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Early Angiopoietin-2 Levels after Onset Predict the Advent of Severe Pancreatitis, Multiple Organ Failure, and Infectious Complications in Patients with Acute Pancreatitis

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BACKGROUND: Acute pancreatitis is a severe condition that requires early identification of patients at risk of developing potentially lethal complications. Current clinical scoring systems and biochemical parameters are insufficient. In this study, we aimed to assess whether early plasma Angiopoietin-2 (Ang-2) is associated with adverse outcomes in patients with predicted severe acute pancreatitis (SAP).

STUDY DESIGN: This analysis is a substudy of the PROPATRIA trial (probiotics vs placebo in patients with predicted SAP). The Ang-2 levels were measured prospectively in plasma in the first 5 days after admission in 115 patients.

RESULTS: Early Ang-2 levels were higher in patients who developed SAP: 6.4 vs 3.1 μg/L ($p < 0.001$) and also were higher in patients who developed multiorgan failure in the first week ($p = 0.001$) and after the first week ($p = 0.049$). Furthermore, high Ang-2 levels were associated with infectious complications in the first week ($p < 0.001$) and after the first week ($p < 0.001$). Finally, plasma Ang-2 was significantly higher in patients who died ($p < 0.001$) and in patients who developed bowel ischemia ($p < 0.001$). As a predictor of adverse outcomes, plasma Ang-2 was superior to a number of current scores, such as the APACHE II score, the Imrie score, C-reactive protein, lipopolysaccharide binding protein, and procalcitonin.

CONCLUSIONS: In the setting of this randomized controlled trial, early plasma Ang-2 was found to be an accurate predictor of SAP, multiorgan failure, and infectious complications. As a biomarker, it did outperform all of the investigated conventional predictors that are currently used in clinical practice. (J Am Coll Surg 2014;218:26–32. © 2014 by the American College of Surgeons)

Acute pancreatitis is an inflammatory disorder with a mild course in the majority of the patients. Approximately one-fifth of all patients with pancreatitis will, however, develop a severe form of disease, characterized by pancreatic necrosis and frequent organ failure. Morbidity and mortality rates in this group of patients are high, mainly due to infectious complications and/or multiorgan failure (MOF). Early identification of patients at risk for these adverse outcomes would allow appropriate clinical management to reduce complications.

To date, the most frequently used scoring systems to stratify patients with acute pancreatitis into risk groups include the Acute Physiology And Chronic Health Evaluation II (APACHE-II) and the Imrie score.[1] Acute phase proteins such as C-reactive protein,[2] procalcitonin,[3] interleukin (IL)-6, IL-8,[4] blood urea nitrogen,[5] and lipopolysaccharide binding protein[2] have previously been assessed for their capability to predict adverse outcomes

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Abbreviations and Acronyms

Ang-2	= Angiopoietin-2
IL	= interleukin
IQR	= interquartile range
LBP	= lipopolysaccharide-binding protein
MOF	= multiorgan failure
NPV	= negative predictive value
PPV	= positive predictive value
PROPATRIA study	= probiotics vs placebo in patients with predicted severe acute pancreatitis study
ROC	= receiver-operator curves
SAP	= severe acute pancreatitis

in acute pancreatitis, with varying diagnostic accuracy. One recent study stated that C-reactive protein remains the most useful single biochemical marker in acute pancreatitis.[6]

Recently, Angiopoietin-2 (Ang-2) has been identified as a promising biomarker in plasma to predict severe acute pancreatitis (SAP).[7] Ang-2 is 1 of 4 angiopoietins (Ang-1 to -4); Ang-1 and Ang-2 play an important role in the autocrine regulation of vascular stability and permeability as well as in the inflammatory balance. Both Ang-1 and Ang-2 are produced by endothelial cells. Ang-1 is produced and immediately released at a constant rate. In contrast, Ang-2 is presynthesized and stored in Weibel-Palade bodies. These endothelial-specific storage granules also store von-Willebrand factor, P-selectin, CD63, IL-8, endothelin-1, and tissue plasminogen activator.[8] The content of Weibel-Palade bodies is rapidly released on activation of the endothelial cell in response to, among others, thrombin, histamine, serotonin, and superoxides,[9-12] and in turn, leads to inflammation, coagulation, and angiogenesis.

