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(RESEARCH ARTICLE)

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Roles of soluble TAM receptors, ligands for these receptors, and shedding enzymes in patients with acute pancreatitis

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Abstract

The goal of the study was to measure the plasma levels of soluble TAM receptors (sTAMRs: Tyro3, Axl and Mer), growth arrest-specific 6 (Gas6), free-PROS1 (Protein S), and C4bp-protein and two disintegrin metallopeptidase domains (ADAM 10 and 17) in patients with acute pancreatitis (AP).

The subjects were 56 patients, including 14 cases of severe AP and 42 cases of mild AP, and 20 healthy normal controls. Plasma levels of the three sTAMRs, Gas6, free PROS1 and C4bp-protein, and two ADAMs were measured using ELISA kits.

The levels of the sTAMRs and ADAM10 and 17 increased markedly with greater severity of AP. Three sTAMRs increase rate became the order of sTyro3, sMer and sAxl, while ADAM10 was higher than ADAM17. The platelet count and PROS1 significantly decreased in AP but there are differences between C4bp-protein, but these are not significant. sTAMRs levels increased in the early stage in almost all AP patients, but then gradually normalized. However, sTAMRs in non-survivors increased again in the late stage. The fluctuations of ligands (Gas6 and free-PROS1) levels in AP patients showed progress like three sTAMRs. The correlation coefficients of sTAMRs with ligands were stronger for free-PROS1 in non-survivors and patients with severe AP, whereas Gas6 was more strongly correlated with sTAMRs in patients with mild AP.

Marked increases of sTAMRs and ADAMs were found with increased severity of AP. Gas6 and free-PROS1 in the TAM system may have complementary roles in local and systemic lesions, respectively.

Keywords: TAM receptor; Tyro3; Gas6; PROS1; ADAM10; ADAM17

1. Introduction

Acute pancreatitis (AP) is a potentially fatal disease that shows early pathologic findings of necrosis, apoptosis and microthrombosis in many tissues. Important mild local and severe systemic factors are associated with disorders of vascular endothelial tissue, microcirculation due to leukocyte migration to tissues, and activation of coagulation [1, 2]. Monocytes and macrophages, two important phagocytes, use surface receptors for phagocytic uptake of apoptotic cells. Tyro3, Axl, and Mer are integral membrane proteins that constitute TAM receptors. TAMRs play an important role in anti-inflammatory responses through modulating the function of macrophages. This signaling occurs through two bridging molecules, PROS1 and Gas6, which are vitamin K-dependent proteins. TAMRs are widely expressed in several tissues, and particularly in immune cells (macrophages, dendritic, and natural killer cells), platelets, endothelial cells, osteoclasts, sertoli cells, and the retinal pigment epithelium [3, 4].

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TAMR signaling has important regulatory roles in vascular smooth-muscle homeostasis, platelet function and thrombus stabilization, and erythropoiesis [4]. Gas6 can bind to all three TAMRs, while PROS1 only binds to Tyro3 and Mer. Axl has the highest affinity for Gas6 in vitro (K_d=1.0 nM), followed by Tyro3 with roughly equal affinity, whereas Mer affinity for Gas6 is at least 10-fold lower [5]. ADAM10 cleaves Axl (and possibly Tyro3) and ADAM17 (tumor necrosis factor-α-converting enzyme: TACE) cleaves Axl and Mer to form soluble forms in blood [5, 6]. Such sTAMRs generated by proteolytic cleavage or alternative splicing can quench ligands, which limits TAM signaling, and are transported from the membrane for recycling.

TAMRs, their ligands, and cleavage enzymes are negative regulators of immune function at the interface of innate and adaptive immunity [6]. M_1 macrophages contribute prominently to systemic immune response syndrome (SIRS), which is associated with multi-organ dysfunction syndrome (MODS) and increased mortality. SIRS and multi-organ failure (MOF) often develop in patients with severe AP.

In contrast to M_1 macrophages, the M_2 phenotype is associated with organ regeneration and fibrosis development, and TAM signaling can inhibit polarization of M1 macrophages [4, 7, 8]. In this study, the plasma levels of sTAMRs, their ligands (Gas6 and free-PROS1) and shedding enzymes (ADAM10 and 17) were measured to determine the changes in these levels with increasing severity of AP.

2. Material and methods

2.1. Subjects

Table 1 Clinical background of patients with AP

Item	Severe AP		Severe AP	Mild AP
	Non-survivors	Survivors		
Female	0	1	1	8
Male	6	7	13	34
Alcohol intake	3	5	8	18
Bile stone	1	0	1	11
Idiopathic	1	4	5	10
ERCP	0	0	0	3
S-amylase (u/L)	12647 ± 8943	11566 ± 11383	12174 ± 9349	7249 ± 4175
U-amylase (u/L)	18967 ± 6698	14786 ± 9853	15770±7451	6983 ± 4751
Lipase (u/L)	4478 ± 3471	4021 ± 3482	4102 ± 3375	886 ± 542
WBC (/μl)	1.8374±7986	16743±10453	17513 ± 8514	11923 ± 7382
Platelets (10^4/μl)	16.0±8.4* (3.7-37.2)	17.8±6.2* (7.8-30)	17.3±6.0* 3.7-37.2)	18.6±5.4* (8.7-30.3)
APACHE-Ⅱ	22-6	25-2	25-2	15-1
SOFA	17-4	20-1	20-1	13-0

*p<0.05, ** p<0.01 vs. controls

The subjects were 56 patients with AP, including 42 classified with mild AP and 14 with severe AP, and 20 normal adult controls 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. The severity of AP was assessed using Ranson's criteria [9], APACHE-II criteria, JSS score, criteria for Intractable Disease of the Pancreas issued by the Japanese Ministry of Health, Labour and Welfare, and the criteria of the Revised Atlanta Classification [10, 11,12]. MOF was assessed using APACHE-II[10] and SOPA [13]

criteria at the time of blood sampling. Of the 14 patients with severe AP, 6 died due to sepsis and MOF, and there were 8 survivors. Furthermore, there were seven shock cases (non-survival SAP 4, survival SAP 3).

