Review

Tumor markers in finding recurrent disease in colorectal cancer: a diagnostic review

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Abstract

Aim: In the search for evidence-based follow-up of patients after resection for colorectal cancer, numerous tumor markers have been proposed. This review has evaluated these markers and comments on the diagnostic accuracy in finding recurrent disease in relation to Carcino-Embryonic Antigen (CEA).
Methods: A comprehensive literature review (1985-2010) was performed by two independent reviewers. Sensitivity and specificity of markers mentioned in the articles were checked by recalculation. A validated quality score system was used to estimate study quality.
Results: Seventeen studies focusing on eight different markers were included. Three markers were shown to have comparable or better accuracy than CEA: TPA, CA 242 and CA 72-4 in at least one study. These three markers, from four independent studies, showed a tumor marker sensitivity of > 60% in combination with an outperformance of CEA in follow-up. These results were not confirmed by six other studies investigating the same markers.
Conclusion: This review revealed three tumor markers other than CEA that have been shown to adequately indicate recurrences in colorectal cancer. However, comparability of studies was difficult. Therefore a prospective study of these markers seems necessary to investigate their real value, and to overcome design and inclusion biases.

Introduction

In colorectal cancer (CRC), 30-50% of patients will relapse after primary surgery with local recurrence or metastatic disease, mainly in the first two to three years after resection. After curative treatment, patients will be in follow-up in order to detect recurrent disease as early as possible. Early detection of recurrent tumor activity results in better chances of curation than late detection, and intended curative treatment of metastases is associated with higher survival rates than palliative treatment (Gomez *et al.* 2010).

Because of the wide variation in the follow-up programs used, a systematic review and meta-analysis failed to define the best combination and frequency of clinical visits, laboratory blood tests, endoscopic procedures and radiological investigations (Jeffery *et al.* 2007). This paper focuses on laboratory biomarkers used in follow-up for colorectal cancer. Diagnostic accuracy of a tumor marker depends upon its sensitivity and specificity. In follow-up, tumor markers should ideally have high sensitivity with a low false-positive rate.

The best known serum tumor marker used in follow-up is Carcino-Embryonic Antigen (CEA), discovered in 1965. Several studies showed that the preoperative CEA value correlates with prognosis after treatment (El-Awady *et al*. 2009, Wiratkapun *et al.* 2001). Serum CEA has been the most sensitive diagnostic tool in asymptomatic patients for early diagnosis of recurrent disease in CRC and its use is proposed in several international guidelines, despite ongoing controversy concerning the effect of follow-up on overall survival. The available evidence on the clinical effec-

tiveness of CEA as a tumor marker in the follow-up after curative treatment of CRC is based on four reviews and a Cochrane meta-analysis, with much overlap of included studies (Bruinvels *et al.* 1994, Jeffery *et al.* 2007, Kievit *et al.* 2000, Renehan *et al.* 2002, Tjandra & Chan 2007). The Cochrane review, including only prospective trials, failed to define the best use of CEA in follow-up, but did show that studies with frequent CEA measurements are associated with longer survival. Diagnostic accuracy of CEA in follow -up is influenced by the chosen cut-off or threshold value. Although the best way to use CEA is yet to be defined, the rise rather than the absolute value is an important indicator for recurrent disease activity (Grossmann *et al.* 2011). Currently, CEA is the only recommended tumor marker to be used in follow-up in Europe and the United States *(*Duffy *et al.* 2007, Eche *et al.* 2001, , www.oncoline.nl).

The search for better tumor markers as indicators of recurrent disease is ongoing. Monoclonal antibody technology has permitted the identification of new tumor markers, such as Carbohydrate Antigens (CA 19-9, CA 242, CA 50), which show variable results since their introduction in the 1980's and 1990's. Lately nucleic acid markers, which are markers consisting of tumor-derived circulating DNA in serum, mRNA and microRNA, have become a subject of interest (Schwarzenbach *et al* 2011). These markers are associated with the presence of various solid tumors including CRC (Goebel *et al.* 2005) and preoperative rise in several serum nucleic acid markers has proven to predict both prognosis and metastasis in CRC (Herbst *et al.* 2009, Lecomte *et al.* 2002). Proliferative markers are also applied, such as the protein antigen Tissue Polypeptide Antigen (TPA) which is synthesized by tissues undergoing rapid growth. In addition, the ability of tumor cells to degrade the extracellular matrix (ECM) has been used by measuring analytes involved in ECM function as tumor markers (Golovkov 2009).

