

## A Study of Intestinal Permeability in Relation to the Inflammatory Response and Plasma Endocab IgM Levels in Patients with Acute Pancreatitis

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**Background:** There is scarce information regarding intestinal permeability (IP) in patients with acute pancreatitis (AP) and its relationship with systemic inflammatory response and bacterial translocation (BT).

**Aims:** To study IP in patients with mild and severe forms of AP as compared with controls and the presumed correlations between IP, the inflammatory response, and endotoxin.

**Patients and methods:** Sixty-eight patients with AP and 13 healthy controls were included. IP was assessed by means of the lactulose/mannitol (L/M) test, at admission (LMR1), and at the 15th day (LMR2). The presence of endotoxin was assessed by means of endotoxin-core antibodies type IgM (EndoCab IgM), at admission and 15 days later in patients with severe AP. Plasma levels of interleukins 6, 8, 10, and tumor necrosis factor  $\alpha$  were tested within the first 72 hours from the onset of pain.

**Results:** Both LMR1 and LMR2 were significantly higher in patients than in controls, and in patients with severe versus mild forms of AP. Plasma levels of Endocab IgM increased significantly in patients with severe AP. Basal plasma levels of pro- and anti-inflammatory cytokines were significantly higher in patients with severe AP. A significant correlation was found between LMR2 and Endocab IgM levels in patients with severe AP ( $r = 0.73$ ,  $P = 0.02$ ).

**Conclusions:** Patients with AP show an increased IP when compared with controls, being more relevant and persistent in severe cases. This seems related to an increase of endotoxemia late in the

course of the disease, but not with an exacerbation of the systemic immune response.

**Key Words:** acute pancreatitis, intestinal permeability, lactulose, mannitol, endotoxemia, gut barrier, inflammatory response, cytokines

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Acute pancreatitis (AP) is a common disease with an overall mortality ranging from 10% to 20%. However this figure increases dramatically to roughly 80% when sepsis secondary to an infection of a peri- or pancreatic necrosis occur.<sup>1,2</sup>

Gram-negative bacteria (GNB) of intestinal origin are the most frequently isolated microorganisms in this setting,<sup>3,4</sup> which likely supports the contention of associated gut barrier dysfunction. Bacterial translocation (BT) is defined as the passage of microorganisms of intestinal origin, or their products through an apparently intact intestinal wall reaching mesenteric lymph nodes and other territories. Although the route of migration is not completely known, a dysfunction of the gut barrier, together with bacterial colonization of mesenteric lymph nodes and other organs has been demonstrated in animal models of AP.<sup>5–7</sup> Endotoxin is a key component of the wall of GNB,<sup>8</sup> and endotoxin translocation from the intestinal lumen to the systemic circulation has been associated with the development of the systemic inflammatory response syndrome (SIRS), multiorgan failure, and mortality.<sup>9,10</sup>

The concept of permeability is related to the property of a membrane that enables the passage of a solute by unmediated diffusion.<sup>11</sup> Intestinal permeability may be investigated in vivo by the oral administration of different probes, such as ethylene glycol polymers, radio labeled substances, and oligo- and monosaccharides. The current knowledge of permeability suggests that macromolecules are absorbed through pores of different sizes, mostly by a paracellular route.<sup>11,12</sup> An increased IP has been shown to occur in several disorders of the digestive tract such as celiac disease, inflammatory bowel disease, and cirrhosis.<sup>12–14</sup> Similarly, studies suggest that permeability is

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increased in patients with AP, especially in severe forms,<sup>15–20</sup> and a close correlation between an increased IP and the urinary excretion of nitric oxide metabolites, as a marker of systemic inflammation, has been recently reported<sup>21</sup> in severe forms of AP.

We then consider that AP may be accompanied by a gut barrier dysfunction and increased IP. This might be followed by BT and secondary development of local and systemic complications. According to these concepts, the aims of our study are first to study IP in patients with severe and mild forms of AP as compared with healthy controls, together with IP variations in the clinical course of the disease, and the possible correlations between IP, SIRS, and endotoxemia.

## MATERIALS AND METHODS

Sixty-eight consecutively admitted patients with AP were included in the study (51 mild AP and 17 severe AP). Acute pancreatitis was defined as the presence of compatible abdominal pain, a rise in plasma amylase  $\times 3$  above normal levels, and evidence of acute pancreatic inflammation obtained using imaging techniques, once other causes of abdominal pain had been ruled out. Patients in whom IP could not be determined within the first 72 hours after the onset of symptoms were excluded. We also excluded patients with pre-existing conditions associated with an abnormal IP such as advanced chronic liver disease, inflammatory bowel disease, sepsis, burns, multiple injuries, celiac disease, on chemotherapy, diabetes mellitus affecting the intestine, schizophrania, cystic fibrosis, major surgery, or cardiopulmonary bypass.

