Intestinal permeability and systemic endotoxemia in patients with acute pancreatitis

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BACKGROUND: *The bacterial contamination of pancreatic necrosis in acute pancreatitis is supposed to occur through translocation of intestinal bacteria. The aim of this clinical study was to evaluate intestinal mucosa permeability and endotoxemia in patients with acute pancreatitis.*
METHODS: *Sixtythree patients with acute pancreatitis were studied. Classification 42 patients had mild and 21 patients severe pancreatitis. Intestinal permeability was assessed at day 0, 1, 3, 7, 9 and 11 using the lactulose/mannitol differential absorption test. Serial venous blood samples were taken at 0, 30, 60, 90, 120, and 180 minutes, at 12, 24 hours, and at days 3, 7, 9 and 11 for endotoxin measurement RESULTS:Patients with severe pancreatitis had higher intestinal barrier dysfunction compared with patients with mild pancreatitis, the L:M ratio being 0.36 ± 0.15 and 0.051 ± 0.013 respectively (p< 0.05). The systemic endotoxin concentration were higher in patients with severe pancreatitis as regards mild pancreatitis (p < 0.05). A significant correlation was observed between the maximum systemic endotoxin concentration and intestinal permeability measured at day 7 in patients with mild (r_s = 0.721; p = 0.001) and severe (r_s = 0.956; p= 0.001) pancreatitis.*
CONCLUSION: *Gut permeability is increased in patients with acute pancreatitis. Patients with severe pancreatitis may be more exposed to impaired gut barrier function. Moreover the pancreatits (especially severe) can lead to systemic endotoxemia. This agrees with the hypothesis that the splanchnic hypoperfusion, during the pancreatitis, may impair intestinal mucosal barrier function and contribute to the systemic inflammatory response and multiorgan failure.*

KEY WORDS: Acute pacreatitis, Endotoxemia, Intestinal permeability

Introduction

The intestinal epithelium undergoes a continuos renewal process and consist of cells at various stages of differentiation. The integrity of the intestinal epithelium is critical to health [1], and any damage to the cells can effect proliferation and differentiation, leading to altered cell population and functional changes in the intestine. The intestine acts as a barrier to the luminal contents, which include bacteria and endotoxins. The gut barrier is altered in certain pathologic conditions such as shock, trauma or surgical stress, resulting in bacterial or endotoxin translocation from the gut lumen into the systemic circulation [2]. This has been implicated in postoperative complications, such as systemic inflammatory response syndrome (SIRS) and multiple-organ dysfunction syndrome (MODS) [3].

In acute pancreatitis, splanchnic hypoperfusion has been found to be caused by early hypovolemia [4,5]. Splanchnic

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hypoperfusion with following ischemia / reperfusion injury and gut mucosal damage is the responsible mechanism of increased gut permeability [6], and an influence on the course of the disease.
Infectious complication derived from contaminated pancreatic necrosis is the main reason of death in acute pancreatitis, and gut origin-bacteria are the most common microbes found in infected necrosis [7]. Reduction of pancreatic infection by selective digestive decontamination[8] suggests that bacterial translocation does occur from the bowel in acute pancreatitis. In experimental pancreatitis the bacterial translocation has been shown to occur via different routes, such as transmurally through large bowel, transperitoneally through lymphatic ductus or haematogenously [9-13]. In human pancreatitis, the proof of bacterial translocation is based on indirect evidence of intestinal permeability [14,15]. The aim of this study was to evaluate intestinal mucosa permeability and endotoxemia in patients with acute pancreatitis.

Patients and Methods

From April 2008 to August 2015, we studied 63 patients consecutively (38 men, 25 women; mean age, 61.4 years) with acute pancreatitis. The diagnosis of acute pancreatitis was based on clinical symptoms of acute pancreatitis and at least 2.5-fold increase in serum amylase concentration with CT scan proved acute pancreatitis [16]. Sixty-tree healthy volunteers were used as controls for the permeability studies.
This study was approved by the research Ethics Committee of the University of L'Aquila. All patients gave written consent.
For determination of the severity of disease contrast-enhanced CT scan was done within 72h after admission, and again if the clinical condition needed. The patients were classified into mild and severe pancreatitis following the Atlanta classification [17].