Angiopoietins are ligands to the Tie-receptors (Tie-1 and Tie-2), which are almost exclusively expressed by endothelial cells and hemopoietic stem cells.[13] Binding of Ang-1 to the Tie-receptor results in stabilization of the endothelium. In contrast, Ang-2 destabilizes the blood vessels and enhances vascular leakage mainly by priming the endothelial cells to other cytokines.[9]

Elevated plasma Ang-2 has been associated with a range of conditions such as acute myocardial infarction, acute respiratory distress syndrome, liver cirrhosis, and trauma.[14] Ang-2 has been linked specifically to endotoxemia in an experimental human endotoxemia model.[15] This is supported by the finding that Ang-2 levels were higher in septic than in nonseptic critically ill patients.[16] The relationship between endotoxemia and Ang-2 makes it an especially interesting biomarker in SAP, considering previously reported findings that early intestinal barrier dysfunction and bacterial translocation are associated with bacteremia, infected necrosis, organ failure, and mortality.[17]

For Ang-2 to be of interest for daily practice, it needs to compare favorably with common currently used predictive clinical scoring systems and serum markers of inflammation. The aim of this study was to assess the value of Ang-2 in predicting the occurrence of SAP, MOF, and infectious complications.

METHODS

Study design

This study was a substudy of the PROPATRIA trial[18]; a double-blind randomized placebo-controlled trial that assessed the effects of probiotic prophylaxis in patients with predicted SAP. After the PROPATRIA trial had begun to enroll, an amendment to collect plasma samples in all patients was submitted to the local ethics board. As soon as approval was granted, a plasma sample was obtained from all patients enrolled from that moment. The samples were collected in the first week after admission and were stored at −80°C.

Patients

Inclusion criteria for the PROPATRIA trial were the diagnosis of acute pancreatitis and an APACHE II score of 8 or more, Imrie/modified Glasgow Coma Scale score of 3 or more, or C-reactive protein over 150 mg/L (normal < 8 mg/L). Patients were included only if the onset of symptoms was less than 72 hours before randomization. Patients were excluded if any of the following criteria were present: pancreatitis after endoscopic retrograde cholangiopancreaticography; suspected malignancy of the pancreas or biliary tree; nonpancreatic infection or sepsis caused by a second disease; diagnosis of pancreatitis first made at operation; or a medical history of immune deficiency. For the current analysis, patients were included if the plasma samples for Ang-2 measurement had been collected within the first 5 days after admission. As healthy controls, preoperative Ang-2 measurements from 20 living kidney donors were used.

Biochemical analysis

Ang-2 levels were assessed using a human Ang-2 enzyme-linked immunoassay (ELISA) test kit (R&D Systems). Besides Ang-2, lipopolysaccharide binding protein (LBP) and procalcitonin measurements were performed on the same plasma sample. The LBP measurements were also performed using an ELISA test kit (HyCult Biotechnology) for human LBP. The procalcitonin levels

were measured using a fully automated random-access immunoassay system (BRAHMS Kryptor).

Endpoints

The first endpoint was the association between Ang-2 levels and the occurrence of SAP. The second endpoint was the association between Ang-2 levels and presence of MOF. The third endpoint was the occurrence of any infectious complication. In addition to these endpoints, the associations between the Ang-2 levels and mortality and bowel ischemia were assessed.

Definitions

Severe acute pancreatitis was defined as the presence of organ failure and/or pancreatic parenchymal necrosis on CT scan. Organ failure was defined as PaO_2 below 60 mmHg despite FIO_2 of 30% or the need for mechanical ventilation (pulmonary insufficiency), plasma creatinine over 177 mmol/L (normal 53 to 117 mmol/L) after rehydration, or need for hemofiltration or hemodialysis (renal failure), and systolic blood pressure below 90 mmHg despite adequate fluid resuscitation or need for vasopressor (mainly noradrenalin and dopamine) support (cardiocirculatory insufficiency), adapted from the Atlanta classification. Multiorgan failure was defined as the failing of at least 2 systems on the same day.

