Laboratory screening markers in gastroenterology - state of the art

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Introduction. Screening tests for gastrointestinal diseases acceptable for population with a high sensitivity and high specificity can now be offered by clinical laboratories. This paper summarizes major recent advances in this area of laboratory medicine.
Methods. Relevant articles published within the last 5 years in the NLM (National Library of Medicine) PubMed - Medline database covering the three gastrointestinal diseases - colorectal cancer, coeliac disease, and atrophic gastritis were included for this overview.
Results. In Europe, colorectal cancer (CRCA) is the second most frequent malignant disease. Quantitative immunochemical analysis of the stool for haemoglobin provides the best screening test to date, with both sensitivity and specificity approaching 95%. Even though coeliac disease (CD) affects approximately 1% of the general population, it remains largely unrecognised. Recommended methods for screening currently involve the detection of IgA and IgG antibodies against tissue transglutaminase and deamidated gliadin peptide. Evaluations of screening are now discussed for other diseases of the gastrointestinal tract - such as chronic atrophic gastritis (CAG), and inflammatory bowel disease (IBD). Detection of infection by Helicobacter pylori and stomach-specific plasmatic biomarkers, especially pepsinogen I/II ratio, could help with the prevention of gastric carcinomas. The use of faecal calprotectin as a screening test could substantially reduce the number of invasive methods necessary for the diagnostic work-up of patients with IBD.
Conclusions. Screening tests for CRCA and CD have been used worldwide for many years. Screening strategies for gastrointestinal diseases are suggested in the text, based on recent basic science, clinical papers as well as our own experience.

Key words: gastroenterology, screening, colorectal cancer, coeliac disease, chronic atrophic gastritis, inflammatory bowel disease

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INTRODUCTION

A screening strategy was defined in the 1940s as identification of an unrecognised disease by the application of one or more simple procedures. The benefits of screening for disease were first demonstrated while identifying individuals with tuberculosis by the use of mass miniature radiography[1]. The first comprehensive review of the principles of screening was published in 1961 (ref.[2]), however, the general rules, principles, and practices of screening for diseases were published as a WHO monograph in 1968 (ref.[3]). At present, criteria for disease screening, as clearly defined by WHO (ref.[1]), are followed. These are summarized in Table 1. There are different approaches to screening - mass (population-based, national based) screening, selective or targeted screening - i.e. the identification of the disease in subjects who are at high risk for the condition, opportunistic screening - screening, which may be offered to patients who are being examined for other reasons. There has been much discussion about the ways of organizing especially CRCA screening in the European Union[4]. In the Czech Republic the National colorectal neoplasia screening programme was launched in 2002, whereas the guidelines for CD targeted screening were published by the Ministry of Health in 2011.

Table 1. WHO defined criteria[1] for disease screening.

Criteria for disease screening
1. The condition screened for should be an important one.
2. There should be an acceptable treatment for patients with the disease.
3. The facilities for diagnosis and treatment should be available.
4. There should be a recognised latent or early symptomatic stage.
5. There should be a suitable test or examination which has few false positives (specificity) and few false negatives (sensitivity)
6. The test or examination should be acceptable to the population.
7. The cost, including diagnosis and subsequent treatment, should be economically balanced in relation to expenditure on medical care as a whole

This review summarizes some aspects of the laboratory methods used for screening gastrointestinal diseases. The work includes papers published on a global scale in the last few decades, as well as our own experiences in the Czech Republic. When searching documents published

Table 2. Numbers of PubMed - Medline articles (with reference to reviews and screening) on colorectal cancer, coeliac disease and atrophic gastritis, having been published in the last 5 years.