Blood Sampling

Serial venous blood samples were taken at 0, 30, 60, 90, 120, and 180 minutes, at 12, 24 hours, and at days 3, 7, 9 and 11. Blood was collected in pyrogen-free tubes and centrifuged at 4°C and 2000 rpm for 10 minutes and aliquoted into sterile cryotubes (NUNC 36341, Intermed, Denmark) and stored at -80 °C until analysis for subsequent determination of endotoxin.

Intestinal Permeability

Intestinal permeability was assessed at day 0, 1, 3, 7, 9 and 11 using the lactulose/mannitol differential absorption test [18-20]. This test is well validated in our department. It is nontoxic, noninvasive, simple to perform, relatively inexpensive, and reproducible. It has become accepted as a reliable method for assessing small intestinal permeability. A pretest sample of urine was collected after 6 hours of fasting for baseline urinary sugar measurement. After the pretest sample was obtained, patients were given 10 g of lactulose and 5 g of mannitol dissolved in 100 mL of water orally or via nasogastric tube. Urine was collected for 6 hours, aliquoted, and stored at -20 °C until assayed. Urinary lactulose and mannitol concentrations were determined by an enzymatic technique [19,20]. Mannitol excretion was corrected by subtraction of baseline values determined in the pretest samples and lactulose/mannitol excretion ratios (L/M ration) were calculated.

Endotoxin Measurement

Endotoxin was quantified in duplicates using a modified chromogenic Limulus amoebocyte lysate assay (Quadratech, Epson, U.K.). Test plasma samples and standard were diluted 1: 10 in pyrogen-free water and heated to 75 °C for 10 minutes to remove plasma inhibitors. The concentration of entotoxin in the sample was taken as the average of the duplicates calculated from a standard curve. The assay has a sensitivity of 8 pg/mL and is linear in the range 8 to 100 pg/mL. Each aliquot was assayed for endotoxin only once. If the assay gave poor duplicates or very high values, indicating possible contamination, a fresh aliquot of the same sample was retested.

Statistical Analysis

Results are expressed as mean ± SEM. Statistical analysis was performed using the Mann-Whitney *U* test or Spearman's rank-correlation coefficient (r_s) where appropriate with significance taken at the 5% level.

Results

The test and control groups were comparable in terms of age and gender.
Out of 63 patients, 35 patients (55.5%) had gallstones and 22 (34.9%) were alcoholics. One patient (1.58%) had recurrent episode of pancreatitis secondary to hyperlipidemia. The remaining 5 (7.9%) patients had idiopathic acute pancreatitis.
According to the Atlanta score, 42 (66.6%) patients had mild pancreatitis and 21 (33.3%) had severe pancreatitis. 31 patients (49.2%) developed complications (Table I). Six patients (9.5%) died in this study. Four patients had multiple-organ dysfunction syndrome as a cause of their death. The remaining 2 patients died because cardiopulmonary failure, and acute respiratory distress syndrome.

TABLE I - *Complications during course of acute pancreatitis: 31 patients (49.2%).*

Complications	No
Pseudocyst	4
Necrosis	8
Infected necrosis	3
Hepatic	6
Renal	3
Cardiac	2
Respiratory	2
Gastrointestinal	1
Haematological	1
MODS	5
Death	6

MODS: Multiple-Organ Dysfunction Syndrome

INTESTINAL PERMEABILITY

Out of 63, 49 patients (77.7%) had altered permeability, whereas 14 patients (22.2 %) had unaltered permeability. Out of 49 patients with altered permeability, 30 patients (61.2 %) had mild pancreatitis and 19 patients (38.7 %) had severe pancreatitis. Out of 14 patients who had unaltered permeability, 12 (85.7%) had mild pancreatitis, whereas 2 patients (14.2%) had severe pancreatitis.

