CHAPTER 24

24. Laboratory Diagnostics in Gastroenterology

Authors: MUDr. Kocna Petr CSc.

Reviewer: MUDr. Milan Dastych

Laboratory diagnostics in gastroenterology includes targeted diagnostic methods, specific screening programmes and non-invasive function test programmes employing modern methods of analyte detection in faeces or using breath tests.

24.1. Screening programmes

Screening programmes focus on early diagnosis of diseases which would otherwise remain unrecognized at this early stage. Screening programmes are carried out in asymptomatic individuals (i.e. individuals without signs or symptoms of the disease). Defined high-risk groups are subject to preventive medical care programmes. Gastroenterology screening currently includes two basic programmes: screening for celiac disease and screening for colorectal tumours.

24.2. Function tests

They form an important part of the clinical and diagnostic process in gastroenterology. They supplement imaging examination results with an essential piece of information, namely, how the organ is functioning, i.e. its ability to react to stimulation. Function tests include precisely defined stimulation and the result is interpreted as the organ's response to such stimulation, taking baseline analyte values prior to stimulation into account. In addition, the interpretation of indirect function test results must also consider the function of other organs or systems that take part in the process.

24.2.1. Modern, non-invasive function diagnostics

Modern diagnostics focuses on breath tests based on measuring hydrogen or carbon ^{13}C concentrations in exhaled air. H_2-breath test applications cover a wide area, from differential diagnostics of malabsorption syndrome, syndrome of small intestinal bacterial overgrowth (SIBO) and gastrointestinal motility testing, to oral-caecal transit time (OCTT) or the quality of colon preparation before endoscopy. The time of transit through the gastrointestinal tract is an important piece of information for evaluating and interpreting other function tests, and it is therefore often combined with additional breath tests such as the $^{13}C/H_2$ - lactose test, where enzymatic cleavage of lactose is evaluated (the marker is carbon ^{13}C), and at the same time bacterial cleavage in the colon is used to correct motility and transit (the marker is H_2). The reliability and evaluation of H_2 breath tests are increased not only by hydrogen assays, but also combinations with methane assays, enabled by GC (Gas Chromatography) analyzers and NDIRS (Nondispersive Isotope-Selective Infrared Spectroscopy) technologies.

24.3. Laboratory diagnostics of gastric pathologies

This domain includes serological tests of the gastric mucosa: gastrin-17 level, pepsinogens I and II (pepsinogens A and C) ratio, Helicobacter pylori infection, Hp-antibodies, CagA and VacA antigens, the gastric acidity (HCl) function test and the gastric motility breath test (OABT).

24.3.1. The diagnosis of Helicobacter pylori infection

The diagnosis of HP includes invasive tests, requiring the collection of gastric or duodenal mucosa biopsy specimens, or non-invasive tests. The culture test has the highest sensitivity and specificity. However, the considerable sensitivity of the Helicobacter pylori bacterium to oxygen is a drawback since the test requires special conditions for

collection and transport. The rapid urease test (CLO) is based on the chromogenic detection of urease activity (the surface marker of Helicobacter pylori), and is a routine test performed at the time of endoscopy. More recent tests determine Helicobacter pylori in biopsy specimens by immunological detection – the iRUT method. Molecular biology and PCR methods are facilitating other modern methods of detecting Helicobacter pylori in biopsies and stool samples. The breath test with ^{13}C - labelled urea (UBT) is the gold standard of Helicobacter pylori infection diagnostics; the test can also be performed by endoscopy with the administration of 20 mg labelled urea and the collection of gastric gas content using the bioptic channel of the endoscope. A reliable variant of Hp detection is non-invasive Hp antigen detection in the stool.

24.3.2. Gastric acidity tests

Gastric acidity tests are based on the stimulation of parietal cells, withdrawal of gastric juice and the determination of free and total HCl. Pentagastrin is optimal for stimulation, but also histamine (Lambling test) and insulin can be used. Functional test evaluation depends on the type of stimulation, which means that results vary depending on gastrin, histamine or insulin administration. Hypochlorhydria (hypoacidity) or even achlorhydria (anacidity) is a significant symptom of pernicious anaemia or suspected malignancies (gastric carcinoma at the early stages exhibits hyperacidity or normal acidity, however). The gastric acidity test is important for diagnosing Zollinger-Ellison syndrome, where high basal and maximum secretion is determined (BAO > 15, MAO > 60); a BAO/MAO index > 0.60 is found in more than 50% of Zollinger-Ellison syndrome cases. Pentagastrin stimulation in the test can also be used for mucin analysis. Latest studies use the bQRT test (Blood Quininium Resin Test) to detect hypochlorhydria. An endoscopic variant of the function test is stimulation by 4 µg tetragastrin/kg body weight subcutaneously with a 10 - minute secretion collection (20 - 30 minutes after stimulation), and theacidity in mEq/10 minutes is measured by titration. The correlation with MAO-BAO is r = 0.92 and the reproducibility of the test is CV = 5.6%. A breath test with labelled calcium carbonate was the latest development in 2009.

