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Increased plasma soluble major histocompatibility complex class I polypeptide-related sequence A/B (sMICA/B) and related biomarkers in patients with pancreatitis

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Abstract

Plasma soluble MHC class I chain-related gene (sMIC) A/B, sTAMRs (sTyro3, sAxl, and sMer), Gas6 and free-PROS1, ADAM 10/17, sFas, and severity markers (APACHE-II and SOFA) were measured in 55 cases (severe 14 and mild 41) of acute pancreatitis (AP), 20 cases of chronic pancreatitis (CP), and 20 cases of normal control (NC).

Methods: These markers were measured by ELISA-kit.

Results: sMICA/B, ADAM10/17, sFas, sTAMRs and both ligands were significantly higher in the pancreatitis group than in the NC. The non-survival SAP group showed the highest levels for these markers, and the peaks of sMICA/B levels were seen at the onset of disease in many cases and they damped. However, sMICA/B values in the non-survival SAP group showed another peak at onset and 14-30 days after onset.

The correlations between these markers among groups showed significant good correlations except for the non-survival SAP and CP groups.

Regarding the correlations between sMICA/B and APACHE- II in the non-survival SAP and CP group were inconsistent, whereas significant correlations were observed in other pancreatitis groups. The correlations between APACHE-II, and Gas6 and free-PROS1 showed significant correlation with most of the groups, whereas free-PROS1 showed significant correlations with the severe group.

Conclusions: The higher the sMICA/B value, the higher the severity of the disease. In addition, non-survival SAP and CP suggested impaired efferocytosis. Furthermore, while Gas6 acts regardless of the symptoms of pancreatitis, free-PROS1 seems to act mainly in severe cases.

Keywords: sMICA; sMICB; sFas; Gas6; PROS1; sTAMRs

1. Introduction

Acute pancreatitis (AP) is among the three most common benign gastrointestinal diseases, with a mortality rate of 0.9%, while chronic pancreatitis (CP) is characterized by gradual irreversible damage to the endocrine and exocrine parenchyma caused by inflammation and subsequent replacement of these tissues with fibrotic tissue and atrophy [1,2]. AP is a common indication for hospital admission, and is increasing in incidence, including in children, pregnant women,

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and elderly patients. Moderately severe AP (SAP) with fluid and/or necrotic accumulation causes substantial morbidity, and severe disease with persistent organ failure causes significant mortality. The main causes of death are multiple organ failure (MOF) and disseminated intravascular coagulation. More than 50% of patients with CP have a history of AP. Recurrent AP can present without symptoms between episodes, but ongoing episodes eventually lead to morphologic changes and functional deficits of CP. Thus, the two disorders are believed to be part of a disease continuum [1,2].

The major histocompatibility complex (MHC) class I-related chain A/B (MICA/B) is a stress-inducible cell surface molecule. MICA/B labels malfunctioning cells for recognition by cytotoxic lymphocytes such as natural killer (NK) cells. The extracellular domain of MICA/B can be shed from the cell surface by metalloproteases such as ADAM10/17 to generate a soluble form that acts as a soluble decoy receptor (sMICA/B) or in $CD4^+$ T-cell expansion [3,4]. MICA/B is released from the surface of tumor cells and infectious cells of epithelial origin. MICA/B expressed on the cell surface stimulates the immunoreceptor NK group 2, member D (NKG2D), but sMICA/B downregulates NKG2D activity, thus allowing the tumor to escape immunosurveillance by NKG2D-expressing cells [3,4].

Macrophages play critical roles in tissue remodeling in normal physiology and resolution of inflammation and tissue injury. Clearance of apoptotic cells (ACs), which is referred to as efferocytosis, is critical in this process [5, 6]. Efferocytosis reduces inflammation by preventing necrosis, and ACs activate receptor-mediated signaling pathways in macrophages that suppress inflammation and promote resolution and repair. Failed efferocytosis is emerging as a key mechanism driving development and progression of chronic inflammatory diseases, including atherosclerosis, obesity, diabetes, heart failure, chronic lung disease, neurodegenerative disease and cancer. Thus, therapeutic strategies aimed at improving efferocytosis may improve inflammation.

NKG2D is believed to induce apoptosis via ligands such as MICA/B and Fas-FasL. The process of efferocytosis that suppresses inflammation involves integrated, but mechanistically distinct, pathways, including the Tyro3, Axl, and Mer receptors (TAMR)-mediated pathway. Activation of TAMRs suppresses NF-κβ signaling; thus, TAMRs act as immunoregulatory receptors that dampen inflammation [7,8]. TAMRs are three homologous type I receptor tyrosine kinases (RTKs) that are activated by two endogenous ligands: PROS1 and Gas 6. These ligands can activate TAMRs as soluble factors, or opsonize phosphatidylserine (PtdSer) on ACs and bridge between ACs and TAMRs [8-10].

Gas6 and PROS1 are vitamin K-dependent proteins present in plasma that have regulatory functions in response and repair to damage. The proteins interact with TAMRs through a C-terminal sex hormone-binding globulin (SHBG) domain, inducing dimerization and intracellular signal transduction. The Gla domain of the proteins allows calcium-dependent binding of TAMR ligands to PtdSer in ACs, activated platelets and certain enveloped viruses (apoptotic mimicry). Therefore, the Gla domain plays a key role in TAMR function, bridging PtdSer-exposed cells to enhance efferocytosis or viral entry. The PtdSer-Gla interaction intensifies receptor signaling and changes receptor affinity [11,12].

