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ORIGINAL ARTICLE

Protease-Related Predictors of Acute Pancreatitis Severity: A Systematic Review of the Literature

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ABSTRACT

Context Acute pancreatitis is a protean disease with wide and varied presentation from mild to critical with no accurate predictors of severity available. **Objective** Since pancreatic and leucocyte proteases are the early markers to rise in acute pancreatitis, we hypothesised that these mediators may be early predictors of pancreatitis severity. **Methods** The literature was searched for all studies that evaluated proteases, protease precursors, anti-proteases and protease-anti-protease complex in the prediction of acute pancreatitis severity from January 1985 to December 2014. A study was included in this systemic review if it had a prospective design and provided sensitivity, specificity or allowing the derivation of true positive, false positive, false negative and true negative results. A random-effects model was used to calculate the pooled estimates. **Results** There were 44 studies with 9 different serological protease-related markers with seven of these studies yielding data on more than one marker. Serum polymorphonuclear elastase at 24 hrs had a diagnostic odds ratio of 70.4 (21-235.7) with a positive predictive value of 80% followed by serum carboxypeptidase activation peptide with diagnostic odds ratio of 18.4 (5.1-66.7) and positive predictive value of 66%. **Conclusions** Serum polymorphonuclear elastase may be a very early and sensitive predictive marker of acute pancreatitis severity.

INTRODUCTION

The early and accurate prediction of acute pancreatitis severity is important for clinical decision making [1] and patient outcome [2]. It aids the triage of patients for transportation to regional centres and admission to intensive care units as well as for decisions about fluid resuscitation and early ERCP. A delay in identifying severe disease can be associated with a four-fold increase in the risk of death [3].

Many different approaches have been taken to the early prediction of AP severity and the conduct of clinical trials. Two recent reviews [4, 5] highlighted the universal problem of a relatively low sensitivity and specificity. An accuracy of 70-80% means that 20-30% of patients will be misclassified, which severely limits the utility of these predictors in managing individual patients. Other limitations include the need to delay 48 hours (e.g. Ranson's and Glasgow criteria) and the number of parameters to be collected (e.g. eleven in APACHE II and five in BISAP score) [6]. Also some of the methods for severity prediction (e.g. APACHE II) require invasive and constant monitoring and are more suited to the intensive care unit (ICU) setting. Combinations of predictors appear to improve accuracy, but this can be cumbersome which limits clinical use [4]. The current approaches to severity prediction in acute pancreatitis appear to have hit a ceiling and there appears to be two broad ways forward [1, 7]. There either needs to be better ways to use existing predictors of severity (e.g. sequencing, combinations or neural networking) [1] or there needs to be the discovery of new biomarkers of severity that reflect critical outcome determining pathophysiology directly early in the disease course, and preferably on admission to hospital.

Since Chiari proposed the "autodigestion" theory a century ago [8] a key concept in the pathophysiology of acute pancreatitis has been the role of pancreatic proteases. The premature activation of trypsinogen within the acinar cell is considered a sentinel event in AP [9, 10] and the basolateral extrusion of activated trypsin, phospholipase and other proteases into the pancreatic interstitium drives local and systemic inflammation [11, 12, 13, 14]. Once released into the pancreatic interstitium, retroperitoneum, peritoneal cavity and the circulation, these proteases cause pancreatic and peri-pancreatic fat necrosis [15, 16]. The recruitment of inflammatory cells to the injured tissues and activation of zymogen secretions from these granulocytes, such as polymorphonuclear elastases (PMN elastase) compound the injury by pancreatic proteases [17] and help drive the systemic inflammatory response syndrome (SIRS). This is a predictor of severity [18] and the prodrome of multiple organ dysfunction and failure [19], the leading cause of death from severe AP [20] **(Figures 1)**. The natural defense against the destructive

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effects of prematurely activated and dislocated proteases are a range of anti-proteases [21] which form a protease-antiprotease complex [22]. Anti-protease defense mechanisms can be overwhelmed [23]. The important early role that pancreatic and leucocyte proteases play in the pathogenesis of AP is supported by finding that their serum levels raise within 24 hours of the onset of AP [24, 25, 26]. On this basis protease related markers, from the pancreas and leucocytes, represent logical candidates for predictors of acute pancreatitis severity.

The aim of this study is to systematically review the clinical literature and determine summary estimates of the absolute and relative value of pancreatic and leucocyte protease related markers, and their precursors, in the prediction of severity early in acute pancreatitis.

MATERIAL AND METHODS

Search Strategy

The literature was searched for all studies that evaluated proteases, protease precursors, anti-proteases and protease-anti-protease complex in the prediction of AP severity. The search contained data between Jan 1985 and December 2014. The search strategy for MEDLINE was "sensitivity and specificity"(All Fields) OR "false positive"(All Fields) OR "false negative"(All Fields) OR "accuracy"(All Fields) OR ""predictive value of tests"(All Fields) OR "likelihood ratio"(All Fields) OR "reference values"(All Fields) OR "ROC analysis"(All Fields) "acute pancreatitis"(MeSH Terms) OR "acute pancreatitis, severe"(MeSH Terms) AND "human"(MeSH Terms). The search terms for EMBASE included "acute pancreatitis" OR "acute pancreatitis, severe" AND "prognosis" OR "severity" AND (humans)/lim. Additionally, the references of the primary and review articles were secondarily searched to identify publications not retrieved by electronic searches. We also searched SCOPUS to get additional articles missed out from MEDLINE and EMBASE. Finally, we tried to retrieve any imminent or unpublished material relevant to this study using the Clinical Trials Search and ClinicalTrials.gov databases. Titles and abstracts of all citations were screened independently by two reviewers (STS and MSP). Language restrictions were not applied to the search strategy.

Study Inclusion Criteria

A study was included in this systemic review if it had a prospective design and provided both sensitivity and specificity of a serological marker (index test) of pancreatic or leucocyte protease activation, activation peptides, protease anti-protease complex or anti-proteases for predicting the severity or prognosis of acute pancreatitis, or when it provided the data on individual study subjects allowing the derivation of true positive, false positive, false negative and true negative results.

The other inclusion criteria for the studies required that they had to include a grading of severity either by CT scan (CTSI), clinical scoring criteria's (Ranson's, Marshal's and APACHE) or during laparotomy. Eligible outcomes included severe pancreatitis which was defined by the Atlanta criteria [27], Japanese criteria of severity [28], multiple or single organ failure, presence of local or systemic complications, pancreatic necrosis (infected or not), need for intervention, survival, and hospitalization length. These studies also had to include serial assessment of markers at 24, 48 and/or 72 hours from the onset of symptoms or admission to hospital. Studies were excluded if they correlated postulated prognostic markers but did not examine an eligible outcome. As described the index test did not form part of the reference standard for selection. If publications used over-lapping study populations, we selected the study with the largest number of patients enrolled.

Data Extraction

The number of patients, clinical setting, study population, the prevalence of acute pancreatitis and severity, study design and the cut-off level used for each serological marker were extracted from the literature independently by two investigators (S.T.S., M.S.P.). For each included study, the true positive, false positive, true negative and false negative results for each of the markers were abstracted and recorded in data collection sheets.

Assessment of Study Quality

Quality assessment was performed by one reviewer (M.S.P) and checked by the second reviewer (S.T.S). Included studies were assessed for methodological quality using the list of QUADAS items [29].