2.2. Measurement of sTAMRs, ligands and related protein, and shedding enzymes

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 DuoSet® (R&D Systems, Minneapolis, MN, USA) for Gas6, and a Protein S test Teijin® (Teijin Diagnostics, Osaka, Japan) for free PROS1 and total PROS1. Values of C_4bp-PROS1 were calculated using the formula C_4bp-PROS1= total PROS1-free PROS1. The Intra- and inter assay coefficients of variation for the measurements were 7.9-11.2%.

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

Serum and urine amylase, white blood cell counts, and platelet counts in patients and controls were measured by routine laboratory procedures. Venous blood samples were taken on admission (days 0-1: within 24 h of pain onset), and 3, 7, 14, and ≥30 days after admission. Totals of 62 and 48 blood samples were collected in patients with severe and mild AP, respectively. 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 $P<0.05$ was considered significant.

3. Results

3.1. Plasma levels of sTAMRs (sTyro3, sAxl, and sMer)

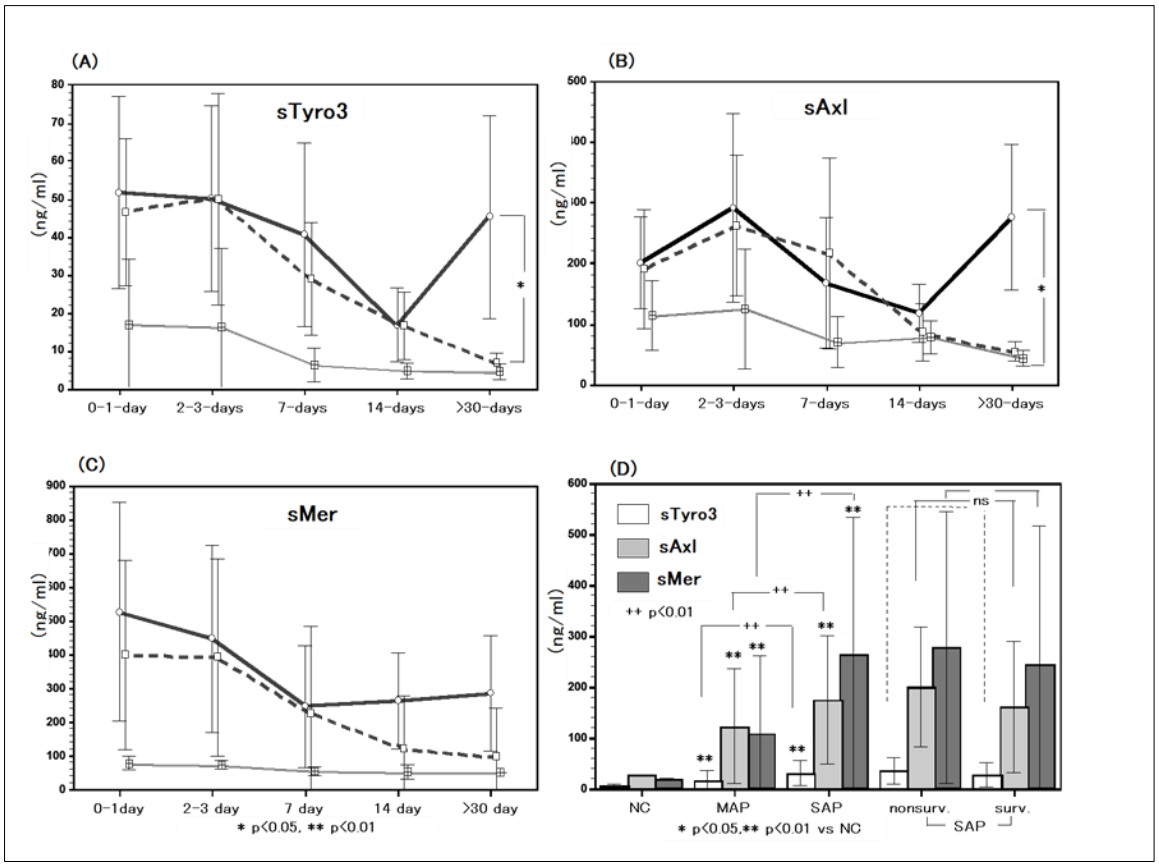

Figure 1 Serial levels of sTAMRs in patients with AP

sTAMR levels were significantly higher in patients with severe and mild AP, compared to controls, and were higher in non-survivors than in survivors among patients with severe AP, although this difference was not significant [Figure 1D].

sTAMR levels in almost all patients showed a peak during hospitalization at 0-3 days, but slowly decreased and normalized thereafter. However, the sTAMR levels in non-survivors elevated again at 14 days after admission [Figure 1]. Similarly, sAxl and sTyro3 levels at ≥30 days after admission differed significantly between survivors and non-survivors [Figure 1 A-C]. The increase in sTAM Rs was quantitatively in the order of sMer> sAxl> sTyro3, but the rate of increase relative to the average value of the healthy subject group was in the order of sTyro3> sMer> sAxl.

3.2. Changes and fluctuations in plasma levels of Gas6 and free-PROS1

The Gas6 level in patients with AP was significantly higher than that in controls, and increased with disease severity. The free-PROS1 level in patients was significantly lower than that in controls, and decreased with disease severity. There were also significant differences in Gas6 and free-PROS1 levels in survivors and non-survivors [Figure 2].

Gas6 levels in survivors and patients with mild AP peaked at hospitalization day 3 and decreased and normalized thereafter, but those in non-survivors continued to elevate in hospitalization. The free-PROS1 levels in survivors and patients with mild AP changed with progression and then normalized, but decreased in non-survivors after hospitalization for 14 days [Figure 2].

