New Trends in Early Detection of Colorectal Cancer

D. Dimitroulopoulos, D. Xinopoulos, K. Tsamakidis, E. Paraskevas

SUMMARY

Current practice guidelines recommend annual fecal occult blood-test screening for colorectal cancer in the average-risk population over the age of 50. Efficacy of fecal occult blood-test screening is supported by several recent, controlled, prospective trials and case-control studies.

On the other hand, a new generation of fecal tests based on immunocytochemical and RT-PCR methods recently has been developed.

There is strong evidence that colon screening of asymptomatic, average-risk subjects can reduce colorectal cancer mortality. Endoscopic screening with sigmoidoscopy can reduce mortality associated with left-sided cancers due to discovery of early curable cancers and the detection and removal of premalignant adenomas. But, with this technique, only the superficial mucosa is visualized and at relatively low magnification. Thus, new imaging systems, based on light-tissue interactions, such as optical coherence tomography, light-induced fluorescence spectroscopy, time-resolved fluorescence, light-scattering spectroscopy, chromoendoscopy and magnification endoscopy are being developed to advance endoscopic diagnosis of colorectal cancer.

INTRODUCTION

Colorectal cancer (CRC) is the fourth most common malignancy that occurs worldwide with an estimated 678,000 new cases diagnosed in 19851 and the second most common cause of death from malignant disease2. It affects men and women almost equally, with approximately one-third of a million new cases diagnosed in each gender group each year. The disease is most frequent in occidental and economically “developed” countries.

Because of its orderly natural history and location within a readily accessible organ, CRC appears ideally suited for preventive intervention. Over the past 30 years, fecal occult blood test (FOBT) screening has been widely practised as an empiric approach to CRC prevention in community-based programs, promoted by a number of advocacy groups, and incorporated by many physicians into the periodic health examination3. Despite a large number of publications, the effectiveness of FOBT in preventing CRC is still an unanswered question. In a recently published randomized trial, Jorgensen et al, using FOBT, showed a 30% reduction in risk of death for CRC when compared with controls4. This study along with 2 other large randomized trials from Minnesota, USA, and Nottingham, UK, demonstrate the efficacy of FOBT as a screening tool for CRC5,6.

On the other hand, a new generation of fecal tests has been developed recently and a new era in prevention of CRC has come. This new method is based on stool detection of exfoliated markers that arise from neoplasms per se and are released continuously. Neoplasms exfoliate luxuriant populations of viable colonocytes, unlike the sparse and largely apoptotic cells shed from normal mucosa7. Theoretically, any colonocyte constituent that reflects a dysplastic lineage could qualify as a candidate marker from this class. Whole colonocytes have been recovered from stool and fecal leukocytes are greater in numbers in stools from patients with CRC than in those from healthy persons8. Isolated focal colonocytes can also be incorporated into assay systems using immunocytochemical methods or RT-PCR display of genes that are overexpressed by neoplasms9.
Neoplasm-specific DNA alterations have been well characterized and represent especially intriguing markers. DNA appears to be stable in stool and amplification techniques permit detection of minute amounts of analyte. Several investigators have recovered mutant DNA in stools from patients with cancer or large adenomas. But this method is based on mutant K-ras detection and unfortunately this mutation is expressed in approximately 50% of all colorectal neoplasms. On the other hand, not all DNA alterations are neoplasm specific and K-ras mutation may arise from other non-neoplastic sources.

Recently, the feasibility of a new multi-target DNA-based assay system has been reported. Following recovery of human DNA from stool using a sequence-specific hybrid capture technique, assay components targeted point mutations at any of 15 mutational hot spots on K-ras, APC and p53 genes; mutations on Baf-26 and “long” DNA.

Despite the small number of tested subjects (28 persons with endoscopically normal large bowel, 11 with large adenomatous lesions and 22 with CRC) the authors reported a sensitivity of 91% for cancer and 82% for adenomas and an initial specificity of 93%.

In this study “long” or high-molecular weight DNA proved to be the most informative component marker from the assay panel. This marker appears to reflect the presence of non-apoptotic colonocytes, which are characteristically exfoliated from neoplasms.

Other most recent studies for specific molecular genetic stool testing using the genetic targets TP53, BAT26, K-ras together, BAT26 alone and APC also reported promising results.

As used in present-day practice, colonoscopy represents an extended form of physical diagnosis for CRC. But, with this technique, only the superficial mucosa is visualized and at relatively low magnification. The microscopic, cellular and biochemical changes that constitute the pathophysiology of the disease remain beyond the reach of the endoscopist. Although endoscopic ultrasonography has greatly extended the diagnostic accuracy in the upper gastrointestinal tract, in diseases of the colorectum it is still limited in the staging of rectal carcinoma. Only one study suggests that endoscopic ultrasonographic staging of rectal cancer is similar in sensitivity to that of other luminal cancers. Thus, new imaging systems, based on light-tissue interactions, are being developed to advance endoscopic diagnosis of CRC.

**I. Optical Coherence Tomography (OCT):** OCT is a new imaging method for cross-sectional, subsurface imaging of biological tissues with high spatial resolution.