Year	Papers (all)	Review	Screening	Screening review
Colorectal cancer				
2011	9223	1308	5575	791
2010	9169	1368	6036	847
2009	8202	1222	5333	738
2008	7819	1209	5089	754
2007	7434	1323	4751	809
Coeliac disease				
2011	862	150	509	95
2010	781	140	515	96
2009	802	154	512	94
2008	769	168	489	110
2007	701	130	473	90
Atrophic gastritis				
2011	156	31	95	15
2010	159	20	107	6
2009	143	23	99	14
2008	151	23	111	16
2007	155	36	125	31

within the last 5 years in the NLM (National Library of Medicine) PubMed - Medline database, there were more than 50 thousand papers covering the three gastrointestinal diseases - CRCA, CD, and CAG (Table 2). The NLM PubMed Medline database comprises some 6000 journals. The embodiment of other databases such as Web of Science, Scopus or Google Scholar could increase the number of hits more than 10-times.

Screening tests acceptable for populations and having high sensitivity and high specificity for gastrointestinal diseases can now be offered by clinical laboratories; additionally, they can be performed non-invasively as well as inexpensively. Biochemical markers may be analysed from peripheral blood, exhaled air as well as from stool samples. New and highly accurate biochemical tests involving a single sample of faeces are a valuable alternative to invasive screening tests. Currently, there are complete automatic analysers available for routine determination of many faecal analytes including haemoglobin, haptoglobin, calprotectin, or transferrin. The External Quality Assurance Services (EQAS) programmes should be used to monitor all these laboratory methods, with the aim to increase the quality and screening accuracy.

COLORECTAL CANCER

CRCA is the second most frequent malignant disease in Europe. Every year 412 000 subjects are diagnosed with this condition and 207 000 patients die of it[5]; furthermore it was estimated to cause 49 920 deaths in the US in 2009 (ref.[6]).

The pathogenesis of CRCA ordinarily occurs in a staged progression from normal mucosa, through adenoma, and finally to carcinoma over a period of approximately 7-10 years[7]. This provides an excellent opportunity for the utilization of screening tests for the early detection of pathology; the goal being the reduction of cancer deaths by the removal of pre-malignant adenomas and early localized cancer prior to the onset of more advanced stages.

The introduction of population-wide national screening programmes is a priority for the healthcare policies of individual nations. European Union (EU) administrators are also addressing this issue at the highest levels. A national screening programme, of one sort or another, has been implemented in 19 out of 27 European countries. The most frequently applied method is testing the stool for occult bleeding (faecal occult blood test, FOBT). In the Czech Republic CRCA screening programmes were launched in 1994 (ref.[8]), and population-based national screening with FOBT was commenced in 2002. The involvement of general practitioners has been found to improve patient compliance with bowel cancer screening[9].

The first generation of FOBT were guaiac-based methods (gFOBT). They are still used in many countries with reference to evidence based-medicine, and are recommended as one of many faecal screening methods by the American College of Physicians[10]. Twenty years of experience with gFOBT in the UK have recently been published[11]. Guaiac methods are not specific for human haemoglobin. The gFOBT test is based on the oxidation of guaiac gum impregnated on a card by hydrogen peroxide catalysed by the pseudoperoxidase activity of haemoglobin. This oxidative reaction may also be catalysed by any peroxidase found in the faeces (e.g. plant peroxidases), or by certain chemicals. Being lower than 30% the sensitivity of gFOBT for colorectal cancer accounts for its replacement by immunoanalytical methods in most countries.

The second generation of FOBT tests is based on immunoanalytical methods (iFOBT, FIT), which employ the antibody against human globin (the protein component of human haemoglobin). The iFOBT are more sensitive than the gFOBT methods[12]. Various qualitative immunoanalytical methods differ in both sensitivity and specificity for colorectal tumours. The values range from 29 to 72% (ref.[13]), owing to different sampling devices and different stabilities of the haemoglobin extract in the sampling buffer. Additional biochemical markers, such

as haptoglobin and transferrin, are sometimes used as a second analyte detected in these test, in order to increase screening accuracy[14,15].