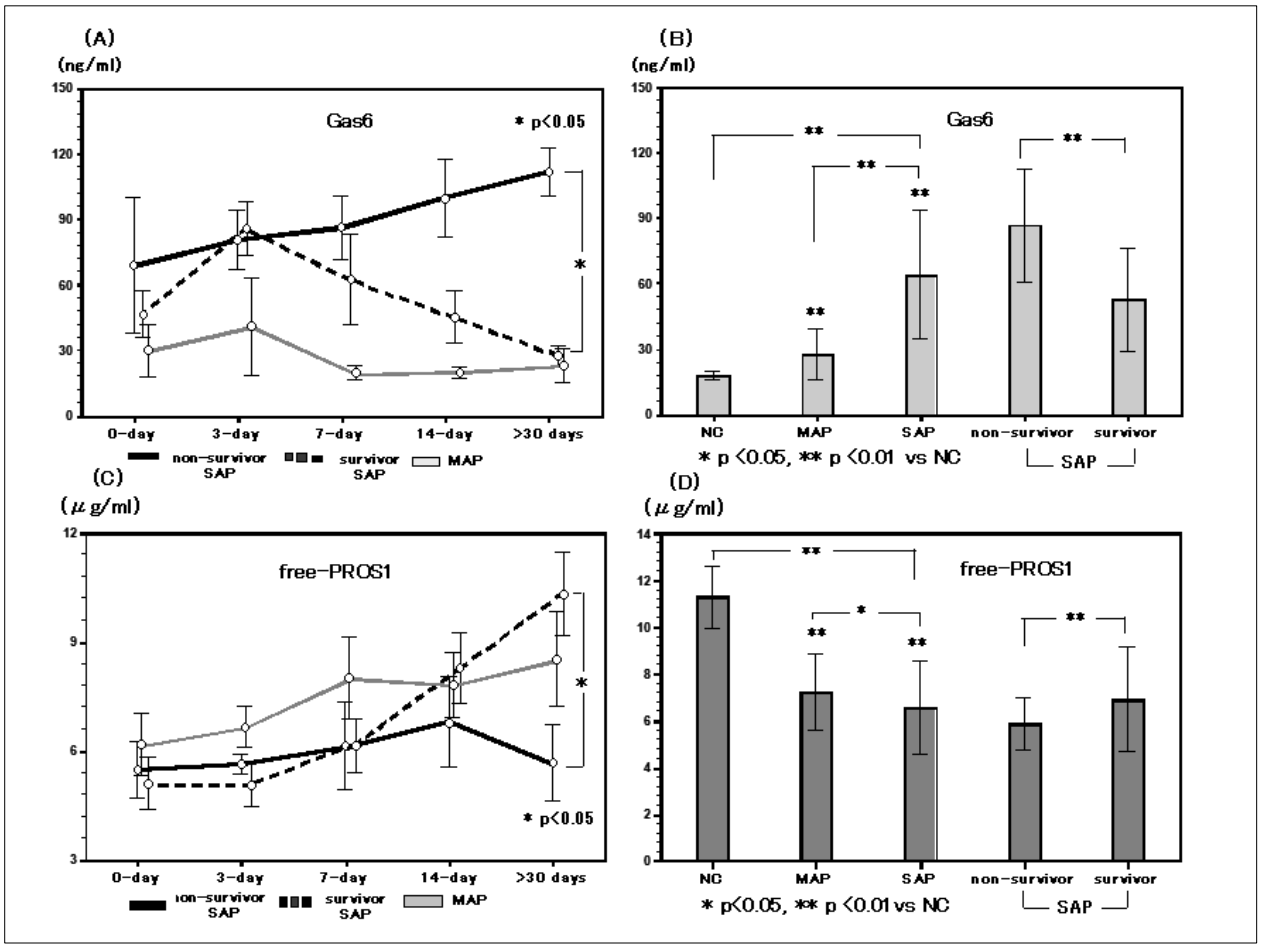

Figure 2 Changes and fluctuations in plasma levels of Gas6 and free-PROS1

3.3. Change and fluctuation in plasma levels of C4bp protein

The change of C4bp-protein in AP decreased for early days and gradually increased before long, but the change was limited to the normal range [Figure 3 A] The PS significantly decreased in AP and there are significant differences between AP and NC, nonsurvivor SAP and survival SAP. However, C4bp-protein and the difference between these were not significant. Also, in the non-survival SAP group, there was not the significant difference than survival SAP and MAP group, but showed an increase tendency [Figure 3 B].