Statistical Analysis

Two-by-two contingency tables were constructed for all serological markers reported in the included studies. The analyses were done with Revman software (version 5) [30]. If two or more studies investigated the same index test, their results were summarized by pooling estimates of sensitivity, specificity, likelihood ratio for positive index test (LR+), likelihood ratio for negative index test (LR-), diagnostic odds ratio (DOR), and their corresponding 95% confidence intervals. We added 0.5 to each cell of all two-by-two tables that included at least one zero cells [31]. A random effects model (DerSimonian and Laird) was used to calculate all summary estimates [32]. MetaDiSc version 1.4 was utilised for generating summary receiver operating characteristic (SROC) and DOR forest plot curves [33]. Sensitivity was defined as the proportion of patients who developed severe acute pancreatitis among those who had a positive index test result. Specificity was the proportion of patients without severe acute pancreatitis among those who had a negative index test result. LR+ is the ratio of the true positive rate to the false positive rate (sensitivity/100 – specificity). The LR- is the ratio of the false negative ratio to the true negative ratio (100 – sensitivity/specificity). The pre-test prevalence was derived from the data provided in the studies from which positive and negative post-test probabilities were derived (http://www.med.wisc.edu/pds/ebm/calculators/pp-calc.html).

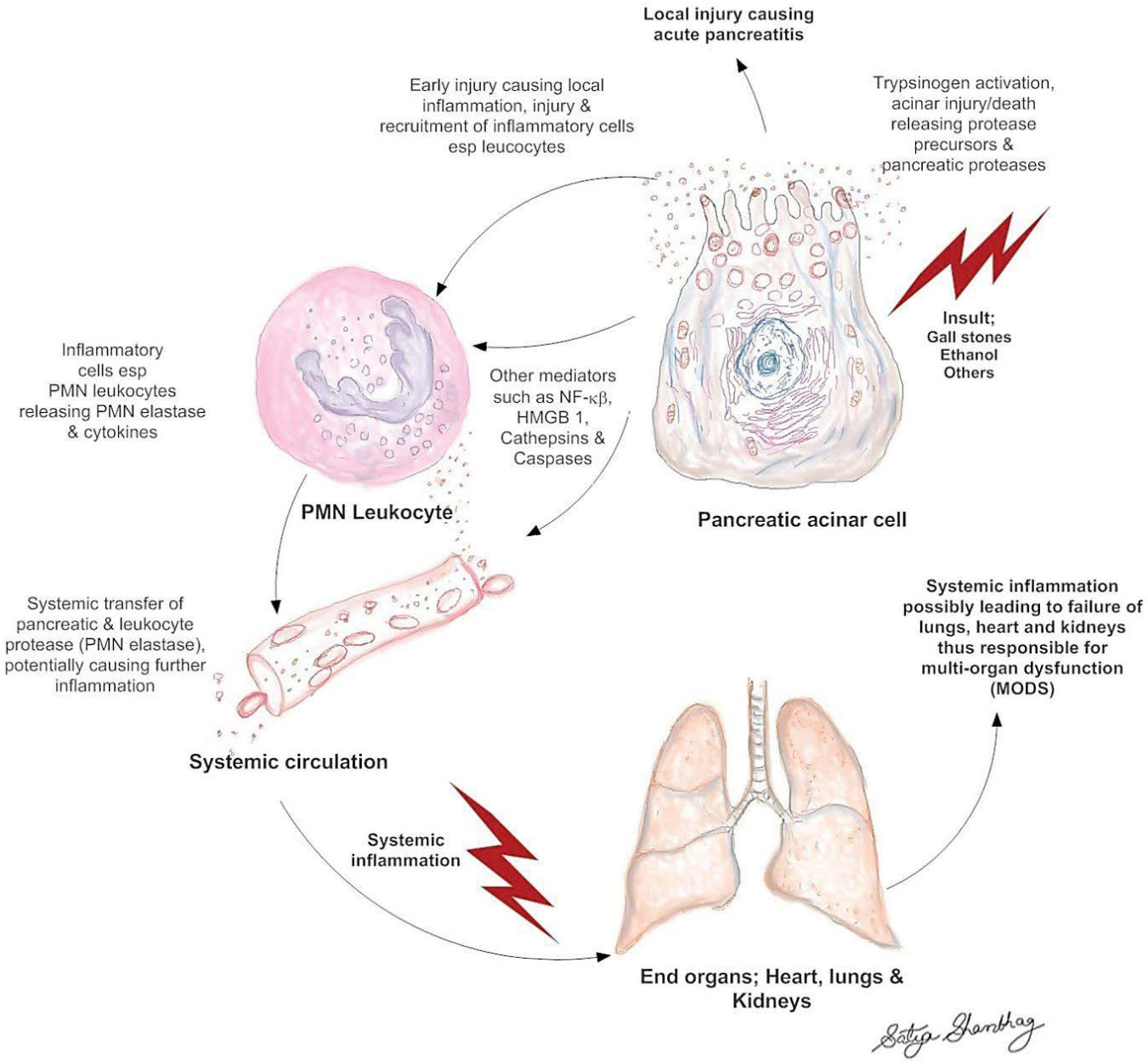

Figure 1. Overview of the role of protease activation (pancreatic and leucocyte) in the systemic inflammatory response and multi-organ dysfunction syndromes.

The DOR was defined as the ratio of the odds of the test being positive if the subject had a disease relative to the odds of the test being positive if the subject did not have the disease.

RESULTS

Study Characteristics

From the initial literature search, we identified and screened 486 abstracts **(Figure 2)**. Ninety-three articles were considered for inclusion and the full text was retrieved for detailed evaluation. Forty nine of these 93 articles were subsequently excluded from the review (34 studies did not satisfy inclusion criteria, 9 were based on the same study population, for 5 studies 2 X 2 contingency could not be constructed and one study had data from peritoneal fluid). The remaining 44 studies had a suitable prospective design and yielded usable data.

Serological Protease-Related Markers

There were 9 different serological protease-related markers investigated in 2924 patients in these 44 studies **(Table 1)** with seven of these studies yielding data on more than one marker. There were 9 markers which can be subdivided into 4 classes: protease precursors, proteases, anti-proteases and protease/antiprotease complexes **(Table 1)**. The summary data is presented in a similar format in **Tables 2 to 7** for the protease related markers and as pooled results **(Table 8)**. This summary data includes the definition that was used for severity assessment (original Atlanta, Ranson's criteria, and/or CT Severity Index), source of sample (urine or serum), sample timing (24, 48 or 72 hours after symptom onset or hospital admission), cut-off value used for the predictor and the results of decision analysis (sensitivity, specificity, true positive, false positive, true negative, and false negative).

