Laboratory diagnosis of acute pancreatitis: in search of the Holy Grail

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Abstract
Acute pancreatitis is an acute inflammatory condition of the pancreas, which might extend to local and distant extrapancreatic tissues. The global incidence varies between 17.5 and 73.4 cases per 100,000 and the pathogenesis recognizes alcohol exposure and biliary tract disease as the leading causes, ahead of post-endoscopic retrograde cholangiopancreatography, drugs and abdominal trauma. The diagnosis of acute pancreatitis is substantially based on a combination of clinical signs and symptoms, imaging techniques and laboratory investigations. Contrast-enhanced computed tomography is the reference standard for the diagnosis, as well as for establishing disease severity. The assessment of pancreatic enzymes, early released from necrotic tissue, is the cornerstone of laboratory diagnosis in this clinical setting. Although there is no single test that shows optimal diagnostic accuracy, most current guidelines and recommendations indicate that lipase should be preferred over total and pancreatic amylase. Although a definitive diagnostic threshold cannot be identified, cut-offs comprised between ≥ 2 and ≥ 4 times the upper limit of the reference interval are preferable. The combination of amylase and lipase has been discouraged as although it marginally improves the diagnostic efficiency of either marker alone, it increases the cost of investigation. Some interesting biomarkers have been also suggested (e.g., serum and urinary trypsinogen-1, -2 and -3, phospholipase A2, pancreatic elastase, procalcitonin, trypsinogen activated protein, activation peptide of carboxypeptidase B, trypsin-2-alpha1 antitrypsin complex and circulating DNA), but none of them has found widespread application for a variety of reasons, including the inferior diagnostic accuracy when compared with the traditional enzymes, the use of cumbersome techniques, or their recent discovery. The promising results of recent proteomics studies showed that this innovative technique might allow the identification of changes characterizing pancreatic tissue injury, thus highlighting new potential biomarkers of acute pancreatitis.

Keywords: Acute pancreatitis, laboratory testing, lipase, amylase, diagnosis

Abbreviations: 2-DE, two-dimensional electrophoresis; APACHE II, acute physiology and chronic health evaluation II; ARDS, adult respiratory distress syndrome; AST, aspartate aminotransferase; CAPAP, activation peptide of carboxypeptidase B; CAPB, carboxypeptidase B; CCK, calcium-sensing receptor; CCK, secretin, cholecystokinin; CECT, contrast-enhanced computed tomography; CFTR, cystic fibrosis transmembrane conductance regulator; CRP, C-reactive protein; CTRC, chymotrypsinogen C; ED, Emergency Department; EPS, ethylidene protected substrate; ERCP, post-endoscopic retrograde cholangiopancreatography; GRP, gastrin-releasing peptide; HL, hepatic lipase; HPL, include pancreatic lipase; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; iTRAQ, isobaric tags; LC, liquid chromatography; LDH, lactate dehydrogenase; LPL, lipoprotein lipase; MRI, magnetic resonance imaging; MS, mass spectrometry; PLRP2, pancreatic lipase related protein 2; proCAPB, procarboxypeptidase B; SIBioC, Italian Society of Clinical Biochemistry and Clinical Molecular Biology; SPINK1, serine protease inhibitor Kazal 1; TAP, trypsinogen activation peptide; trypsin-2-AAT, trypsin-2-alpha1 antitrypsin complex; TOF, time of flight; URL, upper limit of the reference interval; US, ultrasonography; VIP, vasoactive intestinal peptide; WBC, white blood cell
Epidemiology of acute pancreatitis

The global incidence of acute pancreatitis varies between 17.5 and 73.4 cases per 100,000 worldwide. The most comprehensive and recent data on the epidemiology of acute pancreatitis in Europe can be taken from the 2006 systematic review based on reported population-based studies of Yadav and Lowenfels. The authors identified eighteen full-length original articles (8 from the UK and 10 from other European centers) published from 1966 to June 2005 containing reliable data on the epidemiology of first-attack acute pancreatitis. Overall, the annual incidence of first-attack (all causes) in the years 1990–2005 was between 10 and 40 cases per 100,000. The annual incidence of first-attack acute gallstone pancreatitis and acute alcoholic pancreatitis ranged from 8 to 16 and from 4 to 10 cases per 100,000, respectively. The global case fatality for first-attack acute pancreatitis was between 3 and 10%, but varied widely according to the pathogenesis (e.g., <10% for sterile versus ~25% for infected pancreatic necrosis). Interestingly, an increase in the annual incidence for first-attack acute pancreatitis was evident in 10 of 12 studies with longitudinal data. The incidence of disease and mortality also increased steadily with patient age. Gallstone pancreatitis was more common in female subjects, whereas alcoholic pancreatitis was more prevalent in middle-aged male subjects.

Although the case fatality has decreased over time, the overall population mortality rate has remained unchanged. To investigate the epidemiology of hospital admissions for acute pancreatitis in the U.S., Fagenholz et al. examined data from the 1988–2003 National Hospital Discharge Survey. Hospital admissions increased from 40 per 100,000 in 1998 to 70 per 100,000 in 2002 in the U.S. population. The average age of patient admission was 53 years, with a slight predominance of men (51%). The overall mortality was 2%. Increasing patient age along with the male gender were independent risk factors for death. A national investigation of the epidemiology of acute pancreatitis in Japan reported that the total number of patients affected in 1998 was 19,500 (i.e., 15 cases per 100,000; 65% males). In 55% of cases the pancreatitis was mild, in 20% moderate and in 25% severe.

Pathophysiology of acute pancreatitis

Acute pancreatitis is a common cause of abdominal pain in the Emergency Department (ED), and represents both a diagnostic and therapeutic challenge. The clinical presentation may vary from mild abdominal pain to refractory shock, and shares several signs and symptoms with other abdominal and extra-abdominal disorders. The pancreas, which comprises only 0.1% of total body weight, has 13-times the protein-producing capacity of liver and reticulo-endothelial system combined, both of which account for 4% of total body weight. Enzymes are produced as proenzymes (typically single-chain polypeptides) within the pancreatic acinar cells, then packaged into storage vesicles called zymogens and released via the pancreatic ductal cells into the pancreatic duct, where they are finally secreted in the small intestine. Under physiologic conditions, up to 15 different types of digestive enzymes are produced and stored as proenzymes, including chemotrypsinogen, trypsinogen, proelastase, procarboxypeptidase B (proCAPB), prophospholipase A2, and procolipase.

When a meal is ingested, the vagal nerves, vasoactive intestinal peptide (VIP), gastrin-releasing peptide (GRP), secretin, cholecystokinin (CCK) and enkephalins stimulate an enzymatic release into the pancreatic duct. The proenzymes move to the brush border of the duodenum, where they are activated by cleavage of a lysine- or arginine-containing peptide bond in the N-terminus of the protein. The initial conversion of trypsinogen to trypsin promotes and further catalyzes the conversion of the other pro-enzymes to active form. Elevated levels of trypsin decrease CCK and secretin levels, thus limiting additional pancreatic secretion.

