

616.37–002–02:616.981.45]–074/–078

### **The laboratory diagnostic of pancreatitis of Yersinia etiology**

Y. A. Voskresenskaya

*Pasteur Institute of Epidemiology and Microbiology of Rospotrebnadzor, Saint Petersburg, Russia*

**Key words:** acute pancreatitis, chronic pancreatitis, *Y. pseudotuberculosis*, *Y. enterocolitica*, polymerase chain reaction

During last 5–7 years pancreatitis has taken leading place in frequency of hospitalization: it accounts for 43.3% of the total number of hospitalized patients with acute surgical pathology. There are various pathogenetic factors in its pathogenesis base. Acute pancreatitis is able to progress after any operation on abdomen organs, and the frequency of this disease is variable and high enough at the same surgery.

It is thought, that microorganisms such as in the etiology of acute pancreatitis also play a role microorganisms, particularly pathogens yersiniosis and pseudotuberculosis. The literature contains descriptions of sporadic cases of pancreatitis in the genesis of which a key role is played *Yersinia enterocolitica* *Y. pseudotuberculosis*.

According to some authors, the share of these types of bacteria in the etiology of acute pancreatitis is not more than 1%.

Polymorphism of clinical symptoms in these diseases, due to the presence of pathogens in a variety of pathogenic factors of plasmid and chromosomal nature, involvement in the pathological process of many organs and systems, including the pancreas, often the causes for such patients in the surgical hospital. In most cases, yersinia pseudotuberculosis and remain undiagnosed, masquerading as a different pathology - diseases of the digestive system, musculoskeletal system, connective tissue, nervous system, skin, etc. It is important to note that infections caused by *Yersinia*, characterized by high frequency of chronic process, which will certainly contribute to late diagnosis and inadequate treatment. Therefore early detection of pathology caused by yersinia becomes important.

**Aim of investigation** — to define effectivity of diagnostic techniques for revealing pancreatitis

#### **Materials and techniques**

101 patients of St.-Peterburg emergency clinic by I. I. Dganelidze from 2007 to 2009 were investigated with the help of laboratory technique complex.

76 patients had pancreatitis of various stage (main group). The age of patients - 17–81 years; there were 54 men, and 22 women.

The control group was comprised from 25 patients with other types of urgent surgery pathology (acute cholecystitis, acute intestinal obstruction, perforated duodenal ulcer (DU), bleeding from the ulcer of the stomach or duodenum). The average age is  $53 \pm 15$  (from 18 to 88 years); number of men is 14, women is 11.

To identify the etiology of diseases of *Yersinia* in all patients for clinical laboratory tests were taken material in accordance with the rules given in the methodological guidelines. On admission to hospital patients conducted serological blood tests. Used agglutination (PA) with corpuscular antigens. They used agglutination (PA) with corpuscular antigens *Y. pseudotuberculosis* serotype O: 1, *Y. enterocolitica* serotype O: 3, O: 4, O: 5.27, O: 9 (production NIIEM them. Pasteur) and ELISA using commercial test kits *Yersiniosis* ELISA-IgA, *Yersiniosis* ELISA-IgG, ELISA-*Yersiniosis* IgM (produced by "Omniks", St. Petersburg) for the detection of antibodies of A, M and G to the outer membrane proteins encoded by the *Yersinia* virulence plasmid pYV. Statement and account of the Republic of Armenia and the ELISA was performed in accordance with the instructions.

By polymerase chain reaction (PCR) investigated feces (admission) effusion (Horizontal packing bags and pancreatic cysts, exudate from the peritoneal cavity) to identify chromosomal regions of genes encoding virulence factors *Yersinia* - superantigen toxin YPM *Y. pseudotuberculosis* and protein adhesion / invasion Ail *Y. enterocolitica. pseudotuberculosis* IP 33156 and *Y. enterocolitica* IP 1105 obtained from the National Reference Center for *Yersinia* (Institut Pasteur, Paris) were positive control samples. For bacteria lysate grown on Hottinger agar was prepared slurry with a concentration of  $10^8$  l / ml in sterile distilled water, 100 l of the slurry was boiled 5 minutes, centrifuged at 10 000 rev / min for 1 min, the supernatant was used [10]. Applied primer pairs produced by "Syntol" (Moscow) to sites of genes *ypmA / C* (Forward: 5'-SACTTTTCTCTGGAGTAGCG-3', Reverse: 5'-ACAGGACATTTTCGTCA-3', product size 422 bp). [10] and *ail* (Forward: 5'-TAATGTGTACGCTGCGAG-3', Reverse: 5'-GACGTCTTACTTGCACTG-3', product size 351 bp). The reaction mixture for PCR was prepared using standard reagents production «Fermentas» according to the instructions 2.5 ul 10x Taq-buffer (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2 mmol / L MgCl<sub>2</sub>, 0,2 mmol / L each dNTP, 0,4 uM / L forward and reverse primers, 0,625 U Taq-DNA polymerase with addition of 4 l of the sample to a final volume of 25 microliters. PCR was performed in a programmable thermostat TA4-01-PCR "Tertsik" manufactured by JSC "APF DNA technology" using the following program: 1 cycle 95 ° C - 2 min 30 cycles 94 ° C - 45 seconds, 55 ° C (for primers to *ypmA / C*) or 57 ° C (for primers to *ail*) - 45 s, 72 ° C - 45 seconds, 1 cycle of 72 ° C - 10 minutes. To take into account the results of electrophoresis of PCR was performed on a 1.5% agarose gel at constant voltage

75 V in TBE buffer (0.9 M Tris, 0.9 M boric acid, 20 mM EDTA • Na<sub>2</sub>) with ethidium bromide staining and subsequent photographing in UV light transmitted.