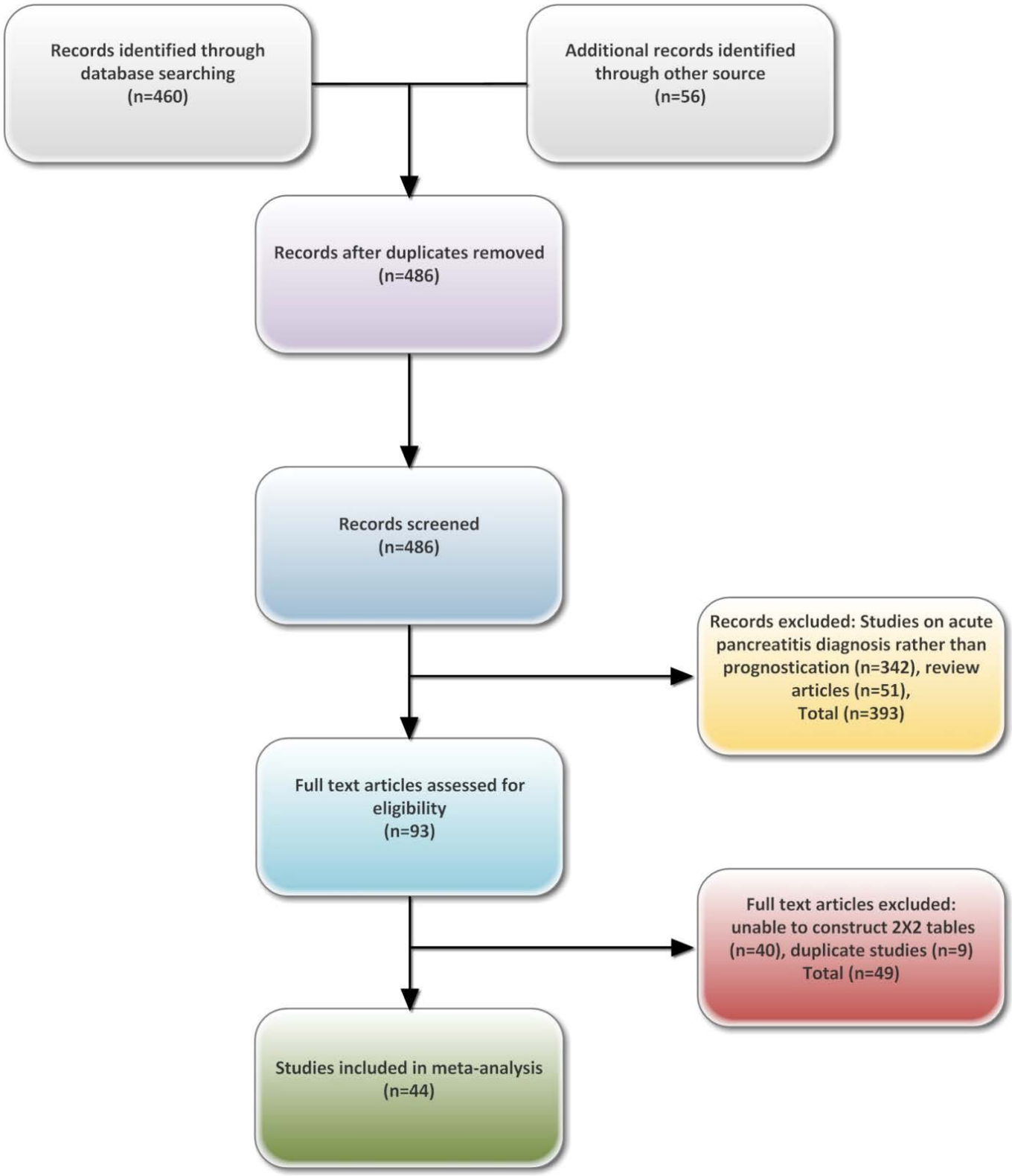

Figure 2. PRISMA chart of selection of studies.

Protease precursor (trypsinogen activation peptide, TAP): Table 2 provides the summary data from the 18 studies that evaluated TAP as a predictor of AP severity. Severity was determined by Atlanta criteria, CT scan or by organ failure (OF) in these studies. There was also a range of cut-off values as shown. Ten of these studies [16, 26, 34, 35, 36, 37, 38, 39, 40, 41, 42] tested urinary and one serum TAP [43]. Nine of the studies tested urinary TAP at 24 hours and six studies at 48 hours [26, 35, 37, 38, 39, 40, 41, 43, 44]. There were six studies [26, 36, 37, 39, 40, 42] evaluating TAP >35nmol in urine. The summary data shows that the mean sensitivity is 74 (95% confidence interval 70-78) and specificity is 77 (CI 75-79). The mean false positive rate is 12 (range 7 - 18), and the mean false negative rate is 4(range 3 - 5). These results improve when only data for 48 hours from the onset of symptoms are considered **(Table 8)**.

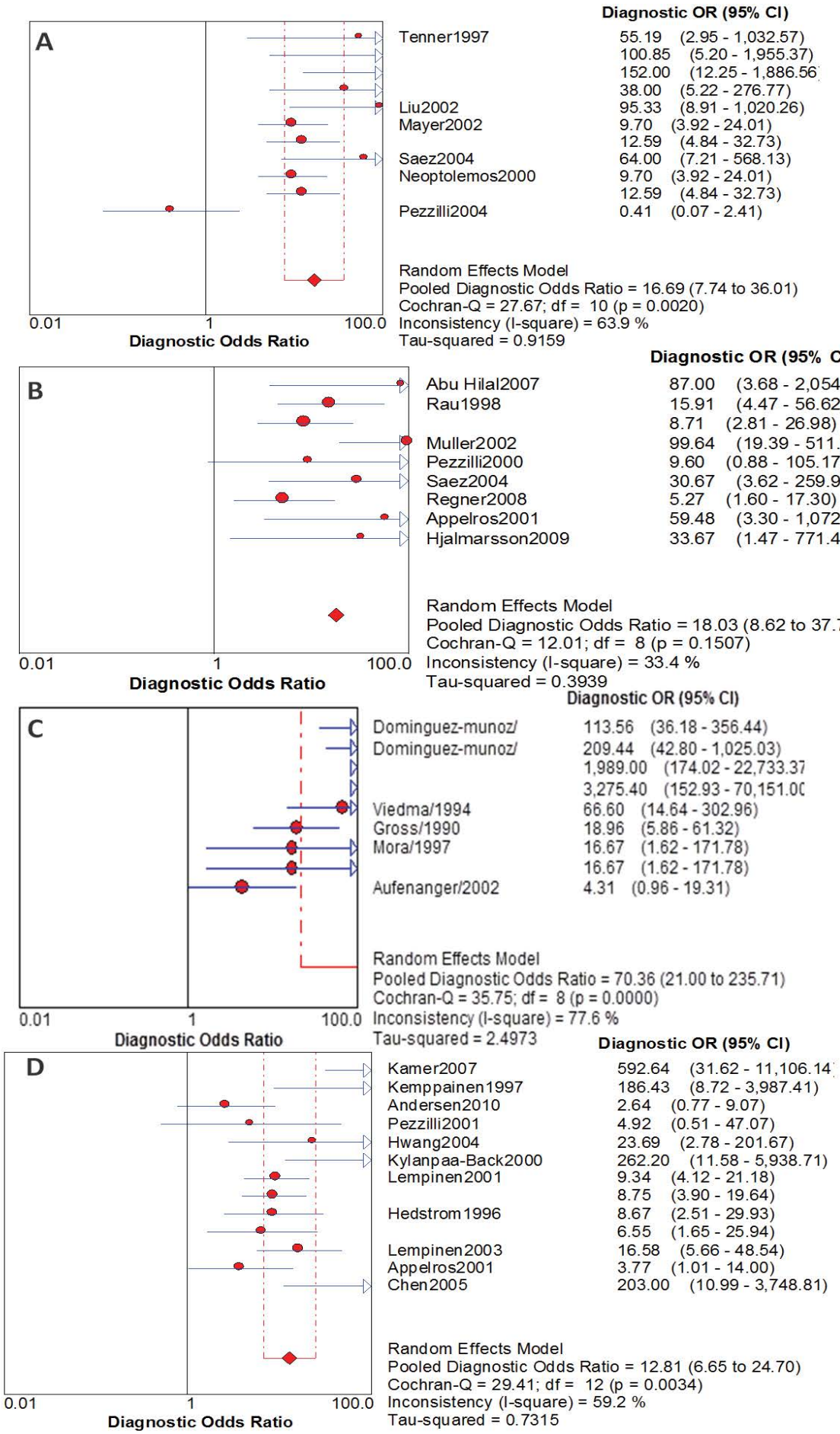

Figure 3. Diagnostic odds ratio (DOR) for the major pancreatic and leucocyte proteases and protease activation markers from the study. In the figure A is for TAP (urine), B is CAPAP (serum), C is PMN Elastase (serum) and D is T_2 (urine).