Premature activation of pancreatic enzymes within the pancreas may cause organ injury and, as such, several mechanisms exist to limit this occurrence. First, proteins are secreted in form of inactive proenzymes. Second, post-translational modification in the Golgi apparatus allows their segregation into the unique subcellular zymogen compartments where the proenzymes are packaged in a paracrystalline arrangement together with protease inhibitors. Third, zymogen granules have an acidic pH and a low calcium concentration, which prevent their premature activation. Under various conditions, these protective mechanisms might be disrupted, resulting in intracellular enzyme activation.

The observation that acute pancreatitis is an “auto-digestive” phenomenon due to inappropriate activation of digestive enzymes within the pancreatic tissue is as old as a century, when the Czech pathologist Hans Chiari first proposed that pancreatitis represents a disease in which “the organ succumbs to its own digestive properties”. A vast number of clinical studies have confirmed Chiari’s original hypothesis, and it is now acknowledged that premature trypsin activation, mediated by cathepsin B, takes place in membrane-bound compartments of the pancreas. The newly generated trypsin further boosts the process by activation of other digestive enzymes in a similar way as occurs in the duodenum, where the process is catalyzed by the enzyme enterokinase.

It is acknowledged that the intra-pancreatic activation of proenzymes may occur when factors involved in maintaining cellular homeostasis are imbalanced. Upon initiation of cellular injury, cellular membrane trafficking is impaired, leading to a variety of detrimental consequences: (i) lysosomal and zymogen granule compartments fuse, allowing the activation of trypsinogen to trypsin; (ii) intracellular trypsin triggers the entire zymogen activation cascade; and (iii) secretory vesicles

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are extruded across the basolateral membrane into the interstitium, where molecular fragments act as chemoattractants for inflammatory cells.

Activated neutrophils further amplify the damage by releasing superoxide or additional proteolytic enzymes such as collagenase, elastase, cathepsins B, D and G. Macrophages also contribute to this injury, by releasing cytokines such as (tumor) necrosis factor-alpha, interleukin-6, and interleukin-8, that further trigger a local (and, in severe cases, systemic) inflammatory response\(^{6,11,12}\). These cytokines mainly induce increased pancreatic vascular permeability, which leads to hemorrhage, edema and pancreatic necrosis.

Upon permeation of these mediators into the circulation, systemic complications develop, including bactemia due to gut flora translocation, acute respiratory distress syndrome, pleural effusions, gastrointestinal hemorrhage, as well as renal failure. A systemic inflammatory response syndrome might also occur, which is a predisposing condition for the onset of systemic shock\(^{6,11,12}\).

Although there are many distinct etiologies for acute pancreatitis, the specific mechanism remains unclear (Figure 1). A variety of factors (e.g., endotoxins, toxins, ischemia, infections, and anoxia) may trigger the activation of proenzymes. This noninfectious damage to the pancreatic parenchyma rapidly causes a local inflammatory reaction that further contributes to the vascular dilatation, permeability and edema. Acute pancreatitis is distinguished from other intra-abdominal diseases by its predisposition to cause remote systemic effects\(^{13}\), and this probably represents an extension of the localized process into a generalized systemic inflammatory response\(^{10,14-16}\).

Alcohol exposure and biliary tract disease are the leading causes of acute pancreatitis. However, in 10–30% of cases the cause remains unknown, although some studies have suggested that up to 70% of cases of idiopathic pancreatitis might be secondary to biliary microlithiasis\(^{17}\).

Biliary stone disease is the most common cause of acute pancreatitis in developed countries, occurring in up to 40% of cases, with the transit of gallstones into the bile duct and their temporarily lodging at the sphincter of Oddi the underlying mechanism. The risk of a stone causing pancreatitis is inversely proportional to its size, and occult microlithiasis is probably responsible for most cases of idiopathic acute pancreatitis as already explained\(^{17-19}\).

Alcohol is the second leading cause of acute pancreatitis, occurring in ~35% of cases. The disease frequently occurs in patients with habitual alcohol ingestion for over 5–15 years, but occasionally develops in patients with a weekend “binging” habit. It is, however, unknown as to whether certain individuals are more predisposed for developing acute pancreatitis than others who ingest similar quantities of alcohol\(^{6}\).

Post-endoscopic retrograde cholangiopancreatography (ERCP) is the third leading cause (~4% of cases), ahead of drugs (~2%) and abdominal trauma (~1.5%). No medications, with the exception of aggressive pre-intervention intravenous hydration, have been proven to prevent post-ERCP pancreatitis in randomized studies\(^{6}\).

Although several drugs have been associated with the onset of acute pancreatitis (e.g., azathioprine, sulfonamides, sulindac, tetracycline, valproic acid, didanosine, methyldopa, estrogen, furosemide, 6-mercaptopurine, pentamidine, 5-aminosalicylic acid compounds, corticosteroids and others), the small number of patients affected by drug-induced pancreatitis as compared with the relatively large number who receive potentially toxic drugs makes this form of pancreatitis a relatively rare occurrence, probably attributable to genetic predisposition\(^{6,10}\).

Genetic mutations contribute to a wide range of pancreatic disorders and six pancreas-targeting factors have recently been associated with susceptibility to acute (and/or chronic) pancreatitis. These include cationic trypsinogen (PRSS1), anionic trypsinogen (PRSS2), serine protease inhibitor Kazal 1 (SPINK1), cystic fibrosis transmembrane conductance regulator (CFTR), chymotrypsinogen C (CTRC) and calcium-sensing receptor (CASR)\(^{20}\).

Pancreatic injury occurs more frequently in penetrating injuries (e.g., from knives or bullets) than in blunt abdominal trauma (e.g., from steering wheels, horses, bicycles).\(^{6,10}\) Infection, hereditary enzymatic defects, hypercalcemia, developmental abnormalities of the pancreas, hypertriglyceridemia, tumors, toxins, autoimmunity, vascular abnormalities, each account for <1% of acute pancreatitis\(^{6,10}\).

**Signs and symptoms of acute pancreatitis**

The major symptom of acute pancreatitis is pain in the epigastrium or left upper quadrant, which is most commonly described as a constant, tedious ache which often radiates to the back, as well as to the flanks, chest, or lower abdomen. Although the intensity is usually described as severe, it can be highly variable and does not reflect the severity of disease. Colicky discomfort is atypical and suggests a different etiology. Nausea and vomiting are common, as well as abdominal bloating from gastric and intestinal hypomotility\(^{6,10}\).

The physical examination findings can vary with the severity of the disease. Fever (76%) and tachycardia (65%) are common abnormal vital signs. Abdominal tenderness, muscular guarding (68%) and distension (65%) can also be observed, with a minority of patients manifesting jaundice (28%)\(^{10,12}\). Some patients also experience dyspnea (10%), which may be caused by diaphragm irritation (due to underlying inflammation), pleural effusion, or a more serious condition, such as adult respiratory distress syndrome (ARDS).

