diagnostic accuracy for the diagnosis of PDAC, including CA 19-9, CEA, CA 125, CA 153, serum sCD163 level, ROCs curves were generated in the entire cohort, and differences among the areas under the curve (AUC) were compared. A $p < 0.05$ was statistically significant. All statistical analyses were performed by SPSS software version 22.

## Results

### Patient characteristics

Patient's clinicopathological characteristics are presented in Table 1 and Table S1. In patients with benign tumor, the median age of patients was 52 years (range 16-69) and all the patients had low level of CA 19-9 (< 37 U/ml). In PDAC patients, the median age of patients was 60.5 years (range 35-85). Thirty-two (59.26%) had tumor located in head of pancreas. The medium tumor diameter was 3 cm. Thirteen patients (24.07%) had low level of CA 19-9 (< 37 U/ml). Most patients had TNM III (19 cases) and IV (15 cases) stage. Thirty-five (64.81%) PDAC patients received operation. Twenty-one (38.89%) PDAC patients received postoperative chemotherapy.

### The high level of serum sCD163 in patients with PDAC

Serum sCD163 level was measured in patients with PDAC, benign tumor and healthy controls. The median value of serum sCD163 level in patients with PDAC, patients with benign tumor, and health controls were 40.62 ng/ml (range 7.67 - 280.44), 19.82 ng/ml (range 12.39 - 64.31), and 20.62 ng/ml (range 10.99 - 49.11). As shown in Figure 1, the serum level of sCD163 was obviously higher in patients

with PDAC, compared to the patients with benign tumor or healthy controls. However, serum sCD163 level in patients with benign tumor was not statistically significant compared with health controls.

### Receiver operating characteristics curves of sCD163, CA 19-9, CEA, CA 125, and CA 153

To evaluate the diagnostic accuracy of serum sCD163 parameters for PDAC diagnosis, ROCs curves analyses for CA 19-9, CEA, CA 125, CA 153 and serum sCD163 level were performed in entire cohort. The AUC values of serum sCD163 level (0.7670) were lower than CA 19-9 (0.8615) and CEA (0.8115), whereas higher than CA 125 (0.6820) and CA 153 (0.5387; Fig. 4 A-E). These results indicated that the utility of sCD163 as a diagnostic biomarker in PDAC patients may be limited. However, the AUC for the combination of sCD163 and CA19-9 was 0.9485 (Fig. 4 F), with a p value of $< 0.05$ compared with CA19-9 alone or $< 0.001$ compared with sCD163 alone (Fig. 5).

### Correlation between serum sCD163 and clinicopathological characteristics in patients with PDAC

Patients with PDAC were stratified into high and low groups by the median value of serum sCD163 level ($< 40.62$ / $\geq 40.62$ ng/ml). Correlation between the serum sCD163 level and the clinicopathological characteristics was shown in Table 1. The serum sCD163 level was associated with tumor location ($p = 0.001$), NLR ($p = 0.027$) and LMR ($p = 0.002$), whereas not associated with gender ($p = 0.268$), age ($p = 0.564$), tumor diameter ($p = 1.000$), TNM stage ($p = 0.468$) and CA 19-9 levels ($p = 0.340$).

### Prognostic value of serum sCD163 level in PDAC patients

The median follow-up time of the PDAC patients was 8 months, and the median OS was 12 months. Univariate analysis showed that variables associated with OS included tumor diameter (HR = 2.292, $p = 0.050$), TNM stage (HR = 5.912, 8.691, 16.467; $p = 0.105, 0.038, 0.008$), patients received operation (HR = 0.433, $p = 0.034$; Table 2). We found that high level of serum sCD163 was associated with worse OS compared to low level of serum sCD163 ($p = 0.027$; Fig. 2). However, gender, age, tumor location, CA 19-9 levels, NLR, LMR and patients received postoperative chemotherapy were not statistical factors for OS. We next performed multivariate analysis to explore if serum sCD163 could remain an independent predictor of OS. Tumor diameter, TNM stage and serum sCD163 were used in the multivariate analysis. We found that serum sCD163 remain an independent factor for prognosis (Table 2).