24.3.3. Serum gastrin level

Gastrin level determined by RIA or ELISA immunochemical detection is 25 - 100 ng/L. A 10 to 1000 - fold increase in gastrin level is detected in Zollinger - Ellison syndrome (gastrinoma, pancreatic tumour with gastrin overproduction); however, this level fluctuates widely even during the day; normal gastrin level can be found in 20 - 40% of cases. As there are three forms of gastrin, the test result depends on the type of antibody used in the test. Gastrin test methods have been standardized to synthetic gastrin G-17; G-34 and G-13 tests depend on cross-reactivity with the relevant antibody. The normal proportion of the forms G-13:G-17:G-34 is 8:2:1; G-34 is higher in a fasted state, and G-17 and G-13 are higher after meals. The gastrin test is part of the gastrin stimulation test, where a 90 - minute profile is determined (in 9 serum specimens) following insulin, secretin or Ca-gluconate stimulation.

24.3.4. Pepsinogen A

Pepsinogen A is a mucosal atrophy marker and is used in genetic studies as a subclinical marker of duodenal ulcer disease. Pepsinogen C is used as a marker for the state of gastric mucosa (or the PG-A/PG-C ratio), and also as a marker of Helicobacter pylori infection eradication. Electrophoresis permits the separation of 8 gastric mucosa proteases in agar gel: pepsinogens PG1 - PG5 form a group of immunologically identical proteins – pepsinogen I (PG-I, PGA), pepsinogens PG6 and PG7 form a group of pepsinogen II (PG-II, PGC), and the last protein is cathepsin E (SMP, Slow Moving Proteinase). A decrease in the pepsinogen A level is tested in patients with achlorhydria, for example in pernicious anaemia. The latest studies have proven a significant decrease in pepsinogen-I with a concurrent increase in IgA antibodies against Helicobacter pylori in gastric carcinoma. The detection of pepsin in saliva/sputum is also indicated as a simple screening for gastroesophageal reflux disease (GERD) with extra-gastric signs of the disease. The PG-I/PG-II ratio decreases significantly depending on the histological risk or the presence of a vacA+ positive Helicobacter pylori infection. The combination of pepsinogen-I/II, gastrin-17 and Helicobacter pylori antibody tests is used as a „serological biopsy", referred to as the GastroPanel, in the differential diagnosis of gastritis. Screening testing of the risk of atrophic gastritis or gastric carcinoma in connection with positive Helicobacter pylori is another area of screening for gastrointestinal tract tumours. Pepsin determination in the insulin test is also clinically important.

24.3.5. The ^{13}C-octanoic acid breath test

The acid breath test is a non-invasive test of gastric evacuation, which can be used for a differential diagnosis of functional dyspepsias, reflux diseases and for the indication of some modern drugs (prokinetics). Octanoic acid does

not absorb in the stomach, but is absorbed quite quickly in the duodenal mucosa. Metabolic activity in the liver tissue produces $^{13}CO_2$, which is then determined in exhaled air. The range of normal values is 110 - 160 minutes for solid food and 91 - 155 minutes for semi-solid food. Gastric evacuation rate assessment using the ^{13}C-octanoate breath test exhibits a high correlation with the scintigraphic method.