This background indicates the importance of efferocytosis for tissue homeostasis and organ development. The process is mainly orchestrated by phagocytes, and aberrant efferocytosis has been associated with inflammatory diseases. Regulation of this process by TAMRs is crucial for tissue homeostasis [8-12]. In this study, we examined pancreatitis from the viewpoint of efferocytosis, with a focus on the relationships of sMICA/B with its shedding enzymes, as well as TAMRs, Gas6 and PROS1, and the severity of the disorder.

2. Material and Methods

2.1. Subjects

The subjects were 71 patients with AP and CP, including 41 cases with mild AP (MAP), 14 with severe AP (SAP), 20 with CP, and 20 normal controls (NCs) matched by age. The patients with AP had a mean age of 53.2±15.7 years. The baseline characteristics of these patients are shown in Table 1. Diagnosis of AP was based on abdominal signs associated with high pancreatic enzymes and morphological abnormalities consistent with AP on contrast-enhanced computed tomography and ultrasonography carried out within 24 h of admission. Diagnosis of CP was based on the diagnostic criteria of the Japanese Pancreatic Society (2012) [13]. Severity of AP and CP was assessed using Ranson's criteria [14], APACHE-Ⅱ, JSS score, criteria for Intractable Disease of the Pancreas issued by the Japanese Ministry of Health, Labour and Welfare, and the Revised Atlanta Classification criteria [15,16]. MOF was assessed using APACHE-Ⅱ and SOFA at the time of blood sampling [15,17]. Six of the 14 SAP cases died due to sepsis and MOF, and 8 survived. Shock occurred in 7 patients. The clinical stages of 5 patients with pancreatic cancer were III-IV.

2.2. Measurement of sTAMRs, ligands, and shedding enzymes

Measurements of sMICA/B and sFas were performed using a sandwich Human MICA and MICB kit (Dia clone SAS. Besancon Cedex, France), and a soluble (not for proteolytic membrane) FAS kit (MBL. Nagoya. Japan). Measurements of sTAMRS, ligands, and enzymes were performed using sandwich ELISA kits: Tyro3 ELISA Kit (Human), MERTK ELISA Kit (Human), ADAM 10 ELISA Kit Human, ADAM 17 ELISA Kit Human (Aviva Systems Biology. San Diego. CA. USA), Human AXL ELISA Kit (Thermo Fisher, Vienna, Austria), a Duo Set® (R&D Systems, Minneapolis, MN, USA) for Gas6, and a Protein S test Teijin® (Teijin Diagnostics, Osaka, Japan) for free PROS1 (fPROS1) and total PROS1. The intra- and inter assay coefficients of variation for the measurements were 6.5-9.8%.

2.3. Determination of white blood cell counts, platelet counts, and pancreatic enzymes

Serum and urine amylase, white blood cell (WBC) counts, and platelet counts in patients and controls were measured by routine laboratory procedures. Venous blood samples were taken at admission (days 0-2: within 48 h of pain onset), and 7, 14, and 14-30 days after admission. A total of 110 blood samples were collected in patients with severe and mild AP. Plasma samples were collected in 3.8% citric acid (1/10) and frozen immediately for storage at -75 ℃ until analysis.

2.4. Statistical analysis

Values are expressed as mean ± standard deviation (SD). An unpaired Student t-test was used for comparison of mean values, with $P<0.05$ considered to be significant.

3. Results

3.1. Serum pancreatic enzymes, WBC counts, platelet counts, and disease severity

Serum and urine amylase, serum lipase and WBC counts increased in order of SAP, MAP and CP cases, whereas platelet counts decreased in this order. The mean APACHE- Ⅱ and SOFA scores were 25-2 and 15-1 in pancreatitis cases [Table 1]. More severe cases had elevated pancreatic enzymes and WBC counts, decreased platelets, and higher APACHE II and SOFA scores [Table 1].

Table 1 Clinical background of patients with pancreatitis

	Severe Acute Pancreatitis		SAP	Mild AP	Chronic Pancreatitis
	non survival	survival			
Female	0	1	1	8	5
Male	6	7	13	33	15
Alcohl	3	5	8	18	11
Bile stone	1	0	1	10	4
Ideopathic	1	4	5	10	5
ERCP	0	0	0	3	0
serum-Amylase (iu/L)	12647 ±8943	11566 ±11383	12174 ±9349	7249 ±4175	649 ±178
urine-Amylase (iu/L)	18967 ±6698	14786 ±9853	15770 ±7451	6983 ±4751	878 ±216
Lipase (u/L)	4478 ±3471	4021 ±3482	4102 ±3375	886 ±542	203 ±151
WBC (/μL)	18374 ±7986	16743 ±10453	17513 ±8514	11923 ±7582	7058 ±4431
Platelet (10^4/μL)	16.0 ±8.4	17.8 ±6.2	17.3 ±6.0	18.6 ±5.4	21.9 ±5.7
APACHE-II	25~4	25~2	25~3	15~3	13~2
SOPHA	23~3	23~1	25~1	13~1	9~1