Aim

Given the gain in survival which can be obtained by finding recurrences in an early stage, there is a need for a tumor marker that indicates recurrent disease. The aim of this diagnostic review article is to examine the current literature on quantitative tumor markers in human blood samples which have been serially measured for use in follow-up in CRC and to compare their clinical value with CEA measurements. Therefore, we analyzed all available literature on quantitative markers that were serially measured during follow-up of CRC.

Materials and Methods

Systematic literature search and primary outcomes

A comprehensive review of the literature (1985 - 2010) was performed using multiple electronic search engines including PubMed, Embase and the Cochrane Database. The MeSH search term [tumor marker] AND [Colorectal neoplasm] were used, limited to 'English language', 'Humans', and 'Adult', and with exclusion of "Chemotherapy". The 'related articles' function in PubMed was also used. Additional relevant references found in articles were included. Review articles and letters were used as a reference but not included in the analysis. Abstracts were selected on available information concerning the use of tumor markers in follow-up after curative resection of CRC.

Inclusion criteria were [1] curative treatment of any stage of CRC, [2] postoperative surveillance with serial tumor marker measurements in addition to CEA itself, i.e., the marker of interest was quantitatively measured more than once during follow-up, [3] availability of sensitivity data of the tumor marker in indicating recurrent disease and [4] quality score ≥ 4 (Figure 1).

Criterion	Points if Yes	Points if No
1. Is the population under study defined with in- and exclusion criteria?	1	0
2. Were patient data prospectively collected?	1	0
3. Are the main prognostic patient and tumour characteristics presented?	1	0
Is the antibody used specified?	1	0
Are control samples and a cut-off value for positive expression specified?	1	0
5. Is the study endpoint defined?	1	0
6. Is the time of follow-up specified?	1	0
7. Is loss during analysis or follow up described?	1	0

Figure 1. Criteria for quality assessment of a study. The maximum score is 8.

Table 1 (continued on page 59). Characteristics and quality score of reviewed studies.

Author and year	Trial type	Marker	No of patients	Follow-up sSchedule [a]	Length of follow-up [b]	Elevation [c]	Short Conclusion	Quality score
Morita 2004	retrospective	CA 19-9	118	Every 3-6 months from resection; at least 3 postoperative values measured	Median 48 months	2 consecutive elevations beyond cut-off value	No routine use of CA 19-9 in surveillance of colorectal cancer patients	5
Filella 1994	prospective	CA 19-9	370	Trimestral determination	Median 14.5 months Mean 25 months Range 3-47	Elevation above cut-off value, followed by confirmation 15 days thereafter	CEA is the best marker	3
Fucini 1986	prospective	TPA CA 19-9	52	3-monthly for 2 years, 6 -monthly thereafter for 2 years; at least four consecutive assays	Median 47 months Range 17-72	Elevation beyond cut-off value	Routine TPA surveillance does not seem to be justified	6
Guadagni 1993	prospective	CA 72-4 CA 19-9	51	3-monthly for 3 years	Range 2-37 months	Elevation beyond cut-off value	CA 72-4 is recommended in follow-up and has additional value to CEA surveillance	5
Hall 1993	prospective	CA-242	149	3-monthly for 2 years, 6 -monthly thereafter	Median 24 months	Elevation twice beyond cut-off value, or three successive elevations above cut-off value	CA-242 should be routinely measured because it has complementary value to CEA	6
Engaras 2003	prospective	CA 50 CA 242	132	1-monthly for 1 year, at 18 months and at 24 months	Median 77 months	Elevation above cut-off value	CA>30 U/mL1 month after surgery is indicator for recurrence	4
Fernandes 2005	prospective	TPA	81	6-monthly for 2 years, 1 -yearly thereafter	Mean 22 months Max 60 months	Elevation above cut-off value	Follow-up with serial cytokeratins assay allows early detection of recurrences	4
Yakabe 2010	prospective	CA 19-9	227	3-monthly for 3 years 6 monthly thereafter for 2 years	Max 72 months	Elevation above cut-off value; comparison with preoperative value 30 days before resection	The efficiency of CA 19-9 surveillance is low	6
Barillari 1992 [d]	prospective	TPA CA 19-9	54	At least 6 consecutive assays with a maximum of four months in-between	Median 64 months Range 34-78	Elevation above cut-off value	TPA and CA-19-9 surveillance are useful and give better results than sole CEA surveillance	5
Barillari 1991	prospective	TPA CA 19-9	103	3-monthly, at least 6 consecutive assays	*Unknown*	Elevation above cut-off value, followed by confirmation 10 days thereafter	TPA and CA-19-9 are useful in finding recurrences and give better results than sole CEA surveillance	7

[a] Follow-up schedule: the frequency in which the marker of interest was serially measured
[b] Length and median of follow-up differed per study, and range was not always mentioned
[c] Elevation: the definition of elevation in tumor marker as indicated in the article
[d] In this article, only patients with rectal cancer were investigated
[e] For the markers CA 72-4 and CA-195, 71/90 patients

Table 1 (continued from previous page).