The third and so far the latest generation of FOBTs are quantitative methods of faecal haemoglobin determination with automated analysers (qiFOBT). This approach has elevated the sensitivity and specificity of the screening to 90 - 95%, enabling the setting of country-specific optimal cut-offs, and practical checking of the methods by the EQAS programmes. The European Group on Tumour Markers recommends the use of qiFOBT with an adjustable cut-off value to all new centres undertaking FOBT[16]. The organized faecal immunochemical test screening has been associated with an increase in annually detected CRCA (ref.[17]).

Three analytical systems for qiFOBT (Magstream HT, OC-Sensor/DIANA, FOB Gold/SENTiFOB) were compared as to their diagnostic accuracy, analytical sensitivity, sample stability, as well as sample consignment by tested subjects[18-21]. Quantitative FOBT was found to be superior in compliance and CRCA detection, when compared with both gFOBT and flexible sigmoidoscopy[22]. The collection of two stool samples improves the qiFOBT´s diagnostic yield, but it also doubles the screening costs[23,24]. Recent analyses of the cost-effectiveness of qiFOBT were published by Dutch working groups[25,26]; the optimal cut-off value was found to be 50 ng Hb/mL. It is notable that the identical value was selected for screening in the Czech Republic based on a pilot study with OC-Sensor[27].

Faecal immunochemical test results may be expressed as haemoglobin concentration in the sampling device buffer and, sometimes, albeit rarely, as haemoglobin concentration per mass of the faeces. The current lack of consistency in regard to the units used for the reporting of haemoglobin concentration may result in misleading clinical interpretations, reinforcing the need for standardization[28,29].

Stool-based DNA testing for CRCA is becomming a favoured alternative to the existing DNA screening tests. The basis for DNA screening is the identification of genetic alterations in the initiation of a sequenced progression from adenoma to carcinoma, such as mutations in APC, K-ras, DCC, and p53 genes[30-33]. Rapid advancement of proteomics and associated techniques provides a new promising route for the diagnosis of precancerous laesions[34]. Hypermethylation of the plasma septin-9 gene may be considered as a potential non stool-based screening tool[35,36]. Blood-based biomarkers are not likely to be established as an alternative to FOBT-based CRCA screening, but can be used in combination with iFOBT to increase colorectal screening accuracy[37-39]. Tumour pyruvate kinase M2 (M2-PK) is a key enzyme in the altered metabolism of tumour tissues. It is present in high concentrations in malignant tissues, plasma, and other body fluids[40,41]. Together with other tumour markers plasmatic and faecal levels of M2-PK could be used for prognosis. M2-PK is not applicable for screening, even though its diagnostic efficiency is similar to that of gFOBT (ref.[42]).

COELIAC DISEASE

CD has a prevalence of nearly 1% in both the USA and Europe. However, diagnosed cases of CD only have a prevalence of only about 0.27%, or less. The risk of CD in various autoimmune diseases is approximately 5 - 10% (ref.[43,44]). Increased risk of complications in untreated CD patients include malignancy and severe malabsorption. Thus, early diagnosis of CD and subsequent adherence to the gluten-free diet may prevent the development of other autoimmune diseases[45,46] and decrease the risk of mortality[47].

CD is a chronic small bowel disorder of autoimmune origin occurring in both children and adults. It is one of the most commonly underdiagnosed diseases in the general practice with an incidence of 1:100 (ref.[48]). The disease is genetically determined, has a strong HLA association with DQ2 (DQA1*0501/DQB1*02), and gliadin peptides derived from wheat gluten were identified as its precipitating factors[49].