### Correlation of serum sCD163 level with tumoral CD163 expression in PDAC patients

Both serum sCD163 level and tumoral CD163 expression showed poor prognosis in different types of cancer [17-19]. It is necessary to explore whether these two parameters were connected. Unfortunately, there are 19 advanced PDAC patients in our cohort, who were not considered for surgical resection, small biopsies were collected and insufficient tissues were available for IHC staining. Hence, only 35 PDAC tumor tissues were collected for IHC staining. As shown in Figure 3, serum sCD163 level was not associated with tumoral CD163 expression ($r = - 0.085$, $p = 0.627$). However, we found that serum sCD163 level was negatively correlated with LMR in 54 patients with PDAC ($r = -0.428$, $p = 0.001$, Fig. 6).

# Discussion

Serum sCD163 as a prognostic biomarker in patients with multiple myeloma, classical hodgkin lymphoma, melanoma, hepatocellular carcinoma and cutaneous T cell lymphoma [5, 11-13, 18]. The elevated serum sCD163 level is correlated with disease progression and worse OS in those cancers. To the best of our knowledge, serum sCD163 has not yet been investigated in pancreatic cancer. We found that higher serum sCD163 level was significantly associated with poorer OS in patients with PDAC. Our data suggest that serum sCD163 could be a valuable biomarker for prognosis of PDAC.

In addition, we found that the level of serum sCD163 in PDAC patients was higher than this in patients with benign tumor and healthy controls. This suggests that serum sCD163 elevated appears to be a warning signal in patients with PDAC. To evaluate if this parameter might be useful diagnostic marker for PDAC, we performed the ROCs curves analyses. The AUC values of the serum sCD163 were lower than CA 19-9 and CEA. While, when both sCD163 and CA19-9 were used in combination, the results were even more favorable compared with using CA19-9 or sCD163 alone. This indicate that the serum sCD163 might not be a better diagnostic marker than CA 19-9 and CEA in PDAC patients, but the combination of sCD163 and CA19-9 may improve the diagnostic accuracy in PDAC patients.

Tumor site TAMs derive from peripheral blood monocytes [4] and differentiate into $CD163^+$ macrophages within the tumor microenvironment under the stimulation of tumor-derived chemotactic factors [20]. These $CD163^+$ macrophages generate the immunosuppressive tumor microenvironment and promote tumor progression and metastasis [2]. The CD163 is also an exclusive biomarker expressing on circulating monocytes [5]. Hence, serum sCD163 may derive from circulating monocytes or tumor site $CD163^+$ macrophages. Our data demonstrate that serum sCD163 level was correlated with circulating monocytes but not tumoral CD163 expression. This indicates that serum sCD163 is a systemic inflammatory response marker to predict the proportion of monocytes in circulation. Further study could focus on the positive correlation of tumor-derived chemotactic factors, inducing alternatively activated $CD163^+$ macrophages with serum sCD163, which may further predict the subtype of macrophages at tumor site and estimate the immune status and prognosis of PDAC.

The main limitation of our study is the small, retrospective, single-center study, which may have involved various biases. Validation in larger and prospective cohorts in multi-center is needed to verify our preliminary results.

# Conclusion

Our results demonstrated that the serum sCD163 might serve as a novel and promising biomarker to predict the prognosis in PDAC patients.

# Abbreviations

sCD163: soluble CD163; PDAC: pancreatic ductal adenocarcinoma; OS: overall survival; ROCs: receiver operating characteristics; AUC: areas under the curve; NLR: neutrophil to lymphocyte ratio; LMR: lymphocyte to monocyte ratio; TAMs: tumor-associated macrophages; IHC: immunohistochemistry; HPF: high-power field; ELISA: enzyme-linked immunosorbent assays; CA 19-9: carbohydrate antigen 19-9; CEA: carcinoembryonic antigen; HR: hazard ratio; CI: confidence interval.