Table 1. The nine serological protease-related markers selected for this review, in 4 classes, including the reference studies from which the data was extracted.

Class	Mediators	References
Protease precursors	Trypsinogen activation peptide (TAP)	22, 51, 52, 54, 55, 56, 57, 58, 59, 60, 62
	Carboxypeptidase activation peptide (CAPAP)	36, 56, 57, 58, 63, 64, 65, 66
	Pro-phospholipase A2 peptide (PLAP)	35, 52
Proteases	Trypsinogen 2 (T_2)	36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 49, 57, 84
	Polymorphonuclear elastase (PMN elastase)	30, 31, 32, 33, 34, 35
	Phospholipase A_2 (PLA_2)	35, 47, 48, 50, 51, 52
Anti-proteases	α_2 Macroglobulin	31, 69
	β_2 Macroglobulin	70, 71
Protease/anti-protease complex	T_2/α_1 antitrypsin	31, 45, 72

Table 2. Summary of the studies evaluating trypsinogen activation peptide (TAP) as a predictor of AP severity, including definition of severity, timing of sample, source of sample, cut-off levels, and the result of clinical decision analysis.

Authors/year	Severity Definition	Sample Timing	Sample Source	Cutoff level	Sensitivity	Specificity	TP	FP	TN	FN
Khan *et al.* (1973)	AC, CT	At 24 hrs	urine	>35 nmol/L	100	77	19	9	30	0
Tenner *et al.* (1997)	CT	at 48 hrs	urine	5 ng/mL	100	75	9	10	30	0
		at 48 hrs	urine	10 ng/mL	100	85	9	6	34	0
		at 48 hrs	urine	15 ng/mL	89	95	8	2	38	1
		at 48 hrs	urine	20 ng/mL	67	95	6	2	38	3
Zhi-Su Liu *et al.* (2002)	AC	at 24 hrs	urine	25 nmol/L	100	76	12	7	22	0
		within 48 hrs	urine	35 nmol/L	92	90	11	3	26	1
Gudgeon et al. (1990)	OF	at admission	urine	>2 nmol/L	80	90	12	4	36	3
		at 24 hrs	urine	>2 nmol/L	80	85	12	6	34	3
Mayer *et al.* (2002)	AC	at 48 hrs of symptom	urine	>35 nmol/L	81	71	28	40	97	7
		at 48 hrs of hospital	urine	>35 nmol/L	83	72	29	38	99	6
Saez *et al.* (2004)	AC	at 24 hrs	urine	14.1 nmol/L	100	69	10	5	11	0
		at 48 hrs	urine	18.1 nmol/L	92.3	80	16	7	28	1
Lempinen *et al.* (2003)	All	at admission	urine	>35 nmol/L	64	82	18	18	80	11
		at 24 hrs	urine	>35 nmol/L	52	92	15	8	90	14
Neoptolemos *et al.* (2000)	AC, CT	at 24 hrs of symptom onset	urine	>35 nmol/L	58	73	20	37	100	15
		at 48 hrs of symptom onset	urine	>35 nmol/L	81	71	28	40	97	7
		at 24 hrs of admission	urine	>35 nmol/L	68	74	24	36	101	11
		at 48 hrs of admission	urine	>35 nmol/L	83	72	29	38	99	6
Johnson *et al.* (2004)	All	at 24 hrs	urine	25 nmol/L	62	73	16	44	120	10
		at 24 hrs	urine	35 nmol/L	46	80	12	33	131	14
Pezzilli *et al.* (2004)	AC, CT	at 24 hrs	serum	0.005 OD	16.7	77	1	3	10	5
		at 48 hrs	serum	0.005 OD	20	62	2	8	13	8
Saez *et al.* (2002)	AC	at 24 hrs	urine	18.1 nmol/L	92.3	80	16	7	28	1
Summary of results (Mean ± 95% confidence interval)					74 (70-78)	77 (75-79)	11 (7-14)	12 (7-18)	40 (30-51)	4 (3-5)

AC Atlanta Criteria; All includes CT, AC, OF and on laparotomy; APACHE acute physiology and chronic health evaluation; CT CT scan; FN false negative; FP false positive; OF organ failure; TN true negative; TP true positive

Protease precursor (carboxypeptidase precursor activation peptide, CAPAP): Table 3 provides the summary data from the 8 studies that evaluated CAPAP as a predictor of AP severity [38, 45, 46, 47, 48, 49, 50, 51]. Severity was determined by Atlanta criteria, CT scan or by organ failure (OF) in these studies with the range of cut-off values as shown. Eight studies evaluated serum and five studies [38, 45, 46, 50, 51] evaluated urinary CAPAP at 48 hours respectively. The summary data shows that the mean sensitivity is 75 (CI 70-79) and specificity is 77 (CI 75-79). The mean false positive rate is 11 (range 7 - 16), and the mean false negative rate is 4 (range 3- 4). CAPAP at 48 hours from urine showed better specificity (82, range 77-86) than serum CAPAP at 48 hours (73, range 68-77, Table 8).

Protease precursor (phospholipase A_2 activation peptide, PLAP): Table 6 provides the summary data from the 2 studies that evaluated PLAP as a predictor of AP severity [36, 52]. The summary data shows that the mean sensitivity is 64 (CI 52-74) and specificity is 59 (CI 53-65). The mean false positive rate is 42 (range 11 - 58), and the mean false negative rate is 10 (range 4 - 15).

Protease (Trypsinogen2, T_2): Three proteases have been tested as predictors of AP severity in 26 studies. Thirteen

Table 3. Summary of the studies evaluating procarboxypeptidase B (CAPAP) as a predictor of AP severity, including definition of severity, timing of sample, source of sample, cut-off levels, and the result of clinical decision analysis.