In addition, out of 21 severe cases, 19 (90%) had altered permeability, and out of 42 mild cases, only 30 (71%) had altered permeability. The L:M ratio was 0.018 ± 0.008 in controls. It was 3 times higher in patients with mild acute pancreatitis (0.051 ± 0.013). In patients with severe pancreatitis, it was 22 times the control (0.36 ± 0.15) (p< 0.05) (Table II). The L:M ratio increased gradually over the period of time duration and course of disease and was maximum at day 7 (p < 0.05) (Figs. 1, 2). The L:M ratio directly correlated with the outcome. The L:M ratio was 0.018 in the control group. Patients who died had an L:M ratio 0.41 ± 0.18, whereas patients who developed complications had an L:M ratio 0.086 ± 0.024. The L:M ratio of patients who resolved without complications was 0.062 ± 0.016 (p< 0.05) (Table III).

TABLE II - *Permeability alteration correlating with severity of acute pancreatitis.*

Severity	No	L:M ratio	SEM
Control	63	0.018*	0.008
Mild	42	0.051*(3 times)	0.013
Severe	21	0.36* (22 times)	0.15

**p <0.05*

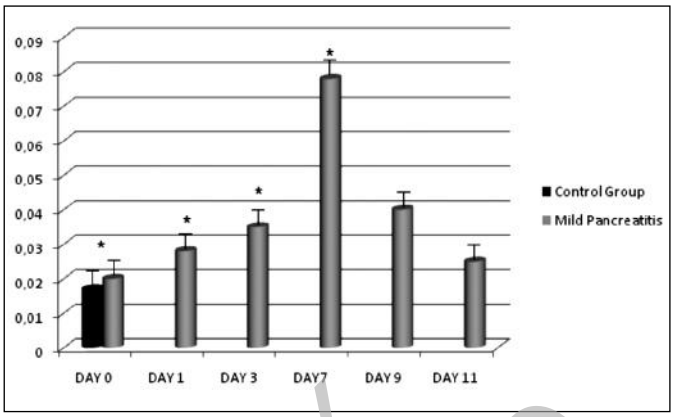

Fig. 1: Intestinal permeability measured by lactulose/manitol excretion ratio (L/M ratio) over the first week of the hospitalization in patients suffering from mild pancreatitis. *p< 0.05

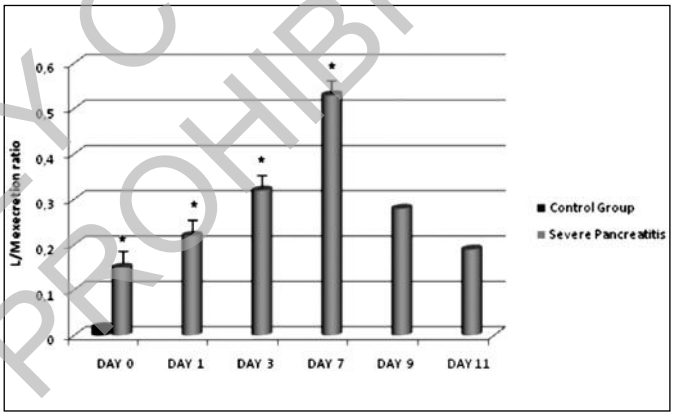

Fig. 2: Intestinal permeability measured by lactulose / mannitol excretion ratio (L/M ratio) over the first week of hospitalization in patients suffering from severe pancreatitis. * P < 0.05

TABLE III - *Permeability alterations correlating with the outcome.*

Outcome	No	*L/M ratio*	SEM
Control	63	0.018*	0.008
Death	6	0.41*	0.18
Survivors with complications	25	0.086*	0.024
Survivors without complications	32	0.062*	0.016

** p <0.05*

ENDOTOXIN

The systemic endotoxin concentration rose significantly in patients with acute pancreatitis as regards control group (p < 0.05) (Figs 3, 4). Systemic concentrations of endotoxin were higher in patients with severe pancreatitis as regards mild pancreatitis (p < 0.05). The

