Demonstrating Infection in Severe Acute Pancreatitis: A Role for Polymerase Chain Reaction and Gene Sequencing?*

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Sepsis in acute pancreatitis (AP) carries a high mortality rate and accounts for approximately 80% of all deaths from this disease (1). In severe AP with extensive necrosis, the infection rate may be as high as 55% to 70% (2). The mechanism of such infection has been the subject of considerable investigation. Animal studies have implicated bacterial translocation from the gastrointestinal tract (3). In humans, however, definitive evidence for such intestinal barrier dysfunction is lacking (4, 5), although the predominance of Gram-negative aerobic species in cultured samples and the presence of circulating endotoxin lend credence to this hypothesis. Based on this, there have been a host of studies investigating the ability of probiotics, antibiotics, and selective decontamination of the digestive tract to reduce the prevalence of pancreatic superinfection (3, 6).

Infection in AP demands appropriately targeted antibiotic therapy, with best guess empiric regimens contributing to drug resistance, a shift in the microbiologic profile of infection (opportunistic fungal infections and Gram-positive bacteria), and ineffective treatment (1, 2). However, obtaining a definitive diagnosis with identification of the specific organisms remains problematic. Blood cultures can demonstrate bacteremia—a useful marker to gauge the probability of infected necrosis. However, cultures are slow, fail to address the urgency of pathogen detection, and lack sensitivity (1). Fine needle aspiration of the necrosis is more specific for pancreatic infection, but there continue to be concerns about its sensitivity and the introduction of organisms into the pancreas. A rapid, sensitive, and specific test to diagnose infection in AP would be invaluable to guide management, including the need for antibiotic therapy and even surgical intervention.

*See also p. 1938.

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In this issue of *Critical Care Medicine*, Li et al (7) further investigate the role of gut organisms in the pathophysiology of infection in pancreatitis. Taking blood samples from 48 patients diagnosed with AP, they compared bacteriologic results obtained from standard culture techniques with those results obtained from polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE), finding PCR with DGGE to be three times more sensitive than blood culture in the detection of bacteremia (68.8% vs 21.9% positive results). They demonstrate a positive correlation between bacteremia, particularly polymicrobial, and disease severity.

A handful of human studies have investigated the use of PCR technology in an effort to establish a faster and more sensitive way to analyze blood samples for infection in AP. Universal primers exploiting the conserved 16S or 18S ribosomal RNA (rRNA) region of microorganisms allow detection of the presence of bacteria and fungi, respectively, while further sequencing techniques are necessary to precisely identify the specific infecting organism (8–13). Results have been variable with PCR detection rates ranging from 0% (8) to 33.4% (13), with better sensitivity compared with cultures in the latter study. In the report where no samples were found to be positive for bacterial DNA, this was attributed to the presence of inhibitory substances in the blood interfering with PCR chemistry. Peng et al (11) compared culture techniques with PCR detection in a rat model of pancreatitis and found PCR to be as sensitive for the detection of bacteria as cultures of blood, lymph nodes, pancreas, and liver.

The current study by Li et al (7) is the largest to date using PCR and DGGE to specifically assess infection in patients with pancreatitis. The PCR technique followed by DGGE with gene sequencing using databases, such as GenBank, reveals bacterial profiles similar to those identified in blood cultures run in parallel and mirrors those previously reported using standard blood culture techniques, including *Escherichia coli*, *Pseudomonas* species, and *Klebsiella pneumoniae* (2). The rate of polymicrobial bacteremia (60.4%), however, is higher than reported in various other studies (13 %–42%); this may be due to a shift in spectrum of pathogens following administration of antibiotics.

Despite the use of positive and negative PCR controls, the much higher rate of positive results (68.8%) than in other studies does raise the suspicion of false positives. The fact that samples were taken after administration of prophylactic antibiotics and routinely at certain time points irrespective of whether clinical features of infection were present (e.g., fever, raised white cell count) would certainly lead one to expect a lower detection rate of bacteremia. Given that the sensitivity of PCR to detect bacteremia has been shown to be as high as 10

organisms/mL, bacterial contamination of samples resulting in false-positive results is a major concern with this technique (8).

The authors suggest that they have demonstrated bacterial translocation from the gut, but in fact no measures of intestinal permeability, endotoxinemia, endotoxin core antibodies, or mesenteric lymph node culture were made to justify such claims. Although the presence of Gram-negative organisms may suggest an enteric origin and, by inference, bacterial translocation, other sources of bacteremia, such as the biliary tract or IV catheters, are plausible. With the methods employed in this study, use of 18S rRNA primers against fungal DNA would have been a valuable additional undertaking given the smaller but significant number of mycotic infections. The authors also do not address how antibiotic sensitivity might be determined using this technique.

Although this study adds to the evidence supporting bacterial translocation and PCR and DGGE technology do offer promise in this setting, further studies are required to assess the validity and benefits of these assays. In a study of more than 500 biological samples from patients hospitalized with a variety of diagnoses, the broad-range rRNA PCR technique was found to have 74% sensitivity and approximately 99% specificity. In patients where both cultures and PCR results were positive, there was 83% concordance in the microbiologic diagnosis (14). Döring (15) offers a strong word of caution on the use of nucleic acid amplification techniques to diagnose infection. Specifically, universal primers lack the sensitivity of more targeted primers, positive and negative controls are essential, assays must be assessed for PCR inhibition, risk of bacterial contamination of samples must be minimized, and comparison to current microbiologic gold-standard methods must be made (15). Even if established as a valid and reproducible technique, analysis of pancreatic aspirate samples may prove even more advantageous than analysis of peripheral blood samples.

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