In severe cases, hemodynamic instability is manifest (10%), and hematemesis or melena can occur (5%)\(^{10,12,19}\).
Patients with severe form are also frequently pale, diaphoretic, and listless. Additional physical findings such as the Cullen sign (i.e., a bluish discoloration around the umbilicus resulting from hemoperitoneum) and the Grey-Turner sign (i.e., a reddish-brown discoloration along the flanks resulting from retroperitoneal blood dissecting along tissue planes) may appear in patients with severe necrotizing pancreatitis. The onset of a ruddy erythema in the flanks secondary to extravasated pancreatic exudates and focal subcutaneous fat necrosis frequently accompanies the most severe forms, especially acute hemorrhagic pancreatic necrosis.

Overall, up to 20% of patients with acute pancreatitis have a severe course, and 10 to 30% of those with severe acute pancreatitis experience a fatal outcome. Despite improvements in intensive care treatment during the past few decades, the rate of death has not significantly declined. The local complications include phlegmons, abscesses, or pseudocysts, usually occurring in the first 2 to 3 weeks after the onset of pancreatic injury. Systemic complications include pulmonary, cardiovascular, renal, hematologic, central nervous system, as well as metabolic abnormalities.

The identification of patients at risk of acute pancreatitis is challenging. In 1994, Ranson suggested multiple diagnostic criteria to predict patient outcome on admission, including age >55 years, blood glucose >200 mg/dL (11.1 mmol/L), white blood cell count (WBC) >16×10⁹/L; serum aspartate aminotransferase (AST) >250 U/L; and lactate dehydrogenase (LDH) >700 U/L. The number of factors is then averaged and used to predict mortality. Other scoring systems have been also used such as the Acute Physiology and Chronic Health Evaluation II (APACHE II) score, but complexity and poor sensitivity on initial presentation limit their role in the ED setting.

**Diagnostic imaging**

Imaging provides a significant contribution to the diagnosis as well as to the assessment of disease severity in patients with acute pancreatitis. The radiological imaging workup of patients with acute abdominal pain traditionally begins with a plain abdominal film, which may, however, be unreliable in this setting. Frequent radiological findings such as sentinel loop, colon cut-off sign and obscuration of the psoas margin are non-specific, and, as such, the plain abdominal film is not recommended for the diagnosis. A chest x-ray is commonly performed along with an abdominal x-ray series and might be of value for detecting extra-glandular complications such as pleural effusion and ARDS.

Abdominal ultrasonography (US) is generally acknowledged as an imaging tool of limited utility for diagnosis and severity assessment of acute pancreatitis, due to its poor accuracy in obese patients and in the presence of intestinal gas. This technique is, however, commonplace in the ED because of its low cost, wide availability, and suitability for bedside assessment. US is valuable for visualizing the gallbladder, as well as dilatation of the common bile duct, and is considered the gold standard for detection of gallstones, due to the high sensitivity and specificity (both >95%)..

In acute pancreatitis, US can reveal a diffuse enlargement of the pancreas with decrease of echogenicity as a result of edema, but a normal finding does not rule out the diagnosis. Peripancreatic fluid collections are identified as anechoic collections and when infection or hemorrhage are present, these collections may demonstrate internal echoes and gas bubbles. Ascites is an additional and frequent finding.

Contrast-enhanced computed tomography (CECT) is the standard imaging technique for evaluation of acute pancreatitis and its complications and both intravenous and oral contrast agent should be administered. Protocols vary, but the most critical issue is to obtain thin-section images during the peak of pancreatic arterial perfusion which can usually be achieved by imaging 30–40 s after the administration of iodinated contrast at 3–4 mL/s with multidetector CT.

**Figure 1.** Pathogenesis of acute pancreatitis.

**Figure 2.** Contrast-enhanced computed tomography of the abdomen in a case of acute pancreatitis. The pancreas appears enlarged (arrows) with fluid collection (asterisk).
Typical CECT findings include focal or diffuse enlargement of the pancreas, heterogeneous enhancement of the gland with irregular contour of the margins, increased density of peripancreatic fat planes with thickening of fascial planes, as well as the presence of intraperitoneal or retroperitoneal fluid collections (Figure 2). Fluid collections are most commonly observed in the peripancreatic and anterior pararenal spaces, although they can be extended to the pelvis. Necrosis of pancreatic tissue is the most reliable finding, and is recognized as lack of enhancement after intravenous contrast administration. Balthazar et al. has developed a grading system that correlates with severity of acute pancreatitis (Table 1)31. CECT should be performed 48 to 72 h after the onset of an acute attack. With this delay, the scan yields a higher accuracy in the delineation of necrotizing pancreatitis32.

Pancreatic necrosis is a major concern in staging acute pancreatitis. It is commonly classified into three types: (i) organized pancreatic necrosis, a hypodense collection characterized by encapsulation and inhomogeneous contents of necrotic fatty tissue and solid necrotic pancreatic; (ii) central gland necrosis, due to the involvement of the body and/or tail of the pancreas and resulting in disruption of the pancreatic duct and persistent collections; and (iii) extra-pancreatic necrosis, in which necrosis spread in the peripancreatic tissues in the absence of pancreatic necrosis32.

Complications such as pseudocysts, abscess, necrosis, venous thrombosis, pseudoaneurysms and hemorrhage, can all be detected with CECT. A pseudocyst appears as a round water density collection with a thin or thick wall, whereas a pancreatic abscess has a thick wall with gas bubbles inside. Of note, CECT findings of abscess are not specific, and a percutaneous fine-needle aspiration is generally necessary to confirm the presence of pus for the diagnosis.

Magnetic resonance imaging (MRI) is becoming a viable alternative in situations where CECT is contraindicated, such as in patients with contrast allergy. The morphologic changes of acute pancreatitis with MRI are similar to those of the CECT33. The pancreas may be enlarged focally, or diffusely with peripancreatic inflammation appearing as strands of low signal intensity in the surrounding fat. Hemorrhage is characterized by high signal intensity on T1 sequences with fat suppression. Peripancreatic fluid collections, pseudocysts, and abscesses are easily recognized by their high signal intensity on T2 sequences (Figure 3). Necrotic areas of the pancreas fail to enhance on contrast-enhanced images. MRI is better than CECT in distinguishing the fluid part of the pseudocysts from the solid part of sterile pancreatic tissue, thereby providing more useful information for surgery34. An MRI cholangiography can also be performed, in order to assess the viability of the biliary tree and the presence of stones in the common bile duct35.

### Current diagnostic biomarkers of acute pancreatitis

The diagnosis of acute pancreatitis is substantially based on a combination of clinical signs and symptoms, imaging techniques and laboratory investigations, with the assessment pancreatic enzymes the cornerstone of laboratory diagnosis in this particular clinical setting36.