3.2. Changes in plasma levels of sMICA/B

Plasma levels of sMICA/B were higher in all pancreatitis and pancreas cancer cases compared to NCs. sMICA and B levels in NCs (n=20) were 178.8±65.1 and 54.8±20.8 pg./ml, respectively [Figure 1A, C]. sMICA levels in AP (n=88), CP (n=22),

non-survival SAP (n=24), survival SAP (n=24), SAP (n=48), and MAP (n=40) cases were 1013.8±826.3, 463.9±208.4, 1454.0±697.5, 1037.3±763.8, 1173.8±802.8, and 681.0±769.1 pg./ml, respectively [Figure 1A]. sMICB levels in these respective groups were 168.6±173.8, 76.3 ±44.5, 226.5±207.8, 184.9±207.8, 218.0±207.0, and 92.9 ±46.3 pg./ml [Figure 1C]. sMICA/B levels were significantly higher in SAP compared to MAP, and AP compared to CP, but did not differ significantly between non-survival and survival SAP cases [Figure 1A, C]. sMICA/B peaked during hospitalization at 0-2 days in most patients, and slowly decreased and normalized thereafter. However, sMICA/B in non-survivors elevated again at 14-30 days after admission, whereas these levels in survivors with SAP peaked at 0-2 days and decreased and normalized thereafter [Figure 1B,D]

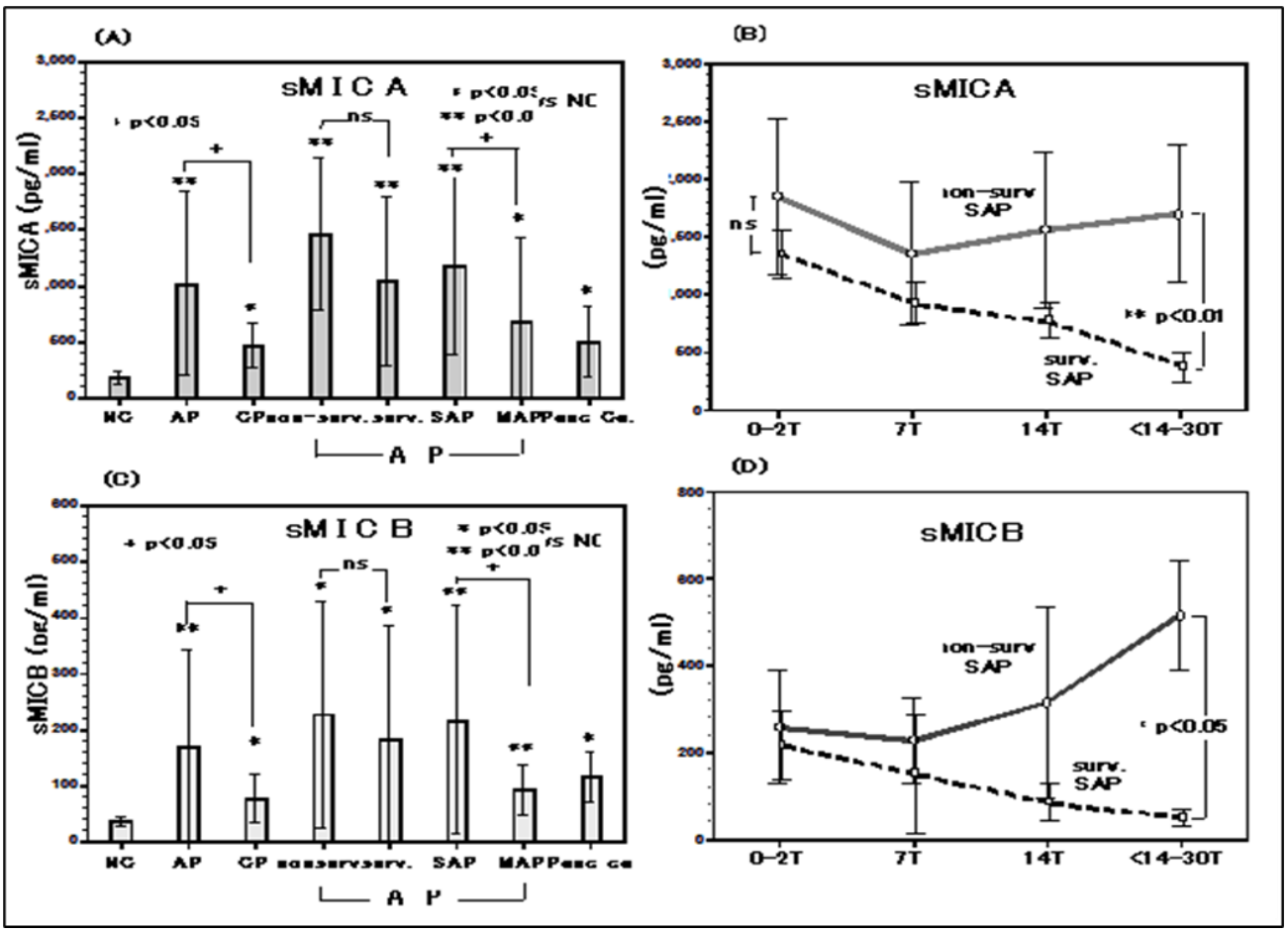


Figure 1 Changes in plasma levels of sMICA/B in patients with pancreatitis

3.3. ROC curves for sMICA/B

ROC curves of sMICA/B levels in non-survival SAP cases are shown in Figure 2. The areas under the ROC curve (AUC) for sMICA and B in non-survival SAP and SAP cases were 0.888, 0.861, 0.869, and 0.872, respectively [Figure 2A, B]. The AUCs for sMICA and B in CP cases were 0.875 and 0.895, respectively.