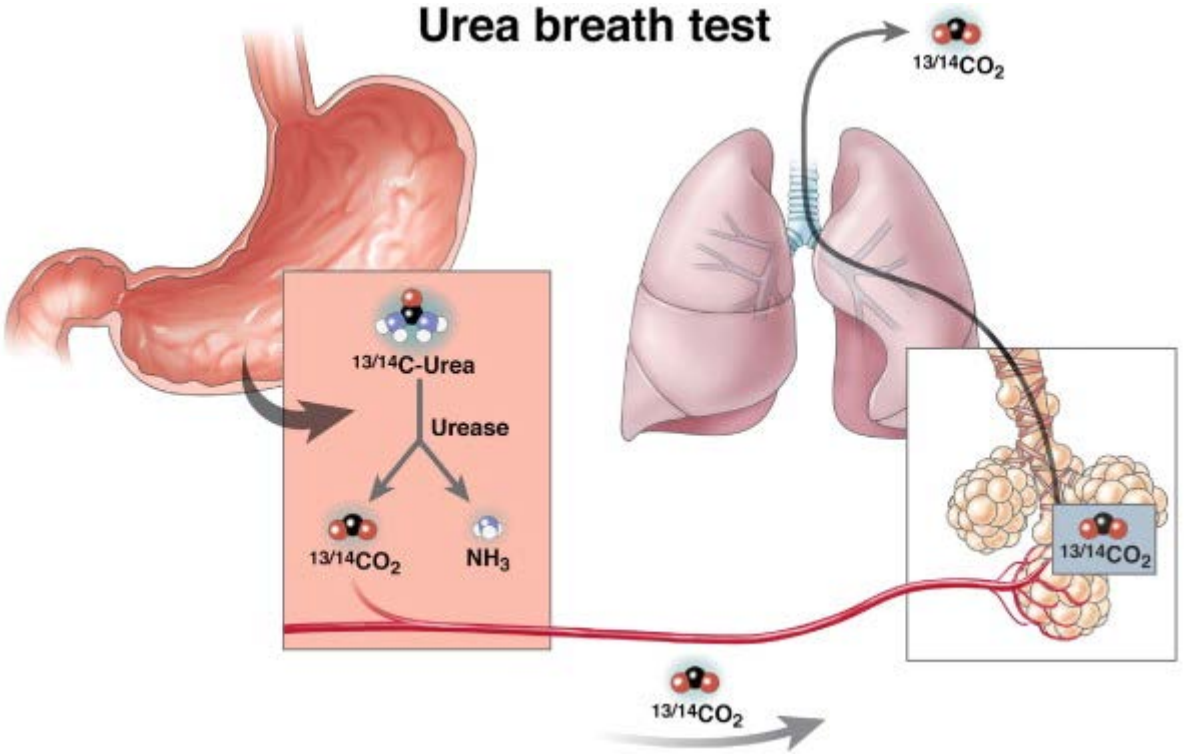

Figure 24.1. A positive answer offers conclusive evidence that the patient is infected with Helicobacter pylori. In the absence of Helicobacter pylori, the administered urea is absorbed from the gastrointestinal tract and subsequently voided.

24.4. The laboratory diagnostics of malabsorption syndrome

The diagnostics of malabsorption includes function tests aimed at clarifying the cause and degree of malabsorption (primary and secondary malabsorption syndrome), and serological tests in connection with the screening and long-term monitoring of patients with celiac disease. Function tests include challenge/tolerance tests with oral administration of D-xylose or vitamin A, and intestinal permeability tests such as the lactulose/mannitol (LA/MA) test. The lactulose/mannitol test can also be combined with the administration of D-xylose, sucralose and other sugars. Each sugar is analyzed using gas chromatography for relative indices of permeability. The average LA/MA index value is 0.016 ± 0.008 and LA/XY 0.013 ± 0.009. The intestinal permeability LA/MA test can be used for monitoring post-operative chemotherapy.

Function tests may also use other substances such as iron and vitamin B12 (Schilling test) or secretion function tests with labelled albumin (^{51}Cr-albumin test). When collecting samples following the administration of tested substances and interpreting results, one should consider not only the transit time but also the different locations in the small intestine (from the duodenum to the terminal ileum) where the tested substrate is absorbed. Non-invasive breath tests include the H_2-lactose breath test or ^{13}C-xylose breath test suitable for detecting small intestinal bacterial overgrowth.

24.4.1. A comprehensive stool analysis

Stool analysis from macroscopic evaluation, determination of volume, structure and pH to an analytical determination of laboratory markers, is considered the basic testing process if malabsorption syndrome is suspected. The qualitative determination of undigested sugars, fats and proteins – a microscopic evaluation of stained faeces is presented by the latest publications of internal medicine, gastroenterology and clinical biochemistry.