Authors/year	Severity Definition	Sample Source	Sample timing	Cutoff level	Sensitivity	Specificity	TP	FP	TN	FN
Abu Hilal *et al.* (2007)	AC	serum	at admission	>6 nmol/L	100	88	7	2	14	0
		urine	at admission	>40 nmol/L	100	96	7	1	15	0
Rau *et al.* (1998)	CT	serum	at day 3	>200 ng/mL	91	64	35	11	20	4
			Overall	>200 ng/mL	86	60	33	12	19	6
Muller *et al.* (2002)	CT	serum	< 48 hrs	3.2 nmol/L	95	87	31	7	45	2
			48-72 hrs	0.4 nmol/L	89	84	29	8	44	4
Pezzilli *et al.* (2000)	AC	serum	Day 1	1.14-1.19 nmol/L	87	40	8	7	4	1
			Day 2	1.14-1.19 nmol/L	89	54	8	5	6	1
			Day 3	1.14-1.19 nmol/L	78	82	7	2	9	2
			Overall	1.14-1.19 nmol/L	85	5	8	4	7	1
Saez *et al.* (2004)	AC	serum	at 24 hrs	4.83 nmol/L	89	47	9	9	7	1
			at 48 hrs	4.97 nmol/L	93	67	16	12	23	1
		urine	at 24 hrs	15.45 nmol/L	89	81	9	3	13	1
			at 48 hrs	9.18 nmol/L	85	66	14	12	23	3
Regner *et al.* (2008)	AC, CT	serum	Cut off level used	1 nmol/L	95	50	15	62	62	1
				2 nmol/L	75	64	12	45	79	4
				3 nmol/L	56	79	9	26	98	7
				15 nmol/L	63	71	10	36	88	6
		urine	Cut off level used	20 nmol/L	63	75	10	31	93	6
				25 nmol/L	56	76	9	30	94	7
Appelros *et al.* (2001)	AC, CT	serum		5.5 nmol/L	100	72	12	14	34	0
		urine		100 nmol/L	92	89	11	5	43	1
Hjalmarsson *et al.* (2009)	AC, CT	serum	0-24 hrs	8 nmol/L	60	82	5	9	41	4
			25-48 hrs	8 nmol/L	22	100	2	0	50	7
			48-72 hrs	8 nmol/L	0	95	0	3	47	9
			0-72 hrs	8 nmol/L	22	94	2	3	47	7
		urine	0-24 hrs	100 nmol/L	40	90	4	5	45	5
			25-48 hrs	100 nmol/L	20	100	2	0	50	7
			49-72 hrs	100 nmol/L	29	98	3	1	49	6
			0-72 hrs	100 nmol/L	27	96	2	2	48	7
Summary of results (Mean and 95% confidence interval)					75(70-79)	77(75-79)	13 (9-17)	11 (7-16)	38(29-47)	4 (3-4)

AC Atlanta Criteria; All includes CT, AC, OF and on laparotomy; APACHE acute physiology and chronic health evaluation; CT CT scan; FN false negative; FP false positive; OF organ failure; TN true negative; TP true positive

studies examined trypsinogen 2 (T_2) as predictive marker in acute pancreatitis either in serum or urine [44, 45, 53, 54, 55, 56, 57, 58, 59, 60, 61] **(Table 4)**. Eleven studies examined urine within 24 hours of symptom onset or admission. Most of the studies utilised the rapid Actim-Strip urine test for this marker in differentiating severity of acute pancreatitis. Only 3 studies utilised serum T_2 level for analysis. The pooled summary data shows that the mean sensitivity is 72 (CI 68-76) and specificity is 77 (CI 75-80). The mean false positive rate is 13 (range 10 to 17), and the mean false negative rate is 6 (range 4 - 9). At 24 hours serum T_2 showed a sensitivity of 78 (CI 68-86) and specificity of 65 (CI 58-72) compared to 78 (CI 72-83) and 78 (75-81) from urinary T_2 respectively (Table 8).

Protease (Polymorphonuclear elastase, PMN elastase): Six studies examined the performance of polymorphonuclear elastase (PMN elastase) as predictive marker in early detection of SAP [52, 62, 63, 64, 65, 66] **(Table 5)**. The pooled summary data shows that the mean sensitivity is 86 (CI 81-90) and specificity is 94 (CI 92-95). The mean false positive rate is 5 (range 1 - 9), and the mean false negative rate is 4 (range 3 - 5).

Protease (Phospholipase A_2, PLA_2): There were 7 studies which examined the performance of phospholipase A_2 (PLA_2) as predictive marker of acute pancreatitis [36, 52, 67, 68, 69, 70, 71] **(Table 6)**. There were two studies that also analysed the activation peptide of PLA_2, pro-PLA_2 (PROP) [36, 52]. The pooled summary data shows that the mean sensitivity is 77 (CI 70-83) and specificity is 81 (CI 77-85). The mean false positive rate is 6 (range 2 -10), and the mean false negative rate is 4 (range 1 - 6).

Anti-proteases (α2 macroglobulin): Four studies evaluated the clinical usefulness of anti-proteases in severity prediction of AP. There were two studies which evaluated α2 macroglobulin [63, 72]. The pooled summary data shows that the mean sensitivity is 67 (CI 54-78) and specificity is 72 (CI 66-76). The mean false positive rate is 31 (range 7 - 44), and the mean false negative rate is 7(range 1 - 11).

Anti-proteases (β2 macroglobulin): There were two studies which evaluated β2 macroglobulin [73, 74] (Table 7) in SAP. The pooled summary data shows that the mean sensitivity is 52 (CI 40-64) and specificity is 77 (CI 68-84). The mean false positive rate is 5(range 4 -7), and the mean false negative rate is 7(range 6 - 9).

Table 4. Summary of the studies evaluating Trypsinogen-2 (T_2) as a predictor of AP severity, including definition of severity, timing of sample, source of sample, cut-off levels, and the result of clinical decision analysis.

Authors/year	Severity Definition	Sample Source	Sample timing	Cutoff level	Sensitivity	Specificity	TP	FP	TN	FN
Kamer *et al.* (2007)	All	urine/Actim strip	Overall	50 mg/L	100	92.4	26	5	61	0
Kemppainen *et al.* -1997	AC, CT	urine/Actim strip	at admission	50 mg/L	100	93	7	3	43	0
Andersen *et al.* (2010)	AC, CT	urine/Actim strip	at 24 hrs	50 mg/L	87	28	26	32	13	4
Pezzilli *et al.* (2001)	AC, CT	urine/Actim strip	at admission	50 mg/L	89	38	8	13	8	1
Hwang *et al.* (2004)	CT	urine/Actim strip		50 mg/L	93	63	14	13	22	1
Sainio *et al.* (1996)	CT, OF	serum	at 24 hrs	1000 µg/L	91	71	18	8	18	2
Kylanpaa-Back *et al.* (2000)	AC, CT	urine/Actim strip		50 mg/L	100	94	9	2	34	0
lempinen *et al.* (2001)	AC	urine/Actim strip	at admission	2000 µg/L	62	87	26	14	94	16
			at 24 hrs	2000 µg/L	62	85	26	16	92	16
		urine/ quantitative	at admission	2000 µg/L	62	77	26	25	83	16
			at 24 hrs	2000 µg/L	71	78	30	24	84	12
Hedstrom *et al.* (1996)	OF	urine/ quantitative	within 24 hrs Preferred	3800 µg/L	68	80	13	8	32	6
			within 24 hrs	1400 µg/L	26	90	5	4	36	14
			within 24 hrs	2100 µg/L	47	80	9	8	32	10
			within 24 hrs	3200 µg/L	42	90	8	4	36	11
Lempinen *et al.* (2003)	All	urine	at admission	>3000 µg/L	72	81	21	19	79	8
			at 24 hrs	>3000 µg/L	82	78	24	22	76	5
Appelros *et al.* (2001)	AC, CT	serum	at admission	1350 µg/L	38	58	5	20	28	7
		urine	at 24 hrs	3500 µg/L	58	74	7	13	35	5
Hedstrom *et al.* (2001)	AC, CT	serum	at admission	1713 µg/L	70	77	15	10	33	6
			at 24 hrs	1158 µg/L	80	65	17	15	28	4
				911 µg/L	90	58	19	18	25	2
Chen *et al.* (2005)	AC	urine/Actim strip	at 24 hrs	>50 mg/L	100	86	17	7	43	0
Summary of results (Mean ± 95% confidence interval					72(68-76)	77(75-80)	16 (13-20)	13(10-17)	45 (33-56)	6 (4-9)

AC Atlanta Criteria; All includes CT, AC, OF and on laparotomy; APACHE acute physiology and chronic health evaluation; CT CT scan; FN false negative; FP false positive; OF organ failure; TN true negative; TP true positive

Table 5. Summary of the studies evaluating polymorphonuclear elastase (PMN Elastase) as a predictor of AP severity, including definition of severity, timing of sample, source of sample, cut-off levels, and the result of clinical decision analysis.

