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OSTEOPROTEGERIN, TRAIL AND OSTEOPROTEGERIN/ TRAIL RATIO IN PATIENTS AT EARLY PHASE OF ACUTE PANCREATITIS

Abstract: Aim: Our aim was to determine serum concentrations of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and its decoy receptor osteoprotegerin (OPG) in patients with mild and moderate to severe acute pancreatitis (AP) in the early phase of the disease.
Materials and methods: We included 40 patients with AP (16 women, 24 men) admitted to I[st] Department of Surgery, Jagiellonian University Medical College, Krakow. Twenty-eight had mild (MAP) and twelve moderate to severe form of AP (SAP). Serum concentrations of OPG and TRAIL were measured by ELISA at admission and on days 3, 5 and 7.
Results: Both TRAIL and OPG were elevated in AP patients as compared to reference values. Starting from day 3 of the study, OPG concentrations were significantly higher in SAP than in MAP. Also, day 3 OPG was higher in patients who died from AP. OPG positively correlated with Glasgow score, C-reactive protein (CRP) concentrations and length of hospital stay. Day 3 OPG cut-off of 713 pg/mL enabled to differentiate between SAP and MAP with sensitivity of 71% and specificity of 80%. Area under ROC curve was 0.795, comparable to that achieved for CRP (0.838; $p > 0.05$). In contrast, serum concentrations of TRAIL were not associated with AP severity.
Conclusions: Determination of serum OPG concentrations may help in early prediction of severity of AP. However, diagnostic utility of the measurements seems too low to use OPG as a single clinically reliable predictor. Serum TRAIL is not useful in the differentiation between mild and severe form of AP.

Key words: acute pancreatitis, osteoprotegerin, TNF-related apoptosis-inducing ligand (TRAIL).

INTRODUCTION

Acute pancreatitis (AP) is a potentially fatal disease. Although most patients recover without complications (mild acute pancreatitis — MAP), in about 20–25% of patients the disease evolve into moderately severe or severe form (SAP), associated with transient or persistent organ failure and local complications. Severe acute pancreatitis is associated with high mortality, reaching 50% [1, 2]. Factors determining the extend of inflammatory response to pancreatic injury and the severity of the disease are not fully understood. However, the earliest events in

AP involve an injury of acinar cells, resulting in apoptosis or necrosis. Apoptosis of acinar cells appears to be associated with mild disease, while in severe form of AP necrosis prevails [2, 3]. As shown in animal experiments, early induction of apoptosis during the course of AP may have beneficial effect on the outcome [4]. On the other hand, the development of systemic complications in SAP may be associated with apoptosis affecting distant tissues and organs, such as lung, kidney, intestine or liver [5–7].

Apoptosis, a programmed cell-death, may be triggered by internal or external factors. The external pathway requires binding of death receptors with their ligands. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), a member of tumor necrosis factor (TNF) superfamily, is able to induce apoptosis via TRAIL receptor 1 (TRAIL-R1) and TRAIL-R2 while TRAIL-R3 and TRAIL-R4 do not transduce the apoptosis signal and may protect cells from TRAIL-induced apoptosis [8]. Soluble protein osteoprotegerin (OPG) has been shown to act as a decoy receptor for TRAIL [9].

In patients with SAP, early initiated intensive treatment is required. However, in clinical practice, early identification of these patients exerts an unresolved problem. Clinical scores such as Glasgow, Ranson or Acute Physiology and Chronic Health Evaluation II (APACHE II) as well as C-reactive protein (CRP) measurements that are routinely used for this purpose, enable reliable diagnosis after 48 hours from the onset of AP [10]. Among various biomarkers studied in this context, the members of TNF superfamily, namely, TNF-α, soluble TNF-R I and II and TNF-like weak inducer of apoptosis (TWEAK) have shown potential usefulness in diagnosis and prognosis of AP [11–13].

