Review paper

Markers of metastatic colorectal cancer

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Gastroenterology Rev 2020; 15 (2): 94–97
DOI: https://doi.org/10.5114/pg.2019.84848

Key words: colorectal cancer, metastasis, circulating tumour DNA.

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Abstract

Metastatic colorectal cancer (CRC) is a major cause of cancer-related death. However, early diagnosis of CRC metastases offers a chance of long-term survival in as much as 40% of patients after curative treatment. Current guidelines are based on clinical examination, carcinoembryonic antigen (CEA) testing, computed tomography scanning, and endoscopic surveillance. Although CEA is the most widely used laboratory test, it has very low sensitivity (30–40%). Moreover, there is no evidence to support the association of CEA testing with improved survival or quality of life. Thus, novel markers with greater specificity and sensitivity are needed. The aim of this review was to define the role of available laboratory markers in early diagnosis of metastatic CRC. We identified novel tests with the highest association to metastatic CRC: circulating tumour DNA, growth/differentiation factor 15, and β6-integrin. We also discuss other promising markers, although most of the studies are preliminary and require validation.

Introduction

Metastatic colorectal cancer (CRC) is a major cause of cancer-related death [1–4]. Resection of limited metastases offers 10-year survival in up to 38% of patients [5]. Moreover, recent development of minimally invasive techniques has reduced the morbidity and improved the cost-effectiveness of liver surgery, similarly to other types of procedures [6, 7]. Thus, early detection of metastatic CRC is of utmost importance for patients who are candidates for surgery [5]. Current guidelines recommend intensive surveillance for the first 5 years after curative treatment of stage II and III CRC. They are based on clinical examination, carcinoembryonic antigen (CEA) testing, computed tomography (CT) scanning, and endoscopic surveillance [1, 2]. Intensive follow-up (every 3–6 months for the first years after primary CRC operation) was shown to be on average three times better in diagnosing resectable CRC metastases than minimum follow-up based on symptoms [8]. Because laboratory tests seem more convenient and objective than clinical examination and carry less risk than CT scanning or endoscopy, in this paper we concentrate on laboratory markers of CRC. Most of them are not useful in differentiation between local recurrence and distant metastases. Hence in this study we focus on markers with the strongest association with metastatic CRC, especially useful in surveillance after surgical treatment. All the markers presented in the study have the potential to detect synchronous or metachronous metastases.

Proteins

Most professional guidelines on surveillance after resected colon and rectal cancer include CEA testing every 3–12 months for the first 3–5 years [1, 2]. This is based on initial studies showing that an increase in CEA levels often precedes the diagnosis using other methods. However, CEA was shown to have very low sensitivity (Table I). Shinkins *et al.* [9] demonstrated that with the threshold of 5 µg/l CEA testing achieves at best 50% of detected CRC recurrences. At the same time, false alarms would be present in 56.7% of patients, reflecting the low specificity of CEA assessment. Lowering the CEA threshold to 2.5 µg/l would reduce the percentage of missed recurrences to 36.5 at the cost of 84.2% of false alarms. The authors concluded that not the single result, but the trend of consecutive CEA measurements should be the basis of clinical decision making [10]. Intensive CEA monitoring has the potential to select a group of patients who could benefit from recurrence treatment [10]. Notwithstanding, this strategy has not been proven to result in survival benefit [11].

Table I. Data on circulating markers of colorectal cancer with potential to detect metastases. Only data with specificity and/or sensitivity > 90% or strong association with metastases were selected from literature