Author and year	Trial type	Marker	No of patients	Follow-up sSchedule [a]	Length of follow-up [b]	Elevation [c]	Short Conclusion	Quality score
Plebani 1996	prospective	PIP PIIIP	32	At 3 months, at 12 months, and at the end of follow-up	Median 24 months Range 3-36 months	Elevation above cut-off value; threshold dependent on fixed specificity	Variations in serum levels postoperative may be a useful index for predicting subsequent recurrences	4
Spila 2001	prospective	CA 242 CA 19-9	50	Not mentioned, but longitudinal for at least 5 years	Range 7-117 months	Elevation above cut-off value	CEA remains the marker of choice, but combined elevation of the three markers shows a complementary on CEA	5
Holubec 2000	retrospective	CA 19-9 CA 72-4	393	At least three times between 1 and 6 months after surgery	Mean 26 months	Different cut-offs were retrospectively determined dependent on fixed specificity	CEA and CA 19-9 are comparable, the sensitivity of CA 72-4 is considerably lower	4
Park 2009	prospective	CA 19-9	700	3-monthly for 2 years, 6-monthly thereafter for 1 year	Median 48 months Range 1-156 months	Elevation above cut-off value	Accuracy of serum CA 19-9 surveillance is lower than that of CEA	4
Nicolini 2010	prospective	CA 19-9 CA 72-4 TPA	108	4- or 6-monthly for at least 1 year, dependent of Dukes stage	Mean 99 months Range 13-179 months	Elevation above threshold followed by 30% rise after 2 weeks	Combinations of markers perform best Inclusion of TPA in tumor marker panel increased CEA sensitivity from 47 to 72%	5
Griesenberg 1999	prospective	TPA CA 19-9 TAG 72	116	3-monthly for 2 years, every 6 months thereafter	Median 21 months Range 3-51 months	Elevation above cut-off value	Data not sufficient to recommend the use of CEA surveillance; sensitivity for the tumor marker panel higher than for CEA alone	5
Nicolini 1995	prospective	TPA GICA CA 72-4 CA 195	90 90 71[e] 71	At least one year follow-up, schedule not mentioned	Minimum 12 months	Different types of elevations are defined (“isolated elevation of 20% above cut-off value, constant elevation and progressive increase).	Combination TPA/CA -195 is the best indicator for recurrence	4

[a] Follow-up schedule: the frequency in which the marker of interest was serially measured
[b] Length and median of follow-up differed per study, and range was not always mentioned
[c] Elevation: the definition of elevation in tumor marker as indicated in the article
[d] In this article, only patients with rectal cancer were investigated
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Sensitivity is the percentage of patients correctly identified as having the condition by rise in the tumor marker, while specificity is the percentage of healthy people correctly identified as not having the condition by no rise in tumor marker. Sensitivity and specificity of the markers investigated in each article were recalculated using the following equations:
(1) Sensitivity = 100% X (true positives) / (true positives + false negatives)
(2) Specificity = 100% X (true negatives) / (true negatives + false positives)
Studies were excluded [1] when the full text of the article was not accessible at our institution, [2] when the investigated marker was only qualitatively reported (i.e. absent or present) or [3] when the study only concerned CEA as a marker.

Study quality assessment
Two investigators (CJV and WHJ) independently extracted data from the included studies. Inconsistencies were resolved by consensus. A standardized characteristics and result abstraction form was used to collect descriptive patient data, type of tumor and tumor stage, study design, follow-up schemes, assays and cut-off values. Study quality was assessed independently by the two investigators applying a predefined form with face validity, which was derived from McShane (McShane *et al.* 2005) and used earlier by de Graeff (de Graeff *et al.* 2009, Figure 1). This resulted in a quality score with a minimum of 0 points and a maximum of 8 points.