In this study, we hypothesized that the serum concentrations of TRAIL and its decoy receptor, OPG, may be associated with the severity of AP. Our aim was to determine the concentrations of TRAIL and OPG and the ratio of TRAIL/OPG in the early phase of AP and to compare them between patients with mild and moderate to severe disease.

MATERIALS AND METHODS

STUDIED GROUP

The study involved 40 patients admitted to Ist Department of Surgery, Jagiellonian University Medical College, Krakow with AP with the symptoms lasting no longer than 12 hours before admission. Twenty-eight patients had mild (MAP) and 12 moderate to severe form of AP (SAP). Basic demographic and laboratory characteristics of patients are presented in Table 1. A diagnosis of AP was based on clinical history, ultrasound scan and serum lipase or amylase activity (at least three times above the reference limit). The progression of morphological changes within the pancreas and in the surrounding tissues was evaluated

Table 1

Characteristics of the study group and laboratory parameters at admission.

	MAP (N = 28)	SAP (N = 12)	p
Female/male, N (%)	14 (50)/14 (50)	2 (17)/10 (83)	0.052
Age, years	51.8 ± 15.3	42.1 ± 11.5	0.1
Etiology: biliary/other, N (%)	17 (61)/11 (39)	2 (17)/10 (83)	0.011
Time of hospital stay, days	6 (5–8)	20 (12–47)	<0.001
Glasgow score, points	1 (0–1)	2 (1–3)	0.024
Mortality, N (%)	0	6 (50)	–
Amylase, U/L	482 (232–1243)	1411 (910–1660)	0.031
Lipase, U/L	925 (663–2400)	1124 (821–5045)	0.4
Albumin, g/L	36.9 ± 4.2	34.6 ± 7.4	0.6
CRP, mg/L	26.9 (10.9–85.5)	106.7 (71.7–235.5)	0.030

MAP — mild acute pancreatitis; SAP — moderate to severe acute pancreatitis; N — number of patients; LDH lactate dehydrogenase; CRP — C-reactive protein

using ultrasound imaging. In all patients ultrasound was performed every day during the study period. All patients with the severe form of AP underwent computed tomography (CT). In a few cases, the CT was performed more than once and showed the evolution of necrotic changes in the parenchyma of the pancreas and in the retroperitoneal and peritoneal spaces. The severity of AP was determined on the basis of repeated clinical examination, laboratory and imaging tests, according to the revised Atlanta criteria (2012) [1]. Patients with formerly diagnosed liver or kidney failure and those who refused to give the informed consent were excluded.

The study protocol has been approved by Jagiellonian University Medical College Bioethics Committee (KBET/86/B/2012). The study was conducted in accordance with the Declaration of Helsinki.

LABORATORY METHODS

The concentrations of OPG and TRAIL were determined in samples collected during first week of hospitalization: at admission (i.e. day 1) and on days 3, 5, and 7 of hospital stay. The samples were taken together with the blood used for routine biochemical tests in order to avoid additional burden for the patients. Twenty minutes after drawing, the blood was centrifuged (4000 rotations/minute; 10 minutes), the serum was frozen and kept in –70°C until assayed. The measurements

were carried out after all the samples have been collected. The following methods were used:

— OPG: ELISA method with the use of Human Osteoprotegerin kit (BIOMEDICA Medizinproducte GmbH&CoKG, Germany). The reference interval established by the manufacturer was 3.77–8.23 pmol/L (75.4–164.6 pg/mL).
— TRAIL: ELISA method with the use of Human TRAIL/TNFSF10 Quantikine ELISA kit (R&D Systems, Minneapolis, USA). The reference interval established by the manufacturer was 28–135 pg/mL and the reference interval determined by the authors in healthy individuals was 71.87–153.82 pg/mL.

The measurements of all ELISA micro-plates was performed using Automatic Reader (BIO-TEK® Instruments Inc., USA).

