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Review

The Role of Circulating Biomarkers in the Early Detection of Recurrent Colorectal Cancer Following Resection of Liver Metastases

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Abstract

On a global scale, colorectal cancer (CRC) is currently the fourth most commonly diagnosed cancer and despite progress in early diagnosis and treatment has the third highest mortality. Patients with oligometastatic disease to the liver may be suitable for liver resection with a curative intent. A sustained progress in perioperative management and surgical techniques, including staged liver resections, has increased the number of patients who may be offered hepatectomy. It is well recognised that early detection of any tumour, including recurrence, leads to a timely initiation of treatment with improved outcomes. Tumour biomarkers have long been desired in the search for a tool to aid cancer diagnosis, prognosis and follow-up. Currently, the only widely used biomarker for CRC, Carcinoembryonic Antigen (CEA), has multiple limitations, clearly illustrating the need for novel biomarkers. It is therefore unsurprising that much research has focused on identifying such markers with the literature being swamped with new and promising biomarkers. The aim of this study is to review the current status and role of circulating biomarkers in patients post hepatectomy for colorectal cancer metastasis including alternative cancer antigens to CEA, extracellular vesicles, circulating microRNA, circulating tumour cells and circulating tumour DNA.

Keywords: colorectal cancer; liver metastasis; hepatectomy; recurrence; circulating biomarkers; review

1. Introduction

Colorectal cancer (CRC) is one of the most common cancers, estimated to affect 150,000 individuals in the United States annually [1]. Over 50% of these patients will develop liver metastases at some point during follow-up. The liver is the most frequent metastatic location. The lifetime risk of developing CRC is currently estimated at 4.3% (1 in 23).

Of the patients who develop liver metastases, only 20% will be candidates for a potentially curative resection of the liver. There has been a dramatic improvement in long-term survival following surgery for colorectal liver metastases (CRLMs), with five-year overall survival (OS) rates doubling from approximately 30% in the 1980s to 1990s to almost 60% in the last 20 years.

For the patients not suitable for liver surgery and who receive systemic chemotherapy alone, five-year overall survival (OS) rates are less than 11%, with only 2% attaining long-term survival [2].

Follow-up after cancer resection is of paramount importance, as early detection of recurrence can improve outcomes. Most cancers have a recommended follow-up regime/program mostly recommended by respective cancer societies. In the UK, patients with non-metastatic colorectal cancer have a structured follow-up regime recommended by NICE [3].

Biomarkers are defined by the national institute of cancer as '*biological molecules found in blood, other body fluids, or tissues that are a sign of a normal or abnormal process, or of a condition or disease. A biomarker may be used to see how well the body responds to a treatment for a disease or condition*©' [4]. Also called *molecular marker* and *signature molecule* they are considered extremely important in diagnosis and surveillance of disease, in particular cancer. They have been described as a holy grail of surveillance [5]. Colorectal cancer follow-up for non-metastatic disease includes testing for CEA, as recommended by NICE [6].

Early detection facilitates early treatment of metastatic disease, improving overall prognosis [7]. In particular, patients who have recurrent disease within the liver are often suitable for further treatment such as further liver resection or liver ablation. Liver transplantation for non-resectable oligometastatic disease to the liver is also emerging as a potential option, although international consensus on this front is still lacking [8].

This review aims to summarise the current status and role of circulating biomarkers in patients who have had a hepatectomy for colorectal cancer metastasis.

2. Biomarkers

Broadly, circulating biomarkers for colorectal cancer can be categorised into proteins, nucleic acids and circulating tumour cells.

2.1 Proteins

2.1.1 Carcinoembryonic Antigen (CEA)

Reflecting the high incidence of colorectal cancer, CEA is one of the most commonly investigated and used tumour markers [9]. A foetal glycoprotein involved in cell adhesion, CEA is one of the first tumour biomarkers, identified in 1965 by Gold and Freedman [10]. It is normally produced by the epithelium of the large intestine of the foetal gastrointestinal tract, but CEA is detectable in very low serum levels of healthy adult individuals.