The severity of AP was classified as mild (MAP), and severe (SAP) according to Atlanta criteria.<sup>22</sup> An initial prognostic assessment was performed using the APACHE II score within the first 24 hours after admission. Initially, all patients were treated by fasting, I.V. fluids and analgesics. In patients with potentially SAP (APACHE II  $\geq 8$ ) artificial nutrition was administered, (parenteral or enteral), for at least 1 week according to the criterion of the physician in charge, together with prophylactic I.V. imipenem 500 mg TID administered for at least 14 days. Infected pancreatic necrosis, as shown by CT scan-guided fine needle aspiration and culture, was considered an indication for surgery. Oral feeding was reintroduced once the inflammatory signs had settled and the intestinal motility recovered. Local complications of SAP were evaluated on abdominal CT scans using intravenous contrast within the first 72 hours after hospital admission. Emergency colangiopancreatography was performed in patients with SAP of biliary origin. The Hospital General Universitario Ethic's Committee approved the study protocol, and all patients gave written informed consent for the inclusion in the study.

### Intestinal Permeability (IP)

Assessment of IP was performed in all patients within the first 72 hours after the onset of symptoms and repeated 15

days later in both groups of patients. IP was measured by means of the lactulose-mannitol test. Patients received 50 mL of water containing lactulose 10 g and mannitol 5 g. The urine was collected in a receptacle with 0.2 mL of chlorhexidin 2% to avoid bacterial overgrowth. After completing the procedure, the total urinary volume was measured, and 2 aliquots of urine 10 mL each were collected and immediately frozen at  $-70^{\circ}\text{C}$  until analysis. No patient presented urinary tract infection when intestinal permeability was assessed.

The levels of lactulose and mannitol were measured by means of the method previously described by Northrop<sup>23</sup> and Lunn.<sup>24</sup> Urinary recovery of lactulose was determined by the spectrophotometric measurement of the amount of NADPH formed in a series of reactions.<sup>23</sup> The amount of NADPH formed is shown by measuring the increased absorbance at 340 nm. Mannitol was determined by measuring the NADH (measured at 340 nm) generated by the action of mannitol-dehydrogenase on the mannitol.<sup>24</sup> Both tests were done using a Roche Cobas Mira photometric auto-analyzer to determine the concentration (mg/mL) of the 2 sugars in the urine. The results are expressed as the percentages of urinary recovery of lactulose and mannitol according to the urinary volume, and the ratio of both percentages (%Lactulose/%Mannitol = LMR). LMR1 and LMR2 are the figures for the ratio of the 2 sugars in the first and second determinations respectively. Both are expressed with median and standard deviation values.

### Determination of Pro- and Anti-inflammatory Cytokines

Blood samples were collected within the first 72 hours of onset of symptoms in heparinized tubes and centrifuged at 3500 rpm for 15 minutes. Plasma was removed and frozen at  $-70^{\circ}\text{C}$  until analysis. Levels of pro-inflammatory (IL-6, IL-8, and TNF) and anti-inflammatory cytokines (IL-10) were measured by means of a standardized enzyme-immune analysis technique (Medgenix Diagnostics S.A, Brussels, Belgium).

### Determination of Antiendotoxin Antibodies

IgM antiendotoxin antibodies (Endocab, Chromogenic, Molundall, Sweden) were measured in all patients using an enzyme-immunoassay technique (ELISA) within the first 72 hours after the onset of AP. In patients with SAP the measurement was repeated 15 days later. The referred intervals were established from figures obtained in a study of 1000 healthy blood donors in which the median anti-core antibody concentration of IgM endotoxin was 149.4 (73.2 – 446.7) median units /mL (MU/mL).

### Statistical Analysis

For the description of the variables we used the median and 25% and 75%iles. The Kruskal-Wallis test was used for comparison of 3 groups, and the Mann-Whitney *U* test for 2 groups (MAP and SAP). Correlations between variables were

performed with the Spearman correlation coefficient. The level of statistical significance was taken as  $P < 0.05$ .

**RESULTS**

The main clinical characteristics of all patients included in the study are detailed in Table 1. No statistically significant differences were observed in age, sex, and etiology of AP between both groups of patients. AP was severe in 17 cases (23.5%) and mild in 51 cases. Six patients developed extrapancreatic infections (of the biliary, urinary or respiratory tracts). Nine (10.7%) and 2 (2.4%) patients received parenteral or enteral nutrition respectively due to suspicion of progression to SAP. Overall, 7 patients with SAP died during the study period (10.3%, see Table 1).

