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Mini-review

Blood autoantibodies against tumor-associated antigens as biomarkers in early detection of colorectal cancer

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ABSTRACT

Multiple studies have shown that cancer patients produce detectable autoantibodies against certain tumor-associated antigens, which might be promising blood biomarkers for early detection of colorectal cancer (CRC). We aimed to provide an overview of published studies on blood autoantibody markers for early detection of CRC and to summarize their diagnostic performance. A systematic literature search was performed in PubMed, ISI Web of Knowledge and EMBASE to find relevant studies published until 23 July 2013. Relevant information, such as study population characteristics, autoantibodies studied, analytical methods and diagnostic performance characteristics was independently extracted by two reviewers. Overall, 67 studies evaluating 109 autoantibody markers were included. Most individual markers showed low sensitivity (below 25%) for detecting CRC, along with high specificity close to 100%. Occasionally reported higher sensitivities for specific antibodies are yet to be replicated in independent studies. Generally, more promising results were seen for combinations of multiple autoantibody markers. But again, these promising results are yet to be replicated in other samples. In conclusion, autoantibody signatures may become a promising approach to noninvasive CRC screening. Optimized marker panels are yet to be developed, and promising results require validation in large screening populations.

1. Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in men and the second in women worldwide [1]. Because of its slow progression from detectable and curable precancerous lesions and the strong dependence of prognosis on stage at diagnosis, early detection of CRC has great potential to reduce the burden of this disease. CRC and its precursors can be most reliably detected by colonoscopy, and in the distal colon and rectum also by sigmoidoscopy. However, use of these invasive procedures is limited by available resources, costs and low compliance [2,3]. Furthermore, sigmoidoscopy and colonoscopy are essentially "unnecessary" (i.e., they do not provide a real benefit) for the majority of people who do not carry any colorectal neoplasms. Therefore, noninvasive tools reliably identifying those at highest risk would be highly desirable but are yet to be developed. Established noninvasive tests, such as guaiac based fecal occult blood tests (gFOBT), suffer from low sensitivity [4]. Also, stool tests might be less accepted than blood tests in population-based screening. Therefore, there is a need for new biomarkers, ideally blood based markers, which could reliably identify early CRC and its precursors, and aid in the selection for colonoscopy only for those who will most likely benefit from it.

In recent years, an increasing number of studies has shown that cancer patients produce antibodies against certain tumor-associated antigens (TAAs) that are detectable in the blood [5–7], suggesting that these antibodies might be useful for cancer screening. In particular, TAAs could be identified even at low levels by the humoral immune system and autoantibodies against TAAs could be detected even at early stages of cancer development [8–10]. Meanwhile, numerous studies have evaluated diagnostic performance of autoantibodies against TAAs for early detection of CRC. The aim of this systematic review is to provide an overview of published studies on blood autoantibodies for early detection of CRC, and to summarize their diagnostic performance.

2. Methods

2.1. Search strategy

A systematic literature search was performed to identify studies assessing blood autoantibodies as biomarkers in early detection of CRC. PubMed, ISI Web of Knowledge and EMBASE databases were searched for eligible articles until 23 July 2013. The following combination of keywords was used: [colorectal (or) colon (or)

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rectum] (and) [cancer (or) neoplasm (or) carcinoma (or) adenoma (or) malignancy] (and) [autoantibodies (or) antibodies] (and) [detection (or) diagnosis] (and) [serum (or) blood (or) plasma]. After getting the initial search result, duplicate articles were deleted. A first round of selection was based on reviewing the titles and abstracts. A second round was based on full-text review. In the latter round, cross-referencing was additionally used as a possible source for identifying studies related to the topic.

2.2. Eligibility criteria

The search was limited to studies on humans published in English. Only full-text reports of original studies were included, because sufficiently detailed information could not be extracted otherwise. Studies without cancer-free controls were excluded. In addition, studies focusing on markers other than autoantibody markers were also excluded. If information regarding diagnostic performance for detection of CRC (i.e., sensitivity, specificity and statistical test results) was not reported or could not be calculated from published results, studies were excluded as well. We only included studies focusing on autoantibody markers against a priori defined targets. Therefore, we also excluded studies if the findings were based on tumor cDNA expression library-based serological techniques (e.g., SERPA, SEREX) only but were not verified in other independent immunoassays.

2.3. Data extraction and statistical analysis

Studies fulfilling the above-mentioned criteria were included in the data extraction procedure which was performed by two independent investigators (HC and SW) using a standardized data extraction form. Any initial disagreement was resolved by further review and discussion among the authors. Information extracted included study population characteristics (number and age of study participants, country and setting), stage distribution of cases, and indicators of diagnostic performance. For studies which defined a cutoff value of blood autoantibodies to distinguish between positive and negative test results, overall and stage specific (where applicable) sensitivity, and overall specificity were selected as main indicators of diagnostic performance, along with results of statistical tests to examine differences in blood autoantibody levels between cases and controls. Furthermore, the area under the receiver operating characteristic curve (AUC) was extracted for quantitative tests when reported. Because stage specific results were reported using different classification schemes (TNM, Dukes, Aster-Ciller, UICC), all stage information was translated into the UICC classification to enhance comparability. Exact 95% confidence intervals of sensitivity and specificity were calculated by applying Fisher's exact test through statistical software R (version 3.0.1) when not reported in studies.