Raised CEA expression is associated with many cancers including breast, respiratory, genitourinary and gastrointestinal cancers [11]. The action of CEA has been shown to be central in the multistep cascade by which malignant cells colonise the liver, including apoptosis inhibition [12], facilitation of malignant cell adhesion [13] and evasion from Kupffer cell detection [14]. Unsurprisingly therefore, high serum levels of CEA in patients with CRC are associated with the development of liver metastases [15].

Following curative-intent surgery for primary CRC, numerous studies have demonstrated that patients following an intensive surveillance regime that includes regular CEA testing had a significantly favourable outcome than patients undergoing surveillance without CEA testing [16]. As such, most expert panels in North America and Europe recommend serial measurements of CEA following curative-intent surgery for CRC [17–19].

In the case of follow-up for liver metastasis, following resection of curative intent, CEA measurement is recommended at 3–6 month intervals for 3 years after surgery [20]. However, CEA measurement alone is inadequate. A series following 314 patients reported that recurrences were detected in 23% through a CEA increase alone without any relevant findings on routine imaging, in 46% through a CEA rise with simultaneous positive imaging, and in 31% by positive imaging alone [21].

A significant elevation is considered to be at least 30% over that of the previous elevation whilst the recommendation is that CEA should be measured 3 monthly for 3 years following metastasectomy [18]. Elevated CEA levels should prompt further investigation in the form of imaging to detect potential metastatic disease.

2.1.2 Carbohydrate Antigen 19-9 (CA 19-9)

Although more commonly associated with pancreatic and biliary tumours, elevated serum CA 19-9 levels have also been linked to CRC. CA 19-9 is a tetrasaccharide carbohydrate also known as sialyl Lewis A and is synthesised by gastrointestinal epithelium. Paradoxically, it was first discovered to be elevated in colon cancer [22], however, due to its low sensitivity and specificity it has not been established as a reliable tumour marker in this setting.

Some studies have shown that elevated CA 19-9 levels have been linked with poorer prognosis [23]. A recent study by Thomsen *et al.* [24], as part of the NORDIC VII trial, investigated the prognostic factor of CA 19-9 in patients with unresectable metastatic colorectal cancer and RAS or BRAF mutations. A total of 566 patients were investigated and elevated CA 19-9 (and CEA) levels were associated with a reduced overall survival, most notably in CRC expressing the BRAF mutation.

We did not identify any studies suggesting a role for CA 19-9 in following up patients with resected primary colon cancer or within the setting of resected CRC liver metastases.

2.1.3 Cancer Antigen 72-4 (CA 72-4)

CA 72-4 is an antigen to tumour-associated glycoprotein 72 (TAG-72), a 48-kDa mucin-like glycoprotein complex first identified in 1981 [25]. CA 72-4 has been found in a variety of human adenocarcinomas including colorectal, gastric, breast, lung and ovarian, but is rarely expressed in normal or benign tissue [26]. In a study of 106 patients (53 colon and 53 rectal) Sing *et al.* [27] found a diagnostic sensitivity of 45.3% and specificity of 95.9% in 53 patients with colon cancer, and 69.4% and 63.9%, respectively, in rectal cancers. However, when used in combination with other tumour markers (TK1, CEA and CA 19-9) sensitivity increased to 86.9% and 96.0% for colon cancer and 80.9% and 96.1% for rectal cancer. Carpelan-Holmström *et al.* [28] studied various tumour markers including CEA, CA 19-9 and CA 72-4 in 102 patients with resected colorectal cancer, in particular their value in detecting recurrence. Only CEA proved to be of diagnostic significance. We did not identify any studies justifying the use of CA 72-4 in the surveillance of resected CRC liver metastases.

2.1.4 DR-70 Testing

The DR-70 test marketed under the brand Onko-Sure is a serum ELISA assay that quantifies fibrin and fibrinogen degradation products in blood. These have been found to be elevated in various cancers including CRC. DR-70 screening is approved for use in many countries including the US and Europe for lymphoma and lung, breast, stomach, liver, ovarian, oesophageal, cervical, trophoblastic, thyroid, brain and pancreatic cancers, as well as CRC. It was approved by the USFDA in 1982 and was the first test cleared for monitoring of CRC after CEA. Although it does not feature in societal guidelines, there is some evidence that it may be useful in patients who have a positive histological diagnosis of CRC with normal CEA levels [29].