LMR1 and LMR2 were significantly higher in the overall group of patients than in controls. LMR1 was significantly higher in patients with SAP than in MAP and controls ( $0.044 \pm 0.188$ ,  $0.026 \pm 0.099$  and  $0.013 \pm 0.07$ , respectively.  $P < 0.05$  in all comparisons) (Fig. 1), a behavior similar to that observed with LMR2 ( $0.040 \pm 0.081$ ,  $0.018 \pm 0.059$ , and  $0.013 \pm 0.07$ , respectively.  $P < 0.05$  in all cases) (Fig. 2). LMR2 decreased, although to a non-significant level, when compared with LMR1 in both groups of patients (Fig. 3). No significant correlation was found between age and LMR1, and the etiology of AP did not significantly influence LMR1 (biliary  $0.032 \pm$

$0.166$ , alcohol-related:  $0.026 + 0.001$ , hypertriglyceridemia:  $0.023 + 0.142$ ; other:  $0.030 + 0.028$ ).

No statistically significant differences were found in Endocab IgM levels at admission between patients with SAP or MAP (52.1 vs. 56.8 MU/mL, respectively). Values of Endocab IgM increased significantly in patients with SAP on day 15 in comparison to those obtained at admission ( $100.8$  vs.  $52.1$  MU/mL; respectively.  $P < 0.01$ ), but this increase was not observed in patients with MAP.

A significant correlation was found between Endocab IgM and LMR2 in patients with SAP ( $r = 0.73$ ;  $P = 0.02$ ), but not between LMR1 and the corresponding Endocab IgM levels.

Patients with SAP showed significantly higher levels of IL-6, IL-8, and IL-10 within 72 hours from the onset of symptoms than patients with MAP. Although TNF $\alpha$  levels were also higher in patients with SAP, these differences did not reach significance ( $P = 0.09$ ) (Fig. 4). No significant correlations were found between LMR1 and any of the studied cytokines.

**DISCUSSION**

In this study we report evidences that patients with AP show an increased IP when compared with controls, being more relevant and persistent in severe forms of AP. This fact seems related to an increase of endotoxemia late in the course

**TABLE 1.** Epidemiological Characteristics, Aetiology and Course of AP

	n	SAP* (17)	MAP* (51)	Controls (13)	p
Sex					
Men (%)		8 (47.1)	22 (43.2)	8 (61.5)	
Women (%)		9 (52.9)	29 (56.9)	5 (38.5)	ns
Age		63.5 $\pm$ 19.2	60.4 $\pm$ 16.4	41.3 $\pm$ 9.3	ns
Aetiology					
Biliary (%)		10 (58.7)	27 (52.9)		
Alcohol (%)		1 (5.9)	7 (13.6)		
Hypertrigl. (%)		2 (11.8)	1 (1.9)		
Others (%)		4 (23.5)	16 (31.4)		ns
APACHE II		9.41 $\pm$ 2.4	3.45 $\pm$ 2.40		
Complications					
MOF** (%)		7 (10.3)			
Pseudocyst (%)		5 (7.4)			
Necrosis (%)		5 (7.4)			
Infect. Necrosis (%)		1 (1.5)			
Abscess (%)		1 (1.5)			
Mortality		7 (41.2)	0		

\*The quantitative variables are expressed as numbers and percentages, and the qualitative variables as means  $\pm$  standard deviation. \*SAP, severe acute pancreatitis; MAP, mild acute pancreatitis.

\*\*MOF, multiple organ failure.

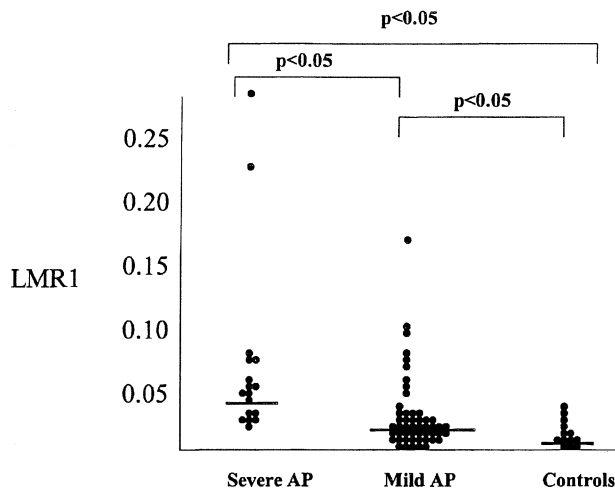


FIGURE 1. Relationship between Lactulose/mannitol test at admission (LMR1) and severity of acute pancreatitis. Line expresses median values.

of the disease in cases of SAP, but not with an exacerbation of the systemic immune response.