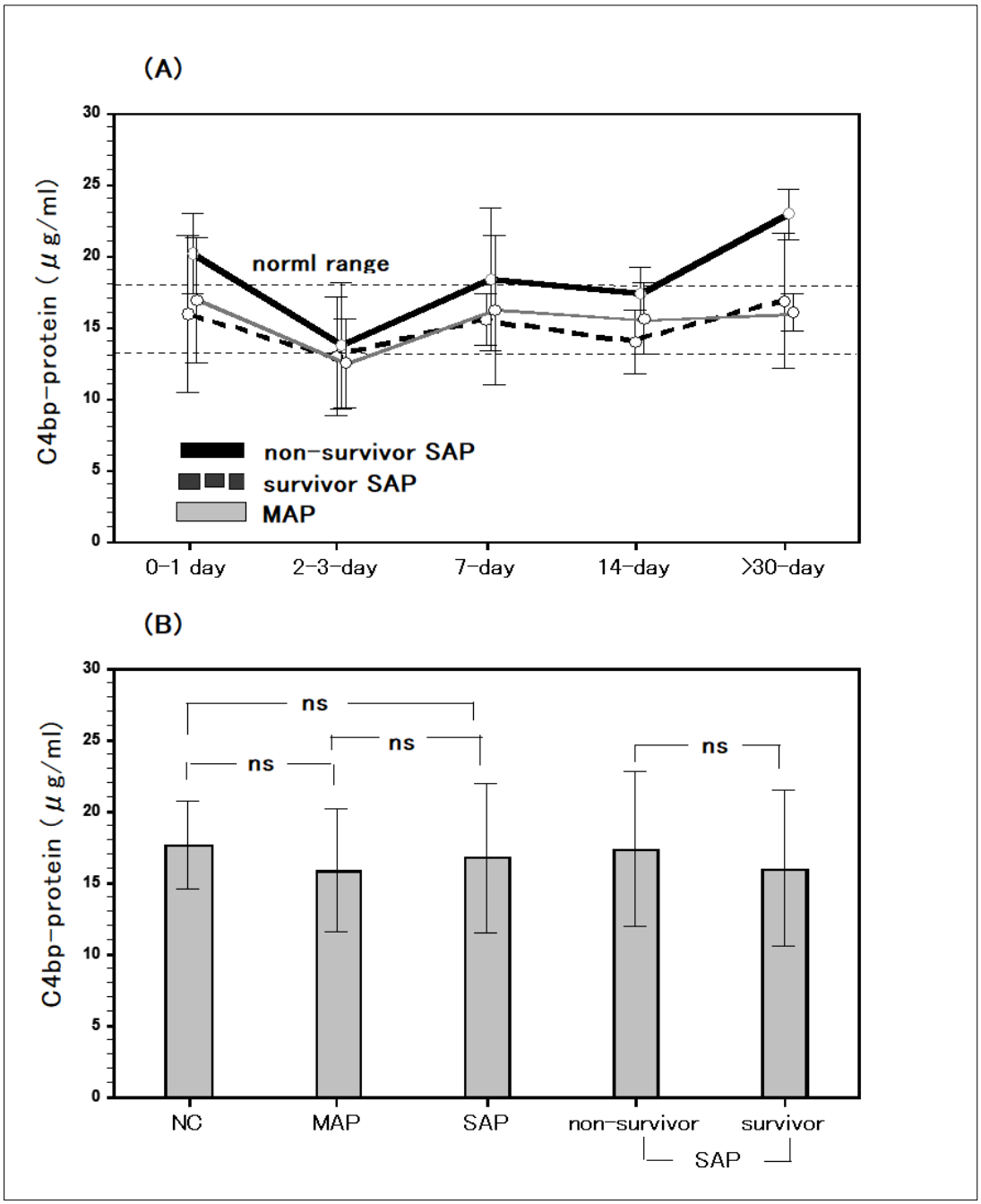

Figure 3 Change and fluctuation in plasma levels of C_4bp protin

3.4. Plasma levels of ADAM10 and ADAM17

ADAM-10 and ADAM-17 showed higher values of ADAM-17 than ADAM-10 in healthy subjects, but ADAM-10 was higher than ADAM-17 in AP cases, and both showed significantly higher values than the healthy subjects.

The ADAM 10 levels in almost all patients showed a peak during hospitalization at 0-3 days, but slowly decreased and normalized thereafter. However, the ADAM10 levels in non-survivors elevated again at 14 days after admission. Those levels at ≥30 days after admission differed significantly between urvivors and non-survivors [Figure 4A].

The ADAM17 levels in MAP and survival SAP showed a peak during hospitalization at 0-3 days, but slowly decreased and normalized thereafter. However, ADAM17 levels in non-survivor SAP showed a peak at 0-1day administration, and slowly decreased at 7 days, and elevated again at ≥30 days after admission. Those levels at ≥30 days after admission differed between survivors and non-survivors. However, the difference was not significant [Figure 4 B].

ADAM-10 and-17 levels were significantly higher in patients with severe and mild AP, compared to controls, and were significantly higher in non-survivors than in survivors among patients with severe AP, ADAM-10 and-17 levels were significantly higher in patients with severe and mild AP, compared to controls, and were higher in non-survivors than in survivors among patients with severe AP, although this difference was significant [Figure 4C].

A shock state was shown in seven cases (non-survivor 4 and survivor 3) of the SAP. ADAM-10 and ADAM-17 values in shock cases in the SAP were 27.8 and 37, 77.6 and 87.5, 84.5 and 54, 77.6 and 87.5, 80.5 and 62, 54 and 78, and 22.8 and 19.4, respectively [Figure 4 C].