Routine measurements (CRP, amylase, lipase, albumin) were performed on the day of blood collection, with the use of routine laboratory methods.

STATISTICAL ANALYSIS

Number of patients (percentage of the appropriate group) are shown for categories. Quantitative data are presented as median (lower-upper quartile) or mean ± SD, according to distribution, as tested by Shapiro-Wilk test. Student t-test or Mann-Whitney test was used to assess differences between groups. Friedman ANOVA with appropriate *post-hoc* tests was used to compare the results of repeated laboratory measurements. Correlations were assessed with Spearman rank correlation coefficient. Receiver operating characteristic (ROC) curves were computed to assess the diagnostic utility of selected laboratory tests; the values of area under curve (AUC) are reported with 95% confidence interval (95% CI). Results were considered significant at $p < 0.05$. Computations were performed with Statistica 10.0 package (Statsoft Inc., Tulsa, USA).

RESULTS

OPG AND TRAIL CONCENTRATIONS AND OPG/TRAIL RATIOS IN PATIENTS WITH SAP AND MAP

Median concentrations of OPG and TRAIL as well as the ratio of OPG/TRAIL in AP patients during the early phase of the disease are presented in Table 2. At admission (day 1), median concentrations of OPG were almost 3 times higher in MAP patients and 4.5 times higher in SAP patients comparing to the upper reference values. On day 3, we observed further increase in OPG concentrations in SAP group ($p = 0.047$), followed by a decrease on day 7 ($p = 0.049$). No significant variability of OPG concentrations was observed in MAP group during the study period. Starting from day 3, OPG was higher in SAP than in MAP patients ($p = 0.031$; $p = 0.048$; $p = 0.029$ on day 3, 5 and 7, respectively).

Table 2

The concentrations of OPG and TRAIL and OPG/TRAIL ratios in patients with mild and moderate to severe acute pancreatitis during first week of the disease.

	Day	MAP	SAP	p
OPG, pg/mL	1	447 (345–642)	716 (587–1149)	0.055
	3	512 (365–654)	924 (539–1387)	0.031
	5	491 (395–724)	953 (483–1924)	0.048
	7	422 (369–550)	754 (573–1132)	0.029
TRAIL, pg/mL	1	647 (322–1003)	834 (530–2569)	0.1
	3	637 (339–1027)	784 (330–920)	0.8
	5	725 (466–1301)	952 (430–1889)	0.6
	7	1149 (698–1742)	1108 (920–1889)	0.6
OPG/TRAIL	1	0.45 (0.32–0.94)	0.64 (0.33–1.28)	0.7
	3	0.42 (0.35–0.83)	0.78 (0.29–3.14)	0.3
	5	0.46 (0.27–0.84)	0.85 (0.26–2.50)	0.3
	7	0.22 (0.20–0.56)	0.63 (0.30–1.23)	0.048

MAP — mild acute pancreatitis; SAP — moderate to severe acute pancreatitis; OPG — osteoprotegerin; TRAIL — tumor necrosis factor-related apoptosis-inducing ligand

Similarly, compared with upper reference values, TRAIL concentrations at admission were about 4 and 5.5 times higher in patients with MAP and SAP, respectively. However, TRAIL concentrations did not differ significantly between patients with MAP and SAP during the early phase of disease. In MAP group, TRAIL concentrations on day 7 were significantly higher than the initial concentrations ($p = 0.032$). Similar trend, although non-significant, was observed in SAP group.

Median OPG/TRAIL ratios were consequently higher in SAP patients as compared to MAP, but the groups differed significantly on day 7 only ($p = 0.048$).