#### Total and pancreatic amylase

Amylase is a glycoside hydrolase primarily produced by the pancreas and the salivary glands, although it can be found in other tissues to a much lower extent. There are three specific amylase isoenzymes, which are designated by different Greek letters (α, β and γ). The α-amylase (chromosome 1p21), also known as 1,4-α-D-glucanohydrolase or glycogenase (EC 3.2.1.1), is the only isoenzyme present in humans. The pancreas and the salivary glands synthesize specific isofoms, with different mobility on isoelectric focusing, and that can also be distinguished and quantified by specific monoclonal antibodies. The leading function of α-amylase is the digestion of starch, glycogen and related poly- and oligosaccharides, by hydrolysis of α-1,4-glycosidic bonds37. After the onset of acute pancreatitis, serum amylase increases rapidly in blood (from 3 to 6 h), exhibits a half-life of 10–12 h, persists elevated for 3-5 days and is finally excreted by the kidneys38.

![Figure 3. Ultrasonography of the abdomen in a case of acute pancreatitis (same case). The sagittal ultrasonography of the epigastrium shows the enlarged pancreas (P) and the fluid collection (asterisks).](image)
The reference interval for serum total amylase varies according to age and gender, but is typically between 20–300 U/L. Following production and certification of an enzyme reference material, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) developed a reference method, which is conventionally called ethyliedine protected substrate (EPS) and is based on nitrophenylmaltoheptaoside (as substrate) and glucosidase (as enzyme) to liberate 4-nitrophenolate. The terminal glucose is blocked by an ethyliedine bridge to prevent glucosidase degradation at the non-reducing end of the substrate. The calibration of catalytic activity is based on an empirically determined, but generally accepted, molar absorption coefficient. Specific techniques for assessment of pancreatic amylase also exist, and those commercially available are mostly based on the inhibition of the activity of the salivary isoform by monoclonal antibodies, which do not interfere with the activity of the pancreatic isoform. The residual (pancreatic) amylase activity is then specifically assessed with the IFCC technique.

Macroamylasemia is a relative rare condition whose prevalence is typically between 0.1 and 0.2% in the general population, but may increase up to 10% in patients with hyperamylasemia. It is typically characterized by a modest increase of serum amylase activity in association with a reduced urine output of the enzyme. The increased amylase levels are due to a complex formation between amylase and immunoglobulins (usually IgA) or hydroxylethyl starch, which decrease the renal clearance and prolong the presence in circulation of the enzyme. Although this condition has no meaningful clinical implications, its identification is, however, important because macroamylasemia might interfere with the interpretation of test results.

Lipase Lipases are involved in a variety of biological processes, from routine metabolism of dietary triglycerides to cell signaling. Human lipase is primarily synthesized in the pancreas and released into the digestive tract, where it exerts a pivotal role for digestion of fats by acting on specific positions on the glycerol backbone of the lipid substrate. The main isoforms of lipase in humans include pancreatic lipase (also known as HPL) and pancreatic lipase related protein 2 (PLRP2), which are both synthesized and released by the pancreas. Additional human isoforms include hepatic lipase (HL), endothelial lipase, and lipoprotein lipase (LPL).

The enzyme pancreatic lipase (EC 3.1.1.3), also known as pancreatic triacylglycerol lipase, is encoded by the PNLIP gene, mapped at 10q25.3. At present, there is no reference method for pancreatic lipase and the current routine techniques include turbidimetry, reflectometry, spectrophotometry. The IFCC Committee is, however, working on a concept for the development of the reference assay (a colorimetric method using 1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6'-methylresorufin) ester or DGGR). Two reference materials have been identified (i.e., BCR 693 and BCR 694), to be used for verifying the accurate implementation of the standardized measurement procedure, to assign values to secondary lipase materials and thereby ensure traceability to the standardized procedure and harmonization of laboratory results.

The serum concentration of lipase increases 3–6 h after the onset of acute pancreatitis, peaks within 24 h, but can persist increased above the upper limit of the reference interval (URL) for 1–2 weeks. At variance with amylase, lipase is reabsorbed by the kidney tubules and, thereby, remains in blood for longer and at higher concentration. Similar to amylase, the reference interval varies according to patient and test factors, but is typically <200 U/L.

Increased concentrations of serum lipase can occasionally be observed in conditions other than acute pancreatitis, including other pancreatic diseases (e.g., ERCP, surgery, trauma, acute cholecystitis, appendicitis, diabetic ketoacidosis), inflammatory bowel disease, intestinal ischemia, obstruction or infarction, malignancies (especially cancers of duodenum, esophagus, gastroesophageal junction, liver, small bowel, stomach and tongue), fat embolism, esophagitis, liver and renal failure, as well as analytical interference due to hypertriglyceridemia or with the presence of macroforms (e.g., benign hyperlipasemia).

Trypsinogen Trypsinogen is the zymogen of the pancreatic enzyme trypsin (EC 3.4.21.4), which undergoes activation by an enteropeptidase (i.e., enterokinase) to produce the final enzyme trypsin. This human protein is encoded by three different genes (i.e., PRSS1, PRSS2, and PRSS3), which are transcribed to produced trypsinogen-1 (also known as “cationic” trypsinogen), -2 ("anionic" trypsinogen) and -3 ("mesotrypsinogen"), and display 90% sequence identity. The main function of the active isoenzymes is to cleave the peptide bond on the carboxyl side of basic amino acids.

Trypsinogen is physiologically secreted into the pancreatic fluid by the acinar cells, so that a small amount of the two main isoenzymes (i.e., trypsinogen-1 and
trypsinogen-2) enter the circulation and are subsequently excreted in the urine under normal conditions. During acute pancreatitis, however, the activation of trypsinogen occurs early and is also accompanied by a remarkable increase of leakage into the bloodstream and consequent increased urine clearance. The serum and urinary concentrations of these biomarkers increase significantly a few hours after the onset of disease and return to baseline levels in 3 to 5 days.

Specific assays for trypsinogen-1 and trypsinogen-2 have been developed. Early data showed that qualitative rapid urine trypsinogen-2 test strip might be a reliable and useful approach for the screening of acute pancreatitis, especially in healthcare settings lacking laboratory facilities. However, recent studies demonstrate that the high specificity is not accompanied by an acceptable sensitivity and has thus hampered its implementation in routine clinical practice.

**Additional biochemical alterations**

In addition to increased concentrations of the most widely used pancreatic enzymes and proteins (i.e., lipase, amylase and other markers discussed in another section of this article), additional laboratory abnormalities that are frequently encountered in patients with acute pancreatitis include hyperglycemia, hypocalcemia, leukocytosis, anemia, as well as a modest increase of bilirubin and liver enzyme test results (e.g., a rise in serum alanine aminotransferase above 80 U/L can be considered highly specific, but poorly sensitive for gallstone pancreatitis).

