Perspectives of tumour markers in gastrointestinal malignancies – an overview

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Received: 5 November 2008 / Accepted: 8 November 2008

Abstract There is an increasing incidence of GI cancers as per the Cancer Registry Data. In such a situation, it is essential to evaluate the patient by standard investigational tools like clinical examination and endoscopy which help in establishing the diagnosis. Determination of tumour markers is useful not only in diagnosis and prognosis of cancer but also monitoring of cancer treatment. Tumour markers in GI oncology include oncofoetal proteins, hormones, enzymes and cancer antigens. Recent advances include application of tumour marker level determination combined with newer radiological and functional imaging such as FDG PET – CT.

Keywords Tumour markers · CEA · GI malignancies

Introduction

Tumour markers are substances that can be detected in higher-than-normal amounts in the blood, urine, or body tissues of some patients with certain types of cancer. Most tumour markers can be elevated in patients who do not have a tumour; and, no tumour marker is entirely specific to a particular type of cancer. The carcinoembryonic antigen (CEA) is one of the first Tumour markers identified in making the diagnosis of colorectal cancer.

Gastrointestinal (GI) malignancies including pancreatic cancers have begun to rise in Asia. There is an alarming rise in the incidence of GI malignancies in our country. According to the PBCR, incidence of colorectal cancer in males is 2.92 and 2.30 [1] in females in Bangalore. The common question posed to the surgeon is – is there a marker in tissue or serum which detects the presence of malignancy without being an invasive procedure? Whether absence of the marker post-treatment ensures “cure”?

Tumour markers

These are cellular products that are abnormally detected in malignancies [2]. These markers can be detected in various body fluids and on the surface of cancer cells. The perfect tumour marker would be one that is –

1. Produced only by the tumour cell.
2. Present in all patients with the said cancer.
3. Levels in the blood correlating with the tumour burden.
4. Detectable in occult disease.
5. Absent in benign disease.

There is no tumour marker which meets the above requirements. The utility of tumour markers depends on its sensitivity, specificity and variability in assays. The measurements that help define the utility of the tumour markers are:

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1. Sensitivity – it is the percentage of patients with cancer who have an abnormal test.
2. Specificity – it is the percentage of people without cancer whose test is negative.
3. Positive predictive value – it is the possibility that patients with an abnormal test actually have cancer.
4. Negative predictive value – it is the probability that a negative test will predict that a person will not have a cancer.

Determination of these tumour markers can be used in:

1. Diagnosis
2. Prognosis of the cancer
3. Monitoring of treatment

Classification of tumour markers

The markers can be classified in several ways: According to their chemical structure, tissue of origin, site of malignancies and functionality. The most common classification is depicted in Table 1.

Usefulness of tumour markers in GI malignancies (site specific)

Table 2 depicts commonly used tumour markers in clinical practice with special reference to diagnosis, prognosis, postoperative follow-up, predicting and monitoring response to therapy in various GI malignancies. There is sufficient evidence to show that tumour markers are useful in clinical practice [3].

Table 1 Classification of tumour markers

1] Oncofetal proteins: These are substances found normally in large amounts during foetal development. Cancer derived in adults from their foetal counterparts elaborate these proteins in increased quantities.	Carcinoembryonic antigen (CEA) Alfa fetoprotein (AFP)
2] Hormones:	B-human chorionic gonadotropin (BHCG)
3] Enzymes:	Prostate specific antigen (PSA) Lactate dehydrogenase (LDH) Neuron specific enolase (NSE)
4] Cancer antigens: These are glycoproteins without defined normal counterparts and expressed only by malignant cells.	CA – 19.9 CA – 15.3 CA – 72.4 CA – 125
5] Biomarkers:	P53 Tenascin-C (Tn-C) ALU repeat sequences CD 144 Vascular endothelial growth factor 2 (VEGFR-2) Carboxypeptidase A Colon cancer specific antigen-2
6] Molecular markers:	Adenomatous polyposis coli (APC) Micro satellite instability MSI

Oesophageal cancer

Neoangiogenesis and lymph angiogenesis represent prognostic factor in human malignancies. Determination of microvessel density [4] (MVD) by immunohistology has been investigated by CD-31 staining and quantification in tumour samples of patients with oesophageal cancer for real time PCR analyses of VEGFR-2, VEGFR-3. It has been found that MVD was significantly linked to the expression of VEGF-A, C, D and Ang 1 and Ang 2 and this represents a novel tool to study the association of oesophageal carcinoma with lymph node metastasis.