Screening strategies and diagnostic algorithms for the detection of CD, especially concerning the serological markers, belong among the research priorities of the European Working Group on Serological Screening for Coeliac Disease. The specificity and sensitivity of serological markers as reported in numerous studies have ranged from 31% to 100%. No single marker may be characterized by either 100% sensitivity or 100% specificity. However, combinations of tests are capable of detecting 100% of all CD cases[50-53]. The diagnostic accuracy of serology for CD has progressively increased in the last few years. IgA antibodies to tissue transglutaminase (tTGA) owing to their high sensitivity have been suggested as the first level test. The diagnostic performance may further be improved by using IgA anti-endomysium antibodies (EmA); IgG tTGA test should be performed in cases with a concomitant IgA deficiency, whereas the determination of IgA anti-gliadin antibodies (AGA) is indicated for children of less than 2 years of age. Therefore CD screening has been based on the combination of IgA tTGA, and IgG deamidated gliadin peptide (DGP) (ref.[54]). The new definition of CD, as well as the latest ESPGHAN (European Society of Paediatric Gastroenterology, Hepatology and Nutrition) guidelines, modify the aforementioned rules. tTGA level 10 times exceeding the cut-off and its confirmation by positive EmA from an independent blood sample, without duodenal biopsy, are the first requirements for a diagnosis in symptomatic children[55,56].

Mass screening for CD, as a public health intervention, is controversial[57]. The main argument against screening is the compliance with gluten-free diet, which might be low in screen-detected patients (even when symptomatic) (ref.[53]). In contrast to the general population, targeted screening in high-prevalence groups may prove to have a favourable cost-benefit ratio. The guidelines for targeted screening for CD in the Czech Republic have been specified in the Bulletin of the Ministry of Health of the Czech Republic (February 2011), indicating these high-prevalence subjects (Table 3). In 2011, new methods for

Table 3. Coeliac disease associated diseases, syndromes, and signs indicating screening.

Associated symptoms and signs	Associated diseases and syndromes
Dermatitis herpetiformis	Type 1 diabetes
Osteoporosis, unexplained fractures	Autoimmune thyroiditis
Chronic diarrhoea with abdominal distension	Autoimmune liver disease
Anemia	Systemic lupus erythematosus
Chronic fatigue syndrome	Primary biliary cirrhosis
Polyneuropathy	Primary sclerosing cholangitis
Cerebellar ataxia, epilepsy	Sjögren syndrome
Spontaneous abortion and fetal growth retardation	Alopecia areata
Growth retardation, pubertal delay	IgA nephropathy
Involuntary weight loss	IgA deficiency
Unexplained anaemia (iron, folic acid)	
Dental enamel hypoplasia	
Recurrent aphthous stomatitis	
Hypertransaminasemia	

CD screening employing salivary anti-transglutaminase autoantibodies were started in Italian primary school children[58].

CHRONIC ATROPHIC GASTRITIS

CAG is an inflammatory condition characterized by the loss of gastric glandular structures, which are replaced by connective tissue (non-metaplastic atrophy) or by glandular structures inappropriate for location (metaplastic atrophy). Diagnosis and screening of CAG with stomach-specific plasma biomarkers might be effective in the prevention of gastric carcinoma, as well. This is because invasive gastric carcinomas are preceded by a cascade of precancerous laesions, multifocal atrophic gastritis, and intestinal metaplasia. The multistep process initiates from Helicobacter pylori-related chronic inflammation of the gastric mucosa, progresses to CAG, intestinal metaplasia, dysplasia, and finally leads to the development of gastric cancer[59,60]. The diagnosis of Helicobacter pylori infections is now simple and easy, with a high sensitivity and specificity 92 - 98%, using both the urea breath test and stool antigen tests[61,62].

Parallel assays of pepsinogens (PGI, PGII), PGI/II ratio, and of amidated gastrin-17 represent an exact and validated set or panel of biomarkers that reflect the degree of mucosal inflammation, the extent and grade of atrophic gastritis in the stomach, as well as the capacity of the existing mucosa to secrete acid and gastrin-17 (ref.[63,64]). The sensitivity and specificity of singular biomarker test panel (commercial test panel - GastroPanel, Finland) have been found to range from 71 to 83% and 95 - 98%, respectively[65]. A high prevalence of more than 3% of advanced atrophic corpus gastritis among Finnish adult volunteers, without specific complaints, was diagnosed last year[66].