**Evidenced-based guidelines and recommendations**

To identify appropriate guidelines and recommendations on laboratory testing for the diagnosis of acute pancreatitis, we searched the MeSH Browser in PubMed, Thomson, Google Scholar and Scopus from the year 2000 to 2011, with the following terms: “acute pancreatitis” AND “diagnosis” AND “guidelines” OR “recommendations”, and with no language restriction. Overall, 9 out of the 172 documents retrieved met the criteria for inclusion, in that the documents were: (a) officially released by national or international organizations or scientific societies, (b) containing recommendations on laboratory testing for the diagnosis of acute pancreatitis.

In 2001, the French Society of Gastroenterology released a document containing consensus recommendations, where it was concluded that lipase assessment should be preferred over total and pancreatic amylase for the diagnosis of acute pancreatitis, with a diagnostic threshold set at 3 times the URL. The assessment of type 2 trypsinogen might also be considered in the ED for the rule out of acute pancreatitis due to its high negative predictive value.

To provide a support to manage acute pancreatitis, the Japanese Society of Abdominal Emergency Medicine developed evidence-based guidelines in 2002. Among the main points of this document, the evidence that lipase is superior to amylase in the diagnosis of acute pancreatitis received a grade A recommendation. Additional emphasis was placed on the fact that neither amylase or lipase are useful for stratifying the severity of disease (grade D recommendation), whereas serum C-reactive protein (CRP) at 48 h after the onset of disease might be useful in stratification of severity of acute pancreatitis (grade A).

In the same year, the Working Group on Enzymes of the Italian Society of Clinical Biochemistry and Clinical Molecular Biology (SIBioC) published recommendations for the routine use of pancreatic amylase measurement instead of total amylase for the diagnosis and monitoring of pancreatic pathology, since the former measurement was considered to be more sensitive and specific for detecting pancreatic tissue injury, easier and faster to carry out in emergency conditions, more accurate for the intended clinical use, more suited for inter-laboratory comparison and characterized by well-defined diagnostic thresholds.

The most recent guidelines issued by a Working Party of the British Society of Gastroenterology, Association of Surgeons of Great Britain and Ireland, Pancreatic Society of Great Britain and Ireland, Association of Upper GI Surgeons of Great Britain and Ireland, establish that lipase estimation is to be preferred over amylase for the diagnosis of acute pancreatitis, although the latter enzyme provides acceptable accuracy of diagnosis (recommendation grade A). It is also clearly stated that diagnosis should not be based on arbitrary limits of values 3 or 4 times greater than the URL, but the increase should be cautiously interpreted according to the time since the onset of symptoms. The preference of lipase over amylase has been justified by the globally better diagnostic performance, which is attributable to the greater specificity (i.e., lipase is only produced in the pancreas), as well as the longer half-life of the former enzyme.

In 2006, the Practice Parameters Committee of the American College of Gastroenterology released Practice Guidelines in Acute Pancreatitis, concluding that it is unnecessary to assess both serum amylase and lipase for the diagnosis of acute pancreatitis, but serum lipase is advisable because it is more sensitive and specific than the total amylase, and remains below the URL in various non-pancreatic conditions that cause a significant elevation of serum amylase (e.g., macroamylasemia, parotitis, some carcinomas). However, a uniform threshold was not reported for both enzymes, as this may vary from ≥2 times to ≥4 times the URL. It was also endorsed that the degree of increase of both enzymes should not be used for assessing the severity of disease and the daily measurement after the diagnosis is of very limited value for follow-up and prognosis.

The official recommendations of the American Gastroenterological Association report that the diagnosis of acute pancreatitis should be established within 48 h of admission and be based on elevations in amylase or
lipase levels greater than 3 times the URL in the absence of impaired renal function. The increase of lipase concentration is also considered more specific and hence preferable.

In 2007, the American Academy of Family Physicians defined the best practices for diagnosing and treating acute pancreatitis, highlighting that the initial laboratory evaluation should include amylase and lipase levels, complete blood count with differential, a metabolic panel (blood urea nitrogen, creatinine, glucose, and calcium levels), triglyceride level, urinalysis and arterial blood gases. For diagnosis, plasma lipase is considered more sensitive and specific than amylase (a specific threshold has not been set), and serial testing is not recommended. Additional investigational biomarkers may include trypsinogen activation peptide (TAP), CRP, procalcitonin, phospholipase A2, and the cytokines interleukin-6 and interleukin-8.

In 2010, the Japanese Ministry of Health, Labour, and Welfare released diagnostic criteria for acute pancreatitis, which report that the measurement of blood lipase is recommended because it is superior to all other pancreatic enzymes in terms of diagnostic accuracy (Grade A recommendation), although the cut-off level could not be set, due to lack of sufficient evidence and consensus. It was also endorsed that pancreatic amylase should be assessed when the measurement of blood lipase is unavailable (Recommendation A).

A comprehensive analysis of the available scientific literature revealed that the sensitivity and specificity of lipase were 82–100%, whereas those of total amylase were between 67–100% and 85–98%, respectively. Interestingly, the usefulness of pancreatic amylase for diagnosing acute pancreatitis was uncertain compared with total amylase, as well as other pancreatic enzymes, with sensitivity and specificity values ranging from 67–100% and 83–98%, respectively. With regards to other pancreatic enzymes, excellent sensitivity (89–100%), but modest specificity (79–83%) were reported for trypsin, as well as for elastase 1 (sensitivity 97–100% and specificity 79–96%).

The main reasons supporting lipase over both types of amylase for the diagnosis of acute pancreatitis included the higher sensitivity (e.g., amylase concentrations might be normal in alcohol-induced acute pancreatitis), the higher specificity (several conditions cause hyperamylasemia other than acute pancreatitis) and the larger diagnostic window (e.g., amylase is characterized by a faster return to values below the URL as compared with lipase). Similar conclusions were reached by the Working Group of the Italian Association for the Study of the Pancreas on Acute Pancreatitis, which endorsed that although amylase is widely available and provides acceptable degree of accuracy, when serum lipase is available, it should be preferred for the diagnosis of acute pancreatitis (recommendation A).

In order to assess the guidelines in modifying the clinical and laboratory practice, the Japanese Society of Abdominal Emergency Medicine recently undertook a survey to assess the degree of adherence to the available recommendations. Interestingly, the number of respondents who used lipase to diagnose acute pancreatitis was roughly equivalent to that of those who used amylase (i.e., 47 vs. 41%), compared with 31 versus 52% before publication of the guidelines. After publication, the frequency of amylase testing alone also decreased significantly for assessing the severity of disease, while its use within severity scores increased. The similar frequency of lipase and amylase testing in clinical laboratories indicates that the implementation of guidelines and recommendations are still far from being optimal.

Taken together, the current guidelines and recommendations indicate that lipase should be preferred over total and pancreatic amylase for the initial diagnosis of acute pancreatitis and that the assessment of should not be repeated over time to monitor disease prognosis. Repeat testing should only be considered when the patient has signs and symptoms of persisting pancreatic or peripancreatic inflammation, blockage of the pancreatic duct, or development of a pseudocyst (Table 2).