Marker	Material	Sensitivity (%)	Specificity (%)	Association with metastases	Number of patients	Type of the study	Reference
CEA	Serum	50	93.3	Poor	104	Prospective	[9]
CA 19-9	Serum	23	96	Poor	Nd	Meta-analysis	[13]
CA 11-19	Serum	98	84	Nd	131	Prospective	[13]
β6-integrin	Serum	69.8	100	Strong	269	Retrospective	[12]
DC-SIGNR	Serum	94.8	98.7	Moderate[1]	242, 81[1]	Prospective	[13, 15[1]]
GDF15/MIC1	Serum	43.8	96.7	Strong	473	Meta-analysis	[13]
Interleukin-8	Nd	70	91	Nd	725	Meta-analysis	[13]
ctDNA	Plasma	Nd	Nd	Strong	101	Prospective	[18]
SDC2 methylation	Serum	87	95.2	Nd	131	Prospective	[13]
methylated SEPT9	Nd	71	92	Nd	2975	Meta-analysis	[13]
ALU115 of cfDNA	Serum	69.2	99.1	Nd	104	Prospective	[13]
lncRNA NEAT1_V2	W. blood	70	96	Nd	100	Prospective	[13]
mi-155 microRNA	Serum	58.2	95	Moderate	146	Retrospective	[13]
mi-1290 microRNA	Serum	70.1	91.2	Moderate	211	Retrospective	[13]

CEA – carcinoembryonic antigen, nd – not discussed, [1]data from publication [14], ctDNA – circulating tumour DNA, cfDNA – circulating free DNA, lncRNA – long non-coding RNA, w. blood – whole blood.

β6-integrin has been recently reported as a novel promising serum marker of CRC. A cut-off value of ≥ 2 ng/ml had 100% specificity for metastatic CRC (Table I). In a study cohort of 269 CRC patients, β6-integrin predicted the onset of metastatic disease. In the same study, the results were prospectively confirmed in 40 CRC patients [12].

CA 11-19 is a glycoprotein marker of early CRC and adenomatous polyps (Table I). Data about association between CA 11-19 and metastatic CRC is lacking [13].

Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), also known as CD209 (cluster of differentiation 209), belongs to the group of lectins, found on the surface of dendritic cells. DC-SIGN can initiate recognition of tumour cells. Decreased serum DC-SIGN was associated with stage I/II colon cancer and short survival. Notwithstanding, DC-SIGN does not seem to be a marker of metastatic CRC [13, 14]. Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin-related protein DC-(SIGNR) is an analogue of DC-SIGN found on endothelial cells in the liver and lymph nodes [15]. In contrast to DC-SIGN, DC-SIGNR has been reported to increase in stage I/II CRC [13, 14]. It has been demonstrated in a mouse model that DC-SIGNR plays a role in hepatic metastasis of CRC. Knocking down DC-SIGNR decreased the liver metastatic potency of CRC and increased survival, while expressing DC-SIGNR enhanced CRC liver metastases. Moreover, in the same paper the authors revealed that patients with metastatic CRC had greater serum DC-SIGNR concentrations than those without metastases (Table I) [15].

Carbohydrates

Carbohydrate antigen 19-9 (CA 19-9) has low sensitivity (Table I). Admittedly, a strong correlation has been reported between CA 19-9 and nodal involvement of CRC, but not with metastases to liver or lungs [13, 16].

Nucleic acids

At present, tissue sampling remains the gold standard in the assessment of tumour genetic features [17]. A fraction of CRC DNA is shed into the blood, and sensitive methods to detect it have now been introduced. Assessment of circulating tumour DNA (ctDNA) has the potential to not only detect CRC recurrence, but also to identify its genetic or epigenetic features. Information about RAS or BRAF mutation, HER2 amplification, or microsatellite instability are especially valuable in order to tailor target treatment of metastatic CRC. With the advent of modern methods allowing the use of plasma for genetic testing, the term "liquid biopsy" was

introduced. Quick and less invasive, it also avoids the bias of tissue sampling, associated with the difficulty of obtaining sufficient material of good quality using fine needle aspiration [18]. A study on 27 patients treated for CRC revealed that all 14 subjects with elevated ctDNA had tumour relapse, whereas among 13 remaining patients with normal ctDNA no relapse was found [19]. Another prospective study on 45 patients with CRC showed significant association of detectable ctDNA with inferior relapse-free survival during 2 years of follow-up. The authors recommended ctDNA testing upon equivocal results of standard investigations [20]. Moreover, a strong correlation between ctDNA and liver metastases has been reported (Table I) [18].