Results

In total, 224 articles were identified using the above keywords and restrictions. Title and abstract review resulted in the exclusion of 187 articles, which means that 37 articles were searched in full. The process is visualized in Figure 2. After applying the quality restrictions described previously, seventeen studies remained, investigating 8 additional markers. In Table 1, an overview of the included studies is shown, focusing on the value of the tumor markers in finding recurrent disease (Barillari *et al.* 1992, 1991, Engaras 2003, Fernandes *et al.* 2006, Filella *et al.* 1994, Fucini *et al.* 1987, Griesenberg *et al.* 1999, Guadagni *et al.* 1993, Hall *et al.* 1994, Holubec *et al.* 2000, Morita *et al.* 2004, Nicolini *et al.* 1995, 2010, Park *et al.* 2009, Plebani *et al.* 1997, Spila *et al.* 2001, Yakabe *et al.* 2010). The different markers are CA19-9, CA242, CA72-4, CA-195, CA-50, TPA (or TPS), C-terminal peptide (PIP), and N-terminal peptide (PIIIP), the latter two being markers of ECM synthesis. The studies comprised 2594 patients in total (range 24-700 per study). Mean quality score was 4.8 points.

Follow up schedules
Patients entered the follow-up program in all studies after curative treatment. Generally, blood samples were drawn at each follow-up outpatient visit, and surveillance was performed on a 3- or 6-monthly basis. This schedule is the common guideline in all countries from which study data were collected. In 2 studies, there was no strict protocol for tumor marker measurements. Patients from these studies were included if they had serial measurements of the marker in follow-up for a pre-defined number of years (Nicolini *et al.* 1995, Spila *et al.* 2001). In 14 studies follow-up schedules were strict and well-described. The length of follow-up differed per study. Unfortunately the numbers of patients lost to follow-up are not mentioned in most studies, which was reflected in our quality score assessment. In Table 1, follow-up schedules are mentioned and further commented on.

Cut-off values and assays
The cut-off values and assays used per tumor marker are shown in Table 2. In this table the recalculated sensitivity and specificity for the marker of interest are also given. For the marker that was most intensively

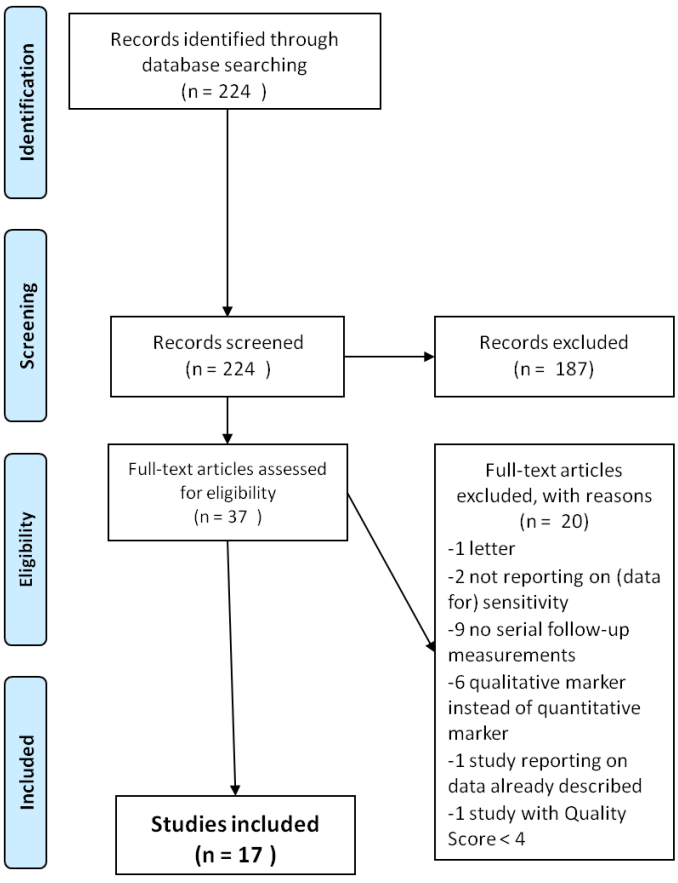

Figure 2. Inclusion form. Adapted from Moher *et al.* 2009.

Table 2 (continued on page 62). Characteristics of investigated tumor markers: Sensitivity, specificity, applied assays and cut-off values.