3. Results

3.1. Literature search result

The initial search yielded 3493 articles using the above-described search terms (Fig. 1 and Supplementary Table 1). After removal of 319 duplicate articles, titles and abstracts of 3174 articles were carefully reviewed. Eighty-two articles seemed to be potentially relevant and underwent full-text review. Fifteen articles were excluded for the following reasons: no inclusion of controls ($n = 7$), studies focusing on markers other than autoantibodies ($n = 4$), no information regarding diagnostic performance provided ($n = 3$) and markers discovered based on the SEREX (serological analysis of recombinant cDNA expression libraries) method only without verification by other independent immunoassays ($n = 1$). Finally, 67 studies met the inclusion criteria and were included in this review. Information regarding sensitivity and specificity was reported or could be calculated in 63 studies. In 4 studies, only the results of statistical tests examining the differences of blood autoantibody levels between cases and controls were reported.

3.2. Study population characteristic

An overview on the studies, their characteristics and results are given in Supplementary Tables 2 and 3. Studies were mostly carried out in East Asia (e.g., 27/67 studies were from China, 8/67 were from Japan), Europe (e.g., 4/67 studies were from Germany, 2/67 were from France) and North America (e.g., 14/67 studies were from USA and 1/67 were from Canada). Patient recruitment was done in hospitals in 61 studies. Colorectal cancer patients ($n = 45$), colon cancer patients ($n = 21$) and rectal cancer patients ($n = 1$) were selected as cases. Case numbers ranged from 15 [11] to 1068 [12] across studies. Tumor stage information was reported in 23 studies. Most studies used healthy controls. However, one study also included patients with colorectal polyps [13], two included patients with benign diseases [14,15], and two used both healthy individuals and patients with benign disease [16,17] as controls. Seven studies matched cases and controls. Matching was done by age and gender [18–21], age only [22,23] and ethnicity and geographic location [24] in 4, 2, and 1 studies, respectively.

3.3. Autoantibody detection methods

Various techniques were used for quantitative detection of blood autoantibodies (Supplementary Tables 2 and 3). ELISA (Enzyme-linked immunosorbent assay, $n = 46$) and Western blot ($n = 10$) were the most frequently used methods for detecting autoantibodies. Newly emerging techniques, which could screen for and detect large numbers of autoantibodies simultaneously, were also used. For example, Babel and colleagues [25] applied a protein microarray to screen 8000 proteins simultaneously and identified six autoantibody markers individually showing the best discrimination capacity between CRC patients and healthy controls. A combination of three individual autoantibody markers yielded the highest diagnostic efficacy, with sensitivity, specificity and AUC of 84%, 71% and 0.85, respectively. In addition, there were also several studies applying other methods, such as phage display, peptides microarray, etc.

3.4. Types of TAAs

Apart from full-length protein antigens selected as the targets of autoantibody markers in most of studies, peptide antigens [23], phage-peptide antigens [19,26,27] and glycopeptide antigens [18] were also chosen. For example, in one study by Pedersen et al. [23] in 2013, the diagnostic potential of autoantibodies against seventy-eight different 15-mer p53 peptides representing the whole p53 protein were evaluated. In another study by Ran et al. [27] in 2008, a panel of six phage-peptide antigens was evaluated.

3.5. Diagnostic characteristics of autoantibody markers

Overall, 10 markers were examined in multiple studies (Table 1). The most commonly assessed markers were autoantibodies against p53 (21 studies) and c-myc (5 studies). Great discrepancy in sensitivity and specificity was observed for these markers across studies, possibly due to use of different cutoffs. For example, the sensitivity of p53 antibodies in detection of CRC ranged from 9% to 46% with specificity ranging from 90% to 100% in 21 studies.

Additional 96 markers were only examined in a single study. They are shown in Table 2 and ordered by reported sensitivity. The majority of these markers (57/96, 59.4%) showed relatively low sensitivity (<25%). Furthermore, the markers with the highest sensitivity (>70%) had rather poor specificity (46–84%). AUC values were reported for 31 markers; levels $\geqslant 0.60$ were found for 15 markers only.

Fig. 2 presents an overview of the diagnostic performance of all markers assessed so far. The horizontal axis represents the false - positive rate (100% – specificity), and the vertical axis represents sensitivity. In general, higher sensitivity went along with lower specificity. Nevertheless, for a few markers, relatively high sensitivity (>60%) were reported along with relatively high specificity (>80%). These include antibodies against the sperm-associated antigen 9 (SPAG9), rabphilin-3A-like protein (RPH3AL), coiled-coil domain containing 83 (CCDC83) and CEA.