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The Application of Occult Blood Tests in the Early Screening of Digestive Tract Diseases

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Abstract. The fecal occult blood test is used to screen for diseases of the digestive tract, especially the key non-invasive methods for colorectal cancer. This article systematically sorts out the detection principle and its measurement methods, clarifying the characteristics and applicable scopes of the two main measurement methods of the chemical method and the immune method. The chemical method is based on the peroxidase activity of hemoglobin, its operation is handy but easily disturbed by diet and medication, and it has low sensitivity to upper gastrointestinal hemorrhage. The immunization method is represented by the double antibody sandwich method, which involves detection conducted through specific anti-human hemoglobin antibodies, and significantly overcomes the interference problem of chemical methods. The result of positive test usually points to lower gastrointestinal hemorrhage which is currently the preferred method for early screening. Neither of the two methods can be used as the final diagnostic basis for it needs to be identified through endoscopic examinations, such as colonoscopy, to determine the cause if positive results. In general, FOB test is especially highly specific immunotherapy method that can be found in early detection of digestive tract diseases, it can reduce mortality and guide subsequent diagnosis and treatment. However, the immune method may also have false negative results, so the chemical method is also an indispensable part of the FOB test, cross-use of them can maximize the success rate of the test.

Keywords: Fecal occult blood, Diseases of the digestive tract, Chemical method, Immunization method, Early screening

1. Introduction

The FOB test is the indispensable importance for the diagnosis and treatment of digestive tract diseases. It is not only the key of non-invasive screening tool for the early detection of digestive tract cancers, especially colorectal cancer, but also can assist doctors in preventing some malignant tumors that develop from precancerous lesions, such as adenomatous polyps [1, 2]. With the development of detection technology, selecting screening methods with higher specificity and less interference has become the core issue for improving the early diagnosis rate. This article aims to review the clinical significance of FOB detection and, systematically, meanwhile expound the principles, characteristics and representative research cases of the two mainstream detection methods.

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2. Occult blood and its detection purposes

Occult blood refers to a microbleed in the digestive tract that cannot be identified by the eyes. It is difficult to directly observe red blood cells with a conventional microscope. The main purpose of the test is to screen individuals who may have gastrointestinal bleeding during the asymptomatic or mild symptom stage, and then confirm the root cause through colonoscopy and endoscopy. Especially for the detection of colorectal cancer and its precancerous lesions, so as to achieve early diagnosis and treatment.

3. The clinical significance of early diagnosis

Early diagnosis of occult blood can provide assistance for the early treatment of various diseases of the digestive tract. The 5-years survival rate for early-stage (stage I/II) colorectal cancer can reach over 90%, but for advanced (stage IV) colorectal cancer, the survival rate drops sharply to around 10% after metastasis occurs. Early-stage cancer can be minimally invasively removed through colonoscopy, featuring minimal trauma, quick recovery, low cost, and usually no need for adjuvant chemotherapy or radiotherapy.

For colorectal polyps, most colorectal cancers develop from adenomatous polyps over a period of 5 to 10 years. Detecting and removing polyps through colonoscopy at the polyp stage is the most effective secondary measure of prevention for cancer. For inflammatory bowel disease, early diagnosis and standardized treatment can effectively control intestinal inflammation and can prevent or delay the occurrence of serious complications such as intestinal stenosis、 perforation and becoming cancerous. For peptic ulcers, early diagnosis and treatment can promote ulcer healing and prevent life-threatening emergencies such as massive gastrointestinal bleeding and perforation. Meanwhile, early biopsy can distinguish the benign and malignant nature of ulcers, if it’s early gastric cancer, it can provide a good opportunity for early treatment.

4. Minnesota test

The Minnesota test is a landmark, large-scale, randomized controlled trial that provides conclusive evidence for the effectiveness of fecal occult blood testing as an early screening tool for colorectal cancer [3]. They recruited 46,551 participants aged 50 to 80 from 1975 to 1977 and randomly divided them into three groups for studying: the annual screening group, the biennial screening group, and the control group [3]. The screening method was to use three consecutive guaiac-impregnated paper (Hemoccult) tests on fecal samples, and approximately 83% of the samples in the study were rehydrated before testing to enhance sensitivity. Those who test positive are recommended to undergo diagnostic evaluations, including colonoscopy [3]. The primary endpoint of the study was the cumulative mortality rate of colorectal cancer during the period of 13 years of follow-up [3].

The research results clearly demonstrate the significant benefits of conducting a fecal occult blood screening per year [3]. After 13 years of follow-up, the cumulative mortality rate of colorectal cancer was 5.88 per thousand people by annual screening group, significantly lower than the control group, which 8.83 per thousand people, with a reduction of 33%. The mortality rate of the biennial screening group (8.33 per thousand people) showed a downward trend compared with the control group, but it was not as significant as that of the annual screening group [3]. The clinical basis for this reduction in mortality rate lies in the early detection of cancer. The stages of colorectal cancer detected in the annual screening group were significantly earlier [3]. Specifically, the number of

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advanced cancers in the control group was twice that of the annual screening group (65 cases versus 33 cases), while the five-year survival rate of advanced patients was extremely low (only 2.4%), while that of early-stage patients was as high as 94.3%. They confirmed that the survival rate of cancer patients identified through screening was significantly higher than that of patients not identified through screening by survival analysis [3].