Infected pancreatic necrosis, bacteremia, pneumonia, urosepsis, or infected ascites, during admission and at 90-day follow-up, were considered infectious complications. All infections were weighted equally; multiple infections in the same patient were considered to be 1 endpoint. Accepted criteria in the literature for the different types of infections were used, as described in the previously published PROPATRIA study protocol.[18,19]

Statistical analysis

Normally distributed data are presented as mean ± standard deviation (SD). Non-normally distributed data are presented as median (25^{th} percentile to 75^{th} percentile). The 2-sided Fisher's exact test was used for all comparisons of proportions. The independent Student's *t*-test was used to compare normally distributed continuous variables. The Mann-Whitney test was used to compare non-normally distributed continuous variables. All statistics were performed using SPSS 16.0 (SPSS Inc).

Diagnostic accuracy was portrayed as the area under the curve (AUC) of receiver-operator curves (ROC). The negative predictive value (NPV) was calculated in percentages as the number of patients who did not develop the investigated endpoint and had Ang-2 levels beneath the cut-off point divided by the total number of patients with Ang-2 levels beneath the cut-off point × 100%. The positive predictive value (PPV) was calculated as the number of patients who did develop the investigated endpoint and had Ang-2 levels above the cut-off point divided by the total number of patients who had Ang-2 levels above the cut-off point.

RESULTS

Patients

Plasma samples of 130 of the 296 patients included in the PROPATRIA trial were collected. Of these samples, 115 were collected within the first 5 days after admission, and were therefore included in this analysis. A baseline and outcomes comparison of the patients in this analysis and the rest of the PROPATRIA cohort is shown in Table 1. Patients in the current analysis resembled the rest of the cohort in all baseline and outcomes variables studied.

Severe acute pancreatitis was accompanied by MOF in 18 of 37 cases (48.6%), and by infectious complications in 27/37 cases (73.0%). Twelve cases of infectious complications occurred in patients with mild pancreatitis.

Angiopoietin-2 measurements

Plasma samples for Ang-2 analysis were collected on median day 3 after admission (interquartile range [IQR] 2 to 4), and on median day 3 after the start of symptoms (IQR 3 to 4). The Ang-2 distribution was right-skewed with skewness 1.9 (moderate right skewness); median Ang-2 was 4.1 μg/L (IQR 2.5 to 6.3). The distribution could be transformed to normal by using the log Ang-2 that had a skewness of 0.183 (no skewness); mean log Ang-2 was 0.61 ± 0.28. The median Ang-2 in the healthy controls was 1.3 (IQR 1.0 to 1.6).

Association between Angiopoietin-2 levels and outcomes

The association between plasma Ang-2 levels and adverse outcomes was assessed (Table 2). Levels of Ang-2 were significantly higher in patients who developed SAP, MOF, any type of infectious complications, bowel ischemia, and in patients who died (all $p < 0.001$). Of note, Ang-2 levels were also higher in patients who developed MOF or infectious complications late (ie, after the first week).

Angiopoietin-2 levels and other predictors of outcomes

Plasma Ang-2 levels were plotted in ROC curves to assess their predictive value of SAP, MOF, and infectious complications (Figs.1 and 2). Also plotted in the ROC curves were the conventional predictors of a severe course of pancreatitis: Imrie score, APACHE-II score, and highest C-reactive protein in the first 48 hours after admission.