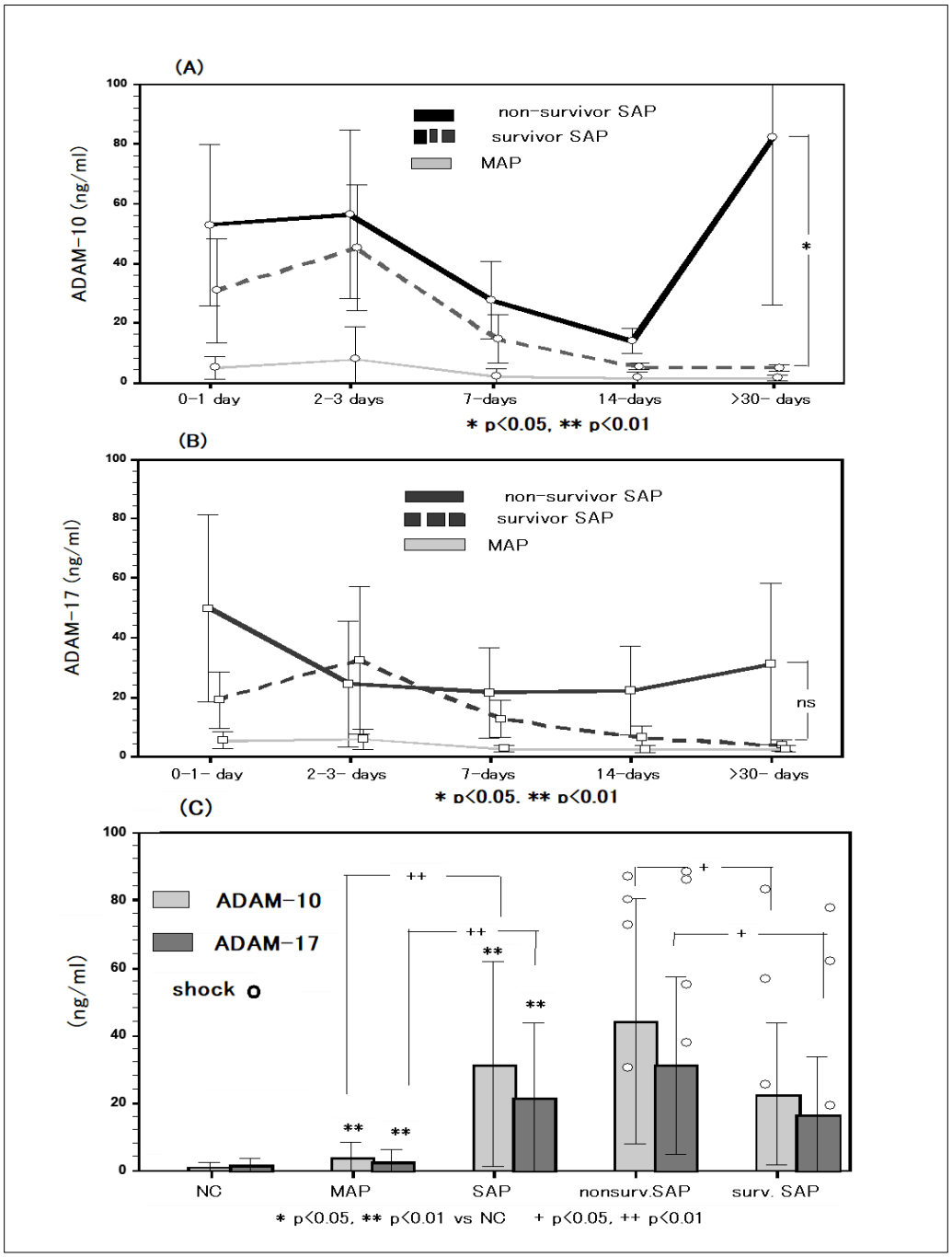

Figure 4 Correlations between sTAMRs, ligands (Gas6 and free-PROS1), and shedding enzymes in patients with AP

3.5. Correlations between sTAMRs, their ligends and shedding enzymes

The correlation of coefficients with Gas6 and TAMRS (sTyro3, sAxl, and sMer) became 0.528, 0.506 [Figure 5 A], and 0.537, respectively.

Likewise, the correlation coefficients with free-PROS! and TAMRs became -0.494 [Figure 5 B], -0.363 and -0.427, respectively. These correlations were significant. ($p<0.05$)

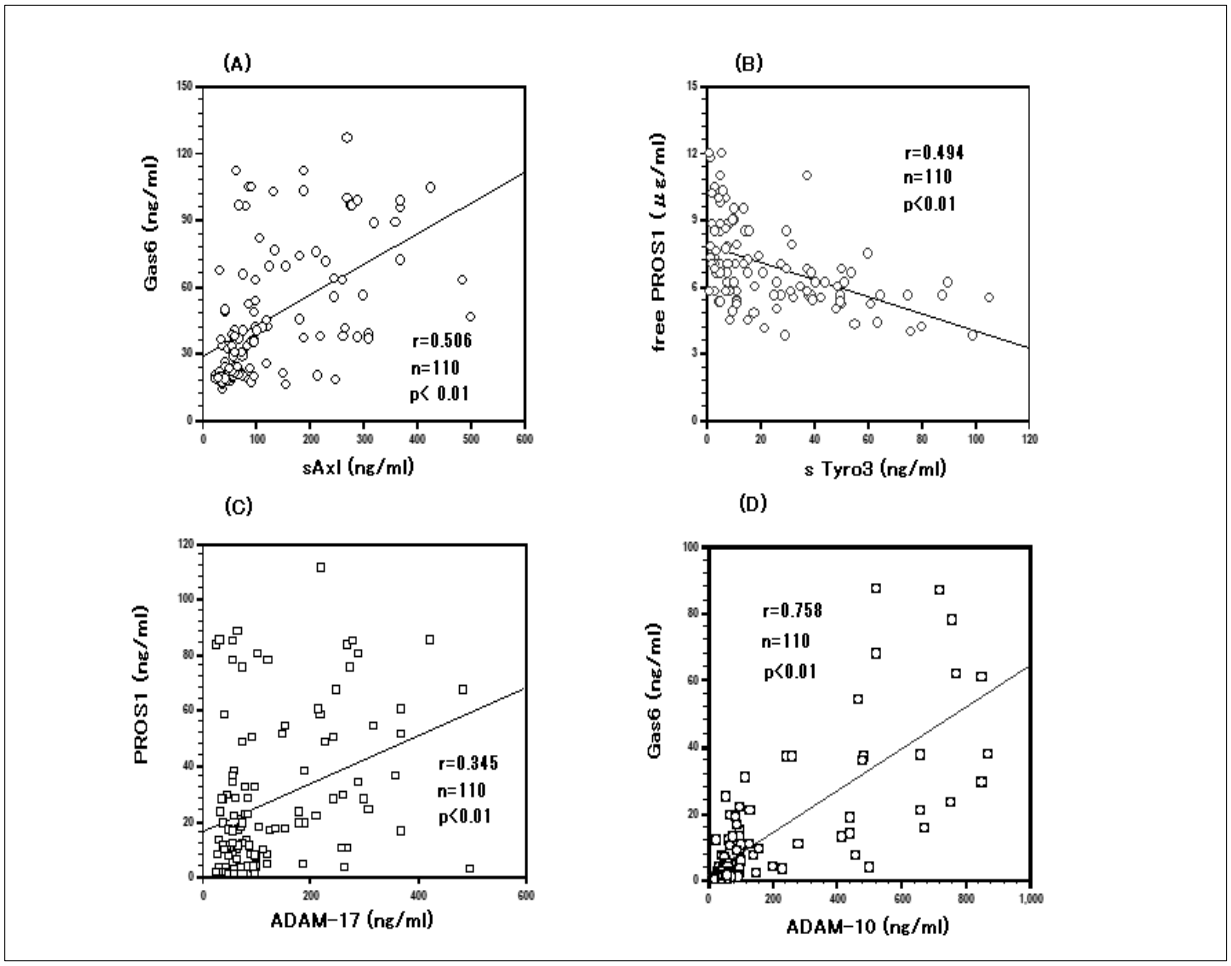

Figure 5 Correlation between sTAMRS, their ligands, and shedding enzymes in AP patients

Each other's coefficients of correlation of Gas6 and free-PROS1 and two shedding enzymes are shown as follows. The coefficients of correlation with ADAM10 and Gas6 and free-PROS1 became 0.638 and -0.471 respectively.