# Declarations

### Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the Helsinki declaration. And all patients whose blood samples were used in this research provided written informed consent, and the study protocol was approved by the Committee for the Ethical Review of Research, Fujian Medical University Union Hospital.

### Consent for publication

Not applicable.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors declare that they have no competing interests.

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### Authors' contributions

QF, YP and conceived the research. QF and YP designed the methodology. QF, YP, XY, TT, XL and RL performed the experiments. QF and YP wrote the manuscript. HH supervised the study. All authors gave final approval of the submitted and published versions of the manuscript.

### Acknowledgement

Not applicable.

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# Additional File

Additional file 1: Table S1. Clinicopathologic characteristics of benign tumor patients.

# Figures

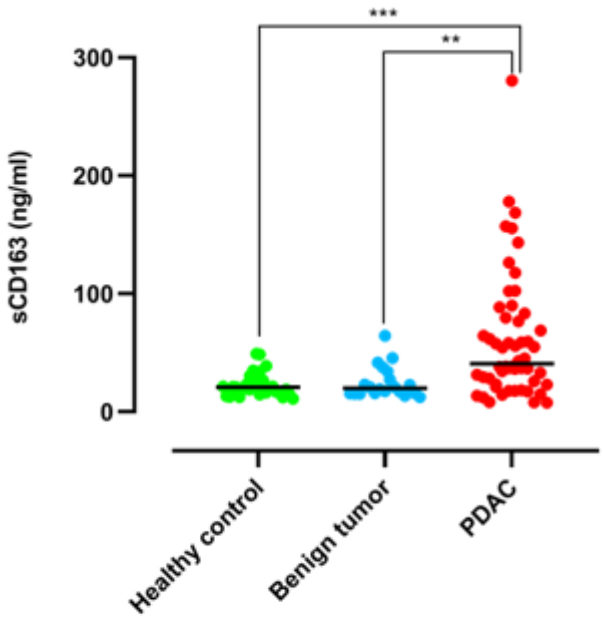



Fig. 1 Comparison of serum sCD163 levels in healthy control, benign tumor and PDAC patients.

***, $p < 0.001$; **, $p < 0.01$.

### Figure 1

Comparison of serum sCD163 levels in healthy control, benign tumor and PDAC patients. ***, $p < 0.001$; **, $p < 0.01$.

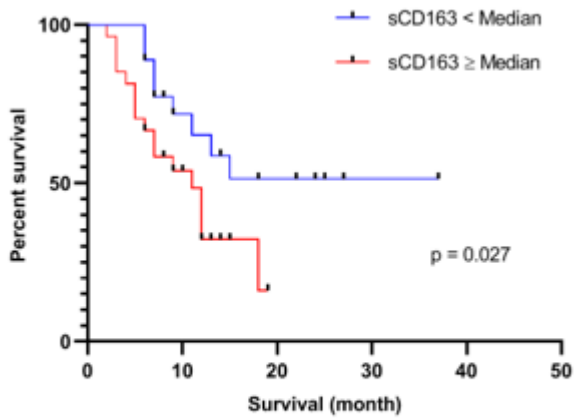
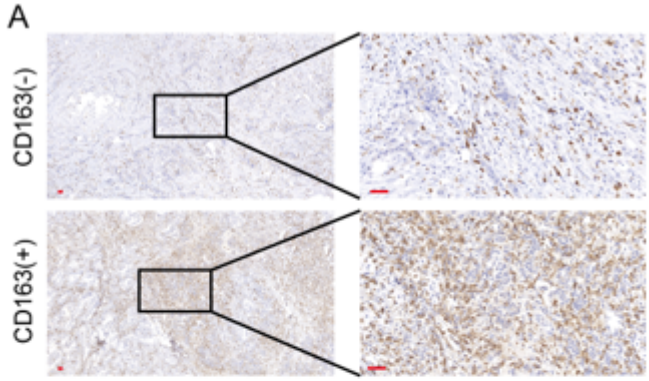



Fig. 2 Kaplan-Meyer plot of overall survival of 54 PDAC patients according to serum sCD163.