It can provide histology-equivalent images of the layers and structures of the gastrointestinal tract wall with a spatial resolution as small as 4-10 μm. Compared with the resolution of the high-frequency endoscopic ultrasonography, computed tomography and magnetic resonance imaging the resolution of this method is 10-25 times higher. The depth of imaging is 2-3 mm and thus the provided information refers the wall layers, the structure and the potentially cellular features of the colon. OCT uses backscattered light as a function of optical delay to create images. The optical technology used is termed low-coherence interferometry. Light from a source (infrared low-coherence diode or femtosecond laser) is sent to an optical fiber splitter that functions as an interferometer and splits the source light into two even beams of light. Half of the evenly split light is carried by a fiber to the tissue while the other half is carried by another fiber to a reference mirror that can change position electronically by microscopic degrees.

OCT images of tissue are based on the reflection of cell membranes, collagen, adipose tissue, muscle tissue etc., because different tissues have different reflection properties.

The initial studies of large bowel OCT, used ex vivo and in vitro tissues, and demonstrated that the method clearly distinguishes mucosa from submucosa in the colon, visualized colonic crypts and glands and also interpreted areas with a high degree of backscattering as indicative of the presence of inflammation. These data indicate that the normal could be differentiated from the malignant colonic tissue by OCT, because malignancy disrupts the normal tissue structure and also present differences in the backscattering properties.

An OCT endoscopic imaging system was tested for the first time in humans in 1997 in esophageal malignancies. This system includes probes designed to pass through the accessory channel of an endoscope.

There are relatively few data concerning OCT in the colorectum. Kobayashi et al have demonstrated the ability of this method in vitro to detect the disappearance of crypts in specimens from patients with ulcerative colitis and the irregular glandular structure of CRC.

Although the depth of penetrating ability of OCT is limited, it is nevertheless adequate for visualization of mucosa and submucosa. Thus, OCT could be useful for
the detection of small, early-stage cancers and perhaps even dysplasia. Thus OCT images are often called optical biopsies.

II. Light-induced fluorescence spectroscopy: Tissue fluorescence and fluorescence endoscopy is a technique based on the absorption and re-emission of light by a wide variety of substances, called chromophores (e.g. water, hemoglobin, melanin, molecules involved in metabolism, structural proteins), within biologic tissues. Whether light is absorbed by a particular chromophore depends on its energy. Fluorescence is a mechanism of photon re-emission and the substances that interact with light in this manner are termed fluorophores. A great number of substances are endogenous gastrointestinal tissue fluorophores. In several studies of colonic tissues under near ultraviolet light excitation, 4 major categories of tissue fluorophores and their emission peaks have been identified.

The initial studies of light-induced fluorescence in the large bowel used laser-induced point fluorescence spectroscopy to differentiate colon adenoma and cancer from hyperplastic polyps. Further studies showed that the method could differentiate normal colonic tissue from hyperplastic polyps and adenomas with an accuracy ranging from 94% to 100%. The data of these laboratory studies were used as the basis for the determination of whether the method could differentiate normal from neoplastic tissue at colonoscopy. The accuracy was found approximately that of histopathologic assessment.

The evolution of the method of ratio fluorescence imaging, has been used primarily in tracheobronchial tree studies. A real-time imaging system based on the principle of ratio fluorescence imaging was developed for the gastrointestinal tract few years ago. Using this system Haringsma et al reported a sensitivity of 96% and a specificity of 70% in the differentiation of dysplastic and nondysplastic colonic lesions. Other investigators also reported similar results.

Wang et al have developed a method of fluorescence imaging that differs from ratio fluorescence imaging. Using this system in vitro reported a sensitivity of 90% and a specificity of 92% for colonic dysplasia.

III. Time-resolved fluorescence: This method is based on measurement of fluorescence lifetimes rather than fluorescence intensities. Mycek et al have demonstrated that time-resolved fluorescence spectroscopy can differentiate adenomatous from nonadenomatous polyps with a high degree of accuracy.

IV. Light-scattering spectroscopy: Light-scattering spectroscopy is based on the detection of a period fine structure in the scattering spectrum of tissue that arises from singly scattered light. Scattered light that emerges from tissue consists of a large diffuse scattered background plus a fine structure component caused by nuclei in the epithelial cell layer. Thus, from the composition of the light-scattering signals, quantitative measures of nuclear enlargement, nuclear crowding and hyperchromasia can be determined.

Mourant et al suggested that CRC could be diagnosed with a sensitivity of 100% and a specificity of 98% using this method.

V. Chromoendoscopy and magnification endoscopy: Chromoendoscopy broadly refers to the addition of an extraneous substance to the surface of the gastrointestinal tract to enhance visualization of one or more features. The vast majority of used substances are chemical dyes that either react with elements within the mucosa or remain within small structures in the mucosal surface.

In the colon, methylene blue staining has been found to be useful to diagnose flat adenoma and carcinoma and to distinguish hyperplastic from adenomatous polyps. Contrast-enhancing dyes are often used together with magnifying endoscopes. The dye is usually sprayed on the mucosa with a special catheter or it can also be injected by the patient. The most commonly used dyes are indigo carmine and cresyl violet. The method has been used mainly for diagnosis of flat adenomas and colonic cancers with a sensitivity of 92% and a specificity of 93%.

Takayama et al used a magnifying endoscope of 40X power together with methylene blue dye, to examine aberrant crypt foci in the left colon and rectum. The number of aberrant crypt foci, their size and the presence of focal dysplasia as imaged by the magnifying endoscope were significantly correlated with the number of adenomas found in the studied patients.

REFERENCES

4. Jorgensen OD, Kronborg O, Fenger C. A randomised study of screening for colorectal cancer using faecal occult blood testing: results after 13 years and seven bienni-