Subsequently the coefficients of correlation with ADAM17 and Gas6 and free-PROS1 became 0.648 and -0.374 respectively. Those correlations were significant ($p<0.01$).

Correlations among the three sTAMRs and two ligands in patients with mild and severe AP are shown in Table 2. In non-survivors, the correlation coefficient of free-PROS1 with sTyro3 was -0.760 ($p<0.05$). All other coefficients with Gas6 and sTAMRs were not significant in these patients.

In patients with mild AP, the correlation coefficients of Gas6 with sTyro3 and sAxl were 0.488 and 0.413, respectively ($p<0.05$). Gas6 was more strongly correlated with sTAMRs than free-PROS1 in mild AP, but free-PROS1 had stronger correlations with TAMRs in patients with severe AP and in survivors. Correlations of ADAMs with sTAMRs increased with greater disease severity [Table 2].

Table 2 Correlations between sTAMRs (sTyro3, sAxl, and sMer), ligands (Gas6 and free-PROS1), and shedding enzymes (ADAM10 and 17) in patients with AP

		Gas6	**free PROS1**	**ADAM 10**	**ADAM 17**
nonsurv. SAP	sTyro3	0.102	-0.760**	0.713*	-
	sAxl	0.033	-	0.669*	-0.004
	sMer	0.332	-0.171	-	0.754*
	T+A	0.049	-	0.754*	-
	A+M	0.298	-	-	0.671*
	T+M	0.327	-0.236	-	-
	T+A+M	0.293	-		-
surv. SAP	sTyro3	0.472*	-0.585**	0.583*	-
	sAxl	0.539*	-	0.621*	0.568*
	sMer	0.356*	-0.405*	-	0.688**
	T+A	0.512*	-	0.637**	-
	A+M	0.465*	-	-	0.690**
	T+M	0.418*	-0.481*	-	-
	T+A+M	0.471*	-	-	-
SAP	sTyro3	0.384*	-0.612**	0.707**	-
	sAxl	0.363*	-	0.602**	0.313*
	sMer	0.372*	-0.370*	-	0.690**
	T+A	0.377*	-	0.660**	-
	A+M	0.427*	-	-	0.639**
	T+M	0.410*	-0.442**	-	-
	T+A+M	0.444*	-	-	-
MAP	sTyro3	0.488*	-0.280*	0.561*	-
	sAxl	0.413*	-	0.523*	0.230
	sMer	0.070	-0.301*	-	0.426*
	T+A	0.464*	-	0.495*	-
	A+M	0.255	-	-	0.159
	T+M	0.537*	-0.318*	-	-
	T+A+M	0.451*	-	-	-
sTyro3 (T), sAxl (A), sMer (M), * p < 0.05, * * p < 0.01					

3.6. Changes in platelet counts and correlation of platelet counts with sTAMR levels

In comparison with the healthy normal controls, the decrease of the platelet counts in AP is regarded as consumption by the thrombogenesis [Table 1].

Correlation coefficients of platelet counts with sTAMRs (sTyro3, sAxl, and sMer) were -0.314 ($p<0.05$), -0.445 ($p<0.01$), and -0.252 ($p<0.05$), respectively, and those for platelet counts with Gas6 and free-PROS1 levels were -0.457 and 0.394,

respectively ($p<0.01$). The platelet counts significantly decreased from patients with mild AP to survivors and then to non-survivors.

3.7. Correlations of AP severity scores with plasma levels of sTAMRs and ligands

The correlation coefficients of Gas6 levels with APACHE-II scores and SOFA scores were 0.650 and 0.654 ($p<0.01$), respectively, and those for free-PROS1 levels with these scores were -0.704 and -0.655 ($p<0.01$), respectively, in patients with AP.

The correlation coefficients of sTyro3, sAxl, and sMer with APACHE-II scores in the patients were 0.613, 0.511, and 0.500 ($p<0.01$), respectively, and those of these sTAMRs with SOFA scores were 0.561, 0.444, and 0.429 ($p<0.01$), respectively.

4. Discussion

The Gas6/PROS1/TAM system is highly pleiotropic and involved in several functions, including regulation of inflammatory response, tissue repair and fibrosis development, and vascular integrity [4, 6]. TAMRs contribute to inhibition of inflammatory responses by initiating phagocytosis through recognition of apoptotic cells, and may be involved in the immune mechanisms of apoptosis and necrosis in severe AP. TAMRs are variably expressed in many tissues and undergo proteolytic cleavage by ADAM10 and 17 to produce sTAMRs in blood. The sTAMRs move from the membrane for recycling and act as decoy receptors for the Gas6 and PROS1 ligands to impair phagocytosis of macrophages [6-8].

Patients with AP had marked increases of sTAMR levels, with particularly high increases in sMer and sTyro3 in comparison with that of sAxl. These levels normalized in most patients with improvement of AP, but sTAMR levels increased again in non-survivors and MOF was the common cause of death [1, 2].