Marker	Reference	Sensitivity - %	Specificity - %	Assay	Cut off value	CEA Sensitivity (%)	CEA specificity (%)	CEA assay	CEA cut-off value ng/mL
CA 19-9	Fucini	20	90	Radioimmunoassay (RIA) Sorin Biomedica	37 U/mL	90	78	RIA (CEA-PR, Sorin Biomedica)	NR[a]
	Holubec	32-59	95	RIA CIS Bio International, France	Calculated for recurrences with a fixed specificity [b]	43-84	95	IRMA, Immunotech, Czech Republic	Calculated for recurrences with a fixed specificity[b]
	Yakabe	33	97	Immunoassay Mitsubishi Chemical Ltd	38 ng/mL[c]	69	95	Latex immunoassay Mitsubishi Chemical Ltd	4.5
	Barillari 1992	60	88	RIA Sorin Biomedica	25 U/l	73	61	RIA (CEA-PR), Sorin Biomedica	3.0
	Barillari 1991	73	87	RIA Sorin Biomedica	25 U/l	73	77	RIA (CEA-PR, Sorin Biomedica)	3.0
	Morita	48	88	Immunoassay; unknown which brand	37 U/mL	93	88	Immunoradiometric (IRMA) & chemiluminescent assay[d]	2.5 – 5 [d]
	Spila	64	71	RIA Centocor Fujirebio Diagnostics	37 U/mL	64	79	RIA, Abbott, USA	5.0
	Guadagni	52	100	RIA Centocor Fujirebio Diagnostics	37 U/mL	78	100	RIA, Abbott	5.0
	Filella	49	83	RIA Sorin Biomedica	37 U/mL	84	NR[a]	RIA, Abbott	5.0
	Nicolini 2010	9	100	Chemiluminescent immunoassay, Abbott	40 U/mL	47	94	Chemiluminescent Immunoassay, Abbott	3.0
	Griesenberg	50	[e]	Enzyme Immuno Assay (EIA) Cobas Care Roche	35 U/mL	78	[e]	Enzymun-Test, ELISA, Boehringer	3.0
	Park	21	96	NR[a]	37 U/mL	76	85	NR[a]	7.0
CA 242	Hall	60	87	Fluoroimmunoassay,Delfia, Finland	20 U/mL	76	86	RIA, Nottingham, UK	3 times >3.0 Or once >10.0
	Engaras[f]	43 / 63	76 / 80	Fluoroimmunoassay Delfia	24.5 U/mL	63 / 79	64 / 82	NR[a]	5.6
	Spila	67	64	EIA Sweden	20 U/mL	64	79	RIA, Abbott	5.0
CA 72-4	Nicolini 2010	9	100	Electrochemoluminescent assay, Roche	6.9 U/mL	47	94	Chemiluminescent ImmunoAssay, Abbott	3.0

[a] NR: Not Reported in article; data could not be extracted from the tables and results of the article
[b] For a fixed specificity of 95% versus No Evidence of Disease, different sensitivities were calculated for groups with different outcomes of follow-up
[c] Optimal cut-off values were calculated using ROC curves and histologically confirmed recurrences
[d] During the study the cut-off value was altered from 2.5 to 5.0 ng/mL since the assay was changed from an immunoradiometric assay to a chemiluminescent assay
[e] No information on elevations in tumor markers for patients without recurrence was given, only patients with recurrences were included
[f] Individual cut-off levels were calculated and compared to standard cut-off levels; as a result two independent values for sensitivity and specificity are given
[g] These cut-off values were set at 3 months postoperative.

Table 2 (continued from previous page).