Authors/year	Severity Definition	Sample Source	Sample timing	Cutoff level	Sensitivity	Specificity	TP	FP	TN	FN
Dominguez-munoz *et al. (2006)*	AC	serum	at 24 hrs	110 µg/L	92	91	46	16	158	4
Dominguez-munoz *et al. (2003)*	OF	serum	at admission	250 µg/L	93	94	26	9	145	2
			at 24 hrs	300 µg/L	93	99	26	1	153	2
			at 48 hrs	300 µg/L	92	100	26	0	154	2
Viedma *et al.* (1994)	CT, OF	serum	at 24 hrs	380 µg/L	84	93	27	3	37	5
Gross *et al.* (1990)	CT	serum	within 24 hrs	400 µg/L	85	76	35	8	26	6
Mora *et al.* (1997)	AC	serum	at day 1	>200 µg/L	77	92	20	1	5	6
			at day 2	>200 µg/L	79	88	20	1	5	6
Aufenanger *et al.* (2002)	OF	serum	within 48 hrs	200 µg/L	60	74	6	8	23	4
Summary of results (Mean ± 95% confidence interval)					86(81-90)	94(92-95)	26(17-34)	5(1-9)	78(24-133)	4(3-5)

AC Atlanta Criteria; All includes CT, AC, OF and on laparotomy; APACHE acute physiology and chronic health evaluation; CT CT scan; FN false negative; FP false positive; OF organ failure; TN true negative; TP true positive

Protease anti-protease complex: There were three studies which examined the performance of trypsin/anti-trypsin complex in severity prediction in AP [60, 63, 75] **(Table 7)**. The pooled summary data shows that the mean sensitivity is 83 (CI 78-87) and specificity is 66 (CI 63 -69). The mean false positive rate is 27(range 18 - 36), and the mean false negative rate is 3 (range 1 - 5).

Pooled data Diagnostic odds ratio (DOR): Table 8 gives the combined diagnostic odds ratio (DOR) for all the serum and urinary markers utilised for severity prediction in AP. PMN elastase with a DOR of 70.4 was the highest of all the markers that was studied here. Also the confidence interval (CI) of DOR for PMN elastase did not overlap with the other markers used **(Table 8)**. Serum CAPAP had a DOR of 18.03

Table 6. Summary of the studies evaluating phospholipase A_2 (PLA_2) and activation peptide of pancreatic phospholipase A_2 (PROP) as a predictor of AP severity, including definition of severity, timing of sample, source of sample, cut-off levels, and the result of clinical decision analysis.

Markers	Authors/year	Severity Definition	Sample Source	Sample timing	Cut-off level	Sensitivity	Specificity	TP	FP	TN	FN
PLA_2	Kemppainen *et al.*(1999)	OF	Serum PLA2	PLA2 II	?	71	41	5	10	7	2
	Mayer et al. (1998)	CT	serumPLA2	tested from 1-4 days	300 ng/mL	89	88	9	3	23	1
					277 ng/mL	80	85	8	4	22	2
					286 ng/mL	80	87	8	3	23	2
	Ignjatovic *et al.* (2000)	CT	serumPLA2		11 U/L	55	100	10	0	40	9
	Nevalainen *et al.* (1985)	OF	serumPLA2	within 24 hrs	>9.2 µg/L	93	63	13	16	28	1
	Aufenanger *et al.* (2002)	OF	Serum PLA2	within 48 hrs	100 U/L	60	84	6	5	26	4
			serum/pancPLA2 or type1	within 48 hrs	7 U/L	100	100	10	0	31	0
	Buchler *et al.* (1989)	Ranson criteria, CT	serum/ catalyticPLA	day 1-5	15 U/L	79	79	28	11	39	7
			serum/ catalyticPLA2	day 1-5	3.5 U/L	75	78	26	11	39	9
Summary of results (Mean ± 95% confidence interval)						77(70-83)	81(77-85)	12(7-18)	6(2-10)	28(21-35)	4(1-6)
Pro PLA_2 (PROP)	Mayer *et al.* (2002)	AC	Urine PROP.	at 48 hrs of symptom.	>1 nmol/L	71	59	25	56	81	10
				at 48 hrs of hospital	>1 nmol/L	56	58	20	58	79	15
	Aufenanger *et al.* (2002)	OF	serum/PROP	within 48 hrs	15 U/L	60	65	6	11	20	4
Summary of results (Mean ± 95% confidence interval)						64 (52-74)	59 (53-65)	17 (6-25)	42 (11-58)	60(20-81)	10 (4-15)

AC Atlanta Criteria; All includes CT, AC, OF and on laparotomy; APACHE acute physiology and chronic health evaluation; CT CT scan; FN false negative; FP false positive; OF organ failure; TN true negative; TP true positive

compared to 18.36 for urinary CAPAP studies. Urinary TAP at 24 hours yielded a DOR of 8.39 as opposed to 16.69 for TAP at 48 hours. Tap >35 nmol yielded a DOR of 8.14, equivalent to TAP at 24 hours but significantly less than TAP at 48 hours. The urinary and serum T_2 studies yielded a DOR of 12.8 and 6.6 respectively. Studies on PLA_2 yielded a DOR of 11.74. These studies also yielded a combined DOR of 10.43 for trypsin/anti-trypsin complex within the first 48 hours of symptom onset/admission.

Positive likelihood ratio, positive and negative predictive value: Table 8 gives a summary of positive likelihood ratio (LR+), pre-test prevalence, positive and negative post-test probability. As a rule of thumb, a test with high predictive value has a positive likelihood ratio over 5, usually closer to 10, and sometimes more [7]. PMN elastase with a LR+ of 14, positive post-test probability of 80% and a negative post-test probability of 4% was the most accurate in predicting severe and non-severe acute pancreatitis. In addition, urinary CAPAP and urinary TAP at 48 hrs also had above average predictive utility.

DISCUSSION

This is the first study to systematically review the clinical prognostic utility of markers related to local pancreatic inflammation by pancreatic protease, anti-protease and subsequent activation of loco-regional inflammation by neutrophils and production of PMN elastase, a leucocyte protease. This study encompasses the patients who were assessed for severity within the first 48 hours from onset or admission with AP. It was found that PMN elastase at a higher level in very early phase is a reliable marker for the progression of AP to a severe disease. The activation peptides in AP, especially TAP and CAPAP have been studied extensively as prognostic markers in this disease. This review has demonstrated that these precursors also have a useful role for the early prognostication of this disease. One of the earliest pancreatic protease to be released in AP which cause the activation of other proteases is trypsinogen [76]. Because of its early release and rapid detection in urine this protease also has a useful role in very early prognostication of AP.