Generally, there is specificity in combinations of TAMRs and ligands: sTyro3 and sAxl act as antagonists by blocking free-PROS1, Gas6 induces TAMR activation, and sMer has weak inhibitory activities toward both ligands [6]. Therefore, the correlation between sMer and Gas6 was highest among the receptor-ligand pairs. TAMRs are expressed predominantly in myeloid-derived hematopoietic cells, and are also found in other cell types, including normal epithelial and endothelial cells. Gas6 and PROS1 are present in the plasma at concentrations of 0.2 nM (18-25 ng/ml) and 350 nM (12-16 μg/ml), respectively. Gas6 is produced mainly in the heart, kidneys and lungs, with little production in the liver. This protein is expressed in endothelial cells, vascular smooth muscle cells and bone marrow, and promotes survival of endothelial and smooth muscle cells upon growth arrest. Most circulating Gas6 is bound to sAxl and is inactive, but endothelial cells and vascular smooth muscle cells secrete Gas6, thereby increasing the local Gas6 level at the vessel wall. Gas6 also activates recycling of Axl receptors in the cell membrane [14-16]**.** The fluctuation of Gas6 levels may be a marker of peripheral events rather than being able to mediate an active signal itself. From these observations suggested that the function of Gas6 is locally restricted rather than systemic [14]. Gas6 regulates tissue factor expression from the endothelium, which participates in thrombus development [17]. PROS1 is present at relatively high levels in plasma, and serves as an essential cofactor for activated protein C (PC), a protease that degrades factor Va and factor VIIIa, thereby inhibiting blood coagulation. PROS1 is mostly produced by hepatocytes and >60% of PROS1 is bound to C4b protein [3,15]. PROS1 functions as anticoagulant by inhibiting FIXa and by serving as cofactor for APC and TFPI. The PROS1-C_4bp complex binds to phosphatidylserine (PtdSer) in the apoptotic cell membrane and inactivates complements such as C3a and C4a. In other words, PROS1 promotes efferocytosis through TAM Rs. Burstyn-Cohen et al. found that approximately 50% of PROS1 in the circulation originates from endothelial/hematopoietic cells [16]. The cytoprotective function of both aPC and PROS1 appears to be mediated by their ability to activate the endothelial PC receptor and TAMRs, respectively, rather than by their roles in the clotting cascade, and the protective function of PROS1 is mediated by Tyro3 [18,19].

All three receptors are present on platelets and vascular smooth muscle cells, and Gas6 and PROS1 have potent trophic effects on these cells, both in vitro and in vivo. Axl and Tyro3 play an important role in platelet activation and thrombosis, and may serve as a better target than Mer for inhibition of thrombosis [18,19]. Our results for the relationships of TAMRs with platelet count had the order of sAxl > sTyro3 > sMer [4, 7], which suggests that there is considerable artery and venous thrombosis in multiple organs in AP. Furthermore, the trends of the three TAMRs and two ligands suggest systemic thrombosis. The relationships among the vascular endothelium, macrophages, platelets, and ligands (coagulation factors) in the TAM system in AP indicate that Gas6 mainly acts in local AP lesions, while PROS1 acts mainly in lesions that are systemic and cause diffuse MOF. [19, 20] The functions of free-PROS1 and Gas6 as TAM agonists may share a role based on severity in development of AP, and the roles of Gas6 and PROS1 in inflammation

may be universal. All three types of TAM receptors can be found in human plasma in their soluble (sTyro3, sAxl e, sMer) forms as a result of membrane receptor cleavage mediated by two metalloproteases (ADAM 10 and ADAM 17). Furthermore, ADAM-17 which increases for severe pancreatitis, activates kinin B1 receptor through an angiotensin converting enzyme 2 (ACE_2) receptor shedding [21]. Furthermore, the endothelial protein C receptor (EPCR) is cleaved by ADAM-10 [22, 23], ACE_2 shedding results in serious hypofunction in neurons, monocytes, pancreatic islets, respiratory systems, cardiovascular systems, kidney etc. The activation of the kinin in those pancreatitis is vasoactive peptides released by enzymatic action of high molecular weight (HMW) and low molecular weight (LMW) kininogens on substrates known as plasma and tissue kallikreins [24]. In contrast, PROS1 expression is upregulated in the interleukin 4-induced Th_2 response, dampening dendritic cell activity via Tyro3 and limiting type 2 immunity [25]. Gas6 interact with TAMRs under hemostatic conditions, they can promote platelet aggregation to maintain thrombosis and platelet stability. During this process, PtdSer will be exposed to activated platelet to participate in production of thrombin and will also bind to Gas6 to activate TAM. PROS1 can be used as anticoagulant in the anticoagulation process of Activation Protein C to inhibit coagulation.Induction of ADAM-10 cleaves endothelial protein C receptor (EPCR), impairs APC formation. and leads to purpura fulminans [22].

This results in the loss of a major feedback inhibitor of endothelial APC system.The generation of APC occurs when the circulating zymogen PC is activated by partial proteolysis by thrombin-thrombomodulin complexes. PROS1 function s as an anticoagulant by directly inhibiting procoagulants, such as FXa, FVa, and FIXa, while as serving as a cofactor for anticoagulants such as Activated PC (APC) and Tissue factor pathway inhibitor (TFPI) By associating with C_4bp-protein, PROS1 has also been shown to minimize the effect of inflammation.PS-C_4bp protein further binds to PtdSer in the apoptotic cell membrane and in activates complements such as C_3a and C_4a [24].

Finally PS promotes efferocytosis through TAMRs. Regarding the origin of MOF in aggravated AP, innate immune activation and disturbances in coagulation to be inseparably linked, with each activity as positive feedback for activation of the orther.

Specific alterations in the plasma levels of sTAMRs and their ligands in patients with AP have been related to disease severity and clinical complications of severe coagulopathies, with strong implication of involvement of immune responses.

Further studies are needed to identify the detailed mechanisms involving the Gas6/PROS1/TAMR system in pancreatic acinar and ductal cells and other tissues at different stages of acute pancreatitis.

5. Conclusion

Regarding the aggravation of AP and the involvement of the TAM system, the increase in ADAM-10 and -17 causes the effects of EPCR and ACE_2 cleavage in the vascular endothelium, while having the background of suppression of Type 2 immunity as shown in the increase in sTyro3. At that time, a role-sharing change occurs from Gas6, which acts at a local site in ligand, to PROS1, which acts systemically.

These can be understood as systemic immune defense reactions that connect the vascular system and organs.