Marker	Reference	Sensitivity - %	Specificity - %	Assay	Cut off value	CEA Sensitivity (%)	CEA specificity (%)	CEA assay	CEA cut-off value ng/mL
	Nicolini 1995	69	90	RIA Centocor	3.8 U/mL	61	96	RIA, Sorin Biomedica	7.0
	Guadagni	78	100	RIA Centocor	6 U/mL	78	100	RIA, Abbott	5.0
	Holubec	30-67	95	RIA CIS Bio International	Not mentioned, but calculated for recurrences with a fixed specificity[a]	43-84	95	IRMA, Immunotech, Czech Republic	Calculated for recurrences with a fixed specificity
	Griesenberg	68	[e]	RIA Sorin Biomedica	3.5 U/mL	78	[e]	Enzymun-Test, ELISA, Boehringer	3.0
TPA	Fucini	60	50	RIA AB Sangtec Medical	85U/L	90	78	RIA (CEA-PR, Sorin Biomedica)	NR[a]
	Fernandes	75	72	Latex ImmunoAssay (LIAmat) TPA-M Prolifigen	72 U/L	50	77	Delfia	5.0
	Barillari 1992	79	61	RIA AB Sangtec Medical	85 U/L	73	61	RIA (CEA-PR), Sorin Biomedica	3.0
	Barillari 1991	73	87	RIA Sangtec Medical	85 U/L	73	77	RIA (CEA-PR, Sorin Biomedica)	3.0
	Nicolini 2010	25	100	RIA Sangtec Medical	95 U/L	47	94	Chemiluminescent Immunoassay, Abbott	3.0
	Nicolini 1995	72	85	RIA Sangtec medical	85 U/L	61	96	RIA, Sorin Biomedica	7.0
	Griesenberg	53	[e]	IRMA Beki Diagnostics Biermann	90 U/L	78	[e]	Enzymun-Test, ELISA, Boehringer	3.0
CA-50	Engaras[f]	46 / 57	77 / 82	Fluoroimmunoassay Delfia, Finland	30,1 U/mL	63 /79	64 / 82	NR[a]	5.6
PIP	Plebani	9	95	RIA Orion	90 ug/L [g] fixed specificity at 95%	NR[a]	NR[a]	NA	NA
PIIIP	Plebani	36	95	IRMA CIS Diagnostici	1.00 U/mL[g] fixed specificity at 95%	NR[a]	NR[a]	NA	NA
GICA	Nicolini 1995	61	88	IRMA CIS Diagnostici	40 U/mL	61	96	RIA, Sorin Biomedica	7.0
CA 195	Nicolini 1995	83	77	EIA Genetic System	10.5 U/mL	61	96	RIA, Sorin Biomedica Biomedica	7.0

[a] NR: Not Reported in article; data could not be extracted from the tables and results of the article
[b] For a fixed specificity of 95% versus No Evidence of Disease, different sensitivities were calculated for groups with different outcomes of follow-up
[c] Optimal cut-off values were calculated using ROC curves and histologically confirmed recurrences
[d] During the study the cut-off value was altered from 2.5 to 5.0 ng/mL since the assay was changed from an immunoradiometric assay to a chemiluminescent assay
[e] No information on elevations in tumor markers for patients without recurrence was given, only patients with recurrences were included
[f] Individual cut-off levels were calculated and compared to standard cut-off levels; as a result two independent values for sensitivity and specificity are given
[g] These cut-off values were set at 3 months postoperative.

investigated, CA 19-9, 6 different assays were used (CIS Biomedical, Abbott, Sorin, Mitsubishi, Centocor, and Bayer) using 2 different cut-off values. In one study cut-off levels were variable, calculated per outcome group with a fixed specificity of 95% (Holubec *et al.* 2000). For this study calculated and recalculated sensitivities and specificities are added.

Best marker

For studies reporting on tumor markers with a sensitivity of more than 60% a separate overview is shown in Table 3. It demonstrates sensitivity and specificity of both markers and CEA as found in the same article. Studies with higher sensitivities for the investigated marker than for CEA are highlighted.

Results in Table 3 show that, according to two studies, TPA is a better marker in finding recurrent disease than CEA. Furthermore, the combination of CEA with an additional marker in several combinations increases the sensivitiy for detection of recurrent disease. Barillari performed a well-described prospective study on TPA, which showed high sensitivity (79%) and low false-positive rates. This study was, however, restricted to rectal cancer (Barillari *et al.* 1992). Fernandes performed a study on TPA with different study design and assay methods, calculating the sensitivity and specificity per rise in TPA with Receiver-Operator Curves (ROC). He found a bigger area under the curve for TPA than for CEA, especially in the first postoperative year (Fernandes *et al.* 2006).

For two other markers higher or similar sensitivity was shown than for CEA: CA 242 and CA 72-4 (Guadagni *et al.* 1993, Nicolini *et al.* 1995, Spila *et al.* 2001). Guadagni performed one of the first prospective studies for CA after introducing monoclonal antibody technology on patients with both benign and malignant disease (n=300). He also performed a sub-analysis of recurrent malignant disease (n=51), thereby finding a sensitivity of 83% for CA 72-4 and a positive predicting value of 100% (Guadagni *et al.* 1993). Spila et al performed a similar longitudinal analysis on CA 242 in which both benign and malignant diseases (n=630) were included with sub-analysis of 50 patients with recurrent malignant disease. Although CA 242 showed a slightly better sensitivity in finding these recurrences than CEA, the overall increase of sensitivity after addition of CA 242 and CA 19-9 to that of CEA alone was about 8% with a false positive rate of 36% (Spila *et al.* 2001).