24.4.2. ß-carotene and vitamin A

Carotene and vitamin A are fat-soluble; ß-carotene is a retinoid – a vitamin A precursor, and their serum level

therefore depends on lipid digestion and absorption. Of the ß-carotene in the circulation, 80% is bound to LDL, 8% to HDL and 12% to VLDL. ß-carotene represents about 25% of serum carotenoids. The very short half-life of ß-carotene, i.e. its rapid conversion into vitamin A, is important from the clinical point of view. The determination of ß-carotene is clinically important primarily as a screening test if malabsorption syndrome is suspected. Increased ß-carotene levels have been described in hypothyroidism, diabetes mellitus, myxoedema, nephrotic syndrome, hyperlipoproteinaemia and pregnant women. Reference values depend on the test procedure; the range commonly specified for the extraction method (i.e. total serum carotenoid assessment) is 0.90 - 4.60 µmol/L, the narrower range of reference values is 1.12 - 3.72 µmol/L. Only the lower limit 0.93 µmol/L is specified for malabsorption syndrome screening in adults, and the reference range 0.37 - 74 µmol/L is described for the HPLC technique specifically assessing ß-carotene. The challenge/tolerance test with vitamin A is evaluated based on serum level increase 3 and 5 hours after administering the test challenge. Normal values 3 hours later are within the range of 3.6 - 12.6 µmol/L, and 7.2 - 24.6 µmol/L 5 hours later. Values < 3.6 µmol/L 3 hours later, or < 7.2 µmol/L 5 hours later indicate a pathological test result.

24.4.3. The D-xylose tolerance test

This test is usually indicated to confirm intestinal malabsorption in gluten-sensitive enteropathy (celiac sprue) and tropical sprue. The urine output result depends on the renal function. A falsely positive result may be found in conditions such as vomiting, dehydration, myxoedema, ascites or oedema. Many drugs such as aminosalicylic acid, acetylsalicylic acid, digitalis, indomethacin and neomycin decrease D-xylose excretion by the kidneys. The ^{14}C-D-xylose breath test and the ^{13}C-D-xylose breath test are variants of the xylose absorption test, and have been used more commonly than urine xylose determination for diagnosing intestinal malabsorption over the last few years. The serum level is 1.40 - 3.80 mmol/L 1 hour after D-xylose administration, 2.13 - 3.86 mmol/L in 2 hours, 1.27 - 2.80 mmol/L in 3 hours, 0.73 - 1.93 mmol/L in 4 hours and 0.40 - 1.20 mmol/L in 5 hours. Values < 1.67 mmol/L 2 hours after the administration of 25 g to adults, and < 1.33 mmol/L after the administration of 5 g to children, indicate a pathological result. In 5-hour urine collection, pathological values after the administration of 5 g D-xylose to children aged 5-12 are < 0.8 g/5 hrs (the broader range of normal values is 0.5 - 1.65 g/5 hrs), and < 4 g/5 hrs after the administration of 25 g to adults (some authors find results < 5 g/5 hrs pathological). The threshold drops to 3.5 g/5 hrs in individuals over 65. If D-xylose is administered based on weight (to small children in particular), the normal range of xylose excreted in the urine over 5 hours is 10 - 33% of the administered amount.

24.4.4. Celiac sprue (CS)

Celiac disease – gluten-sensitive enteropathy, is the primary malabsorption syndrome. Celiac disease is an autoimmune disorder with a genetic predisposition (HLA-DQ2/DQ8) and a specific humoral response to the triggering factor – wheat gliadin (gluten) peptides or storage proteins (prolamins) of the related cereals barley, rye and oats. The diagnosis of celiac disease is based on the histological or histochemical assessment of small intestinal mucosal biopsy specimens. Parameters assessed in celiac disease include inflammatory mucosa alterations, raised intraepithelial lymphocyte count, small intestine mucosa lesions with reduced or lost intestinal villi, hypertrophy of Lieberkühn's crypts, abnormal enterocyte maturation, and a remission of these alterations when on a gluten-free diet.

Figure 24.2. Damage to the intestine is due to a specific immune response triggered by certain peptides derived from gluten proteins in wheat, rye and barley, and sometimes oats. Damage to the intestine generally disappears over months to years after the harmful gluten proteins are completely excluded from the diet. If gluten is accidentally or deliberately reintroduced into the diet, the immune response to gluten is reactivated; many patients experience acute food poisoning and the intestine can be injured if gluten exposure continues.