Table 1. Baseline and Outcomes Comparison Between Patients in this Analysis and the Rest of the PROPATRIA Cohort

Variable	Plasma analysis (n = 115)	Rest of PROPATRIA cohort (n = 181)	p Value
Age, y, mean ± SD	60 ± 16	59 ± 16	0.287
Female sex, n (%)	44 (38.3)	78 (43.1)	0.468
Cause of pancreatitis, n (%)			
Biliary	66 (57.4)	101 (55.8)	
Alcohol	18 (15.7)	37 (20.4)	
Unknown	22 (19.1)	27 (14.9)	
Medication	3 (2.6)	7 (3.9)	
Hypertriglyceridemia	1 (0.9)	6 (3.3)	
Other	5 (4.3)	3 (1.7)	
Severity of pancreatitis			
APACHE II on admission	8.7 ± 4.5	8.4 ± 4.4	0.498
Imrie score in first 48 hours	3.3 ± 1.7	3.4 ± 1.6	0.859
C-reactive protein mg/L, highest in 1st 48 hours	251 ± 186	280 ± 123	0.053
SOFA score on admission	2 (1–3)	2 (1–3)	0.738
MODS on admission	1 (0–2)	1 (0–2)	0.431
Allocation to probiotics, n (%)	61 (53.0)	91 (50.3)	0.721
Severe pancreatitis, n (%)	37 (32.2)	65 (35.9)	0.533
Multiorgan failure, n (%)	18 (15.7)	30 (16.6)	0.873
Infectious complications, n (%)			
Infected necrosis	16 (13.9)	19 (10.5)	0.460
Bacteriemia	27 (23.5)	28 (15.5)	0.093
Pneumonia	17 (14.8)	23 (12.7)	0.606
Urosepsis	1 (0.9)	2 (1.1)	>0.999
Infected ascites	1 (0.9)	3 (1.7)	>0.999
Any infection	39 (33.9)	48 (26.5)	0.192
Bowel ischemia, n (%)	5 (4.3)	4 (2.2)	0.317
Mortality, n (%)	13 (11.3)	20 (11.0)	>0.999

APACHE II, Acute Physiology And Chronic Health Evaluation II; MODS, multiple organ dysfunction syndrome; PROPATRIA, probiotics vs placebo in patients with predicted severe acute pancreatitis study; SOFA, Sequential Organ Failure Assessment.

The ROC curves in Figures 1 to 3 show that Ang-2 is at least as good a predictor as the other predictors. The diagnostic accuracies of procalcitonin and especially LBP (shown in Table 3) were lower than that of Ang-2.

Optimal Ang-2 cut-off values for prediction of SAP, MOF, and infectious complications, based on the ROC curves, were 4.56 μg/L (sensitivity 81.1%, specificity 76.9%), 5.01 μg/L (sensitivity 72.2%, specificity 73.2%) and 4.51 μg/L (sensitivity 79.5%, specificity 76.3%) respectively. These cut-off values yielded the following positive predictive values (PPV) and negative predictive values (NPV): 62.5% PPV and 89.6% NPV for SAP; 33.3% PPV and 93.6% NPV for MOF, and 63.3% PPV and 87.9% NPV for infectious complications.

Effect of probiotics on predictive value of Angiopoietin-2

The Ang-2 levels measured in the first week were similar in the group of patients randomized to receive a specific probiotic composition and in the placebo group: median 4.2 μg/L (IQR 2.9 to 6.2) vs 3.8 μg/L (IQR 2.2 to 6.4) (p = 0.271). Predictive value of Ang-2 for SAP was almost identical in the probiotics arm and the control arm. Predictive value of Ang-2 for MOF and infectious complications were slightly higher in the probiotics arm (Table 3).

DISCUSSION

The major finding of this study was that early Ang-2 levels were able to accurately predict SAP, MOF, and infectious complications. Ang-2 was not only predictive for these events when occurring in the first week of admission, but also for those that presented after the first week.

The association between high Ang-2 levels and all types of infectious complications in pancreatitis is a novel finding. Although infectious complications are often related to SAP, in our study 12 of 39 infectious complications occurred in patients with mild pancreatitis, and 12 of 37 cases of SAP were not accompanied by infectious complications.