## Figure 2

Kaplan-Meyer plot of overall survival of 54 PDAC patients according to serum sCD163.




B

| | Serum sCD163 | | | Spearman's correlation | |
|---|---|---|---|---|---|
| Tumoral CD163 | ≥ Median | < Median | Total | r | p-value |
| Positive | 4 | 5 | 9 | -0.056 | 0.832 |
| Negative | 4 | 4 | 8 | | |
| Total | 8 | 9 | 17 | | |

Fig. 3 Immunostaining of CD163 in PDAC. **A** Negative and Positive staining with an anti-CD163 antibody in human PDAC tissue samples at low (100×) and high magnification (400×). Scale bar = 50 μm (red line at the bottom left). **B** Correlation between serum sCD163 levels and tumoral CD163 expression in patients with PDAC.

## Figure 3

Immunostaining of CD163 in PDAC. A Negative and Positive staining with an anti-CD163 antibody in human PDAC tissue samples at low (100×) and high magnification (400×). Scale bar = 50 μm (red line at the bottom left). B Correlation between serum sCD163 levels and tumoral CD163 expression in patients with PDAC.

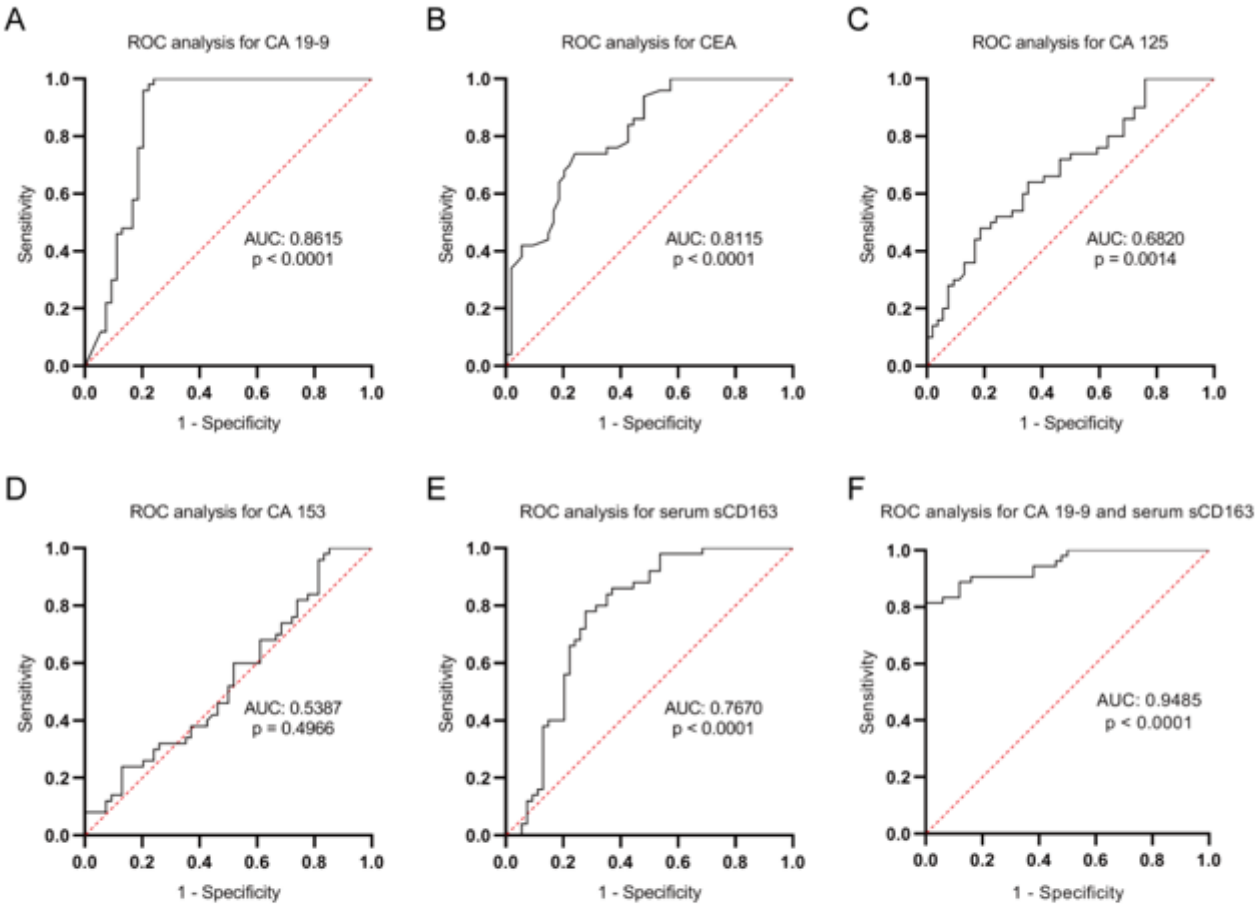