CORRELATIONS OF OPG, TRAIL AND OPG/TRAIL RATIO WITH PARAMETERS RELATED TO SEVERITY OF AP

In the early phase of AP, OPG concentrations positively correlated with Glasgow score in the whole group of patients ($R = 0.50$; $p = 0.001$ for OPG assessed at admission; $R = 0.57$; $p < 0.001$ on day 3; $R = 0.56$; $p < 0.001$ on day 5 and $R = 0.59$; $p < 0.001$ on day 7). Similar although less strong correlations were observed for OPG/TRAIL ratio ($R = 0.41$; $p = 0.009$; $R = 0.48$; $p = 0.002$; $R = 0.39$;

p = 0.013; R = 0.50; p = 0.001, respectively). During the study period, OPG was positively correlated with CRP concentrations (R = 0.38; p = 0.016 at admission; R = 0.43; p = 0.006 on day 3; R = 0.35; p = 0.027 on day 5; R = 0.67; p <0.001 on day 7).

Longer hospital stay was associated with higher OPG concentrations on day 3 (R = 0.46; p = 0.003). Also, OPG concentrations on day 3 were significantly higher in patients who died in the course of AP as compared to survivors [1204 (1021–1387) versus 553 (425–746) pg/mL; p = 0.036].

TRAIL serum concentrations were not associated with the measures of AP severity.

DIAGNOSTIC UTILITY OF OPG IN PREDICTION OF AP SEVERITY

Figure 1 shows ROC curves for OPG measurements used to predict AP severity (i.e. to diagnose SAP versus MAP). OPG measurements at admission and on days

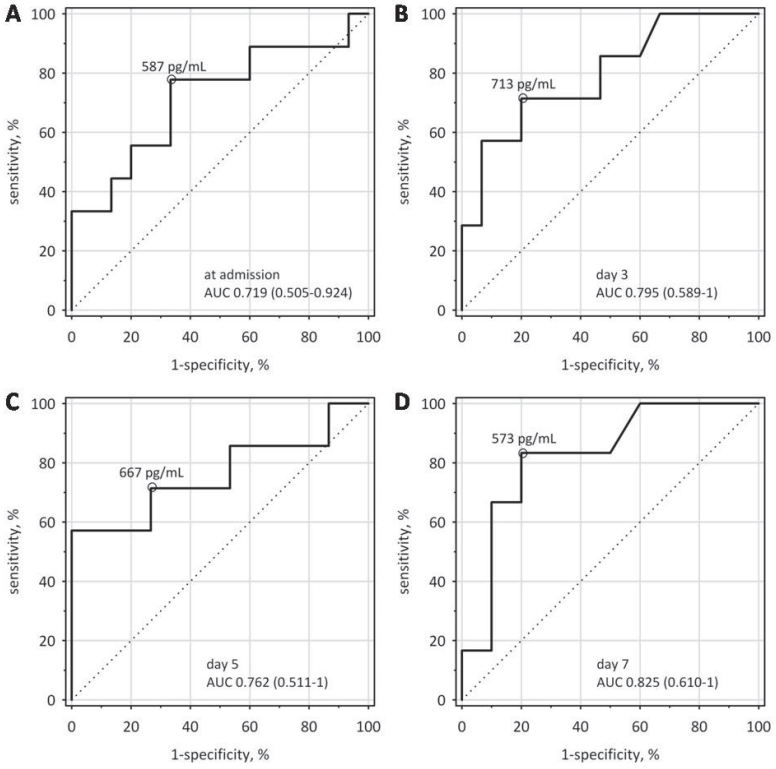

Fig. 1. ROC curves showing diagnostic utility of OPG concentrations measured at the early phase of acute pancreatitis for the differentiation of mild versus moderate to severe disease. OPG concentrations were determined at admission (A), on day 3 (B), 5 (C) and 7 (D). Proposed cut-off values and the values of area under curve (AUC) with 95% confidence intervals are shown on the graphs.