Gastric cancer

CEA level is elevated in approximately one third of patients with primary gastric cancer [5]. The sensitivity of CEA as a tumour marker of gastric cancer is low [6] however if CEA level is elevated it usually correlates with stage of the disease. Combining CEA with other tumour markers like CA – 19.9, CA – 50, CA – 72-4 (TAG 72) can increase sensitivity to CEA alone [6, 7, 8]. CEA was increased in 42.5% of gastric cancer in Indian publication [9].

Gastrointestinal stromal tumours (GIST)

These are histologically heterogeneous group of mesenchymal tumours arising from the GIT (spindle or epitheloid neoplasm). The hallmark of this condition is the expression

Table 2 Commonly used tumour markers in clinical practice

Malignancies	Tumour markers
Oesophageal cancer	VEGFR-2 VEGFR-3 VEGF-A, C, D Ang 1 Ang 2
Gastric cancer	Carcinoembryonic antigen (CEA) CA – 19.9 CA – 50 CA – 72-4 (TAG 72)
Gastro intestinal stromal tumors (GIST)	CD-117 (C-KIT)
Pancreatic cancer	CA 19-9 human telomerase reverse transcriptase (hTERT)
Hepatocellular carcinoma	Alpha fetoprotein (AFP) CEA Vitamin B ₁₂ AFP Ferritin, PIVKA-2
Colorectal cancers	Carcinoembryonic antigen (CEA) CA-19.9 MSI-H Thymidylate synthetase (TS) P53 Tenascin-C ²⁸ Colon cancer specific antigen-2

of CD-117 by immunohistochemical assays [10] and the levels of expression can vary from generally diffuse and strong (most common in the spindle cell subtype) to focal and weakly positive in a dot-like pattern (of the epitheloid subtype). There is only a rare subset (fewer than 5%) of GIST that fail to express CD 117.

Pancreatic cancer

CA 19-9: This is a mucinous glycoprotein detected by antibodies raised in mice against colon cancer. Although it is not useful in evaluating colon cancer, it is the most accurate marker for monitoring pancreatic cancer [11]. CA 19-9 is of limited value as a screening marker [12]; however as approximately 10 to 15% individuals do not secrete CA 19-9 because of their Lewis antigen status. False negative result may be seen when the tumour is small or asymptomatic and false positive result may be seen in benign biliary or pancreatic conditions. Elevated CA 19-9 levels has been demonstrated in the sera of patients with colorectal and gastric cancer. Attempts are being made to combine markers to improve the diagnostic performance of CA 19-9 by combining it with other markers such as EUS and multidetector CT. Serial serum measurements of CA 19-9 correlates well with time to treatment failure (TTF) and overall survival (OS) in patients receiving chemotherapy for metastatic pancreatic cancer. Studies are ongoing to compare CA 19-9 decline and objective radiological response with TTF and OS in pa-

tients of metastatic pancreatic cancer receiving fixed dose rate gemcitabine based chemotherapy.[13]

Newer evaluation biomarkers for early detection

These can be divided into 3 targets – DNA, RNA and proteins. Detection of PC – DNA and PC messenger RNA is more appropriate the analysis of pancreatic juice or FNA. Detection of telomerase enzymatic activity or human telomerase reverse transcriptase (hTERT) subunit may be helpful in differentiating PC from benign pancreatic disease. Protein based biomarkers such as SELDI technique (surface enhanced laser desorption ionisation mass spectrometry) and evaluation of procarboxypeptidase A and carboxypeptidase A as tumour markers for adenocarcinoma of pancreas is being investigated.

Hepatocellular carcinoma

Alpha foeto protein (AFP)