Collectively, these protease related mediators are important because protease activation and inflammation is a very early event in AP with high level of serum markers available for analysis within the first 48 hours of the disease. The protease activation then declines about 48-72 hour of the disease [77]. This early rise means that, in AP these measurements could have prognostic implication in identifying the patient with a severe disease very early in the disease process. This is reinforced by a recent meta-analysis from Huang *et al.* who utilised TAP as a prognostic marker for early severity stratification of AP patients at admission [78]. Our study confirms this expectation, as all the markers utilised are raised within 24-48 hours of disease onset or admission.

The leucocyte protease, PMN elastase, emerged as the strongest early marker and is especially relevant for at least three reasons. Firstly, this leucocyte derived protease

Table 7. Summary of the studies evaluating antiprotease and protease anti-protease complex as a predictor of AP severity, including definition of severity, timing of sample, source of sample, cut-off levels, and the result of clinical decision analysis.

Markers	Authors/year	Severity Definition	Sample Source	Sample timing	Cutoff level	Sensitivity	Specificity	TP	FP	TN	FN
α_2 Macroglobulin	Dominguez-munoz *et al.* (1993)	OF	serum	at 24 hrs	15 mg/dL	61	72	17	44	110	11
				at 48 hrs	30 mg/dL	69	73	19	42	112	9
	Banks *et al.* (1991)	OF	serum	overall 1-7 days	?	87	63	7	7	12	1
Summary of results (95% confidence interval)						67 (54-78)	72 (66-76)	14 (7-17)	31(7-44)	78(12-112)	7(1-11)
β_2 Macroglobulin	Pezzilli *et al.* (1998)	AC, CT	serum		2.1 mg/L	53	82	8	4	18	7
	Pezzilli *et al.* (1995)	AC	serum	Day 1	2.1 mg/L	58	81	9	4	19	6
				Day2	2.1 mg/L	50	77	8	5	18	7
				Day3	2.1 mg/L	43	68	6	7	16	9
				Day4	2.1 mg/L	50	74	8	6	17	7
Summary of results (95% confidence interval)						52(40-64)	77(68-84)	8(6-9)	5(4-7)	18(16-19)	7(6-9)
Trypsin/ antitrypsin	Dominguez-munoz *et al.* (1993)	OF	serum $\alpha_1$protease Inh	at 24 hrs	30 mg/dL	50	71	14	45	109	14
				at 48 hrs	70 mg/dL	69	70	19	46	108	9
	Hedstrom *et al.* (1996)	CT	serum T2/ α_1AT	at 0-12 hrs	288 µg/L	95	65	27	29	53	1
				at 0-24 hrs	267 µg/L	95	64	27	30	52	1
				at 24 hrs	288 µg/L	90	64	25	30	52	3
				at 48 hrs	205 µg/L	95	54	27	38	44	1
	Hedstrom *et al.* (2001)	AC, CT	serum T2/ α_1AT	at 48 hrs	288 µg/L	90	65	25	29	53	3
					836 µg/L	70	72	15	12	31	6
					760 µg/L	80	67	17	14	29	4
					650 µg/L	90	65	19	15	28	2
Summary of results (95% confidence interval) for T/AT2						83(78-87)	66(63-69)	22(19-26)	27(18-36)	50(31-69)	3 (1-5)

AC Atlanta Criteria; All includes CT, AC, OF and on laparotomy; APACHE acute physiology and chronic health evaluation; CT CT scan; FN false negative; FP false positive; OF organ failure; TN true negative; TP true positive

is a potent tissue digesting enzyme which heralds the progression of loco-regional inflammation to a systemic inflammation. Secondly, with the activation of neutrophils and the production of PMN elastase marks the progression of mild pancreatitis to a more severe category [24, 25, 65, 79]. The serum levels of PMN elastase reach significantly higher levels in severe AP than in mild disease at about 12 hours from the onset of symptoms [80]. In this study we found that serum PMN elastase at >300 µg/L within the first 24 hours indicated a severe disease whereas patients with mild AP had serum concentration of <100 µg/L of this protease **(Table 9)**. Lastly, this mediator is early to rise in the progression of acute pancreatitis and easy to measure in the laboratory with peak values even before CRP and other parameters begin to rise [65]. Concentrations rapidly decline in patients with uneventful recovery, while a persistent elevation of this protease is seen in non-survivors [65]. One of the most important findings in this study is that PMN elastase can predict a severe disease reliably and a negative test would reliably confirm the absence of severity. This marker also has a very high DOR for the studies done on the progression of severity. Also the confidence interval (CI) of DOR for PMN elastase did not overlap with the other markers used in this study, suggesting a statistically significant superiority of this marker in predicting severity **(Table 8, Figure 3)** [31]. There has been many studies and narrative reviews elaborating the usefulness of this marker in AP. This current review supports the early prognostication of patients progressing to SAP be done reliably utilizing this serum marker.

The activation peptides in AP, especially TAP and CAPAP, have been studied extensively as prognostic markers in this disease. TAP is a five amino acid activation peptide released from the cleavage of trypsinogen to trypsin. CAPAP and other activation peptides such as PLAP are released after the activation of trypsin. Therefore there is a slightly earlier release of TAP into the serum. The serum levels are elevated in severe disease and peak about 24-48 hours from the onset. Since the first publication about TAP by Gudgeon et al there have been various papers about the clinical utility of this marker in acute pancreatitis severity assessment [35]. A recent meta-analysis on urinary TAP suggests a strong role for them in AP severity stratification at admission [78]. CAPAP is also found in plasma and urine and is more stable than TAP because of its larger size. Both these markers rapidly decline after 72 hours and are thus not useful in delineating severe cases later in the course of this disease [40, 45, 81]. Both these markers have a respectable DOR at 48 hours of the disease **(Table 8, Figure 3)**. These markers would be clinically useful for the prognostication of early onset severe disease.

PLAP is the activation peptide of pancreatic phospholipase A_2. This peptide is also released from activated neutrophils [82]. So the assay of this peptide is

Table 8. The pooled data for serum and urine protease-related markers used to predict the severity of acute pancreatitis at specific time-points. Provided is the mean sensitivity and specificity with the range. The positive and negative likelihood ratios are provided with confidence intervals, with along with the pre-test prevalence allows the determination of the positive and negative post-test probabilities. Also provided is the diagnostic odds ratio with 95% confidence intervals.