Diagnostic tests for celiac disease focus primarily on celiac disease screening and include IgA and IgG class anti-gliadin antibodies (AGA-A, AGA-G), IgA and IgG class anti-endomysial antibodies (EmA) and IgA and IgG class anti-tissue transglutaminase antibodies (atTG-A, atTG-G). Tissue transglutaminase is an enzyme that plays a key role not only in the diagnosis but also in the pathogenesis of the disease itself. Gliadin, gliadin fragments and peptides contain a very high percentage of glutamine (glutamine and proline constitute 30 - 40%), and so gliadin is a very good substrate for the tissue transglutaminase enzyme, which creates a bond to the substrate (gliadin), modifies these peptides, and the neoepitopes that are produced then bind to surface glycoproteins of HLA-DQ2/DQ8-positive immunocompetent cells and induce an immune response in the small intestine mucosa. Studies from 2008 - 2010 recommend detecting antibodies against synthetic gliadin-specific nonapeptides or deamidated gliadin peptides. The sensitivity and specificity of ELISA tests for deamidated gliadin peptides (DGP) is comparable to the reliability of anti-transglutaminase antibodies; DGP antibodies in combination with IgA and IgG are even more reliable. The latest studies recommend tests in combination with IgA atTG and IgG DGP. Molecular biology methods such as PCR permit the detection of specific HLA-DQ2/DQ8 markers. Screening is particularly important in patients with another autoimmune disease. The risk of asymptomatic celiac disease (without clinical signs) in patients with type 1 diabetes is 10 times as high as the risk in the normal population, i.e. the incidence is not 1:200 but 1:20. The increased risk is similar for other autoimmune diseases (autoimmune thyreopathy, hepatitis, rheumatoid arthritis and others).

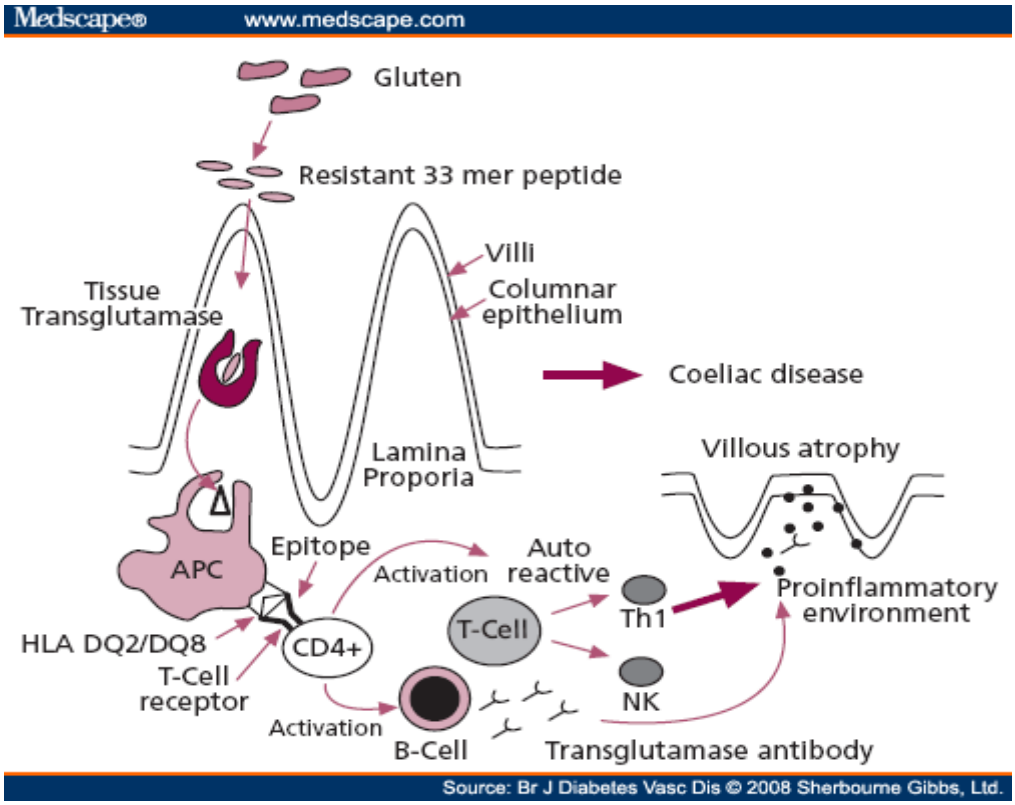

Figure 24.3. Gluten is degraded by gastrointestinal enzymes to a 33 amino acid (33-mer) peptide. The 33-mer peptide is absorbed across the small bowel epithelium to the subepithelial layer in the lamina propria. Tissue transglutaminase deaminates the 33-mer peptide. The deaminated peptides are processed by APC to three epitopes that bind to the HLA-DQ2 or DQ8 molecules. The T-cell receptor on T-cells then cross-react with the HLA molecule leading to the initiation of an autoreactive immune response with subsequent activation of B-cells, CD4+ Th1 cells and NK cells. The resultant proinflammatory environment results in further immune activation and migration of lymphocytes resulting in the characteristic pathological finding of increased intraepithelial lymphocytes and villous atrophy.