3.4. Correlation of sFAS antigen with sMICA/B

sMICA and B levels were significantly positively correlated with sFas levels [Figure 3A, B] and with each other [Figure 3C]. The sFas level in NCs (n=20) of 0.26±0.09 ng/ml was significantly lower than those in AP (n=75), CP (n=18), non-survival SAP (n=23), survival SAP (n=26), SAP (n=49) and MAP (n=33) cases, which were 0.96±0.09, 0.66±0.50, 1.27±1.01, 1.06±0.89, 1.16±0.95, and 0.67±0.41 ng/ml, respectively [Figure 3D]. sFas in non-survival SAP were higher than that in survival SAP, but the difference was not significant. There was a significant difference in sFas level between SAP and MAP cases [Figure 3D].

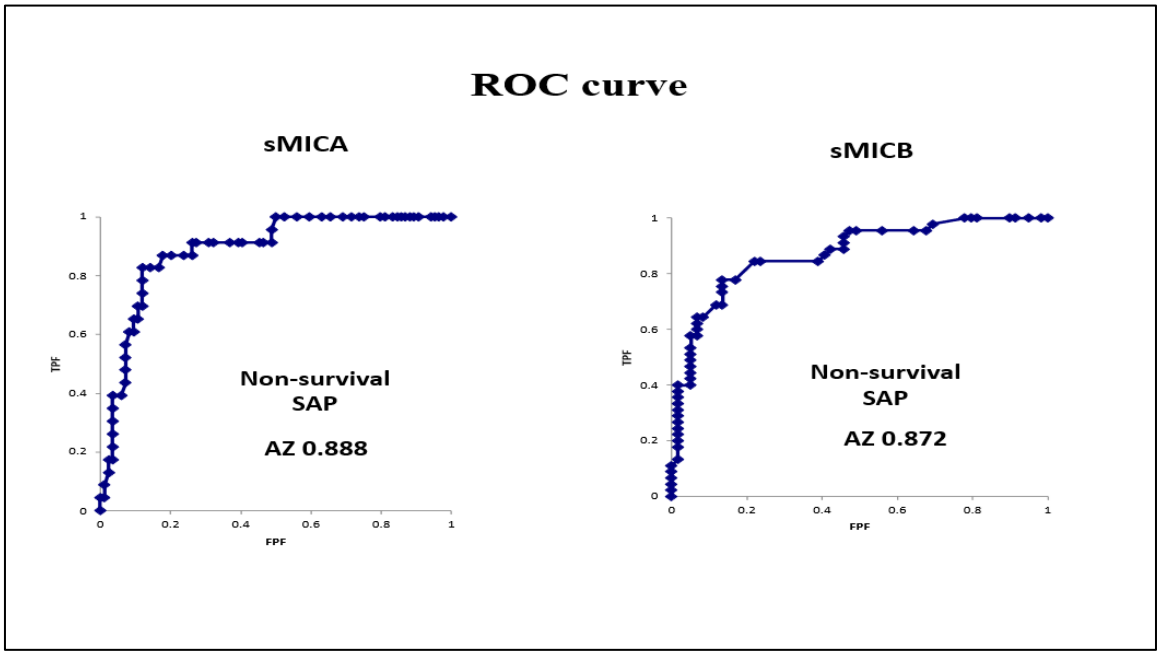


Figure 2 ROC curve for sMICA/B in patients with pancreatitis.

