ANNALS OF GASTROENTEROLOGY 2003, 16(3):230-234

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 visua-lized 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 1985[1] and the second most common cause of death from malignant disease[2]. 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 examination[3]. 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 controls[4]. 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 CRC[5,6].

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 mucosa[7]. 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 persons[8]. Isolated focal colonocytes can also be incorporated into assay systems using immunocytochemical methods or RT-PCR display of genes that are overexpressed by neoplasms[9].

Gastroenterology Unit, "Saint Savvas" Cancer Hospital, Athens, Greece

Author for correspondence:

D. Dimitroulopoulos, 35 Parnassou str., GR 152 34 Halandri, Athens-Greece, Tel. +30 210.6892460, e-mail: dimdim@otenet.gr

Neoplasm-specific DNA alterations have been well characterized and represent especially intriguing markers[10]. DNA appears to be stable in stool and amplification techniques permit detection of minute amounts of analyte[11]. Several investigators have recovered mutant DNA in stools from patients with cancer or large adenomas[12,13]. 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[14]. 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[15] BAT26 alone[16] and APC[17] also reported promising results.

As used in present-day practice, colonoscopy represends 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 encoscopic 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[18]. 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[19,20].

It can provide histology-equivalent images of the layers and structures of the gastrointestinal tract wall[21] 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[21,22,23]. 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[24]. 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[25]. 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[23].

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[20]. 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[27]. 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[28].

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[26].

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[29]. 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[30,31].

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[32]. Further studies showed that the method could differentiate normal colonic tissue from hyperplastic polyps and adenomas with an accuracy ranging from 94% to 100%[33,34]. 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[35,36]. 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[37]. A real-time imaging system based on the principle of ratio fluorescence imaging was developed for the gastrointestinal tract few years ago[38]. Using this system Haringsma et al reported a sensitivity of 96% and a specificity of 70% in the differentiation of dysplastic and non-dysplastic colonic lesions[29,39]. Other investigators also reported similar results[40,41].

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[34].

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[42].

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 heperchromasia 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[43].

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[44,45].

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%[46]. 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 sigificantly correlated with the number of adenomas found in the studied patients[47].

REFERENCES

1. Parkin DM, Pisoni P, Ferlay J, et al. Estimates of the worldwide incidence of eighteen major cancers in 1985. Int J Cancer 1993; 55:594-606.
2. Garfinkel L, Mushinski M. US Cancer incidence, mortality and survival: 1973-1996. Stat Bull 1999; 80:23-32.
3. Simo JB. Occult blood screening for colorectal carcinoma: A critical review. Gastroenterology 1985; 88:820-837.
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-