There is however no published evidence of any role in detection of recurrence after liver resection.

2.1.5 Matrix Metalloproteinases (MMPs) and Tissue Inhibitors of Metalloproteinases (TIMPs)

Matrix metalloproteinases (MMPs), also referred to as matrixins or matrix metallopeptidases are enzymes capable of breaking down extracellular matrix proteins. First de-

scribed in 1962 [30], they have been shown to play a major role in physiological and pathological cell activities such as cell proliferation, migration (adhesion/dispersion), differentiation, angiogenesis and apoptosis [31]; it therefore comes as no surprise that, they have a major role in cancer development and spread [32] in addition to being a potential biomarker of malignancy [33].

As their name suggests, TIMPs, are a family of intrinsic inhibitors of MMPs and play an important role in the regulation of MMP activity. An imbalance between TIMPs and MMPs can result in an altered extracellular matrix in turn, leading to early tumour development and metastasis [34]. Several studies have reported this, including ones specifically investigating CRC [35,36].

Huang *et al*. [37] recently investigated the serum levels of various biomarkers including several MMPs in patients with CRC. This case-controlled study included 112 patients with newly diagnosed CRC at various stages. All cases were confirmed with positive histopathology and were compared to 115 matched controls. They demonstrated that serum MMP-7, MMP-9, MMP-11, TIMP-1, TIMP-2, CEA, and CA19-9 levels were all significantly higher than in the control group.

More recently, Reijonen *et al*. [38] published a study with 419 patients who underwent hepatectomy for CRC metastatic disease. The group investigated the prognostic value of various serum biomarkers including MMP-8 and MMP-9. Pre- and postoperative elevated MMP-8 levels were associated with a worse overall survival at 10 years, whilst MMP-9 did not serve as a prognostic marker.

No studies specifically looking at the diagnostic value of MMPs detected in patients with resected liver metastases from colorectal cancer were identified.

2.2 Nucleic Acids

2.2.1 MicroRNAs (miRNAs)

MiRNAs are a vital component for the non-coding RNA family, that help to shape the expression of most mRNAs. Hundreds of different miRNAs have been isolated in humans and are thought to influence all developmental disease and processes [39]. By binding to tumour suppressor genes or oncogenes, miRNAs regulate a number of oncogenic processes including cell growth, apoptosis, cell differentiation and angiogenesis [39]. MiRNA dysregulation was first reported in chronic lymphocytic leukaemia [40] and has since been associated with various cancers, including CRC.

A wide range of miRNAs have been found to be either up or/and downregulated in patients with CRC [41]. The first report came from Michael *et al*. [42] in 2003 reporting that miR-143 and miR-145 were downregulated in CRC tissue samples compared to normal tissue. Further investigators have reported that patterns of tissue miRNA expression can be associated with prognosis and chemotherapy resistance [43].

Of further interest, however, is that circulating miRNAs found in blood may also be of diagnostic or prognostic value. Han *et al*. [44] used a panel of four miRNAs, namely miR-15b, miR-16, miR-21, miR-31 to create a panel test and reported a diagnostic sensitivity and specificity of 95.06% and 94.44%, respectively, when comparing healthy individuals to patients with CRC. The same panel reported a sensitivity and specificity of 85.19% and 82.09%, respectively, when comparing patients with CRC with patients having colorectal adenomas. Huang *et al*. [45] reported a diagnostic sensitivity of 81.25% and a specificity of 73.33% using a panel of four alternative miRNAs: miR-203a-3p, miR-145-5p, miR-375-3p and miR-200c-3p.

A meta-analysis of 18 studies that included over 2000 patients and controls showed a pooled sensitivity and specificity for circulating miR-21 of 77% and 83%, respectively [46].

Nassar *et al*. [47] investigated a cohort of patients with stage IV CRC to identify miRNA panels that could be used for diagnosis of Stage IV CRC in general, and liver metastasis in particular. They reported that the combination of miR-210 and miR-203 together offers a diagnostic accuracy of 72% for patients with CRC and liver metastases. Nevertheless, despite the above advances, miRNA testing has yet to enter in routine clinical use as a biomarker either in the setting of primary colorectal cancer or in the detection of recurrence after liver resection.