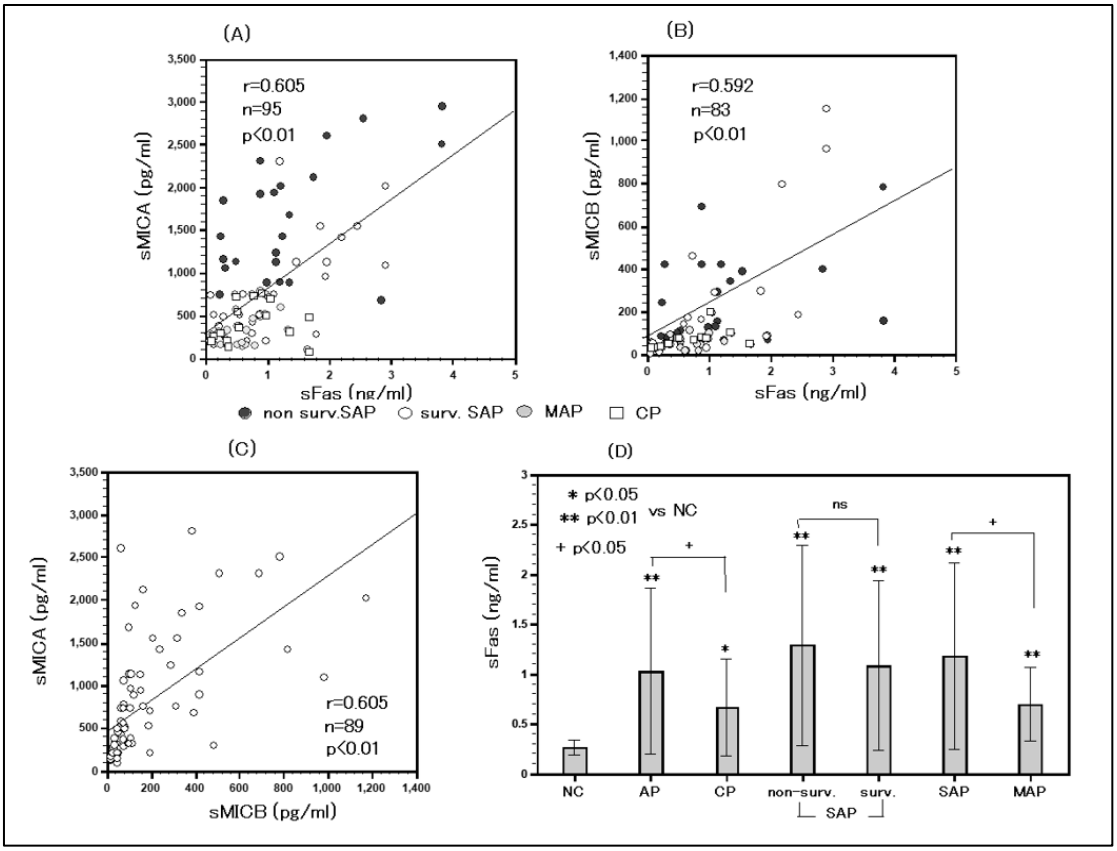


Figure 3 Plasma levels of sFas and correlations of sMICA/B with sFas in patients with pancreatitis

3.5. Plasma levels of sTAMRs, ligands, and shedding enzymes

Plasma levels of the three sTAMRs (sTyro3, Axl, Mer), both ligands (Gas6, fPROS1) and shedding enzymes (ADAM10/17) are shown in Table 2. Most showed a significant increase or decrease compared to NCs. Non-survival SAP cases had peak values for many markers. There were increases in AP compared to CP, in SAP compared to MAP, and in non-survival SAP compared to survival SAP. However, despite the significant difference between ADAM10/17 in non-

survival and survival SAP cases, the three STAMRS and fPROS1 did not differ significantly between these groups [Table 2].

Table 2 Plasma levels of sTAMRs (sTyro3, sAxl, sMer), shedding enzymes (ADAM10/17), and ligands (Gas6, free PROS1) in patients with pancreatitis and normal controls

	sTyro3 (ng/ml)	sAxl (ng/ml)	sMer (ng/ml)	ADAM 10 (ng/ml)	ADAM 17 (ng/ml)	Gas6 (ng/ml)	fPROS 1 (μg/ml)
NC	1.7 ±0.64	26.4 ±11.78	16.2 ±1.55	0.18 ±0.08	0.36 ±0.6	17.8 ±1.2	11.2 ±1.3
AP	24.1** ±23.1	139.1** ±114.4	202.6** ±236.2	19.9 ** ±26.4	14.4 ** ±19.3	54.3 ** ±34.1	6.8 ** ±1.90
CP	8.8* ±13.2 +	73.1 ** ±55.2 ++	58.5** ±22.1 ++	5.0 ** ±5.2 ++	2.7 ** ±2.1 ++	25.2 * ±12.1 ++	7.5* ±1.9
Non-survival SAP	32.5 ** ±26.3	170.1** ±124.9	277.9 * ±256.5	40.2 ** ±36.2	29.0 ** ±25.9	88.0 ** ±38.9	6.1** ±1.7
Survival SAP	29.6** ±129.7	163.8** ±129.7	253.7 * ±273.8	19.1** ±21.0 +	14.3 ** ±16.8 +	53.7 ** ±23.7 ++	6.7 ** ±2.1
SAP	32.2** ±126.0	166.4 ** ±126.0	263.8** ±265.0	28.3 ** ±30.3	20.2 ** ±21.9	67.3 ** ±34.8	6.5 ** ±2.0
MAP	11.7** ±12.6 ++	93.2** ±70.2 +	92.2 ** ±107.1 ++	5.6 ** ±6.8 ++	4.0 ** ±3.1 ++	30.8* ±15.1 ++	7.4 ** ±1.9 ++

NC: normal control, AP: acute pancreatitis, CP: chronic pancreatitis, SAP: severe AP, MAP: mild AP

Mean ±SD * p<0.05, ** p<0.01 vs NC. + p<0.05, ++ p<0.01

3.6. Correlations of sFas, sTAMRs, shedding enzymes, ligands, sMICA/B and severity markers (APACHE- II and SOFA)

Correlation of sFas with the three sTAMRs was significant in all groups except for sTyro3 and sMer in non-survival SAP and CP, and sMer in SAP. Correlations of sFas with ADAM10 and 17 were also significant in all groups except for non-survival SAP and ADAM17 in SAP. The relationships between the three sTAMRs (sTyro3, sAxl, sMer) and their ligands (Gas6, fPROS1) in pancreatitis cases are shown in Table 3. The correlation of Gas6 with the three sTAMRs was significant in all groups except for sTyro3 and sMer in non-survival SAP and sAxl and sMer in CP. The correlation of fPROS1 with sTyro3 was significant only in both SAP groups, while that of sMer with fPROS1 was significant in all groups except for non-survival SAP and MAP. The correlation of sMer with fPROS1 was significant in the survival SAP and CP groups. Correlation coefficients between fPROS1 and STAMRS were higher than those between Gas6 and STAMRS in the other SAP groups. However, while SAP cases showed significant correlations with both ligands, in MAP, mainly Gas6 and sTAMRs had significant correlations [Table 3]. These results suggest that Gas6 exerts its effects in pancreatitis regardless of the severity of symptoms, while fPROS1 has effects mainly in SAP. There were significant correlations of platelet counts (n=102) with Axl, sMer, and sTyro3, with coefficients of -0.495, -0.345, and -0.516, respectively. sMICA/B was also significantly correlated with APACHE-II and SOFA scores in all pancreatitis cases, with coefficients of 0.600 and 0.554 (n=96), and 0.575 and 0.555 (n=85), respectively. However, sMICA/B was not significantly correlated with APACHE-II and SOFA in non-survival SAP cases. There was also no significant correlation between sMICB and SOFA in survival SAP cases, but there were significant correlations in the other groups. These results indicate that the higher the sMICA/B level, the more severe the pancreatitis