An impaired IP has been reported in patients and in experimental models of AP, being associated with the development of complications.<sup>15-21,25</sup> We have also observed an increased IP in patients with AP compared with controls, and this is significantly higher in patients with SAP than with MAP. Our findings seem to confirm a failure of the intestinal barrier in this setting, which may be related in turn, to an impaired intestinal vascular perfusion.<sup>26</sup>

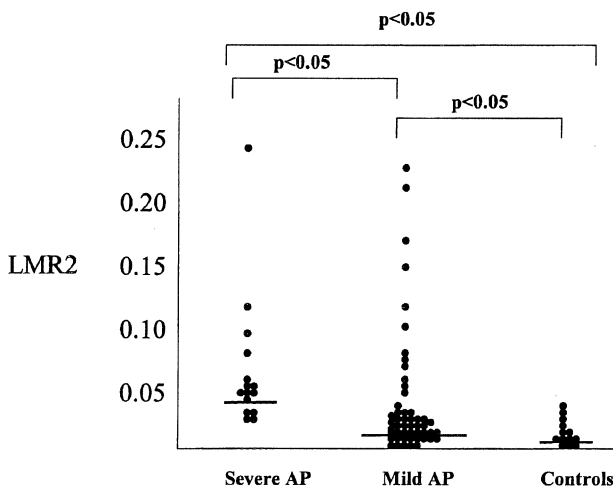


FIGURE 2. Relationship between Lactulose/mannitol test at day 15 (LMR2) and severity of acute pancreatitis. Line expresses median values.

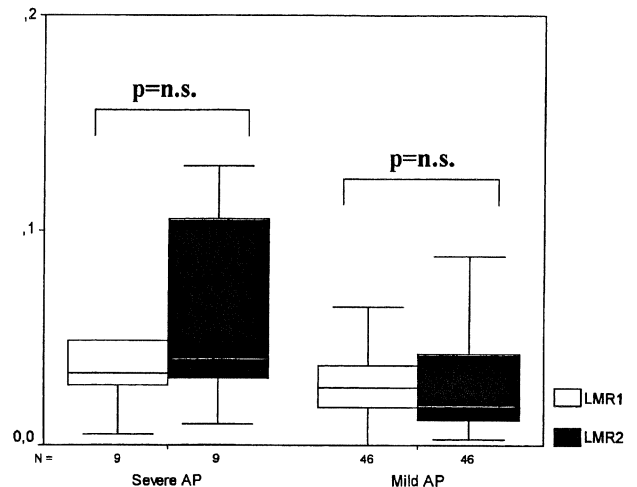
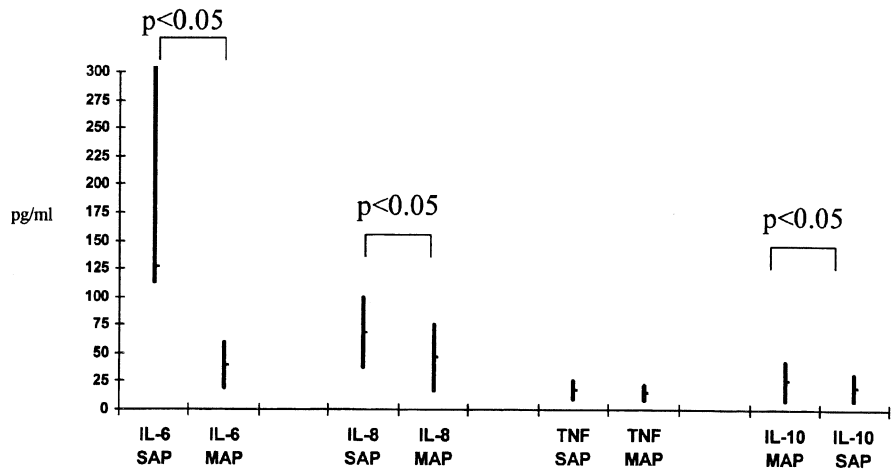


FIGURE 3. Behavior of Lactulose/mannitol test throughout the evolution of mild and severe acute pancreatitis.