Table 4. Recommended laboratory methods for screening in gastroenterology.

Gastrointestinal disease	Recommended screening test
Worldwide used screening	
Colorectal cancer	quantitative immunochemical Hb in stool
Coeliac disease	IgA tTGA and IgG DGP plasma antibodies
Evaluated new screening	
Chronic atrophic gastritis Helicobacter pylori infection	plasma pepsinogen I/II ratio Helicobacter pylori antigen in stool
Inflammatory bowel disease	calprotectin in stool

INFLAMMATORY BOWEL DISEASE

Differentiating patients with inflammatory bowel disease (IBD) from those with irritable bowel syndrome (IBS), poses a diagnostic challenge. Current guidelines suggest performing invasive endoscopy with histological sampling. Consequently, there is a need for a reliable, simple, non-invasive, and inexpensive test that could provide objective evidence of whether the underlying disease is organic or functional. Calprotectin, a neutrophil granulocyte cytosol protein, is a 36 kDa calcium and zinc binding protein expressed by the gene S100 calcium-binding protein A8. Faecal calprotectin concentrations have been shown to reliably differentiate between IBD and non-organic disease in symptomatic patients, and when elevated warrant early endoscopic investigation to rule out IBD and other organic pathologies[67,68]. On the other hand, the use of faecal calprotectin as a screening test

could substantially reduce the number of invasive methods necessary in the diagnostic work-up of patients with suspected IBD (ref.[69]); in adults this would result in a 67% reduction of patients requiring endoscopy[70]. In addition to calprotectin, lactoferrin, M2-PK, and other faecal markers might prove beneficial in the differential diagnostics of large bowel diseases.

CONCLUSION

Screening tests for CRCA and CD have been utilized worldwide for many years. Based on recent scientific and clinical papers, as well as on our own experiences, we may recommend also other screening methods (Table 4).

The best screening test for CRCA uses quantitative immunochemical analysis of human haemoglobin in the stool by automated analysers. The recommended methods for CD screening involve the detection of IgA tTGA and IgG DGP antibodies. Screening of chronic atrophic gastritis (CAG) and inflammatory bowel disease (IBD) are currently under evaluation. The detection of stomach-specific plasma biomarkers, especially the pepsinogen I/II ratio, could help in the prevention of gastric carcinoma. The use of faecal calprotectin might substantially reduce the number of invasive methods in subjects suspected from having IBD. Screening of other gastrointestinal diseases - foremost tumours (such as pancreatic cancer) still remains open for future research.

ABBREVIATIONS

ACG, Atrophic corpus gastritis; AGA, Anti-gliadin antibodies; APC, Adenomatous polyposis coli; CAG, Chronic atrophic gastritis; CD, Coeliac disease; CRCA, Colorectal cancer; DCC, Deleted in colorectal carcinoma; DGP, Deamidated gliadin peptide; DANN, Deoxyribonucleic acid; EmA, Endomysium antibodies; EQAS, External Quality Assurance Services; ESPGHAN, European Society of Paediatric Gastroenterology, Hepatology and Nutrition; EU, European Union; FOBT, Faecal occult blood test; gFOBT, Guaiac based faecal occult blood test; HLA, Human leukocyte antigen; IBD, Inflammatory bowel disease; IBS, Iirritable bowel syndrome; iFOBT, Immunochemically based faecal occult blood test; FIT, Fecal immunochemically based test; IgA, Immunoglobulin A; IgG, Immunoglobulin G; K-ras, Kirsten rat sarcoma; MAG, Multifocal atrophic gastritis; M2-PK, Pyruvate kinase M2; NLM, National Library of Medicine ; PGI, Pepsinogen type I; PGII, Pepsinogen type II; qiFOBT, Quantitative method of faecal occult blood test; tTGA, Antibodies to tissue transglutaminase; UBT, Urea breath test; WHO, World Health Organization.

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CONFLICT OF INTEREST STATEMENT

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