Table 3 Correlations of sFas with sTAMRs (sTyro3, sAxl, sMer) and ligands (Gas6, free PROS1), sTAMRs with ligands, and sMICA/B with severity scores (APACHE-II, SOFA) in patients with pancreatitis

		Non-survival SAP	Survival SAP	SAP	MAP	AP	CP
sFas	sTyro3	0.314 (22)	**0.775 ** (25)**	**0.543 ** (47)**	**0.450 * (31)**	**0.581 ** (78)**	0.244 (18)
	sAxl	**0.774 * (22)**	**0.810 ** (25)**	**0.771** (47)**	**0.681** (33)**	**0.774 ** (80)**	**0.585 * (18)**
	sMer	- 0.157 (22)	**0.673 ** (25)**	0.282 (47)	**0.393 * (31)**	**0.366 * (78)**	0.432 (16)
	ADAM 10	0.495 (22)	**0.671 * (25)**	**0.566 * (47)**	**0.482 * (31)**	**0.603 * (78)**	0.317 (18)
	ADAM 17	- 0.200 (22)	**0.527 * (25)**	0.135 (47)	**0.462 * (31)**	**0.248 * (78)**	**0.566 * (18)**
Gas6	sTyro3	0.305 (24)	**0.482 * (27)**	**0.327 * (51)**	**0.608 ** (33)**	**0.522 ** (84)**	**0.576 * (21)**
	sAxl	**0.831** (24)**	**0.586 * (27)**	**0.483 * (51)**	**0.473 ** (33)**	**0.587 ** (84)**	0.387 (21)
	sMer	0.191 (24)	**0.389 * (27)**	**0.327 * (51)**	**0.498 ** (33)**	**0.393 * (84)**	0.189 (21)
fPROS1	sTyro3	**- 0.772 ** (24)**	**- 0.711** (27)**	**- 0.463 ** (51)**	- 0.279 (33)	**- 0.331 * (84)**	**- 0.435 * (21)**
	sMer	- 0.178 (24)	**- 0.593 * (27)**	**- 0.422 ** (51)**	- 0.013 (33)	**- 0.390 ** (84)**	**- 0.576 * (21)**
sMICA	APACHE-Ⅱ	0.328 (24)	**0.625 * (22)**	**0.482 * (50)**	**0.512 * (33)**	**0.599 ** (83)**	**0.433 * (20)**
	SOFA	0.320 (24)	**0.448 * (22)**	**0.471 * (50)**	**0.531 * (33)**	**0.540 ** (83)**	**0.518 * (20)**
sMICB	APACHE-Ⅱ	0.237 (23)	**0.601* (27)**	**0.429 * (50)**	**0.568 * (33)**	**0.554 ** (83)**	**0.572 * (19)**
	SOFA	0.067 (23)	0.268 (27)	**0.478 * (50)**	**0.618 * (33)**	**0.557 ** (83)**	**0.629 * (19)**

*** P <0.05, **P <0.01**

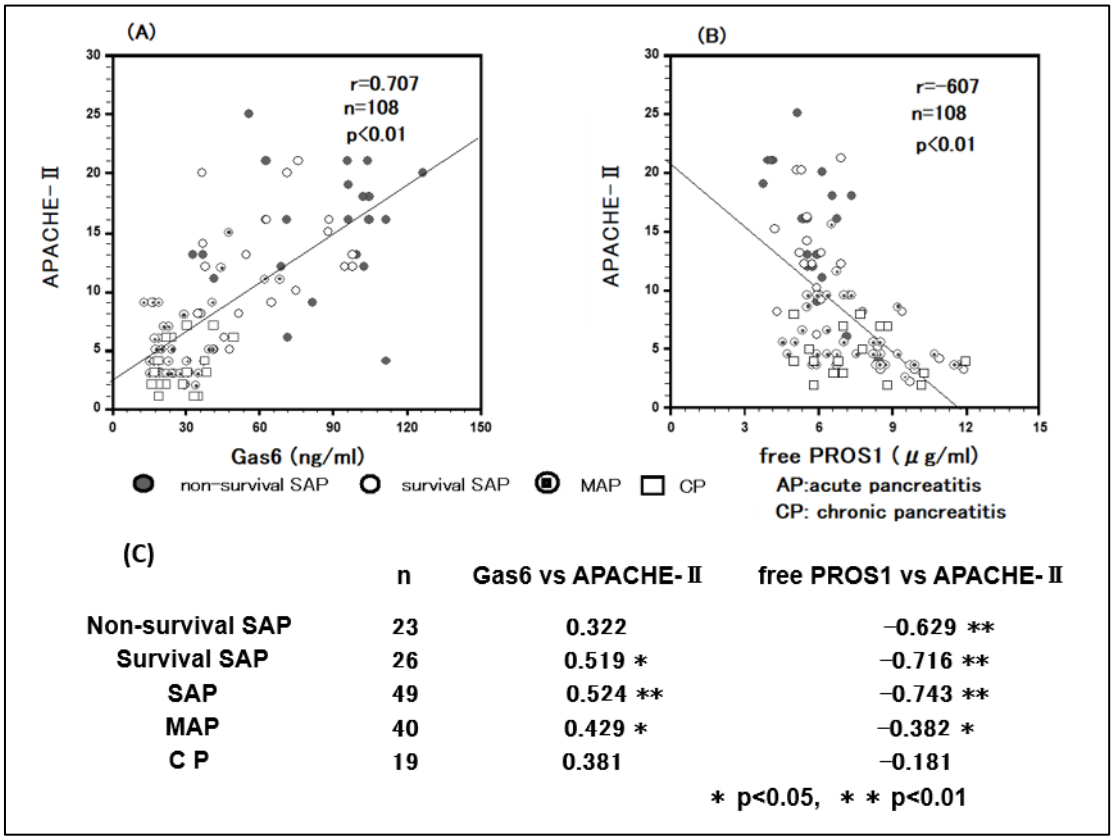
3.7. Correlations of Gas6 and fPROS1 with APACHE-II scores