2.2.2 Circulating Tumour DNA (ctDNA)

Several recent reports have championed the potential use of detecting circulating tumour DNA (ctDNA) in the management of patients with cancer [48]. Although free DNA was first reported in blood in 1948 [49], the categories of body fluids that can be profiled has expanded since to include urine, saliva, cerebrospinal and pleural fluid. As circulating free DNA (cfDNA) is mostly derived from cells undergoing necrosis or apoptosis, higher levels are found in patients with cancer, in addition to insults such as trauma [50], infraction, transplant rejection and infection [51]. The fraction of cfDNA originating from tumour cells is termed ctDNA and along with detection of CTCs is often referred to as a liquid biopsy [52].

Liquid biopsies are broadly categorised into whole genome sequencing and PCR-based tests targeting specific genes. In principle, ctDNA should contain the same genetic defects as the original tumour cells [52]. Detectable Ct DNA has therefore been used to detect mutations and guide treatment such as epidermal growth factor receptor (EGFR) therapy in lung cancer and Ras in colorectal cancer [53,54]. In addition, commercial products that perform whole genome DNA sequencing from liquid biopsy have been licenced by in the US, Japan and EU. Products such as FoundationOne Liquid CDx and Guardant360 CDx [55,56] can perform comprehensive tumour genome profiling from liquid biopsy and provide information about genomic alter-

ations to guide the use of approved targeted therapies and ongoing clinical trials [57].

The CIRCULATE trial, currently recruiting patients, is investigating the role of ctDNA in patients with stage II colorectal cancer and aims to establish if detectable ctDNA can guide adjuvant treatment. As a secondary objective however, it will investigate the potential of ctDNA as a follow-up tool in resected patients [58].

Bolhuis *et al*. [59] investigated 23 patients who had already been enrolled in an ongoing phase 3 trial (the CAIRO5 study) and who had resected colorectal metastatic disease to the liver. This was the first and, to date, only study to analyse the association of postoperative ctDNA in the setting of resected CRC liver metastases. CtDNA analysis was performed at baseline, prior and post liver resection and showed that postoperative ctDNA status is an independent prognostic factor for recurrence. Although this is a small study, the authors suggest that it is a promising biomarker for disease monitoring post liver resection.

To date there have been no trials specifically looking at the diagnostic role of ctDNA detection in patients with resected liver metastases from colorectal cancer, however, this avenue has gained traction and appears to be a promising direction.

2.2.3 Extracellular Vesicles (EVs)

EVs are cell membrane-derived vesicles which are actively secreted by cells. They are broadly subcategorised as microvesicles and exosomes depending on their size, and are a heterogeneous entity; they mediate cell signalling in both physiological and pathological settings. As they are detectable in all body fluids, including blood, they have been lauded as a potential biomarker of disease, including cancer [60]. In addition, they have been considered a therapeutic delivery vehicle or as a target themselves to inhibit pathological cell signaling [61].

EVs are nanosized and comprised of a lipid bilayer surface that carries bioactive cargo in the form of proteins of genetic materials such as DNA, mRNA or miRNA and they form a snapshot of the secreting parent cell [62]. Investigation of EVs involves isolation of EVs from a body fluid, followed by composition analysis [63]. Their relative abundance in blood and stability due to the protective membrane have made EVs of particular interest as biomarkers in many cancers [64].

Silva *et al*. [65] analysed plasma from 91 patients with colorectal cancer. They found that, when compared to blood derived from healthy individuals, patients with colorectal cancer had a statistically significant higher number of detectable exosomes. However, within the cancer group, patients with high levels of exosomes had a similar length of disease-free survival, although they had a shorter overall survival when compared to patients with lower number of exosomes.

There are no substantial studies on human patients specifically looking at the biomarker role of EVs in patients with resected liver metastases from colorectal cancer. Shao *et al*. [66] investigated the role of EVs in promotion of liver metastasis in a murine model and found that miR-21 containing EVs enable this by creating a proinflammatory state by enhancing IL-6 production. As part of this study, plasma from 25 patients with CRC and liver metastases was compared with those of 20 patients without liver metastases, 35 healthy patients and 29 patients with adenomas. EV miR-21 was detected in increasing levels in patients with higher tumour stage.