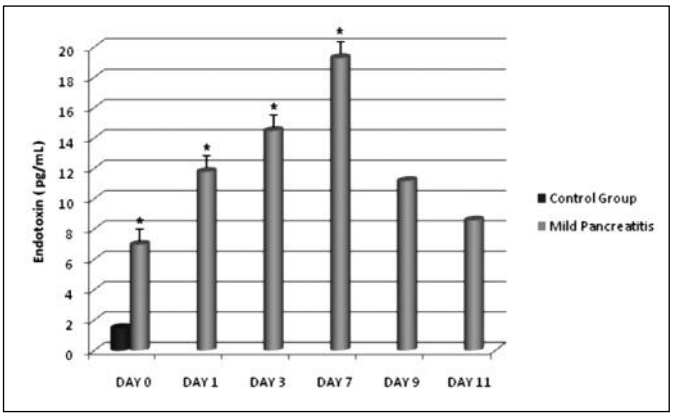

Fig. 3: Systemic endotoxin concentration (mean ± SEM) over the first week of hospitalization in patients suffering from mild pancreatitis. *p < 0.05.

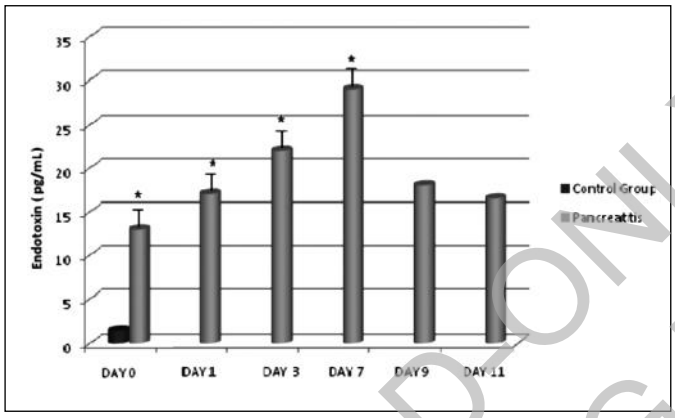

Fig. 4: Systemic endotoxin concentration (mean ± SEM) over the first week of the hospitalization in patients suffering from severe pancreatitis. *p <0.05

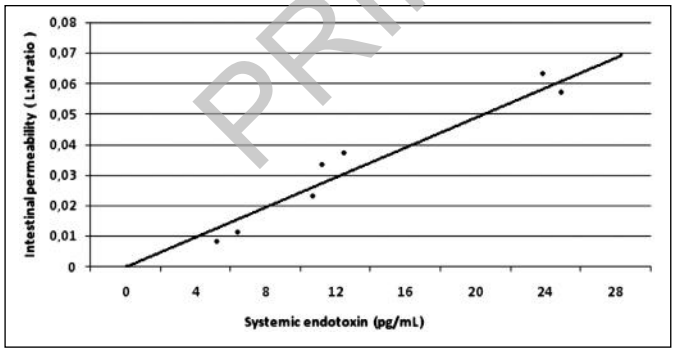

Fig. 5: Correlation between systemic endotoxin concentration and intestinal permeability measured as lactulose/mannitol excretion ratio (L:M ratio) in patients with mild pancreatitis (r_s 0.721; p = 0.001)

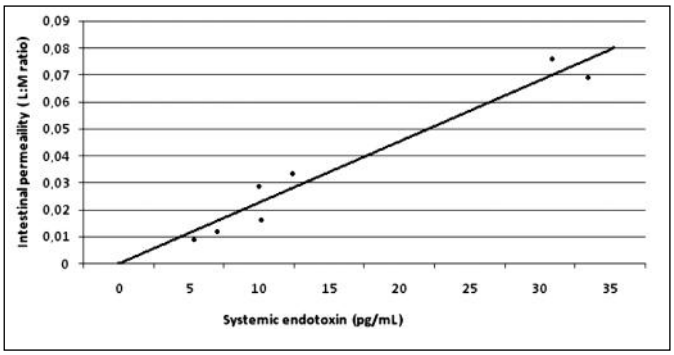

Fig. 6: Correlation between systemic endotoxin concentration and intestinal permeability measured as lactulose/mannitol excretion ratio (L:M ratio) in patients with severe pancreatitis (r_s 0.956; p = 0.001)