Table 2. Association Between Plasma Ang-2 Levels and Adverse Outcomes

Adverse outcome, n (%)	Events, n (n = 115)	Plasma Ang-2, μg/L*		p Value†
		Event	No event	
Severe pancreatitis	37 (32.2)	6.4 (4.6–11.5)	3.1 (2.2–4.5)	<0.001
Multiorgan failure	18 (15.7)	7.4 (4.3–12.1)	4.0 (2.3–5.2)	<0.001
On admission	1 (0.9)	–	–	–
Onset in first week‡	12 (10.4)	8.9 (3.3–12.5)	4.0 (2.3–5.2)	<0.001
Onset after first week‡	5 (4.3)	5.1 (4.3–10.2)	4.0 (2.3–5.2)	0.049
Infectious complications				
On admission§	2 (1.7)	–	–	–
In first week§	17 (14.8)	6.3 (4.9–10.1)	3.2 (2.2–4.5)	<0.001
After first week§	20 (17.4)	6.6 (4.8–11.4)	3.2 (2.2–4.5)	<0.001
Infectious complications				
Infected necrosis	16 (13.9)	6.4 (5.1–8.7)	3.7 (2.4–5.2)	<0.001
Bacteriemia	29 (23.5)	5.7 (3.4–11.2)	4.0 (2.2–5.0)	<0.001
Infected ascites	17 (14.8)	7.0 (4.9–12.0)	3.8 (2.4–5.1)	<0.001
Any infection‖	39 (33.9)	6.4 (4.6–9.8)	3.2 (2.2–4.5)	<0.001
Bowel ischemia	5 (4.3)	11.9 (6.2–14.1)	4.0 (2.5–5.8)	<0.001
Mortality	13 (11.3)	11.9 (5.8–14.1)	4.0 (2.4–5.15)	<0.001

*Numbers in parentheses indicate 25th and 75th percentile.
†p Values were calculated using log Ang.
‡Compared with the 97 patients who never developed multiorgan failure.
§Compared with the 76 patients who never developed infectious complications.
‖Includes 1 patient with urosepsis and 1 patient with infected ascites.

Circulating Ang-2 has previously been identified as a predictor of mortality in critically ill patients.[15] Ang-2 is increased in severe sepsis, and excess circulating Ang-2 is a predictor of mortality in septic critically ill patients.[20,21] In critically ill patients, with and without sepsis, Ang-2 is associated with pulmonary edema and occurrence and severity of acute lung injury and acute respiratory distress syndrome.[16]

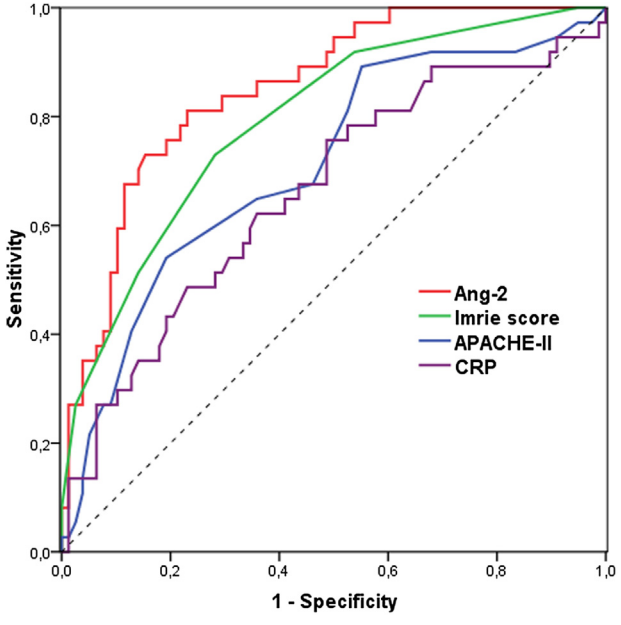

Figure 1. Value of Angiopoietin-2 (Ang-2), Imrie score (first 48 hours), APACHE-II score (admission), and CRP (C-reactive protein) (highest in first 48 hours) in predicting severe acute pancreatitis.