Previous studies have reported conflicting results in this setting. Thus in Ammori series, and others<sup>17,20,21</sup> an increased IP was only found in SAP, with no significant differences observed between MAP and controls. This is similar to that reported by Juvonen et al,<sup>18</sup> who found statistically significant differences in basal IP between patients with MAP and SAP, and with respect to controls. In contrast, McNaught<sup>19</sup> found no differences in IP between SAP and MAP. The discrepancy among the different reported studies and our own data may be related to the different methods used to assess IP: polyethyleneglycol with a molecular weight ranging from 400 to 3350,<sup>17,20</sup> a combination of lactulose, rhamnose, cellulose and methylglucose,<sup>18</sup> or the association of lactulose and rhamnose.<sup>19</sup> Because there is not an universally accepted method to measure IP or even to express the results obtained,<sup>27</sup> it is mandatory to include a control group, and also precludes an adequate comparison among studies.

We have observed a non-significant decrease in IP values obtained on day 15 vs. basal values in patients with SAP and MAP (see Fig. 3). Our findings are in contrast with those from Juvonen et al<sup>18</sup> who observed a normalization of IP in patients with MAP and SAP in a period of 8 and 45 days after the onset of AP respectively. It is likely that differences in the time interval between both studies may explain the discrepancies found.

We have not found any statistical relationship between IP, age, and etiology of AP. Although it has been described that the urinary excretion of both mono- and disaccharides decreases in patients above the 7th decade of life, the ratio between both probes remain constant.<sup>28,29</sup> Similarly, we did not find differences related to alcohol consumption. Although urinary excretion of polyethylene glycol has been reported to be



**FIGURE 4.** Determination of the pro- and anti-inflammatory cytokines in both groups of AP. Horizontal line expresses median values.

increased in patients with both acute and chronic alcohol intake,<sup>30,32</sup> this is not always the case as reported by Pfeiffer et al.<sup>31</sup>

Conflicting data have been reported relating to the influence of the type of artificial nutrition used in patients with AP (enteral vs. parenteral) on IP.<sup>33–36</sup> Although we have not specifically analyzed this aspect, the number of patients studied would have precluded obtaining valuable data.

Endotoxin is the major constituent of the wall of Gram-negative bacteria, its main components being the O-antigenic polysaccharide and lipid A.<sup>37</sup> Endotoxin has been shown to be involved in the development of the systemic inflammatory response syndrome and septic shock.<sup>38,39</sup> Among the mediators associated with the cardiovascular alterations occurring in septic shock, it has been observed that the increased production of TNF- $\alpha$  and nitric oxide play an important role.<sup>39</sup> Experimental studies have shown that intravenous administration of endotoxin is associated with hyperdynamic circulatory changes<sup>39,40</sup> and the increase in the production of IL-2, IL-6 and prostaglandin E2<sup>41</sup>. Endotoxemia has been shown to be directly related to the severity of episodes of AP,<sup>42</sup> being more relevant in patients with SAP.<sup>17,20,43</sup>

The Endocab IgM antibody titer is an indirect marker for endotoxin levels,<sup>44</sup> and endotoxemia peaks coincidentally with the lower levels of Endocab IgM antibody.<sup>45,46</sup> The values reported, however, vary with the different authors. Thus, in Buttenschoen series,<sup>43</sup> after initial similar values of Endocab IgM antibody in patients with MAP and SAP, the level of the antibody became significantly greater in SAP on the 6th day of illness. Our results are similar to those described in the literature.<sup>42,46</sup> We did not observe significant differences in Endocab IgM between MAP and SAP at admission, but a significant increment of values were observed on day 15 in patients with SAP. In concordance with previous studies, we did not observe any significant correlation between Endocab IgM levels and IP

at admission, and this fact likely supports the contention that translocation of endotoxin and IP represent 2 different, and not necessarily related processes, that may be present in patients with AP in different moments of the clinical evolution.

The inflammatory response was studied in relation to the measurement of pro- and anti-inflammatory cytokines IL-6, IL-8, TNF $\alpha$ , and IL-10 respectively. Although IL-8 is usually the first to be detected, the main inflammatory mediator during the acute phase of AP is IL-6.<sup>47,48</sup> An increase in IL-6, IL-1, and TNF $\alpha$  levels has been observed in the initial phases of AP, being significantly greater in SAP and in patients later developing systemic complications.<sup>49,50</sup> As expected, we have observed significantly higher levels of the cytokines IL-6, IL-8, and IL-10 in patients with SAP. We did not find any statistically significant correlation between IP and the levels of the studied cytokines, and this likely suggests that an abnormal permeability is not a prerequisite for the development of an inflammatory response in this setting.

In summary, IP is increased in patients with either MAP or SAP, and keeps abnormal during the clinical course of the disease. This fact is related to high endotoxin levels late in the course of the disease in patients with SAP. Further, the absence of a significant relationship between IP and the inflammatory response suggest that both events, present in patients with SAP in our series, may occur independently.

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