24.4.5. The lactose tolerance test

The lactose tolerance test is an indirect measurement of intestinal lactase activity for the differential diagnosis of the malabsorption syndrome lactose intolerance. The traditional test is blood sugar measurement 15, 30, 60 and 90 minutes after oral administration of 50 g of lactose. Lactase deficiency is demonstrated by a rise in blood sugar of less than 1 mmol/L. A newer lactose test consists in breath tests such as the H_2 test, measuring a rise in hydrogen concentration in exhaled air as a result of bacterial decomposition of unsplit lactose in the colon, or carbon ^{13}C detection after the administration of ^{13}C-labelled lactose. Very accurate results are provided by the combined $^{13}C/H_2$-lactose test. The lactose intolerance diagnosis can be supplemented by specific DNA genotyping – the 13910 T/C variant. Lactose intolerance can also be diagnosed by a duodenal biopsy-based rapid test, which is similar to the CLO rapid test for Helicobacter pylori diagnosis. The incubation medium in the cell for biopsy includes the glucose-oxidase and peroxidase

enzymes and the chromogenic oxidation-reduction substrate. The test takes 20 minutes.

24.4.6. Acute pancreatitis (AP)

Acute pancreatitis is a disease with variable clinical symptoms. Important issues in diagnosis are early diagnosis, severity of the disease, prognosis and monitoring the therapy process. Two stages can be distinguished in severe AP. The first is an extensive, sometimes necrotising inflammation of the pancreas with subsequent development of the Systemic Inflammatory Response Syndrome (SIRS), which may lead to Multiple Organ Dysfunction Syndrome (MODS). The next stage involves bacterial infection of the pancreatic necrosis and a deepening of systemic complications, renal failure, coagulation disorders and other manifestations. Laboratory diagnostics includes the assessment of many specific and non-specific markers, pancreatic enzymes and their precursors in the serum or urine, inflammatory markers and immune reaction markers such as cytokines, interleukins and TNF. The most commonly used parameter is the serum total α-amylase level, elevated values of which may revert to normal 3 - 5 days from the onset of the disease. The total α-amylase level is reliably elevated in 100% of acute pancreatitis cases, but it is also elevated in 80% of all acute abdomen pain cases. Diagnostically much more valuable is the pancreatic isoenzyme assay (P-type, P-AMS), whose level is also increased in 100% of acute pancreatitis cases, but only in 10% of acute abdomen pains. The diagnostic value of a serum pancreatic lipase assay is considerably higher than the α-amylase assay. The serum lipase level remains increased after an acute pancreatitis attack considerably longer than the amylase level (increased lipase activity 14 days later has been described). As serum lipase originates primarily in pancreatic cells, the assay provides considerably higher specificity, comparable with the specificity of the pancreatic α-amylase isoenzyme. A reliable early marker for acute pancreatitis is the pancreatic elastase-1 level post-ERCP and pancreatic carcinoma, where elastase-1 (determined by ELISA techniques) has the highest specificity and sensitivity to pancreatic carcinoma of all pancreatic enzymes. The earliest markers for rapid diagnosis of acute pancreatitis are the detection of TAP and CAPAP activation peptides in the urine. Serum procalcitonin has been studied over the last few years as a marker of bacterial infection of pancreatic necrosis and sepsis.

24.4.6.1. Trypsinogen, trypsinogen activation peptide (TAP) & carboxypeptidase activation peptide (CAPAP)

TAP can be detected in the serum and urine. Clinically, the most commonly used is the urinary trypsinogen-2 level; values 5600 – 10,000 µg/L correspond to a severe, serious form of acute pancreatitis and values 130 – 890 µg/L to a medium to mild form of AP. The product of trypsinogen conversion to active trypsin, trypsinogen activation peptide (TAP), is also determined in the urine. Increased TAP levels are clinically significant for evaluating the seriousness of acute pancreatis: urinary TAP values over 15 nmol/L detect medium pancreatitis, and values over 40 nmol/L a severe form of the disease. The CAPAP is longer than the other peptides released in pancreatic proenzyme activation. It is therefore more stable and more useful for laboratory diagnosis. The normal serum CAPAP level measured by the RIA is 0.8 nmol/L.