Index Test (time point)	Sensitivity (range)	Specificity (range)	Pre-test prevalence	Positive likelihood ratio (CI)	Positive post-test probability	Negative likelihood range (CI)	Negative post-test probability	Diagnostic Odds Ratio (CI)
Serum source								
PMN elastase	86.2	93.8	21%	13.9	80%	0.14	4%	70.4
(at 24 hrs)	(79.7-90.3)	(91.8-95.7)		(4.7-19.9)		(0.1-0.36)		(21-235.7)
CAPAP	86.2	72.9	38%	3.2	66%	0.18	10%	18
(at 48 hrs)	(80.3-90.9)	(68.2-77.2)		(2.2-3.8)		(0.07-0.48)		(8.62-37.7)
PLA_2	75.1	74.5	35%	3.26	64%	0.37	17%	11.74
(all time points)	(68.6-80.9)	(70.4-78.2)		(2.2-4.9)		(0.28-0.5)		(5.8-23.9)
$T2/\alpha_1AT$	83	66	40%	2.38	61%	0.23	13%	10.4
(all time points)	(77.9-87.4)	(62.7-69.2)		(2.1-2.6)		(0.13-0.42)		(5.5-19.7)
T_2	77.9	65	39%	2.23	50%	0.34	18%	6.6
(at 24 hrs)	(68.2-85.8)	(58.0-71.6)		(1.66-3.0)		(0.15-0.78)		(2.4-18.4)
Urine source								
CAPAP	72.1	82.1	31%	4	64%	0.33	13%	18.4
(at 48 hrs)	(59.2-82.9)	(77.0-86.4)		(2.2-8.9)		(0.13-0.84)		(5.1-66.7)
TAP	81.4	75.5	34%	3.3	63%	0.23	10%	16.7
(at 48 hrs)	(75.5-86.4)	(72.4-78.5)		(2.75-4.7)		(0.12-0.47)		(7.7-36.0)
T_2 urine	77.9	78.1	33%	3.5	63%	0.33	14%	12.8
(at 24 hrs)	(72.5-82.7)	(75.0-81.1)		(2.4-5.9)		(0.21-0.5)		(6.6-24.7)
TAP	68.3	77.6	36%	3.06	63%	0.46	20%	8.3
(at 24 hrs)	(61.8-74.2)	(74.7-80.4)		(2.4-3.8)		(0.31-0.67)		(4.4-16.0)
TAP <35 nMol	69.2	76.8	37%	2.95	63%	0.42	20%	8.1
(includes 24 and 48 hrs)	(61.4-76.4)	(73.2-80.0)		(2.2-3.9)		(0.26-0.68)		(3.7-17.9)

DOR diagnostic odds ratio; LR+ positive likelihood, LR- negative likelihood; post-test +ve post-test probability of having a severe disease; post-test –ve post-test probability of not having a severe disease Values in bracket are 95% confidence intervals.

the aggregate impact of pancreatic activation and systemic inflammation [83]. As with other activation peptides, PLAP too declines within 48 hours and therefore would not be ideal to prognosticate later in the course of AP. The assay for PLAP is time consuming and no easy immune linked assay is yet available. Also, with a DOR for PLAP of 11.74, it would be an unlikely candidate versus the widely used TAP and CAPAP urinary assays. There is also not yet a commercial assay for the analysis of phospholipase A_2 available yet, and the analysis is at present confined to the academia and scientific circles [84]. Therefore, currently it is difficult to ascribe any importance to the phospholipase A_2 studies.

There are three trypsinogens secreted from the pancreas, of which trypsinogen-1 or T_1 (cationic) and trypsinogen-2 or T_2 (anionic) [85] are deemed to be of importance. In healthy subjects, these endopeptidases belonging to the chymotrypsin superfamily are partially catalyzed in the duodenum to trypsin by enterokinase [86]. The ratio of T_1 in plasma of healthy subjects is fourfold that of T_2. However, in acute pancreatitis (AP) the serum concentrations of T_2 are more strongly increased, and T_2 has been shown to be a useful marker for AP [85]. In AP, the premature activation of trypsinogen in the pancreatic interstitium is believed to be the first event leading to the auto-digestion of the gland. The mechanisms leading to trypsinogen activation is still unclear. However, ischaemia, hypercalcemia, and activation of cathepsin-D by cholecystokinin have been implicated in their activation [86, 87]. In AP, urinary T2 is elevated rapidly and stays elevated for about a week after the onset of disease [88]. There is urine trypsinogen-2 test strip which gives a rapid result. Also, the DOR for T_2 in our study is 12.81 at 24 hours, which is better than its activation peptide TAP at 24 hours (DOR 8.34, **Table 4, Figure 3)**. This would mean that the urinary T_2 test would be better suited for early prognostication of AP. However at 48 hours of disease progression this marker lags behind its activation peptide in prognostication.

A wide variety of protease inhibitors which inactivate and prevent the trypsin from reaching systemic circulation are altered in AP. The three most extensively studied are α_1-antitrypsin, α_2-macroglobulin and β_2-macroglobulin, of which α_1-antitrypsin is quantitatively dominant one. Strongly elevated concentrations of trypsin-1-α_1-antitrypsin and trypsin-2- α_1-antitrypsin complex levels in the serum of patients with AP within the initial 12-24 hours of admission predicts a severe outcome [75]. The other anti- proteases, α_2-macroglobulin and β_2-macroglobulin also bind to trypsin and other elastases. These complexes are rapidly degraded and eliminated by macrophages from the systemic circulation. Therefore, there is a rapid decrease in α_2-macroglobulin level during severe episode of AP [89, 90]. In this review, the trypsin-2-α_1-antitrypsin complex has a respectable DOR of 15 compared to the other anti-protease complexes. However

Table 9. Serum cut-off value for PMN elastase at 24 hrs in acute (AP) and severe acute pancreatitis (SAP)

	Serum cut off level at 24 hours (µg/L)	
	Acute Pancreatitis	**Severe Acute Pancreatitis**
Gross (1990)	<300	800
Dominguez-munoz (1993)	100	300
Viedma (1994)	79±35	380±95
Mora (1997)	87.3±30	398.5±150
Aufenanger (2002)	-	200
Dominguez-munoz (2006)	68±30	220±90

because of the complicated assay required for the analysis, these anti-proteases currently are not utilised for the rapid assessment of severity in this disease and probably would add little to the predictive value of the other protease markers.

There are a number of possible limitations with this study and these needs to be acknowledged. First, this analysis was based solely on observational studies that might be subject to confounding. While confounders can be best addressed by randomized controlled trials, studies of this design are not available in the literature. Second, there are potential problems with different definitions for severity of AP. The definition of severity has also changed since 1992, after the Atlanta classification was introduced. Most of the studies on severity have been the ones after 1992, and for the ones before this time period, CT scan had been widely used to diagnose and predict severe disease. This issue was addressed by pre-specified subgroup analyses which confirmed the robustness of the findings irrespective of definitions of severity used. Systematic reviews of this nature have to deal with various sources of heterogeneity and biases. In this study we utilised the random effects model to overcome heterogeneity. Last, the timing of the tests and the criteria of severity differed between the studies which will have introduced some heterogeneity.

CONCLUSIONS

In summary, the present systematic review presents the pooled estimates of the protease-related markers in predictive severity assessment of patients with acute pancreatitis. CRP at a level >150 mg/L is considered the gold standard for prognostication of AP >48 hours from disease onset [91]. This study has revealed that PMN elastase, a leucocyte protease marker at a level >300 µg/L within the first 24 hours predicts a severe disease. Thus PMN elastase compares exceptionally well with other more common pancreas derived and inflammatory markers including CRP and also at a much earlier time frame of disease progression. With this study, we expect that there will be renewed interest in further evaluation of this serum marker for early prognostication of AP. The activation markers such as TAP and CAPAP are also supported by this study to be relatively good early severity markers in AP. Further studies in AP are required to analyse the impact of these protease and leucocyte activation markers on the temporal relationship of organ failure, MODS and ultimately death. A better understanding of this temporal relationship with the ideal combination of these severity markers would be the endeavour of further study.

Conflict of Interest

The authors declare no conflict of interest.

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