APACHE-II was significantly correlated with Gas6 and fPROS1 [Figure 4A, B]. The correlation coefficient for Gas6 with APACHE-II was higher than that for fPROS1. The correlations for Gas6 with APACHE-II were significant in all groups except for non-survival SAP and CP, and those for fPROS1 were significant in all groups except for CP. Correlation coefficients for fPROS1 were higher than those for Gas6 in all groups except CP [Figure 4C].

4. Discussion

MICA/B is highly expressed in many infected or transformed human cells. MICA/B generated by inflammation and tumorigenesis is cleaved by ADAM10/17 to generate SMICA/B, which in turn causes evasion of immune surveillance by NK cells and worsens inflammation and tumorigenesis [3,4]. Thus, this dual rule of MICA/B as a target molecule for immunosurveillance by NK cells and impairment of NK cell-effector functions facilitates infection and tumor immune escape, so-called immunoreaction [3, 4, 18, 19].

Recent reports have suggested that sMICA/B levels is a marker for potential confounding clinical conditions in diseases such as cancers, host-graft response, autoimmune disease, myocardial infarction, and bacterial and viral infections [20, 21]. Efferocytosis is the clearance of ACs by professional and non-professional phagocytes. The Fass mechanism is involved in the process. Soluble Fass (sFas), an apoptotic index, is produced in a form lacking 21 amino acid residues containing the transmembrane domain by alternative splicing. sFas inhibits Fass-FasL binding and blockade of Fas-mediated apoptosis. Thus, the increase of sMICA/B may be synonymous with an increase of sFas [22].



(C)

	n	Gas6 vs APACHE-Ⅱ	free PROS1 vs APACHE-Ⅱ
Non-survival SAP	23	0.322	−0.629 **
Survival SAP	26	0.519 *	−0.716 **
SAP	49	0.524 **	−0.743 **
MAP	40	0.429 *	−0.382 *
C P	19	0.381	−0.181

* p<0.05, * * p<0.01

Figure 4 Correlations of APACHE-II scores with ligands (Gas6, free PROS1) in patients with pancreatitis

TAMRs (Tyro3, Axl, Mer) and their cognate glycoprotein ligands (Gas6, fPROS1) are critical regulators of tissue hemostasis and inflammation. Release of the ectodomains of TAMRs by ADAM10/17 generates soluble forms, which can work as decoy receptors, except for sMer [11, 23]. The inflammatory activity of NK cells is also controlled by TAMRs [24, 25]. Therefore, MICA/B may have a close relationship with TAMRs for AC removal via NK cells and by shedding enzymes [26]. Shedding can be induced by inflammatory stimuli (e.g., lipopolysaccharide) leading to the release of the extracellular domain of the receptor and generation of sAxl, sMer, and sTyro3 forms that are able to interact with and sequester Gas6 and PROS1. Gas6 binds to all three receptors, while PROS1 binds to Mer and Tyro3, but not to Axl. Gas6 and PROS1 binds with high affinity to Axl and Tyro3. Completion of efferocytosis requires TAMRs that are attached to the plasma membrane via Ca^{2+} binding to the Gla-domain of Gas6 or PROS1 [27-29].

In pancreatitis, Fas and MICA/B are cleaved from cells by metalloproteases, released into the blood, and suppress inflammation by binding to ACs. This can be inferred from the significant correlation between the two proteins in pancreatitis cases other than non-survival SAP. sMICA/B in survival SAP cases peaked early in the onset of symptoms and then declined, whereas in non-survival SAP cases SMICA/B peaked early and then rose again 14-30 days after onset, indicating inflammatory recurrence in these cases.