24.4.7. Chronic pancreatitis (CP)

CP is defined as a continuing inflammatory process of the pancreas, characterized by irreversible morphologic changes, causing characteristic pain and permanent loss of pancreatic function. The gold standard of functional diagnosis is the secretin-pancreozymin (secretin-CCK) test. Quantitative determination of faecal fat in a 72 - hour collection period is also used as a reference test. Of all the tests available, the secretin-CCK test provides the most accurate information about the secretory activity of the pancreas. Despite the non-standard arrangement, it is considered the “gold standard” of pancreatic function tests, in particular for pancreatic insufficiency assessment. Normal values depend on the method of stimulation, collection and analysis of the duodenum content. The values listed below are just one of the variants: secretin-stimulated secretion volume 165 - 536 mL/hr, HCO_3^- concentration 9.8 - 39.7 mmol/hr, trypsin activity 9.3 - 171 U/20 minutes, amylase activity 34 - 204 U/20 minutes. Foreign literature describes one variant, the Lundh test, in which the pancreas is stimulated by a standardized Lundh meal. The latest studies combine endoscopy, stimulation by CCK or secretin, and the analysis of lipase, bicarbonate and other enzyme concentrations in the pancreatic juice collected at endoscopy, or they recommend assays for other markers such as zinc, which is a more stable analyte compared with pancreatic enzymes. The technological advances made in imaging methods in gastroenterology today offer a combination of morphological imaging with the simultaneous evaluation of some aspects of the tested organ function. The ERCP and MRCP methods can be performed with concurrent stimulation by i.v. administered secretin or cholecystokinin, and are the originators of modern combined testing procedures such as S-MRCP, MRCPQ or ePFT (endoscopic pancreatic function test).

24.4.7.1. Human pancreatic elastase-1

Elastase-1 is synthesized by pancreatic acinar cells. The enzyme is secreted by pancreatic juice to the duodenum, and the protein sequence selected for immunochemical detection is not degraded during intestinal transit. Elastase-1 assessment therefore exhibits greater diagnostic benefit compared to a chromogenic assay for chymotrypsin in stool. The reference range is 200 - 500 µg/g of stool, the borderline range is 100 - 200 µg/g, and serious pancreatic insufficiency is found if values are < 100 µg/g of stool. The immunochemical assay for elastase-1 is not affected by transit through the colon, substitution therapy or other factors affecting the enzyme assay for chymotrypsin in stool. The specificity of the method is 93%, sensitivity is 100% for severe pancreatic insufficiency and 87% for medium and mild forms. This test is commonly used in paediatrics to demonstrate cystic fibrosis with almost 100% specificity and sensitivity. Falsely reduced values can be caused by dilution (water content) during diarrhoea.

24.4.7.2. The breath test with ^{13}C-mixed triglycerides

The principle is the cleavage of this substrate by pancreatic lipase. The ^{13}C-MTG substrate is a triglyceride with ^{13}C - labelled octanoate in position 2 and stearate in positions 1 and 3. Pancreatic lipase cleaves triglyceride, and ^{13}C--octanoate is further oxidized in the liver (beta-oxidation of fats). The amount of $^{13}CO_2$ in exhaled air is measured with a breath test analyzer. The ^{13}C-MTG test is clinically important in the differential diagnosis of malabsorption syndrome, as a function test of exocrine function of the pancreas, and for the long-term monitoring of patients with chronic pancreatitis. ^{13}C breath tests with mixed triglycerides (MTG) or hiolein are indirect function tests of exocrine pancreatic function, and can be used to monitor the success of pancreatic substitution therapy.

24.4.8. Colonic pathologies

Laboratory diagnostics of colonic pathologies focuses primarily on screening for colorectal tumours (CRCA), faecal occult blood tests, the haemoccult guaiac screening test (gFOBT), the more sensitive immunochemical test (iFOBT), and the quantitative faecal occult blood test (qi-FOBT). The activity of inflammatory diseases and tumours can be monitored by many faecal markers, the detection of calprotectin, lactoferrin, pyruvate kinase M2 and the protein S100A12. Progress in molecular biology allows the isolation of DNA from a stool specimen and the assessment of many genetic markers.