2.2.4 Epigenetic Markers

It is widely acknowledged that the modification of gene expression plays an integral role in tumorigenesis and cancer progression. Hence, the search for epigenetic markers of cancer has been another route under investigation for reliable biomarkers in many cancers, including CRC [67]. Although histone modifications have been studied, most of the focus has been on DNA methylation biomarkers [68].

The SEPT9 gene, coding for the Septin-9 protein, has been shown to be methylated in colorectal cancer when compared to normal colon mucosa. The PRESEPT trial was a large prospective study of nearly 8000 asymptomatic patients which showed that screening for CRC by means of detecting circulating methylated SEPT9 DNA (mSEPT9) resulted in a sensitivity of 48.2% and specificity of 91.5% [69]. This led in 2016 to European and FDA approval of the Epi proColon® screening test, the first methylation-based biomarker for CRC [70–72]. It is only indicated, however, in patients above the age of 50 who have an average risk of developing CRC and who cannot or wish not to partake in regular methods of screening.

There is no licence for the use of this test for monitoring patients with resected primary or metastatic CRC. However, recent studies have shown that mSEPT9 levels corelate well with treatment response undergoing hepatectomy. Liu *et al*. [73] studied 51 patients who had had either synchronous or metachronous hepatectomy for CRC with curative intent. The group reported that mSEPT 9 levels dropped considerably following liver resection and patients with higher levels had a poorer prognosis. However, mSEPT9 levels were not used to follow up patients with respect to early detection of recurrence.

Other DNA methylation biomarkers under investigation include Twist-Related Protein 1 (TWIST1) [74], Runt-Related Transcription Factor 3 (RUNX3) [75], Tachykinin-1 (TAC1) [76], Insulin-Like Growth Factor Binding Protein 3 (IGFBP3) [77], Eyes Absent Homolog 4 (EYA4) [78] and Somatostatin (SST) [79], however, none of these has been as widely investigated as SEPT9.

Table 1. Summary of biomarkers and their current status of development.

Biomarker	Category	Current status of development as a biomarker
Carcinoembryonic Antigen (CEA)	glycoprotein	Recommended for postoperative surveillance of surgically treated metastatic disease to the liver [18,20].
Carbohydrate Antigen 19-9 (CA 19-1)	glycoprotein	No published evidence to support use in surveillance of surgically treated metastatic disease to the liver. One ongoing study is measuring CA 19-9 levels after liver metastasectomy as part of a panel of follow-up parameters [89].
Cancer Antigen 72-4 (CA 74-2)	glycoprotein	No published evidence to support use in surveillance of surgically treated metastatic disease to the liver.
DR-70 (Onko-sure)	protein	Licenced by USFDA for use in colorectal cancer monitoring, but no published evidence for use in surveillance of surgically treated metastatic disease to the liver. It does not feature in societal guidelines.
Matrix Metalloproteinases (MMPs)	Protein	No published evidence to support use in surveillance of surgically treated metastatic disease to the liver.
Circulating MicroRNAs	Nucleic acid	No evidence for its use in surveillance of surgically treated CRC or metastatic disease to the liver. Two ongoing studies are measuring MicroRNA levels in patients with primary CRC to establish the relation with survival and recurrence [90,91].
Circulating Tumour DNA	Nucleic acid	No published evidence to support use in surveillance of surgically treated metastatic disease to the liver. However, there are several studies, collectively involving thousands of patients investigating this biomarker and its role in CRC diagnosis and follow-up [92–99].
Extracellular Vesicles	Nucleic acid	No published evidence to support use in surveillance of surgically treated metastatic disease to the liver.
Epigenetic markers	Nucleic acid	No published evidence to support use in surveillance of surgically treated metastatic disease to the liver. One study investigating hypermethylated DNA as a follow-up biomarker for disease recurrence after surgery on primary CRC [100].
Circulating Tumour cells (CTCs)	Circulating cells	One phase 3 trial investigating the use in follow-up CTC monitoring after primary resected colorectal cancer NCT04917289 and one study after metastasectomy [101].