Compliance with ethical standards

Acknowledgments

We would like to thank Teijin Co., Ltd for providing various ELISA-kits.

Disclosure of conflict of interest

None of the authors have a conflict of interest.

Statement of ethical approval

Our study was approved by the ethics committee of Hijirigaoka Hospital and complies with the Treaty of Helenski. All patients admitted to Hijirigaoka Hospital(Hokkaido, Japan) from January 2015 to October 2021 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, K.G.; Resources, materials, data collection and processing: Y.F, T.M.; Analysis and interpretation: K.G.; Literature search and manuscript writing: S.U.; Critical review: K.G.

References

[1] Sah RP, Garg P, Saluja AK. Pathogenic mechanisms of acute pancreatitis. Curr Opin Gastroenterol 2012, 28 (5): 507-15.

[2] Bhatia M. Apoptosis versus necrosis in acute pancreatitis. Am J Physiol Gastrointest Liver Physiol 2004, 286 (2): 189-96.

[3] van der Meer JH, van der Poll T, van't Veer C. Tam receptors, Gas6, and protein S: roles in inflammation and hemostais. Blood 2014, 123 (16): 2460-9.

[4] Rothlin CV, Carrera-Silva EA, Bosurgi L, Ghosh S. TAM receptor signaling in immune homeostasis. Annu Rev Immunol 2015, 33(1) :355-91.

[5] Law LA, Graham DK, Di Paola J, Branchford BR. GAS6/TAM Pathway Signaling in Hemostasis and Thrombosis. Front Med (Lausanne) 2018, 5 (9): 137.

[6] Tsou WI, Nguyen KQ, Calarese DA, et al. Receptor tyrosine kinases, TYO3, AXL, and MER, demonstrate distinct patterns and complex regulation of ligand-induced activation. J Biol Chem 2014, 289 (37): 25750-63.

[7] Lew ED, Oh J, Burrola PG, Lax I, et al. Differential TAM receptor-ligand-phospholipid interactions delimit differential TAM bioactivities. Elife 2014, 29 (3): e03385.

[8] Rothlin, CV, Ghosh S, Zuniga EI, Olistone MBA, Lemke G. TAM receptors are pleiotropic inhibitors of the innate immune response. Cell 2007, 131 (6): 1124-36.

[9] Ranson JHC. The timing of biliary surgery in acute pancreatitis. Ann Surg 1979, 189 (5): 654-63.

[10] Banks PA. Practice guidelines in acute pancreatitis. Am J Gastroentrol 1997, 92 (3):377-86.

[11] Ogawa M, Hirota M, Hayakawa T, et al. Development and use of a new staging system for severe acute pancreatitis based on a nationwide surgery in Japan. Pancreas 2002, 25 (4): 325-30.

[12] Banks PA, Bollen TL, Dervenis C, et al. Classification of acute pancreatitis - 2012: revision of the Atlanta classification and definitions by international consensus. Gut 2013, 62 (1): 102-11.

[13] Vincent JL, Moreno R, Takala J et al. The SOFA (Sepsis-related Organ Failure assessment) score to describe organ dysfunction/failure. Intensive Care Med 1996, 22 (7): 707-10.

[14] Ekman C, Stenhoff J, Dahlbäck B. Gas6 is complexed to the soluble tyrosine kinase receptor Axl in human blood. J Thromb Haemost 2010, 8 (4): 838-44.

[15] Hasanbasic I, Rajotte I, Blostein M. The role of gamma-carboxylation in the anti-apoptotic function of gas6. J Thromb Haemost 2005, 3 (12): 2790–97.

[16] Burstyn-Cohen T, Heeb MJ, Lemke G. Lack of protein S in mice causes embryonic lethal coagulopathy and vascular dysgenesis. J Clin Invest 2009, 119 (10):2942–53.

[17] Laurance S, Aghourian MN, Jiva Lia Z, et al. Gas6-induced tissue factor expression in endothelial cells is mediated through caveolin-1-enriched microdomains. J Thromb Haemosst. 2014, 12(3):395-408.

[18] Zhou J, Yang A, Wang Y, et al. Tyro3, Axl, and Merk receptors differentially participate in platelet activation and thrombus formation. Cell Commun Signal 2018, 16 (1):98.

[19] Angelillo-Scherrer A, Burnier L, Flores N, et al. Role of Gas6 receptors in platelet signaling during thrombus stabilization and implications for antithrombotic therapy. J Clin Invest 2005, 115 (2): 237–46.

[20] Uehara S, Handa H, Gotoh K, Tomita H, Sennshuu M. Plasma concentrations of growth arrest-specific protein 6 and protein S in patients with acute pancreatitis. J Gastroenterol Hepatol 2009, 24(9):1567-73.

[21] Parekh RU, Sriamula S. Activation of kinin B1R upregulates ADAM17 and results in ACE2 shedding in neurons. Int.Mol.Sci.2020, 22 (1):145.

[22] Davis CE, Arnold K. Role of meningococcal endotoxin in purpira.J Exp Med 1974, 140 (1):159-171.

[23] Faust SN, Levin M, Harrison OB et al .Dysfunction of endothelial protein C activation in severe meningococcal sepsis. N Engl J Med. 2001, 345 (6):408-416.

[24] Uehara S, Honjyo K, Furukawa S, Hirayama A, Sakamoto W. Role of the kallikrein-kinin system in human pancreatitis. Adv Exp Med Biol. 1989, 247B (1):643-8.

[25] Chan PY, Carrera Silva EA, De Kouchkovsky D, et al. The TAM family receptor tyrosine kinase TYRO3 is a negative regulator of type 2 immunity. Science 2016, 352 (6281): 99-103.

[26] Lécuyer H, Zoé Virion Z, Barnier JP, Soraya, Matczak S, Bourdoulous S,Bianchini E, Saller F, Borgel D , Nassif X, Coureuil M. An ADAM-10 dependentEPCR shedding links meningococcal interaction with endothelial cells to purpurafulminans. J Thromb Haemost. 2018, 14 (4):1171-82.