The risk of colon carcinoma in the Czech population continues to rise, with 80 cases per 100,000 inhabitants in 2010, which is the highest incidence in Europe. Colorectal carcinoma (CRCA) develops as an adenoma malignization by a sequence of gene mutations over the course of ten years (on average) and most of the tumour tissue is localized intraluminally, at least at the initial stage of the disease. A small proportion of colorectal carcinomas develop in the presence of risk factors such as non-specific inflammatory bowel diseases, familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer and other hereditary syndromes. The carcinoma (and the adenomatous polyp) already intermittently bleeds at the asymptomatic stage of the disease. Screening programmes include primarily laboratory methods of occult blood detection such as faecal occult blood test (FOBT) (*test okultního krvácení ve stolici* – TOKS – in Czech), the detection of genetic markers, and imaging methods such as sigmoidoscopy, colonoscopy, irrigography, virtual computer-aided colonography using computed tomography (CT) or magnetic resonance (MR). Progress in molecular biology and the application of PCR in routine diagnosis are also opening up brand new avenues in GIT tumour screening. The latest screening methods are based on the detection of specific mutations of the DNA isolated from a stool specimen using PCR methods or biochip array technology. APC, K-ras, p53, microsatellite instability (MSI) and other genetic markers can be assessed for the detection of colon tumours from stool specimens. Detection of specific protein (vimentin) methylation may also be used as molecular markers of colorectal cancer, and these DNA alterations can be monitored in the stool and serum of patients with CRCA.

For many years, the only test recommended for screening was the Haemoccult - guaiac test. The test is based on the pseudoperoxidase reaction of haemoglobin and contains a test medium (strip) impregnated with guaiac resin or, in former tests, a derivative of benzidine such as dimethylbenzidine (o-tolidine). Given the chemical principle of the oxidation reaction, tests are affected by the presence of other oxidizers (vitamin C), the presence of haemoglobin from food (meat, blood), and a falsely positive result may also be caused by the presence of vegetable peroxidases (some root vegetables). Defined dietary limitations are therefore recommended depending on how the test is arranged. The sensitivity of first generation gFOBTs is very low, 26 - 30% for cancer detection; however, gFOBTs have almost zero false positivity. Qualitative immunochemical tests (iFOBT) of the second generation are almost twice as sensitive, but their specificity is considerably lower and false positivity is almost 25% for some tests. The third generation of FOBTs, qiFOBT with quantitative faecal Hb detection, provide 90 - 95% sensitivity and specificity by optimizing cut-off values. This is

why quantitative immunochemical tests, qiFOBT, are recommended specifically for CRCA screening at present.

24.4.8.1. Calprotectin

Calprotectin is a 36.5 kDa calcium binding protein with antimicrobial activity; it is composed of two heavy chains and one light chain, and is primarily derived from monocytes and neutrophils. Calprotectin as a marker detected in the stool exhibits lower variability than haemoglobin and is a suitable indicator in the diagnosis and monitoring of therapy for inflammatory bowel diseases, ulcerative colitis and Crohn's disease, or necrotising enterocolitis in children. The determination of concentration in a stool specimen with a cut-off of 30 mg/L exhibits 97% specificity and 100% sensitivity for the differential diagnosis between acute Crohn's disease and irritable bowel syndrome (IBS). Faecal calprotectin is also tested as a colorectal cancer marker. Another inflammatory disease marker measured in the stool is lactoferrin, which has a superior sensitivity to calprotectin.

For more detailed information about each laboratory method, test procedures, links to current NLM Medline abstracts and 100 colour diagrams and photos, refer to the up-to-date on-line GastroLab at the First Faculty of Medicine website - *http://glab.zde.cz*.

Clinical biochemistry

Clinical Biochemistry

Edited by

prof. MUDr. Jaroslav Racek, DrSc.

MUDr. Daniel Rajdl, Ph.D.

Authors

Vladimír Bartoš, Milan Dastych, Milan Dastych jr., Tomáš Franěk, Milan Jirsa,

Marta Kalousová, Tomáš Karlík, Petr Kocna, Viktor Kožich, Michaela Králíková,

Alena Krnáčová, Pavlína Kušnierová, Richard Pikner, Věra Ploticová,

Richard Průša, Jaroslav Racek, Daniel Rajdl, Václav Senft, Vladimír Soška,

Drahomíra Springer, Kristian Šafarčík, Ivan Šebesta, Radka Šigutová,

Zdeněk Švagera, Eva Táborská, Libor Vítek, František Všianský,

Jiří Zadina, David Zeman, Tomáš Zima

Technical collaborator

Martin Navrátil

Published by Charles University

Karolinum Press

www.karolinum.cz

ebooks@karolinum.cz

Prague 2016

First edition

ISBN 978-80-246-3497-5 (pdf)

ISBN 978-80-246-3164-6 (epub)

ISBN 978-80-246-3498-2 (mobi)

The publication has been partly created within project Klinická biochemie – inovovaná, interaktivní výuka e-learningem, reg. number: CZ.1.07/2.2.00/15.0048 and is co-funded by the European Social Fund and the state budget of the Czech Republic.

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