Normal values of serum lipase are extremely rare in patients with acute pancreatitis, so the combined assessment with serum amylase, preferably pancreatic amylase, should be discouraged because it only marginally improves the diagnostic efficiency of either marker alone and increases the overall cost of laboratory investigation. Amylase could be assessed in selected patients with normal lipase values, high clinical suspicion of pancreatitis, but always integrated with appropriate imaging testing.

Although a definitive diagnostic threshold cannot be identified for either enzyme across the different documents, the American College of Gastroenterology and the American Gastroenterological Association have suggested cut-offs between ≥2 and ≥4 times the URL. The clinical interpretation of values less than 3 times the URL is challenging due to the early peak (especially for lipase), so that they should be interpreted in combination with the pretest probability and clinical assessment.

Useful information can also be gathered from critical reviews about lipase and amylase testing in acute pancreatitis. In 2007, Beauregard et al. performed a comprehensive search of the literature to assess which of the diagnostic tests was the best for early and accurate diagnosis of acute pancreatitis in the adult ED setting. Interestingly, only four studies met the inclusion criteria (two retrospective and two prospective; three published in the 1990s and one in 2005), i.e., a separate diagnostic criterion from amylase or lipase for at least a subgroup of patients, as well as sensitivity and specificity data for serum amylase and lipase compared with that standard diagnostic technique. The diagnostic performance of either test varied widely depending on several variables including the gold standard for the diagnosis.

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of pancreatitis, the diagnostic cut-off and the study population. Both enzymes were found to be increased in disorders other than pancreatitis, but the specificity of amylase was lower as it was found to be normal in some cases of pancreatitis. Taken together, the diagnostic accuracy of lipase is better for acute pancreatitis, although the integration of both tests may increase the diagnostic performance.

Huber and Schmid performed a review of recent literature and current guidelines and concluded that the diagnosis of acute pancreatitis can be made on the basis of history, physical examination and serum lipase alone, whereas blood glucose, urea nitrogen and hematocrit can be used for gathering prognostic information74. More recently, Chang and Chung carried out a retrospective study, and assessed serum elastase and total amylase in 3,451 patients who presented to the ED with acute abdominal pain. Both tests showed high accuracy (area under the curve [AUC] of 0.992 and 0.996 for total amylase and lipase, respectively). The sensitivity and specificity at values 3 times the URL were 64% and 99% for amylase and 95% and 99% for lipase75.

**Additional or emerging diagnostic biomarkers of acute pancreatitis**

Although a multitude of prognostic biomarkers (up to 196 including procalcitonin on admission, along with serum interleukin-6, interleukin-8, polymorphonuclear elastase and serum CRP) for assessing the outcome of acute pancreatitis have been proposed over the past decades69,70, the search for diagnostic tests has been less productive. Some interesting biomarkers have been suggested, but none has found widespread application in the diagnosis of acute pancreatitis for a variety of reasons, including overall inferior diagnostic accuracy as compared with lipase, cumbersome technique/s required for their assessment which would make their measurement unsuitable for most clinical laboratories and for rapid diagnosis, or the recent discovery which does not allow to attribute a definitive role in the diagnostic reasoning (Table 3).

Table 2. Synthesis of available guidelines and recommendations for laboratory testing in the diagnosis of acute pancreatitis.

<table>
<thead>
<tr>
<th>Organization/s</th>
<th>Preferred biomarker</th>
<th>Diagnostic threshold</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Société Nationale Française de Gastro-Entérologie</td>
<td>Lipase</td>
<td>≥ 3 times the URL</td>
<td>[56]</td>
</tr>
<tr>
<td>Japanese Society of Emergency Abdominal Medicine</td>
<td>Lipase</td>
<td>Not set</td>
<td>[57]</td>
</tr>
<tr>
<td>British Society of Gastroenterology; Association of Surgeons of Great Britain and Ireland, Pancreatic Society of Great Britain and Ireland</td>
<td>Lipase</td>
<td>Value interpreted according to the time since the onset of symptoms</td>
<td>[59]</td>
</tr>
<tr>
<td>American of Gastroenterology</td>
<td>Lipase</td>
<td>≥ 2 to ≥ 4 times the URL</td>
<td>[10]</td>
</tr>
<tr>
<td>American Gastroenterological Association</td>
<td>Lipase</td>
<td>≥ 3 times the URL</td>
<td>[60]</td>
</tr>
<tr>
<td>American of Family Physicians</td>
<td>Lipase</td>
<td>Not set</td>
<td>[61]</td>
</tr>
<tr>
<td>Working Group of the Italian Association for the Study of the Pancreas</td>
<td>Lipase</td>
<td>Not set</td>
<td>[63]</td>
</tr>
</tbody>
</table>

URL, upper limit of the reference interval.

Table 3. Laboratory biomarkers for the diagnosis of acute pancreatitis.

- Serum lipase
- Serum total and pancreatic amylase
- Serum and urinary trypsinogens (trypsinogen-1, trypsinogen-2 and trypsinogen-3)
- Phospholipase A2
- Pancreatic elastase
- Urine trypsinogen activated protein (TAP)
- Carboxypeptidase B (CAPB)
- Activation peptide of carboxypeptidase B (CAPAP)
- The trypsin-2-alpha1 antitrypsin complex (trypsin-2-AAT)
- Circulating (cell-free) DNA

**Phospholipase A2**

Phospholipase A2 is a key enzyme in the metabolism of arachidonic acid, since it catalyzes the generation of prostaglandins and leukotrienes. It exists in three major isoforms, namely phospholipase A2 group I (pancreatic phospholipase A2), phospholipase A2 group II (non-pancreatic, synovial, or platelet phospholipase A2), and phospholipase A2 group III (cytosolic phospholipase A2)76. The initial enthusiasm from earlier studies about the potential diagnostic usefulness of pancreatic phospholipase A2 in acute pancreatitis has gradually faded as the pathogenetic mechanisms of acute pancreatitis have been revealed77, and the role of traditional or innovative biomarkers characterized by better diagnostic accuracy has been demonstrated78. As such, the potential advantage (if any) provided by phospholipase A2 assessment does not counterbalance its inherent technical limitations (e.g., major cost, cumbersome technique, methods unavailable on automated and high throughout laboratory instrumentation)74.

**Pancreatic elastase**

Flamion et al. assessed serum elastase-1, as well as amylase, lipase, and trypsin-like immunoreactivity in patients with acute pancreatitis79, reporting that the concentration of all biomarkers were elevated within 24 h from the onset of symptoms. Although the diagnostic sensitivity was similar, the assessment of serum elastase did not improve the diagnostic accuracy, and the measurement
of more conventional enzymes outweighed elastase for the faster, easier and less expensive techniques. Further studies demonstrated that serum elastase remains elevated for up to one week after the onset of acute pancreatitis, so that its clinical application might only be limited to those cases with delayed admission. Nevertheless, a serum assay is not widely available and therefore cannot be routinely used.