The discussion section delved deeply into the significance of these findings. Firstly, the consistency among the reduction in mortality rate、 the improvement in survival rate and the advancement of cancer staging strongly demonstrates that screening through early detection and intervention changed the natural course of the disease [3]. Secondly, although sample rehydration significantly enhanced the sensitivity of the test (from 80.8% to 92.2%) and the positive rate, it also reduced specificity, leading to an increase in false positives and a rise in the volume of colonoscopy examinations [3]. The benefits of screening need to be weighed against the increased costs and consumption of medical resources [3]. In addition, the increase in polyp removal rate in the screening group during the study period may have a potential positive impact on the long-term cancer incidence rate, although this effect has not been fully manifested during this analysis period [3].

In conclusion, this study provides crucial evidence for the core role of fecal occult blood testing in the early screening of colorectal cancer. It has been confirmed that annual screening can effectively reduce the mortality rate of colorectal cancer by approximately one-third. The core mechanism lies in moving forward the "window" of cancer detection, identifying the disease at an earlier and more curable stage [3]. This case clearly demonstrates the public health value of early screening strategies is in improving the prognosis of digestive tract cancers and in reducing the disease burden.

5. Testing method of fecal occult blood

5.1. Chemical method

The core principle of the measurement is to utilize the peroxidase-like activity of heme in hemoglobin. This activity can catalyze the decomposition of hydrogen peroxide, releasing nascent oxygen, which in turn oxidizes colorless substrates (such as gum guaiac), which showed blue or green [2]. The equipment and operation process: Taking the test strip method as an example, the detection area of the test strip, which is the T area, is pre-fixed with gum guaiac and urea peroxide. During the test, the fecal sample is applied to the sample area. After adding the buffer chromogenic solution, the hemoglobin in the sample reacts with the reagent, causing a color change in the T zone. The interpretation time costs 1 to 2 minutes. If the diagnosis result is positive, it indicates that there may be bleeding in the digestive tract, but the location of the bleeding cannot be distinguished. Upper gastrointestinal bleeding may lead to false negatives due to the degradation of hemoglobin by digestive enzymes. Lower gastrointestinal bleeding, such as colorectal cancer and polyps, is relatively easy to detect. The main limitations lie in the interference of food and drugs. For instance, red meat containing animal hemoglobin, some vegetables and fruits containing plant peroxidase and iron agents can lead to false positives, and large doses of vitamin C can cause false negatives due to their reducing properties [4, 5]. Secondly, the limited sensitivity of chemical methods may lead to low sensitivity to degraded hemoglobin in upper gastrointestinal bleeding, and intermittent bleeding may be missed. In addition, uneven samples or improper storage can affect the accuracy of the results.

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The core innovation of the chemical fecal occult blood test lies in its pioneering nature. As a colorectal cancer screening tool which be introduced over 30 years ago, it simplifies one kind of complex laboratory test (detecting the peroxidase activity of hemoglobin in feces) into a card test that can be done at home. Its greatest historical contribution lies in making large-scale, low-cost and non-invasive screening of colorectal cancer populations possible for the first time, laying the foundation for the basic model of fecal screening.

In the study "Randomized Comparison of Chemical and Immunological Fecal Occult Blood Tests for Colorectal Cancer in Screened Populations", it was found that the relative proportion of false positives detected by colonoscopy in the population determined positive by the chemical method was slightly lower [6]. This proves a traditional and long-term application advantage of the chemical method, which is relatively conservative and can to some extent, reduce the unnecessary volume of colonoscopy examinations. Meanwhile, its false positive rate was not statistically different from that of the immunoassay, indicating that in the false positive results, the results of the two methods were almost the same [6, 7]. However, the severe deficiency of chemical method sensitivity leads to its positive rate and detection rate being much lower than those of immunoassay [6-8]. The research conclusion clearly indicates that the chemical method seriously underestimates the true prevalence of advanced adenomas and cancers in the screened population, which exposes its most fundamental limitations, namely the methodological non-specificity (reacting to peroxidase in animal blood and certain foods) and the inability to achieve quality control [6].

5.2. Immunization method

The core measurement principle is specific to the binding of antigens and antibodies. This method uses specific monoclonal or polyclonal antibodies against human hemoglobin (rather than its enzymatic activity) for detection, and thus is not interfered with by non-human hemoglobin or peroxidase. For taking the double antibody sandwich method as an example, dilute the fecal sample and add it to the addition area of the test strip. The human hemoglobin in the sample binds to the colloidal gold-labeled antibody on the binding pad to form a complex. When the complex is chromatographic to the detection line (T line), it is captured by another fixed capture antibody, forming an "antibody-antigen-antibody" sandwich structure, which aggregates gold-labeled particles and shows color. The quality control line (C line) is used to verify the effectiveness of the testing process. Because human hemoglobin is completely degraded and inactivated by gastric acid and protease when passing through the upper digestive tract, a positive immunoassay result, both the T line and the C line on the test strip show positive color, usually indicates bleeding in the lower digestive tract (ileocecal region and below), which is commonly seen in colorectal cancer、polyps、 inflammatory bowel disease、 diverticular disease and hemorrhoids etc. The advantage of the immunization method lies in its high specificity and its immunity to interference from diet and vitamin C. No special dietary preparation is required, and patients have good compliance. It has a high sensitivity to lower gastrointestinal bleeding. The limitation of the immunoassay is that it usually cannot detect upper gastrointestinal bleeding. Extremely high concentrations of hemoglobin may cause the "hook effect", resulting in false negatives (modern techniques have reduced this phenomenon), or improper sample handling (such as long-term storage) may affect antigen stability [5].