Despite its potential utility for early diagnosis of metastatic CRC, ctDNA testing is associated with some disadvantages: limited evidence for treatment selection in advanced cancer and incomplete correlation with cellular phenotype or histology. To increase the sensitivity and specificity of ctDNA assessment, tests to detect multiple mutations during one study have been introduced [18]. So far, ctDNA assessment has not been validated for surveillance of patients with CRC. However, dynamic development in methodology and extensive studies might establish the role of ctDNA in this domain.

Another method of DNA assessment in blood is cell free DNA (cfDNA), the total concentration of DNA in serum. However, the association of cfDNA with CRC metastases is much weaker than for ctDNA [18].

The best studied marker in recent years was SEPT9 methylated DNA. Its sensitivity showed great variability (48.2–95.6%) and specificity (80–98.9%) (Table I). Until it becomes more repetitive, the clinical utility of SEPT9 methylated DNA remains doubtful. Other promising nucleic acid-based tests are the following: NEAT-v2 non-coding RNA, DC-SIGN/DC-SIGNR, SDC2 methylated DNA, ALU115 of circulating free DNA, mi-155, and mi1290. However, until they are studied in multicentre prospective trials, no clinical conclusions can be drawn [13].

Genetic information about CRC can also be obtained from microRNA (miRNA) analysis. miRNAs are stable, small, non-coding sequences of RNA, responsible for post-transcriptional control of gene expression and important for CRC proliferation, invasion, and metastasis formation [18, 21, 22]. However, microRNA testing has low to moderate (for mi-1290 and mi-155) power to discriminate metastatic advanced from early CRC (Table I) [13].

Cytokines

Growth/differentiation factor 15 (GDF-15) is a macrophage inhibitory cytokine (MIC-1) belonging to the transforming growth factor-β superfamily. GDF-15 testing sensitivity is low and comparable to CEA (Table I). However, in contrast to CEA, GDF-15 has been reported to correlate strongly with the extent of liver metastases of CRC, so it may be of use if/when confirmed in further studies [13].

Interleukin-8 is a chemokine, involved in the proliferation, angiogenesis, and migration of cancer cells. Interleukin-8 testing in patients with CRC has high specificity and sensitivity; however, its clinical utility remains to be established (Table I) [13].

Many other substances important for cancer and metastasis have been studied, such as interleukin-6, with no evidence of added benefit to the current guidelines [23, 24].

Perspectives

The invasiveness of a testing tool is an important factor influencing patients' adherence to guidelines. Saliva has been suggested as preferable to blood in terms of technical simplicity and potential diagnostic usefulness, demonstrated for assessment of miRNA: miR-21 [22, 25]. Preliminary urinary tests have also been performed, showing urinary ctDNA to be 90% concordant with tissue testing [26]. This compares favourably to blood testing and may open a new perspective in studies on CRC markers.

The metastatic potential of CRC is regulated by mechanisms involving several types of signalling, influencing cancer cell mobility, invasiveness, angiogenesis, intercellular adhesion, and lipid metabolism [27–30]. The search for novel metastatic CRC markers is being continued.

Conclusions

Tumour markers are an important element of intensive surveillance in patients after resection of early CRC. CEA remains the best documented diagnostic tool suggesting CRC recurrence; however, it has low sensitivity and specificity. To increase the value of CEA testing, a trend of consecutive measurements should be analysed rather than a single absolute concentration. Among novel markers, the strongest association with CRC metastases was reported for ctDNA, GDF15, and β6-integrin. Further prospective multicentre studies are needed to validate and define the clinical utility of metastatic CRC.

Conflict of interest

The authors declare no conflict of interest.

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Received: 24.03.2019
Accepted: 8.04.2019