AFP is a glycoprotein of 70,000 Daltons containing cysteine disulphide bridges and sialic acid [14]. This was the first serological assay for detection and clinical follow up of patients with HCC [15]. AFP can also be elevated in patients with hepatic parenchymal inflammation and necrosis and in second and third trimester of pregnancy. Although AFP is

elevated in 70% of individuals in Asian countries bearing HCC, it is only increased upto 50% in patients in US and Europe [16]. In patients with liver cirrhosis and no HCC, serum AFP levels may be normal or elevated with values ranging from 30 – 460 ng/ml (median 30–70). In patients with HCC, the serum AFP concentrations may range from 30–7000 ng/ml (median 275). Measurement of AFP fractions L3, P4 and P5 (different sugar chain structures) may allow the differentiation of HCC from cirrhosis in some cases. Prospective studies for screening of hepatomas in high risk population have generally concluded that USG is more sensitive than AFP elevations [16, 18]. AEP correlates well with response to chemotherapy [19, 20] and in patients being treated with surgical resection, prompt decline follows a good response while slow or incomplete declines accompany an incomplete response. The other most widely used assay is that of des-gamma-carboxy-prothrombin (DCP) protein induced by vitamin K abnormality (PIVKA-2) [17]. It is elevated in 80% of patients with HCC and even has prognostic value [21]. In a patient presenting with a new hepatic mass or recent hepatic decompensation, the following tests should be asked for – measurement of CEA, vitamin B₁₂, AFP, ferritin, PIVKA-2 and standard liver function tests.

Colorectal cancers

Carcinoembryonic antigen (CEA)

CEA is a foetal glycoprotein of 180,000 Daltons found on cell surfaces. It was discovered by Gold and Freedman in 1965. It contains 50–60% carbohydrate by weight. It is chemically related to immunoglobulins. It is produced in the foetal GIT, pancreas and liver and is present in small quantities in the blood and on cells of many normal adult tissues. CEA is a useful marker for monitoring breast, ovary, colon and small cell lung cancers. CEA is increased in patients with a variety of other endothelial malignancies, where its utility is generally limited.

CEA estimation in 450 patients was carried out to study the incidence of CEA as marker in cancer in the Indian pop-

ulation [9]. The incidence of CEA was 41.7% in colorectal cancers, 42.5% in stomach and breast cancer, in the lower range in other cancers.

Elevated levels of CEA (<10 ng/ml) are found in smokers and patients with COPD, inflammatory or peptic bowel disease, liver inflammation or cirrhosis of any cause, renal failure and fibrocystic breast disease.

CEA is too insensitive and non-specific to be valuable for screening of colorectal cancer. However, elevation of serum CEA levels does correlate with histologic differentiation, stage of the disease and visceral involvement. CEA level above 5 ng/ml preoperatively is considered a category I poor prognostic indicator by the CAP consensus panel [22]. Patients in whom elevated CEA levels fail to normalise after an adequate curative resection are particularly at risk. Hence given the independent prognostic significance of CEA, it would be ideal to recommend all patients to undergo serum CEA levels before a curative resection.

Managing an increasing CEA level

About 85% of colorectal recurrences are evident within 3 years after surgery. Serum CEA levels regress after 6 weeks of curative surgery. On follow up a rising CEA levels calls for further investigation to identify the site of recurrence.

Managing patients with an elevated CEA level after resection should include colonoscopy, chest, abdominal and pelvic CT scans, and physical examination. If imaging study results are normal in the face of a rising CEA, repeat scans are indicated every 3 months until either disease is identified or CEA level stabilises or declines. The opinion of the panel on the usefulness of PET scan in the scenario of an elevated CEA with negative good quality CT scans was divide (i.e. some panel members favoured use of PET in this scenario while others noted that the likelihood of PET identifying surgically curable disease in the setting of negative good quality CT scans is vanishingly small). Use of PET scans in this scenario is permissible within these guidelines [23, 25]. This is found to be more sensitive to find metastatic lesion than conventional assessment by 47.6%. Also response to chemotherapy in metastatic setting is better as-

Table 3 Protocol for surveillance following definitive treatment [24]

Pathologic stage	Surveillance
Tis, T1N0M0, T2N0M0 T1-3, N1-2, M0 Or T4, N1-2, M0	CEA every 3–6 months × 2 years, then every 6 months × 5 years
Synchronous resectable Liver or lung mets	CEA every 3–6 months × 2 years, then every 6 months × 5 years
Synchronous unresectable Liver or lung mets	CEA every 3–6 months × 2 years, then every 6 months × 5 years

sessed with serial monitoring of CEA levels and PET scan using RECIST criteria.

The panel does not recommend a so called “blind” or CEA directed laparotomy or laparoscopy for patients whose work up for an increased CEA level is negative [26]. CEA based ‘second look’ laparotomies data reveal that in asymptomatic patients with rising CEA titers 60% of patients were potentially resectable, 55% were resectable with curative intent and 60% of these developed recurrence. 10–12% of patients did not show tumour hence CEA based second look surgery is not recommended routinely.