**Procalcitonin**

Several studies have assessed the diagnostic utility of serum procalcitonin for predicting the development of severe acute pancreatitis and infected pancreatic necrosis, especially at patient admission in the ED. Mofidi *et al.* performed a systematic review of the literature and reported AUCs of 0.87 (72% sensitivity and 86% specificity) for predicting the development of severe acute pancreatitis and 0.91 (80% sensitivity and 91% specificity) for predicting the development of infected pancreatic necrosis. The main diagnostic role of this biomarker would be the identification of patients with mild form of pancreatitis, who are less likely to benefit from more aggressive and antibiotic therapy. However, to the best of our knowledge, no reliable clinical study has assessed the diagnostic performance of procalcitonin in the early assessment of acute pancreatitis.

**Trypsinogen activated protein**

TAP, which is released upon conversion of trypsinogen into trypsin, is the most studied activation peptide in acute pancreatitis. An increase in TAP concentration is typically observed 6 to 12 h after onset of symptoms and represents a useful index for predicting severe acute pancreatitis.

Neoptoloemos *et al.* studied the concentration of TAP in 172 patients with acute pancreatitis (35 with severe disease) and 74 controls, and found that its concentration was significantly higher in patients with severe (37 nmol/L; interquartile range 17–110 nmol/L) and mild (15 nmol/L; interquartile range 5–35 nmol/L) acute pancreatitis, as compared with controls (6 nmol/L; interquartile range 3–18 nmol/L; both p < 0.001) after the onset of symptoms (p < 0.001). Nevertheless, the AUC for the predicting acute pancreatitis 24 h after symptoms onset was only marginally significant (0.69; 95% CI 0.51 to 0.88).

Lempinen *et al.* found a significant correlation between disease severity and TAP concentration in both plasma and urine during the early phase of acute pancreatitis, although the assessment of this biomarker did not allow to reliably distinguish controls from patients with mild pancreatitis.

Pezzilli *et al.* measured serum TAP in 34 patients with acute pancreatitis (22 mild, 12 severe) and 12 patients with non-pancreatic acute abdominal pain. The sensitivity and specificity of serum TAP were 23% and 92%, respectively. These findings led the authors to conclude that TAP is of limited value in assessing acute pancreatic damage.

In a further study Sáez *et al.* investigated whether the concentration of urinary TAP could help in the diagnosis of acute pancreatitis and found that the sensitivity and specificity of this urinary biomarker were only 69% and 40%, with poor positive and negative predictive values (73% and 35%, respectively).

Due to its low diagnostic accuracy as compared with either lipase or amylase, along with the limited availability of validated assays on the market especially on automated laboratory instrumentation, the interest around this biomarker has gradually faded and it has now almost disappeared.

**Activation peptide of carboxypeptidase B**

Carboxypeptidase B (CAPB) is an exopeptidase synthesized as an inactive proenzyme proCAPB by acinar cells along with other pancreatic enzymes. The mechanism leading to activation of proCAPB involves the tryptic release of the 1-81, 9.4 kDa N-terminal amino acid peptide, known as activation peptide of carboxypeptidase B (CAPAP). The large size of this peptide and the ability to obtain high-affinity antibodies gave support to several studies which investigated its prognostic role in acute pancreatitis; two of them also assessed its diagnostic accuracy.

Müller *et al.* investigated the serum concentration of CAPAP in patients with acute pancreatitis and acute abdominal pain of non-pancreatic origin, and found that CAPAP concentrations were elevated in patients with acute pancreatitis, although the diagnostic accuracy (81% sensitivity, 83% specificity 0.850 AUC) was lower than that of proCAPB (95% sensitivity and specificity, 0.990 AUC) and amylase (90% sensitivity, 87% specificity, 0.902 AUC) due to the low levels observed in several patients with edematous pancreatitis.

Sáez *et al.* assessed the usefulness of serum and urine concentrations of CAPAP in the diagnosis of acute pancreatitis and found that this urinary biomarker was more reliable than TAP and urinary tripsinogen 2 for the diagnosis of acute pancreatitis, being characterized by 67% sensitivity and 95% specificity (positive and negative predictive values of 97% and 57%, respectively), with a 14.6 positive likelihood ratio for a cut-off value of 2.3 nmol/L. The clinical value of this biomarker for the early diagnosis of acute pancreatitis is, however, still unclear and its measurement is absent in all diagnostic guidelines.

**Trypsin-2-alpha1 antitrypsin complex**

Trypsin-2-alpha1 antitrypsin complex (trypsin-2-AAT) increases starts within hours after the onset of acute pancreatitis, remains elevated for longer than amylase and the magnitude of increase correlates with the severity of disease. Only a few studies have assessed the usefulness of this biomarker in the diagnosis of acute pancreatitis.

Hedström *et al.* showed that trypsin-2-AAT measurement on admission allowed the differentiation of patients
with acute pancreatitis from controls with high accuracy (AUC 0.948), although its diagnostic performance was comparable to that of trypsinogen-2 (AUC 0.960), lipase (AUC 0.947) and amylase (AUC 0.930). For discrimination between severe and mild acute pancreatitis, trypsin-2-AAT exhibited slightly better performance than trypsinogen-2 (AUC 0.948 versus 0.792)\textsuperscript{84}.

Andersén et al. studied 67 consecutive patients with acute pancreatitis and showed that this biomarker displayed better diagnostic accuracy (AUC 0.838) for discriminating biliary and alcohol-induced acute pancreatitis than trypsinogen-1 (AUC 0.600), trypsinogen-1 (AUC 0.682), amylase (AUC 0.748) and lipase (AUC 0.623)\textsuperscript{85}.

Lempinen et al. argued that the kinetics of trypsin-2-AAT might be unsuitable for the early diagnosis of acute pancreatitis, since they found that its concentration increased much more slowly than that of other conventional biomarkers (i.e., the peak is only reached 24 h after ERCP-induced pancreatitis)\textsuperscript{86}.

**Circulating (cell-free) DNA**

The assessment of circulating (cell-free) DNA in serum or plasma has been proven as a reliable diagnostic tool in a variety of clinical conditions. Gornik et al. investigated whether the necrosis of pancreatic tissue might be associated with an increase of circulating (cell-free) DNA in patients with acute pancreatitis\textsuperscript{87}, and observed that circulating DNA levels were non-significantly higher than those found in a control group, except when patients who developed severe disease were considered separately (AUC 0.97 for prediction of severe acute pancreatitis).

**Trypsinogen-3**

Trypsin-3, the minor trypsin isoenzyme present in the pancreatic fluid (4 to 10% of total trypsin activity), differs from its counterparts -1 and -2 being more resistant to the effect of inhibitors such as pancreatic secretory trypsin inhibitor (PSTI) or α1-protease inhibitor and has a different substrate specificity\textsuperscript{88}. Oiva et al. recently produced monoclonal antibodies to be incorporated within a sandwich-type immunoassay.