Discussion

Main results

Our review comprised the available literature considering the follow-up of CRC, focusing on serially and quantitatively measured tumor markers. Four studies concluded that markers other than CEA had higher sensitivities than CEA itself (Barillari *et al.* 1992, Fernandes *et al.* 2006, Guadagni *et al.* 1993, Spila *et al.* 2001), with sensitivities higher than 60%.

The finding of TPA as a tumor marker was a surprising finding; in the Netherlands most studies have focused on immunological rather than proliferative markers and measurement of TPA serially is unusual. In two independent studies TPA showed to have higher sensitivity than CEA for recurrence of CRC (Barillari *et al.* 1992, Fernandes *et al*. 2006). TPA is a constituent of the epithelial cells of many hollow organs, and is found in tissues undergoing rapid growth, such as tumor cells. Measurement of serum TPA is relatively cheap, TPA is measured by an easily accessible technique, and is therefore broadly available. However, both studies have been performed more than 5 years ago. Recently (in 2010) Nicolini failed to establish the accuracy of TPA. When used as an individual tumor maker, TPA's level was increased in 8/32 recurrences (sensitivity 25%). When integrated in a tumor marker panel together with CEA, TPA resulted in an increase of CEA sensitivity from 46% to 79% (Nicolini *et al.* 2010).

A well-performed large study on CA 19-9 failed to show higher recurrence detection than with CEA as a tumor marker (sensitivity 43% vs. sensitivity 63%). However, its sensitivity was increased when individual cut-off values were applied, based on the lowest postoperative value corrected for inter-assay variation (Engaras 2003). As for CA 72-4, newer studies did not confirm the clinical use of this marker (Carpelan-Holmstrom *et al.* 2004, Holubec *et al.* 2000).

Points of discussion

In all patients included in our review postoperative serial measurements were performed, independent of the preoperative marker level (which was in some studies not measured at all). The relationship between the preoperative value of CEA and the secretion of CEA by recurrences is still under debate. Several studies conclude that postoperative surveillance with CEA is useful regardless of the preoperative value. These studies strengthen our conviction that serial measurements of other markers of interest are also useful when the preoperative value is not known (Grossmann *et al.* 2007, Zeng *et al.* 1993).

The currently emerging class of molecular tumor markers includes circulating nucleic acids, epigenetic alterations, gene-expression profiles and analysis of circulating cancer cells. We realize that by exclud-

Table 3. Sensitivity and specificity per tumor marker compared to CEA and marker panels used in the same study.

Marker	Reference	Sensitivity marker (%)	Specificity marker (%)	Sensitivity CEA (%)	Specificity CEA (%)	Marker panel	Sensitivity tumor marker panel (%)	Specificity tumor marker panel (%)
CA 19-9	Barillari 1992	60	88	73	61	NA		
	Spila	64	71	64	79	CEA - CA 19-9	72	64
	Guadagni	52	100	78	100	CEA - CA 19-9 CEA – CA 19-9 – CA 72-4	78 87	100 100
CA 242	Hall	60	87	76	86	CEA - CA-242	88	78
	Spila [a]	67	64	64	79	CEA - CA 242	72	50
CA 72-4	Guadagni [a]	78	100	78	100	CEA - CA 19-9 CEA – CA 19-9 – CA 72-4	78 87	100 100
	Holubec	30-67	95	43-84	95	CEA - CA 72-4	73	95 (fixed)
	Nicolini 1995[a]	69	90	61	96	1. TPA – GICA 2. TPA – CA 195 3. TPA – GICA – CA 72.4	1. 88 2. 100 3. 92	1. 75 2. NR 3. NR
	Griesenberg	68	?	78	?	CEA – CEA 19-9 – TPA	84	?
TPA	Fucini	60	50	90	78	NA		
	Fernandes[a]	75	72	50	77	NA		
	Barillari 1992[a]	79	61	73	61	NA		
	Barillari 1991	73	87	73	77	NA		
	Nicolini 1995	72	85	61	96	1. TPA – GICA 2. TPA – CA 195 3. TPA – GICA – CA 72.4	1. 88 2. 1003. 92	1. 75 2. NR 3. NR
GICA	Nicolini 1995	61	88	61	96	1. TPA – GICA 2. TPA – CA 195 3. TPA – GICA – CA 72.4	1. 88 2. 100 3. 92	1. 75 2. NR 3. NR
CA 195	Nicolini 1995	83	77	61	96	1. TPA – GICA 2. TPA – CA 195 3. TPA – GICA – CA 72.4	1. 88 2. 100 3. 92	1. 75 2. NR 3. NR