2.3 Circulating Tumour Cells

Circulating tumour cells (CTCs) are cells detectable from sampling the peripheral circulation of cancer patients. They are detectable in the majority of metastatic cancer patients and are of negative prognostic significance. In a meta-analysis, Rahbari *et al.* [80] demonstrated that detection of CTCs confers a poor prognosis in patients with CRC.

Lalmahomed *et al.* [81] investigated the prognostic value of CTCs in patients immediately before CRC liver resections. CTCs were measured preoperatively in 151 patients, however, no corelation was found between presence of CTCs and early recurrence after curative-intent liver resection.

In addition to prognosis, CTCs may be of diagnostic potential. Yu *et al.* [82], in a case-control study with 59 patients with colorectal cancer showed that CTC detection had a diagnostic sensitivity of 83%, higher than other serum biomarkers, a figure that rose to 91.5% if CTC detection was combined with CEA level. Nevertheless, CTCs are not yet used in routine clinical practice and have not been investigated in patients with resected colorectal liver metastases.

3. Other Biomarkers

In addition to those listed above, there are many other candidates such as Thymidine kinase 1 (TK1) [27], soluble CD26 [83] and dermokine [84] that have been investigated and put forward as potential biomarkers. However, research on these has been overall at a relatively earlier stage of investigation.

4. Discussion

In 1968, Wilson & Junger delivered the landmark publication outlining the principles of modern screening, which were adopted by the World Health Organisation [85]. Although not strictly speaking a screening test, postoperative surveillance for oncological surgery of curative intent should follow similar principles.

Colorectal cancer is an important global health problem with a natural history that warrants early intervention. Advances in surgical and oncological therapies offer ever more effective treatment even in the setting of recurrent disease. Circulating biomarkers offer an acceptable, convenient and attractive mode of diagnosing and monitoring these patients.

At present, there are only a few biomarkers used in clinical practice for colorectal cancer, largely due to the low sensitivity and specificity of the alternatives studied to date. In addition, there are no additional biomarkers recommended specifically for patients after liver metastasectomy, in fact the ones in current clinical use are mere extrapola-

tions of their use in primary colorectal cancer.

It therefore comes as no surprise that on a global scale, much research has focused on identifying suitable markers. The various directions this research has followed reflects advances in our understanding of cancer biology, laboratory techniques and the routes pursued for similar biomarkers in other malignancies.

Broadly, potential biomarkers have fallen into three main categories: proteins, nucleic acids and circulating tumour cells. None of these tests can be performed at the bedside at present, however, they have certain pros and cons. Protein testing is relatively cheap, costing a few dollars for an established protein like CEA, and can be taken as part of routine blood test sampling in most laboratories. Sample turnaround is fast, with reliable results within a few hours. On the other hand, circulating nucleic acid testing is currently much more expensive when compared to protein testing. For example, ctDNA tests have been estimated to be approximately $500 per test [86] and turnaround time is considerably longer, at 1–2 weeks [87]. In the right setting, circulating tumour cell testing can be processed in a matter of hours, however, the costs required are considerably higher with an initial investment of $220,000 and then processing costs of $1000 per run [88].

Of the various biomarker candidates presented in this review, the emerging concept of the liquid biopsy appears to be the one that is gaining most traction, in particular ctDNA surveillance. There are several studies currently recruiting patients both in the setting of primary colorectal cancer and for follow-up in patients with resected disease which can be seen summarized in Table 1 (Ref. [18,20,89–101]).

5. Conclusions

Current expert panel recommendations for follow-up of patients who have liver resection for CRC metastases continue to be testing for CEA levels and CT scans at intervals of 3–6 months during the first 3 years post metastasectomy.

Although this field continues to grow and many potential candidates have been discussed in this review, the likelihood is that no single biomarker will be sufficient for postoperative surveillance. Emerging data suggest that it is more likely that panels of combined biomarkers will be established as the most accurate diagnostic tests.

There is a distinct lack of published research addressing the question of biomarkers specifically looking at patients with resected liver metastases from CRC. Clearly, there is an urgent need for further investigation to bring potential candidates to clinical use.

Author Contributions

SP drafted the original manuscript, did the literature search and conducted the literature review; SP, RHB and VKM conceptualised and designed the study, prepared the final draft and approved the final version; RHB and VKM made critical revisions.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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