The use of anti-CEA radiolabelled scintigraphy is not routinely recommended. In the event that surgically curable metastatic disease is identified on CT or MRI, it is not routinely recommended to do a PET before surgical resection to look for evidence of additional metastasis that may change the status of patient respectability.

CA-19.9 – may be of value in monitoring recurrences and complement CEA.

Newer biomarkers colorectal cancer

Micro satellite instability (MSI)

Micro satellites are sections of DNA in which a short sequence of nucleotides (most commonly a dinucleotide) is repeated several times [27]. MSI is a situation in which a micro satellite has gained or lost repeat units and so has undergone a change in length resulting in frameshift mutations. The phenotype with this defect is called high frequency MSI phenotype (MSI-H). Patients with MSI-H colorectal cancer have a better long-term prognosis than patients with cancers exhibiting micro satellite stability (MSS) [28]

Thymidylate synthetase (TS) and P53 are important prognostic markers in colorectal cancers. Patients having at least one of these tumour markers positive are more likely to have lymph node metastasis [29]. This may have an impact on recurrence in node negative patients with colorectal cancer. However this needs further investigation.

Tenascin C [30]

Tenascin C (Tn-C) is a large hexameric, extracellular matrix protein that is involved in remodelling processes, forming various type of high or low molecular variants by alternative splicing. The plasma Tn-C levels are measured by ELISA with the Tenascin-C ELISA kit (IBL, Japan) along with evaluation of CEA and CA 19-9. Negative conversion of plasma Tn-C level following resection suggested completely curative resection thus showing that plasma Tn-C appears an independent marker and is particularly useful as a postoperative monitoring marker for colorectal cancer patients.

Biomarkers requiring further evaluation

Integrity of circulating DNA

The length of free circulating DNA in serum can be used to discriminate healthy volunteers from patients with colorectal malignancies. Careful detection of short and long ALU repeat sequences has potential for detection and monitoring of colorectal malignancies in serum and should be evaluated in large cohort GI studies.

Colon cancer specific antigen – 2 (CCSA-2)

This is a distinct novel colon cancer marker identified using indirect immunoassay which has high sensitivity and specificity however further validation studies are required.

BRAF mutations expression assay

Mutations in the *BRAF* gene limit the response to anti-epidermal growth-factor receptor (anti-EGFR) therapy in patients with metastatic colorectal cancer, researchers reported at the 20th Annual European Organisation for Research and Treatment of Cancer (EORTC)–National Cancer Institute (NCI)–American Association for Cancer Research (AACR) Symposium on Molecular Targets and Cancer Therapeutics, in Geneva, Switzerland. *BRAF* mutation status can therefore help identify patients who are most likely to benefit from treatment with the monoclonal antibodies cetuximab (*Erbitux*; ImClone, Merck) and panitumumab (*Vectibix*, Amgen).

Patients in their study who had tumours containing *BRAF* mutations did not respond to anti-EGFR treatment. Conversely, none of the patients who responded to treatment had *BRAF* mutations.

Panitumumab and cetuximab are effective in an estimated 10% – 20% of chemotherapy-refractory metastatic colorectal cancer patients. Mutations in the *KRAS* gene are associated with resistance to both cetuximab and panitumumab account for approximately 30–40% of resistant patients, but treatment with the *BRAF* inhibitor sorafenib (*Nexavar*, Bayer) restored sensitivity to the anti-EGFR treatment. Combining cetuximab with sorafenib produced a synergistic effect that was associated with a massive induction of caspase-mediated apoptosis in *BRAF*-mutated cells.

Novel gene signature predicts response to FOLFIRI

11-gene signature that could be used to separate patients who will respond to the FOLFIRI chemotherapy regimen (leucovorin fluorouracil, and irinotecan) from those who will not. It is not predictive for the other treatment options.

Conclusion

The biological markers are gaining more importance in the management of various GI malignancies. The serum markers like CEA, AFP etc are helping in diagnosis, prognosis, monitoring of therapy. No Tumour markers are useful in screening. No proper evidence regarding when to do, how often to do, in various malignancies. The genetic markers have remained an investigative methodology for screening, diagnosis, prognosis. This has been very useful in high risk group hereditary cancers syndrome like HNPCC.

Acknowledgements Special thanks to Dr. Ravi Chandra J B, Dr. Amarendra and Mr. Suresh J for contributions in preparing the manuscript.

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