During cell death, PtdSer binding to TAMR ligands enables bridging of apoptotic bodies with phagocytic cells to promote debris removal. Regulation of this process of efferocytosis, by TAMRs is crucial for tissue homeostasis. TAMRs are RTKs that are expressed by multiple immune cells, including NK cells. Although RTKs typically enhance cellular functions, TAMR ligation blocks NK cell activation. The mechanisms by which RTKs block NK cell signaling downstream of activating receptors are unknown [29-31].

In this study, we measured sMICA/B and efferocytosis-related markers in pancreatitis cases and investigated their roles based on correlations. All markers were significantly increased in patients with pancreatitis compared to NCs. Non-survival SAP cases had the highest marker levels and SAP cases had significantly higher levels than MAP cases. ADAM10/17 and sMICA/B had a positive correlation in pancreatitis cases, as expected, but the correlation coefficients tended to be lower in non-survival SAP compared to other groups.

Correlations between the three sTAMRs and two ligands were significant in most AP groups. There were also significant correlations between Gas6 and sAxl, and fPROS1 and sTyro3 in non-survival SAP, but neither correlated with sMer. The significant correlation between TAMRs and both ligands (even taking into account the decoy receptors) suggests that efferocytosis is facilitated by these proteins. Disparate correlations in non-survival SAP cases indicate failure of efferocytosis and relapse of inflammation, while the partially significant correlation in CP suggests partial residual chronic inflammation.

Failure of efferocytosis in non-survival SAP cases may have led to formation of damage associated molecular patterns (DAMPS), which cause relapse of inflammation and MOF due to the associated necrosis. In severe pancreatitis, injured parenchymal cells release DAMPs. These are signals 1 and 2 for respective receptors in DAMP-sensing cells, which then produce IL-1β and IL-18, as effector cytokines that stimulate pro-inflammatory responses in other immune cells, promoting further cell death in the pancreas and immune injury in distant organs. Thus, these cases progress to inflammatory relapse and organ derangement [32,33].

The partially significant correlations among sTAMRs and ligands, ADAM10/17, and sMICA/B in CP cases (although only a small number of cases) suggests chronic inflammation and progression of fibrosis (SLE, liver fibrosis, and IgG4-RD disease) due to partial defects in efferocytosis [34-36]. TAMRs are essential for phagocytosis of apoptotic cells, and TAMR activation is associated with immunosuppressive responses. Impaired TAMR function is associated with activity of pancreatitis in humans, and plasma levels of soluble TAMRs generated by proteolytic cleavage and TAMR ligands may be biomarkers for development and prognosis of AP and CP. Encouraging results have been obtained supporting a therapeutic role for TAMRs in AP and CP.

Gas6 showed a significant correlation in all AP groups, but fPROS1 only showed this correlation in severe disease. Gas6 is a secreted 75 kDa glycoprotein with vitamin K-dependent function that is expressed in many cell types and tissues. The Gas6 plasma level is significantly lower than that of fPROS1. About 60% of circulating fPROS1 is bound to complement C4bp, which is thought to increase during inflammation, and inhibits the cofactor function of Protein C and the function of Tyro3 in vitro. However, Gas6 does not have such a structural site and has no such action. Thus, Gas6 may exert its effects from the early stage of pancreatitis (inflammation), while fPROS1 may act mainly when the disease spreads systemically.

The actions of TAMRs and ligands may also be relevant to sepsis, which is pathologically similar to pancreatitis. There was a significant correlation between the severity indicators APACHE-II and sMICA/B in pancreatitis cases. From these results, we conclude that in pancreatitis, the higher the sMICA/B level, the more severe the disease, with Gas6 acting regardless of the disease state, but fPROS1 acting mainly in severe disease [37]. The fact that Axl and Tyro3 play important roles in platelet activation and thrombosis, and may be better than Mer as targets for thrombosis inhibition, may indicate differences in binding between each TAMR and Gas6 and fPROS1, and this needs further investigation [38,39]. A high APACHE-II score was related to abnormally high Gas6 and fPROS1 levels, with fPROS1 having a stronger correlation than Gas6. This indicates a need to determine the detailed mechanisms in efferocytosis, including in inflammation and tumors.

5. Conclusion

We measured sMICA/B and efferocytosis-related markers in pancreatitis cases and investigated their roles from their correlations. All markers were significantly increased in the pancreatitis groups compared to the NC. The correlations with various markers showed significant correlations in the survival SAP and MAP groups, but disparate correlations in the non-survival SAP group, indicating failure of efferocytosis and relapse of inflammation, while the partially significant correlation in the CP group suggested chronic inflammation. Gas6 shows a significant correlation in all AP groups, but free-PROS1 shows a significant correlation only with the severe disease group.

These results support the potential biomarker and therapeutic role of measuring sMICA/B, three sTAMRs, and both ligands in acute and chronic pancreatitis as potential biomarkers for their pathogenesis and prognosis.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

None of the authors have a conflict of interest.

Statement of ethical approval

The study was approved by the ethics committee of Hijirigaoka Hospital and Evergreen Hospital, and performed compliance with the Treaty of Hilinski. All patients admitted to Hijirigaoka Hospital and Evergreen Hospital from January 2016 to February 2023 were included in the primary analysis.

Statement of informed consent

All participants gave signed informed consent before enrollment.

Author Contributions

Concept, design, and supervision: S.U., T.M.; Resources, materials, data collection and processing: Y.F., TM, S.U., KG.; Analysis and interpretation: K.G.; Literature search and manuscript writing S.U., Y.F.; Critical review: K.G., S.U.

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