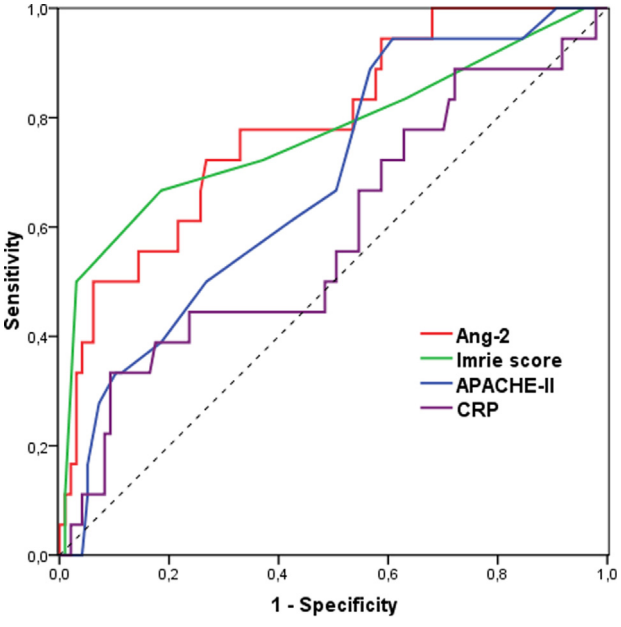

Figure 2. Value of Angiopoietin-2 (Ang-2), Imrie score (first 48 hours), APACHE-II score (admission), and CRP (C-reactive protein) (highest in first 48 hours) in predicting multiorgan failure.

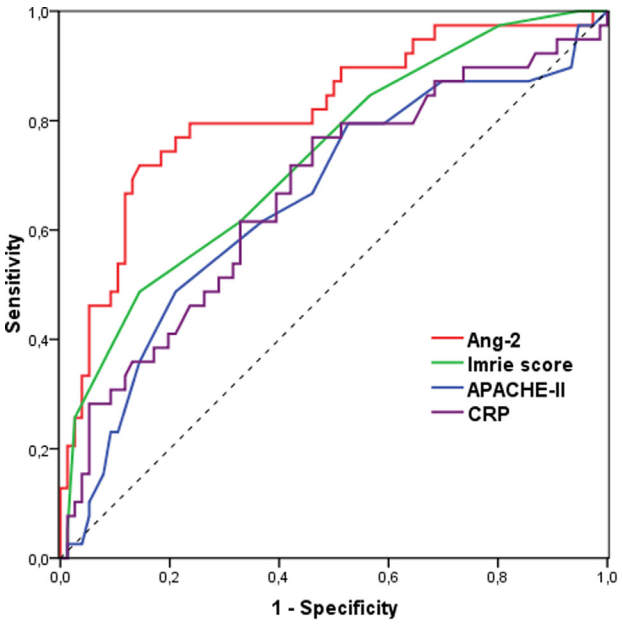

Figure 3. Value of Angiopoietin-2 (Ang-2), Imrie score (first 48 hours), APACHE-II score (admission), and CRP (C-reactive protein) (highest in first 48 hours) in predicting any type of infectious complication.

Recently, Whitcomb and colleagues[7] assessed the predictive value of Ang-2 in 2 independent patient populations with acute pancreatitis in the United States and Germany. Rather than investigating all patients with acute pancreatitis, our study focused on the subgroup of patients who have a higher risk of developing SAP, using serum C-reactive protein, Imrie score, and APACHE score for assessment of severity. The results of our study showed a rate of SAP of 37 of 115 (32.2%) compared with 20 of 151 (13.2%) found by Whitcomb and associates.[7] The areas under the curve in both studies were similar, with optimal cut-off points yielding slightly lower sensitivity (81% vs 90%) but higher specificity (77% vs 67%) in our study. The ratio of the optimal cut-off point to the mean healthy control value in this study was substantially higher than that reported by Whitcomb and coworkers[7] (3.5 vs 1.44 μg/L). Both studies were essentially post-hoc analyses. As such, the predictive value of Ang-2 will need to be validated in a prospective cohort before a definite conclusion can be reached. This study examined a subgroup of patients with higher a priori risk of SAP; a validation study would need to include all patients with acute pancreatitis.

To our knowledge, angiopoietin is not yet offered as a routine clinical measurement. The costs of the test kits will depend on whether they are purchased in package deals with other tests and in what quantity.