endotoxemia was detected mainly during the sixth hour and increased gradually over the period of time duration and course of disease and was maximum at day 7 (p <0.05) (Figs. 3, 4). A significant correlation was observed between the maximum systemic endotoxin concentration and intestinal permeability measured at day 7 in patients with mild (r_s = 0.721; p = 0.001) and severe (r_s= 0.956; p= 0.001) pancreatitis (Figs 5, 6).

The systemic endotoxin concentration directly correlated with the outcome. The systemic endotoxin concentration was 1.30 ± 1.18 pg/mL in the control group. Patients who died had an systemic endotoxin concentration 38.21 ± 8.9 pg/mL, whereas patients who developed complications had systemic endotoxin concentration 31.42 ± 7.6 pg/mL. The systemic endotoxin concentration of patients who resolved without complications was 26.76 ± 8.1 pg/mL (p<0.05) (Table IV).

TABLE IV - *Systemic endotoxin concentration correlating with the outcome.*

Outcome	No	Endotoxin Concentration (pg\mL)	SEM
Contro	63	1.30*	1.18
Death	6	38.21*	8.9
Survivor with complications	25	31.42*	7.6
Survivor without complications	32	26.76*	8.1

*P< 0.05

Discussion

A major function of the gut is to prevent the absorption of toxins, antigens, protease and micro-organisms across the intestinal wall. Epithelial cells cover the surface of the gastrointestinal tract, serving as barrier between the luminal and tissue compartments [21,22]. Hypovolemia after acute pancreatitis [4,5] results in a condition of incomplete ischemia of the gut. The ischemia of the small intestine leads rapidly to an impairment of the protective barrier function of the mucosa [6]. Our study demonstrated a clear increase of gut permeability in patients with acute pancreatitis compared with the control group. The L:M ratio was used to measure intestinal mucosal permeability. Mild cases had only approximately 3-fold increase in intestinal permeability compared with controls, whereas severe cases had an approximately 22-fold increase in intestinal permeability. The disruption of gut mucosal barrier during acute pancreatitis contributes to the genesis of infection[(23)]. Various experimental studies [24,25] have shown the increased intestinal permeability in course of acute pancreatitis. Runkel et al. [25] have shown gut as an important source and bacterial translocation as mechanism for sepsis in acute pancreatitis in a rat model. Kazantsev et al.[26], have confirmed bacterial translocation in acute pancreatitis by plasmid labeling. Hotz et al.[24] showed that increased intestinal permeability is considered as a promoter for bacterial translocation. They have also suggest that there was no significant change in intestinal permeability in the early stages of acute pancreatitis, suggesting a window of opportunity for therapeutic intervention to improve intestinal microcirculation and prevent the late observed increase in intestinal permeability. Nagpal et al. support this fact [15] in a clinical study. They concluded that the permeability increased gradually in patients with acute pancreatitis and was maximum at day 7. Juvonen et al. [14] by using lactulose : rhamnose as a probe marker have concluded that gut absorption capacity is decreased and gut permeability is increased in patients with acute pancreatitis. In addition, patients with severe acute pancreatitis may be more exposed to gut barrier dysfunction. This correlated with our study, which also showed increased intestinal permeability in patients with acute pancreatitis. Moreover our results are consistent with a clinical study where increased gut permeability had also been observed in severe pancreatitis and especially with these patients who developed multiple organ failure.