Fig. 4 Comparison of the areas under the ROC curves for diagnosis among CA 19-9, CEA, CA 125, CA 153, serum sCD163 levels and combination of sCD163 and CA19-9 in patients with PDAC, healthy controls and patients with benign tumor.

## Figure 4

Comparison of the areas under the ROC curves for diagnosis among CA 19-9, CEA, CA 125, CA 153, serum sCD163 levels and combination of sCD163 and CA19-9 in patients with PDAC, healthy controls and patients with benign tumor.

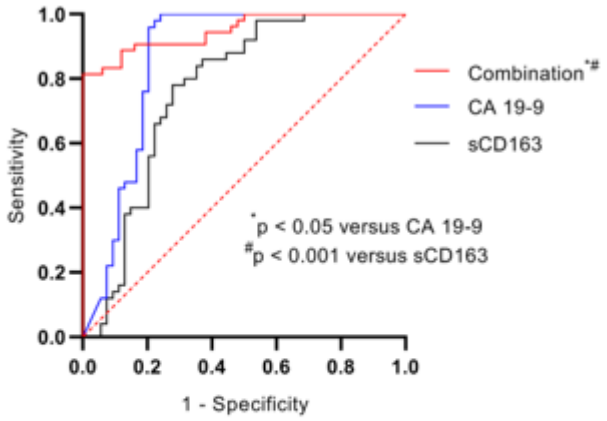



Fig. 5 ROC curves comparing pancreatic adenocarcinoma to healthy controls and patients with benign tumor for combination sCD163 plus CA19-9, sCD163, and CA19-9.

### Figure 5

5 ROC curves comparing pancreatic adenocarcinoma to healthy controls and patients with benign tumor for combination sCD163 plus CA19-9, sCD163, and CA19-9.

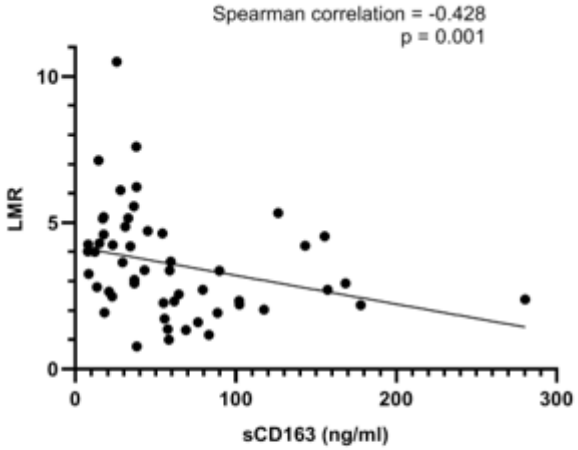



Fig. 6 Correlation between serum sCD163 and LMR in patients with PDAC.

### Figure 6

Correlation between serum sCD163 and LMR in patients with PDAC.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplement1.pdf
- supplement2.pdf
- supplement3.pdf