The need for accurate clinical scoring systems to predict the outcomes of acute pancreatitis is high. In the first week after onset, it is often difficult to predict whether the clinical course will be mild or severe. In case of mild pancreatitis, the disease is self-limiting and medical intervention is minimal: fluid management and early enteric feeding followed by cholecystectomy during primary admission in case of acute biliary pancreatitis will lead to satisfactory results. Severe acute pancreatitis warrants a different management. In case of SAP, a timely admission to a medium or intensive care unit is required, with invasive monitoring of different organ systems, and aggressive intervention is frequently needed to prevent or correct multiple organ failure. Necrosectomy may be necessary in the later stages of SAP and cholecystectomy in case of biliary etiology is deferred until complete recovery after SAP. Reliable diagnostic markers that discriminate a mild course of pancreatitis from SAP will allow clinicians to optimize monitoring and treatment. Furthermore, an accurate diagnostic tool is useful to select and stratify patients in randomized trials to increase homogeneity of the included patients and to allow comparison of different cohorts.

In addition to using Ang-2 purely as a diagnostic marker, targeting of angiopoietin signalling has been suggested as a potential therapeutic intervention. It has been described previously that manipulation of Ang-2 is complex because it must be captured immediately after release from the Weibel-Palade bodies to prevent its

Table 3. Diagnostic Accuracy Defined as the Area under the Curve of Angiopoietin-2, Imrie Score, APACHE-II Score, and Other Acute Phase Markers in Predicting Outcomes

Outcome	Ang-2	LBP	PCT	CRP*	Imrie†	APACHE-II‡
Severe acute pancreatitis	0.851	0.649	0.727	0.661	0.794	0.710
Multiorgan failure	0.784	0.575	0.674	0.586	0.770	0.682
Infectious complications	0.816	0.699	0.756	0.667	0.732	0.655
Bowel ischemia	0.895	0.709	0.761	0.634	0.795	0.684
Mortality	0.865	0.474	0.793	0.495	0.744	0.683

*Highest C-reactive protein in first 48 hours.
†Highest Imrie score in first 48 hours.
‡Highest APACHE-II score on admission.
Ang-2, Angiopoietin-2; CRP, C-reactive protein; IL-6, interleukin 6; LBP, lipopolysaccharide binding protein; PCT, procalcitonin.

proinflammatory autocrine effects.[14] Furthermore, under certain circumstances, Ang-2 has anti-inflammatory effects, and insufficient data are available on possible adverse effects of Ang-2 blockade.

A limitation of our study is the fact that plasma measurements were performed at different points in time during the first 5 days after admission, rather than all on day 1 or day 2. The protocol demanded collection of blood samples in the first 5 days to allow for some logistic flexibility in the high number of participating centers: for example, to allow time for communication between the attending physician and the research laboratory where the samples were stored. Whitcomb and colleagues[7] showed that differences in Ang-2 between patients with severe and mild pancreatitis are most pronounced on days 1 and 2 after admission. So it is plausible that the diagnostic accuracy of plasma Ang-2 levels could be even greater than we have demonstrated with this series if we had been able to perform the measurements in the same window of time in all patients.

CONCLUSIONS

In summary, this analysis showed that in the setting of a randomized controlled trial, plasma Ang-2 is an accurate predictor of SAP, MOF, and infectious complications, outperforming all other conventional predictors currently used in most centers. These findings need to be validated in a prospective cohort.

Author Contributions

Study conception and design: Buddingh, Koudstaal, Leuvenink, Ploeg, Nieuwenhuijs

Acquisition of data: Koudstaal, Van Santvoort, Besselink, Timmer, Rosman, Van Goor, Nijmeijer, Gooszen, Leuvenink, Ploeg, Nieuwenhuijs

Analysis and interpretation of data: Buddingh, Koudstaal, Ploeg, Nieuwenhuijs

Drafting of manuscript: Buddingh

Critical revision: Van Santvoort, Besselink, Timmer, Rosman, Van Goor, Nijmeijer, Gooszen, Leuvenink, Ploeg, Nieuwenhuijs

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