The mucosa of the intestine and the endothelium of blood vessels contain enzymes capable of synthesizing prostaglandins, production of which may be initiated by neural, ischemic, toxic, or mechanical stimuli [27,28]. The generation of these vasoactive prostaglandins can therefore induce splanchnic ischemia with subsequent disruption of mucosal integrity and increased intestinal permeability. Therefore, hypovolemia may lead to the endotoxemia detected in the systemic circulation in patients with acute pancreatitis. The results of the present study concur with this suggestion, as endotoxin was found in the systemic circulation in patients with acute pancreatitis. Regardless of the etiology of impaired mucosal barrier function, permeation of luminal contents across the bowel wall does occur and results in endotoxemia [29-34]. Endotoxin is a potent stimulator of release of cytokines, such as interleukin-6 and tumor necrosis factor. These inflammatory mediators play an important role in the pathogenesis of systemic inflammatory response syndrome [35,36] and multi-organ dysfunction syndrome [37]. However, the significance of the correlation between systemic endotoxin concentration and increased intestinal permeability remains unclear. In our study, the endotoxemia was detected mainly during the sixth hour, but the increased intestinal permeability was observed only 24 hours after acute pancreatitis. Although it would be easy to suggest that the endotoxemia was due to an increase in bowel permeability, one may develop independently of the other. Indeed, endotoxemia is known to reduce splanchnic blood flow and cause disruption of mucosal barrier function [38].

In conclusion, gut permeability is increased in patients with acute pancreatitis. Patients with severe pancreatitis may be more exposed to impaired gut barrier function. The lactulose/mannitol differential absorption test is nontoxic, noninvasive, simple to perform, relatively inexpensive, reproducible, and quite reliable to measure gut permeability.

Moreover the findings of this study suggest that the pancreatits (mild and especially severe) can lead to systemic endotoxemia. This agrees with the hypothesis that the splanchnic hypoperfusion, during the pancreatitis, may impair intestinal mucosal barrier function and contribute to the systemic inflammatory response and multiorgan failure.

Riassunto

La contaminazione batterica che si verifica nella necrosi pancreatica in corso di pancreatite acuta si pensa sia dovuto alla traslocazione batterica intestinale. Lo scopo di questo studio, a carattere clinico, è stato quello di valutare la permeabilità della mucosa intestinale e l'endotossemia in pazienti con pancreatite acuta. Sono stati studiati 63 pazienti con pancreatite acuta. In 42 pazienti la pancreatite era di grado moderato e in 21 di grado severo. La permeabilità intestinale fu valutata al giorno 0,1,3,7,9, e 11per mezzo del test di assorbimento al lattulosio/mannitolo. Per il dosaggio della endotossemia sono stati eseguiti prelievi di sangue venoso a 0,30,60,90,120, e 180 minuti, a 12,24 ore, e nei giorni 3,7,9, e 11.

I pazienti con pancreatite acuta di grado severo hanno riportato un danno al livello della barriera intestinale di

maggiore entità rispetto ai pazienti con pancreatite acuta di grado moderato essendo in rapporto L:M di 0.36 ± 0.15 e di 0.051± 0.013 rispettivamente (p < 0.005). La concentrazione sistemica dell' endotossemia fu piu elevata nei pazienti con pancreatite acuta severa in confronto con pancreatite acuta moderata (p <0.005). una correlazione significativa è stata osservata tra la massima concentrazione sistemica della endotossemia e la permeabilità intestinale misurate al giorno 7 nei pazienti con pancreatite moderata. (rs = 0.721; p = 0.001)e severa (rs = 0.956; p= 0.001).

In conclusione possiamo affermare che la permeabilità intestinale è aumentata in pazienti con pancreatite acuta. I pazienti con pancreatite acuta severa vanno incontro ad un maggiore danno a livello della barriera intestinale. Inoltre la pancreatite (in particolar modo quella severa) è responsabile dell' aumento della endotossemia. Questi risultati confermano l'ipotesi che ipoperfusione splancnica, che si verifica durante la pancreatite, può danneggiare la funzione della barriera mucosa intestinale, contribuire alla risposta infiammatoria sistemica e quindi all'insufficienza multi-organo.

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