Serum trypsinogen-3 concentrations were assessed in 82 patients with acute pancreatitis and 63 patients with upper abdominal pain who served as controls (URL 4.4 μg/L)\textsuperscript{89}. Trypsinogen-3 concentrations were found to be markedly increased in patients with mild, as well as severe acute pancreatitis (median values of 9.5 and 15.0 μg/L, respectively), as compared with controls (median <1.0 μg/L). The overall AUC for diagnosing all cases of acute pancreatitis was 0.90 (0.88 and 0.93 in mild and severe pancreatitis, respectively). Interestingly, no significant association was observed between serum trypsinogen-3 and amylase in patients with acute pancreatitis, suggesting that the increase of trypsinogen-3 would reflect a different pathological mechanism than amylase.

**Proteomics**

In analogy with pancreatic cancer and chronic pancreatitis\textsuperscript{90,91}, proteomics might be a valuable resource for studying the basic mechanisms of disease and identifying novel and potentially useful diagnostic biomarkers in acute pancreatitis. Lassout et al. carried out a peptidomic analysis of low molecular weight proteome in rat pancreatic tissue extracts in the course of acute pancreatitis by centrifugal ultrafiltration, isoelectric focusing and liquid chromatography (LC) – mass spectrometry (MS)/MS analysis without prior enzymatic digestion\textsuperscript{92}. The results showed a substantial modification – most frequently an increase – of several inflammatory and stress proteins. Significant variations were also identified for two functional proteins, i.e., murinoglobulin-1 and CAPB.

Similar results were obtained by Fétaud et al., who studied a comparative proteomic analysis of pancreatic tissue extracts from rats with acute pancreatitis and healthy rodent controls by two-dimensional electrophoresis (2-DE) and MS/MS\textsuperscript{93}. The comparative analysis of diseased and healthy animals allowed to identify 42 proteins or peptides differentially expressed, some of which – newly described in the course of acute pancreatitis – belong to the family of cellular metabolism enzymes (e.g., ATP synthase β subunit), digestive proteases (e.g., leukocyte elastase inhibitor A, carboxypeptidase A1, A2 and B), inflammatory biomarkers (e.g., CRP, α1-macroglobulin, complement C3, α2-macroglobulin), apoptotic (e.g., 14-3-3 Proteins ε, θ and ξ), oxidative (e.g., peroxiredoxin-3) as well as cell stress proteins (e.g., pancreatitis-associated protein 2 and lithostathine, endoplasmin).

Chen et al. quantitatively compared the protein compositions of pancreatic rough endoplasmic reticulum between normal and acute pancreatitis male Wistar rats using isobaric tags (iTRAQ) and 2-DE LC – matrix-assisted laser desorption/ionization (MALDI) – MS/MS, and detected 469 unique proteins belonging to a large number of functional categories including ribosomal proteins, translocon subunits, chaperones, secretory proteins, and glyco- and lipid-processing enzymes\textsuperscript{94}. Interestingly, 37 of these proteins (i.e., 25 unique in arginine-induced pancreatitis, 6 unique in caerulein-induced pancreatitis, and 6 common in both models of acute pancreatitis) were significantly modified in animals with pancreatitis and the six most informative protein patterns were identified to be pancreatic triacylglycerol lipase precursor (decreased), Erp27 (decreased), prolyl 4-hydroxylase beta polypeptide (decreased) and fibrinogen alpha, beta and gamma chains (increased).

Finally, Yu et al. in two separate studies investigated the differentially expressed proteins in cerulein-treated pancreatic acinar cells; an in vitro rat model for acute pancreatitis\textsuperscript{95}. The protein patterns were further separated by 2-DE and identified by MALDI – time of flight (TOF)/MS analysis. Fifteen proteins (Orp150 protein,
Table 4. Novel putative biomarkers of acute pancreatitis identified in proteomic studies.

- 14-3-3 Proteins ε, θ and ξ
- 3-mercaptopyruvate sulforubranse, 78 kDa glucose-regulated protein precursor
- α1-macroglobulin
- α2-macroglobulin
- Adenosylhomocysteinase
- Aldehyde reductase 1
- ATP synthase β subunit
- Carboxypeptidase A1, A2 and B
- Complement C3
- dnaK-type molecular chaperone hsp72-ps1
- Endoplasmin
- Erp27
- Fibrinogen alpha, beta and gamma chains
- Heat shock protein 8, 27, 70 and 90
- Heterogeneous nuclear ribonucleoprotein H1
- Leukocyte elastase inhibitor A
- Lithostathine
- Mitochondrial ATP synthase subunit D
- Mitochondrial ATP synthase β chain precursor
- Mitochondrial glutamate dehydrogenase
- Orp150 protein
- Pancreatic triacylglycerol lipase precursor
- Pancreatitis-associated protein 2
- Peroxiredoxin-2 and -3
- Prolyl 4-hydroxylase beta polypeptide
- Protein disulfide isomerase related protein
- RuvB-like protein 1
- Similar to chaperonin containing TCP-1 β subunit
- Triosephosphate isomerase 1
- Tubulin β chain
- Vasolin-containing protein

protein disulfide isomerase related protein, dnaK-type molecular chaperone hsp72-ps1, mitochondrial glutamate dehydrogenase, similar to chaperonin containing TCP-1 β subunit, RuvB-like protein 1, heterogeneous nuclear ribonucleoprotein H1, aldehyde reductase 1, triosephosphate isomerase 1, peroxiredoxin 2, heat shock protein 90, mitochondrial ATP synthase β chain precursor, tubulin β chain, 3-mercaptopyruvate sulforubranse, mitochondrial ATP synthase subunit D) were found to be up-regulated in acute pancreatic rats, whereas four proteins (vasolin-containing protein, 78 kDa glucose-regulated protein precursor, heat shock protein 8, adenosylhomocysteinase) were found to be down-regulated.

Conclusions

Despite technological advances and continuous progress in our knowledge of the intricate biology and pathogenesis of acute pancreatitis, the laboratory diagnosis of this severe disease remains challenging. The gold standard for both clinicians and laboratory professionals remains diagnostic imaging, since a final diagnosis of acute pancreatitis cannot be made according to the result of a single blood test, no matter how accurate and predictive. Although the search for the “biochemical” Holy Grail of acute pancreatitis must continue, serum lipase can be considered the best available biomarker to date, whose measurement should be integrated within a process of clinical assessment and decision-making. The promising results of recent proteomic studies confirm that this innovative technique might allow the identification of changes characterizing pancreatic tissue injury, thus highlighting new potential biomarkers of acute pancreatitis (Table 4).

Declaration of interest

The authors stated that there are no conflicts of interest regarding the publication of this article.

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