In order to justify the diagnosis of acute pancreatitis Yersinia etiology and determine its severity into account medical history, precipitating factors (alcohol, errors in diet), comorbidity, clinical examination of patients. Perform general clinical and instrumental examination on admission and in the dynamics of the disease<sup>1</sup>.

We compared the clinical manifestations of the disease and laboratory examination of the data in patients with acute and chronic pancreatitis Yersinia etiology and pancreatitis caused by other pathogenic factors.

### **Results and discussion**

Analysis of the results of research it turned out that the nature of Yersinia pancreatitis, confirmed by serological methods, was found in 18 (23.7%) of 76 patients. In 14 (18.4%) patients diagnosed pancreatitis caused by *Y. enterocolitica*, in 2 (2.6%) - *Y. pseudotuberculosis*. More in 2 (2.6%) patients simultaneously antibodies to *Y. pseudotuberculosis* and *Y. enterocolitica*. The diagnosis of "pancreatitis" was confirmed in 4 patients with severe and moderate in 5 cases - in light of its current and in 9 cases - an exacerbation of chronic pancreatitis.

By PCR positive results in the study of fecal samples from 5 people the number of patients with a diagnosis confirmed by serological methods (6.6% of the total number of the patients with pancreatitis): 2 patients with confirmed pancreatitis pseudotuberculosis etiology, in 3 - Yersinia. All patients were admitted to hospital with acute exacerbation of chronic pancreatitis.

Among the 25 patients in the control group antibodies to *Y. enterocolitica* detected in 1 (4%) patients with strangulated hernia, ventral, indicating specificity used laboratory methods. In this study material for this group of patients by PCR in all cases were found to be negative.

For pseudotuberculosis, except abdominal pain, unstable stool and increase the liver, characterized by fever, intoxication, rash, arthralgia, increased peripheral lymph nodes, catarrhal phenomena in the throat. Clinical manifestations of yersiniosis in many ways similar to those of pseudotuberculosis, but is even more diverse. The disease may begin acutely, with phenomena of gastroenteritis, and proceed in a localized form as an acute intestinal infection. In generalized throughout iersinioza disease begins acutely with fever, symptoms of intoxication, rashes on the skin, disorders of the chair, abdominal pain and joint pain, enlarged lymph nodes and liver.

---

<sup>1</sup>We are grateful to associate the 2nd Department of Surgery and Advanced Medical Naval Academy. Kirov S.Y, Polushin for the material for the study

In our study, the clinical manifestations of disease onset were fairly typical of pancreatitis, regardless of its etiology. All patients appeared sharp pain in the upper abdomen, usually herpes nature radiating to the spine, then joined nausea and vomiting, upset chairs, and weakness. A minority of patients (13 patients) was observed paleness or icterus of the skin. Bloating and peritoneal symptoms were observed in 13 patients. In 29 patients with such symptoms occurred in the past.

7 patients with pancreatitis were reported as arthralgia. Rash, typical of yersiniosis and pseudotuberculosis (like "gloves" or "socks"), were absent. Hyperthermia is more than 38 ° C was 23 patients, but the enlarged lymph nodes were found.

The results showed that the infectious etiology of acute pancreatitis is more important than it is now recognized. In our study, yersinia infection found almost one in four of the surveyed patients with pancreatitis (23.7%), while in the control group of patients with yersinia etiology of the disease is not detected. It was also found that pancreatitis caused by *Y. pseudotuberculosis* and *Y. enterocolitica*, may have different variants of the course and severity. It is important to note that one third of patients with acute exacerbation of chronic pancreatitis (9 of 28 patients) are confirmed *Yersinia* nature of the disease, suggesting an underestimation of the potential role of *Yersinia* in the development of pancreatitis.

The clinical picture of acute and chronic pancreatitis in patients with exacerbation of *Yersinia* with the etiology of disease is any difference on the clinical manifestations of infection with the typical course of yersiniosis and pseudotuberculosis lacking. The establishment of the etiology of the disease in patients with gastrointestinal disorders presents considerable difficulties, which indicates the need of screening for *Yersinia* infection.

### **Summaries**

1. The use of specific methods of complex laboratory examination of patients with acute and chronic pancreatitis *Yersinia* helped confirm the etiology of diseases in 23.7% of cases.
2. The most informative in the laboratory diagnosis of acute and chronic pancreatitis with acute exacerbations of *Yersinia* etiology are serological methods (ELISA, PA).
3. The complex methods of laboratory examination of pancreatitis patients should include studies of the biological substrates for *Yersinia*.

## **The laboratory diagnostic of pancreatitis of Yersinia etiology**

Y. A. Voskresenskaya

*Pasteur Institute of Epidemiology and Microbiology of Rospotrebnadzor, Saint Petersburg, Russia*

**Key words:** acute pancreatitis, chronic pancreatitis, *Y. pseudotuberculosis*, *Y. enterocolitica*, polymerase chain reaction.

The effectiveness of diagnostic techniques detecting pancreatitis of Yersinia etiology is discussed. The agglutination reaction and immune-enzyme assay have been applied to detect the outer membrane proteins antibodies of various classes coded by plasmid of Yersinia virulence pYV. The polymerase chain reaction technique was applied to detect sites of chromosomal genes coding the factors of Yersinia virulence — superantigen toxin YPM Y. pseudotuberculosis and protein of adhesion/invasion of Ail Y. enterocolitica. The application of the complex of specific techniques of laboratory examination of patients with acute pancreatitis and chronic pancreatitis with exacerbations permitted to confirm the Yersinia etiology of disease in 23.7% of cases. Then serologic techniques are the most informative in laboratory diagnostics.