al screening rounds. Gut 2002; 50:29-32.
5. Mandel JS, Bond JH, Church TR, et al. Reducing mortality from colorectal cancer by screening for fecal occult blood. N Engl J Med 1993; 328:1365-1371.
6. Hardcastgle JD, Chamberlain JD, Robinson MHE et al. Randomised controlled trial of faecal-occult-blood screening for colorectal cancer. Lancet 1996; 348:1472-1477.
7. Ahlquist DA, Harrington JJ, Burgart LJ, et al. Morphometric analysis of the "molecular layer" overlying colorectal cancer and normal mucosa: relevance to exfoliated stool screening markers. Hum Pathol 2000; 31:51-57.
8. Loktionov A, O'Neill IK, Sylvester KR et al. Quantitation of DNA from exfoliated colonocytes isolated from human stool surface as a novel noninvasive screening test for colorectal cancer. Clin Cancer Res 1998; 4:337-342.
9. Chung KY, Mullick T, Motevalli S et al. Evaluation of sensitivity and specificity of CD44 expression in fecal colonocytes as a noninvasive marker for detecting colonic polyps and cancer. Gastroenterology 1998; 114:A580.
10. Boland CR, Sato J, Saito K, et al. Genetic instability and chromosomal aberrations in colorectal cancer: a review of current models. Cancer Detect Prev 1998; 22:377-382.
11. Poiner HN, Hofreiter M, Spaulding WG et al. Molecular coproscopy: dung and diet of the extinct ground sloth. Notheriops shastensis. Science 1998; 281:402-406.
12. Puig P, Urgell E, Capella G et al. A hghly sensitive method for K-ras mutation detection is useful in diagnosis of gastrointestinal cancer. Int J Cancer 2000; 85:73-77.
13. Villa E, Dugani A, Rebecchi AM et al. Identification of subjects at risk for colorectal carcinoma through a test based on K-ras determination in the stool. Gastroenterology 1996; 110:1346-1353.
14. Ahlquist DA, Skoletsky JE, Boynton KA et al. Colorectal cancer screening by detection of altered human DNA in stool: feasibility of a multitarget assay system. Gastroenterology 2000; 119:1219-1227.
15. Dong SM, Traverso G, Johnson C, et al. Detecting colorectal cancer in stool with the use of multiple genetic targets. J Natl Cancer Inst 2001; 93:858-865.
16. Traverso G, Shuber A, Olsson L, et al. Detection of proximal colorectal cancers through analysis of faecal DNA. Lancet 2002; 359: 403-404.
17. Traverso G, Shuber A, Levin B, et al. Detection of APC mutations in fecal DNA from patients with colorectal tumours. N Engl J Med 2002; 346:311-320.
18. Glaser F, Schlag P, Herfarth C. Endorectal ultrasonography for the assessment of invasion of rectal tumours and lymph node involvement. Br J Surg 1990; 77:883-887.
19. Huang D, Swanson EA, Lin DP et al. Optical coherence tomography. Science 1991; 254:1178-1181.
20. Izatt JA, Kulkami ME, Wang HW et al. Optical coherence tomography and microscopy in gastrointestinal tissues. IEEE J Select Topics Quant Electr 1996; 2:1017-1028.
21. Mallery S, Van Dam J. Endoscopic practice at the start of the new millenium. Gastroenterology 2000; 118:129-147,
22. Brezinski ME, Tearney GJ, Boppart SA et al. Optical biopsy with optical coherence tomography feasibility for surgical diagnostics. J Surg Res 1997; 71:32-40.
23. Tadrous PJ. Methods for imaging the structure and function of living tissues and cells: I. Optical coherence tomography. J Pathol 2000; 131:115-119.
24. Schmitt JM, Knuttel A, Bonner RF. Measurement of optical properties of biological tissues by low-coherence reflectometry. Appl Optics 1993; 32:6032-6042.
25. Bouma BE, Tearney GJ, Compton CG et al. High resolution imaging of the human esophagus and stomach in vivo using optical coherence tomography. Gastrointest Endosc 2000; 51:467-474.
26. Tearney GJ, Brezinski ME, Southern JF et al. Optical biopsy in human gastrointestinal tissue using optical coherence tomography. Am J Gastroenterol 1997; 92:1800-1804.
27. Sergeev AM, Gelikonov VM, Gelikonov GV et al. In vivo endoscopic OCT imaging of precancer and cancer states of human mucosa. Optics Express 1997; 1:432-440.
28. Kobayashi K, Izatt JA, Kulkarni MD et al. Cross-sectional imaging of colon cancer and ulcerative colitis using optical coherence tomography. Gastrointest Endosc 1998; 47:AB33.
29. Haringsma J, Tytgat GN. The value of fluorescence techniques in gastrointestinal endoscopy. Better than the endoscopist's eye? I. The European experience. Endoscopy 1998; 30:416-418.
30. Zonios GI, Cothren RM, Arendt JT et al. Morphological model of human colon tissue fluorescence. IEEE Trans Biomed Engineering 1996; 43:113-122.
31. Bottiroli G, Croce AC, Locatelli D et al. Natural fluorescence of normal and neoplastic human colon: a comprehensive ex vivo study. Lasers Surg Med 1995; 16:48-60.
32. Richards-Kortum R, Rava R, Petras RE et al. Spectroscopic diagnosis of colonic dysplasia. Photochem Photobiol 1991; 53:777-786.
33. Schomacker KT, Frisoli JK, Compton CC et al. Ultraviolet laser-induced fluorescence of colonic tissue: basic biology and diagnostic potential. Lasers Surg Med 1992; 12:63-78.
34. Wang TD, Van Dam J, Crawford JM et al. Fluorescence endoscopic imaging of human colonic adenomas. Gastroenterology 1996; 11:1182-1181.
35. Schomacker KT, Frisoli JK, Compton CC et al. Ultraviolet laser-induced fluorescence of colonic polyps. Gastroenterology 1992; 102:1155-1160.
36. Cothren RM, Sivak MV Jr, Van Dam J et al. Detection of dysplasia at colonoscopy using laser-induced fluorescence; a blinded study. Gastrointest Endosc 1996; 44:168-176.
37. Lam S, McAulay C, Hung J et al. Detection of dysplasia and carcinoma in situ with a lung imaging fluorescence endoscopic device. J Thorac Cardiovasc Surg 1993; 105:1035-1040.
38. Abe S, Izuishi K, Tajiri H et al. Correlation of in vitro autofluorescence endoscopy images with histopathologic findings in stomach cancer. Endoscopy 2000; 32:281-286.
39. Haringsma J, vanlerland-van Leeuwen, Tytgat GNJ. Endoscopic localization of dysplasia using laser induced fluorescence. Gastroenterology 1997; 112:A576.
40. Duvall GA, Saidi R, Kkost J et al. Real-time light induced

fluorescence endoscopy in the gastrointestinal tract. Gastrointest Endosc 1997; 45:AB28.
41. Yano H, Iishi H, Tatsuta M. Diagnosis of early gastric cancers by endoscopic autofluorescence imaging system. Endoscopy 1996; 28:S29.
42. Mycek MA, Schomacker KT, Nishioka NS. Colonic polyp differentiation using time-resolved autofluorescence spectroscopy. Gastrointest Endosc 1998; 48:390-394.
43. Mourant JR, Boyer JD, Johnson TM. Detection of gastrointestinal cancer by elastic scattering and absorption spectroscopy with the Los Angeles Biopsy System. SPIE 1995; 2387:210-217.
44. Axelrad AM, Fleischer DE, Geller AJ et al. High-resolution chromoendoscopy for the diagnosis of diminutive colon polyps: implications for colon cancer screening. Gastroenterology 1996; 110:1253-1258.
45. Kudo S, Kashida H, Nakajima T et al. Endoscopic diagnosis and treatment of early colorectal cancer. World J Surg 1997; 21:694-713.
46. Kiesslich R, von Bergh M, Hahn M et al. Chromoendoscopy with indigocarmine improves the detection of adenomatous and nonadenomatous lesions in the colon. Endoscopy 2001; 33:1001-1006.
47. Takayama T, Katsuki S, Takahashi Y et al. Aberrant crypt foci at the colon as precursors of adenoma and cancer. N Engl J Med 1998; 339:1277-1284.