3, 5, and 7 enabled statistically significant differentiation between mild and moderate to severe AP. We compared these curves with the ROC curve computed for CRP determined on day 3 of AP. The AUC values for OPG (0.719–0.825; Figure 1) were lower than AUC for CRP [i.e. 0.838 (0.651–1)], although the differences were not statistically significant ($p > 0.05$ for all comparisons). OPG concentrations measured at admission enabled the diagnosis of SAP with sensitivity of 78% and specificity of 67% at the cut-off value of 587 pg/mL. On day 3, OPG measurements achieved sensitivity of 71% and specificity of 80% at cut-off value of 713 pg/mL.

DISCUSSION

In the present study, we investigated serum concentrations of TRAIL and its decoy receptor OPG during the early phase (first week) of AP. Starting from day 3 of the study, we found significant differences in OPG concentrations between patients with MAP and SAP, enabling the differentiation between the forms of AP with the diagnostic accuracy comparable with that achieved by CRP measurements. Also, OPG concentrations were higher in patients who died from AP and were significantly associated with measures related to disease severity, such as Glasgow score, CRP concentration and the length of hospital stay. In contrast, serum concentrations of TRAIL were not associated with AP severity. OPG/TRAIL ratios were weakly associated with the severity of AP and did not enable efficient differentiation between MAP an SAP patients in the early phase of AP.

To our best knowledge, this is the first study regarding serum concentrations of TRAIL and OPG in AP. Recently, TRAIL mRNA expression has been studied in experimental AP in rats [14], but the study was inconclusive as to the role of TRAIL in acinar cells apoptosis. TRAIL-mediated apoptosis has been associated with chronic pancreatitis [15]. However, serum concentrations of TRAIL cannot be directly correlated to local cell expression of TRAIL. Also, cellular actions of TRAIL depend on the local expression pattern of various TRAIL receptors [8]. Evidence exist, for example, that TRAIL may exert proliferatory and protective effect on pancreatic beta cells [16]. On the other hand, decreased soluble TRAIL has recently been associated with incidence and severity of sepsis [17]. To the contrary, our AP patients (including those who died) had higher TRAIL concentrations comparing to healthy individuals. The discrepancy may be associated with different pathology of the disease states, and partially explained by the fact that the majority of our patients had mild disease.

OPG has been first described as a decoy receptor for receptor activator of nuclear factor kappa B ligand (RANKL), involved in a critical signaling pathway regulating bone remodeling [18]. Later, OPG has been shown to bind TRAIL, blocking its proapoptotic functions. OPG expression has been shown to be induced by various up-stream inflammatory mediators, such as interleukin 1 and

TNF-α [19, 20]. Elevated serum OPG has been documented in various conditions associated with inflammatory state, i.e. atherosclerosis, where it seems to be mainly expressed by vascular endothelial and smooth muscle cells [18, 19, 21], chronic kidney disease [22], diabetes [23], or rheumatoid arthritis [24]. Recently, increased OPG has been associated with the extend of ischemic brain injury in experimental model in mice [25]. Also, OPG is expressed in pancreas and has been linked with type 1 diabetes [20] and pancreatic cancer [26]. In type 1 diabetes, OPG may act as an auto- or paracrine survival factor, protecting beta cells from apoptosis [20], although another group demonstrated that it promotes loss of beta cells [27].

The positive correlations between CRP and OPG, that were significant throughout the study period suggests that in AP patients, OPG may be up-regulated due to inflammatory state. From practical point of view, diagnostic sensitivity and specificity of OPG for prediction of SAP is comparable to that achieved by day 3 CRP measurements in our patients, and is also comparable to recent reports regarding the diagnostic utility of CRP in AP [28, 29]. However, our results are limited by the low number of patients studied and should be confirmed by the larger prospective trial.

CONCLUSIONS

Determination of serum OPG concentrations may help in early prediction of severity of AP. However, the diagnostic utility of the measurements, comparable to that of CRP, does not enable to use it as a single clinically reliable predictor. Still, it seems worth to be further studied in a larger group of patients. In contrast, our results suggest that serum TRAIL is not useful in the differentiation between mild and severe form of AP.

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Conflict of interest statement — none declared.

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