The core innovation of the immunization method lies in its targeting and quantifiability. Unlike the chemical method for detecting "peroxidase activity", the immunoassay uses specific antibodies and only targets the globulin portion of human hemoglobin for detection. This fundamental innovation has brought about multiple breakthroughs. The immunization method has completely

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gotten rid of the trouble of dietary restrictions. And on this basis, the measurement of occult blood is quantified and simplified, which can be used for large-scale detection and greatly improves the detection efficiency [9].

In the study, it was found that the most direct advantage of the immunization method is that compared with the chemical method, patients can complete the test in a relatively independent situation, and its detection efficiency is significantly improved [9]. Moreover, the positive rate of the chemical method is higher. In the intention-based screening analysis, the detection rate of advanced adenomas and cancers is significantly higher than that of the chemical method [6-9]. This demonstrates the core advantage of the immunological method. Without significantly sacrificing the positive predictive value, it significantly enhances the overall sensitivity of the screening program, thereby more accurately assisting in the diagnosis of digestive tract diseases. The primary issue with immunological methods also stems from the limitations brought about by their high sensitivity - the specificity for cancer is relatively reduced. Therefore, the use of immunological methods would significantly require additional colonoscopy, increasing the burden on the medical system and causing inconvenience to patients with false positives [7]. Secondly, although quantifiability is an advantage, it also places higher requirements on the laboratory equipment and standardized operations of testing institutions.

5.3. Comparison of the two methods

Early screening for colorectal cancer is of vital importance. FOB testing is a commonly used screening method, mainly including chemical fecal occult blood detection and immune fecal occult blood examination. This section is based on a systematic literature review that compares the accuracy of the two in screening colorectal cancer and advanced tumors (including cancer and advanced polyps).

In terms of testing methods, two samples need to be collected from three consecutive defecations and applied to six test cards for the chemical method of fecal occult blood detection, the result is judged through a chemical color change reaction. Positive criteria vary by protocol (usually one color change requires further examination) [7]. The immunological fecal occult blood test only requires the collection of one sample from a single defecation, which is sent for examination in a dedicated container. The positive result is determined by measuring the hemoglobin concentration and based on a preset threshold [7]. Based on the summary and analysis of data from 63 studies and nearly 4 million people, it is assumed that among 10,000 screened individuals [7]. If there are 100 colorectal cancer patients among them, 24 cases will be missed by the immunoassay fecal occult blood test, and 61 cases will be missed by the chemical assay fecal occult blood test [7]. If there are 1,000 cases of large polyps or colorectal cancer among them, 670 cases will be missed by the immunoassay fecal occult blood test and 850 cases will be missed by the chemical assay fecal occult blood test [7]. Both tests led to approximately 594 people undergoing unnecessary colonoscopy (i.e., positive results but not colorectal cancer) [7].

Based on the above data analysis, in population screening, the immunoassay fecal occult blood test has a higher detection sensitivity for colorectal cancer (with a lower rate of missed diagnosis) compared to the chemical method fecal occult blood test. Moreover, when the false positive rate is similar, its detection ability for advanced lesions is also better. Therefore, the immunoassay fecal occult blood test has more advantages in screening performance [10].

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6. Conclusion

Fecal occult blood testing is the cornerstone for the early diagnosis and intervention of digestive tract diseases, especially colorectal cancer. This article systematically expounds the clinical value of early detection and compares the principles and characteristics of the two mainstream techniques, which are the chemical method and immunization method. Chemical methods (for those verified by the Minnesota Laboratory) laid the foundation for fecal screening to reduce mortality, but their inherent interference problems limit their accuracy. Immunotherapy with its high specificity and convenience, has become the first choice for current screening practices.

Looking ahead, fecal occult blood testing technology will continue to develop in a more accurate and convenient direction. For instance, quantitative immunization methods can balance sensitivity and specificity by setting different thresholds to meet the screening needs of people at different risks. In addition, new technologies such as multi-target fecal DNA testing are emerging. However, no matter how advanced the technology becomes, the core positioning of fecal occult blood testing remains unchanged. It has always been a crucial primary screening tool, and its results are important data that it to help prevent cancer and assist in diagnosing various digestive tract diseases. To ultimately achieve the core goal of reducing the mortality rate of digestive tract diseases and improving public health, it is necessary to enhance public awareness of screening, optimize screening strategies, and, on this basis, combine diagnostic techniques such as endoscopy.

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