Only markers with a calculated sensitivity of > 60% are included.
For variable sensitivities per stage, variable values are given
NA: not applicable, no analysis on the value of tumor marker panels is performed
[a] This studies showed a higher sensitivity for the additional marker than for CEA

ing histologically estimated markers and qualitative markers, most of these new tumor markers have not been included in this review. However, by applying strict inclusion criteria we avoided creating a selection bias and thus, the comparability of studies is more reliable. Furthermore the use of serum measurements in daily practice is common and easy in comparison to the use of histological markers.

Tumor marker panels are relatively new and promising in the follow-up of colorectal cancer. Additive value on indicating recurrence is often found, suggesting panels could outperform routine imaging techniques in follow-up, with favorable financial perspectives. Recent evaluation of an extensive tumor marker panel demonstrated an increase in sensitivity in finding recurrences (Nicolini *et al.* 2010). Sensitivity was raised from 47% to 71% by adding TPA to CEA measurements. In the current review, the additional value of tumor marker panels has also been shown.

Specificity of tumor markers is the number of patients without recurrence who are correctly identified by the tumor marker as *not* having a recurrence. It is known that some tumor markers not only increase in case of recurrent disease, but also in several non-malignant processes such as infection and smoking (van Larebeke *et al.* 2003). The studies showing a high sensitivity for TPA demonstrated specificities of 61 and 72%, which we considered acceptable.

Limitations

Differences in study designs regarding patient and tumor stage selection and follow-up schedules influences our conclusions. The authors recognize that the comparison of different designs is the main weakness of our study. However, criteria for patient selection and follow-up schedules have not yet been standardized. We tried to overcome this bias issue by applying strict study selection using standardized study criteria and two independent reviewers. In addition the review was constructed following the REMARK guidelines (McShane *et al.* 2005). We excluded studies with quality scores that were too low according to these guidelines. Furthermore, it is important to realize that only serially and quantitatively measured follow-up markers were considered in this review. Since nucleic acid markers are qualitative markers, most of these were excluded; they either are present or absent. Consequently, bias resulting from different study designs was diminished.

The "cut-off" level determines sensitivity and specificity of the tumor marker. Therefore, the diagnostic accuracy of the marker depends on the cut-off level applied. In addition, comparability of data is influenced by this cut-off level. A variety of markers measured with several assays were included; cut-off values differed per study, therefore resulting in bias. In all except one study immunoassays were used for quantification of test results. In the one study that did not, various cut-off values were tested to obtain the value with the highest sensitivity. Immunoassays are known to have high analytical sensitivity, which means low concentrations can be measured reliably. Other strengths of immunoassays are the potential of full automation and its practicability, with relative little technical expertise required. The main problem in comparing immunoassay results, however, is the fact that results obtained from each commercial available assay depend on their own antibody with its specific characteristics. This leads to different cut-off values and reference values for a single marker and complicates inter-laboratory comparability. Also the inter- and intra-assay variability of the commercially obtained assays causes difficulties for patient follow-up studies (Wood 2008).

Future perspectives

Although TPA shows to be promising in outperforming CEA in finding colorectal recurrences, no conclusive prospective clinical trial has been performed. A recent study showed an increase in finding curable metastases with intensive surveillance using a tumor marker panel including TPA (Nicolini *et al.* 2010), but we did not find conclusive studies on the value of TPA alone. As there is no definitive consensus regarding the postoperative surveillance after curative resection for colorectal cancer, we propose a large prospective follow-up trial focusing on the true value of TPA in colorectal cancer follow-up.

At this time, a national trial with frequent CEA testing and CEA-triggered imaging in follow-up is performed in the Netherlands (Netherlands Trial Register (NTR) 2182). Serum samples of the included patients in this large trial are being stored in a Biobank, and since clinical conditions of all patients are well-described, this enables us to test sensitivity and specificity of TPA in addition to a tumor marker panel consisting of both CEA and TPA (and other possible new markers of interest). All patient characteristics including tumor stage will be registered. Advantages of the Biobank would be the uniform strategy in which all sera are analyzed with the same assay, and that all patients undergo the same study regimen and design. Based on the results of this review, we could start with TPA measurements and subsequently measure other promising markers.

Conflicts of interest

All authors declare that there was no conflict of interests.

Author Contributions

Charlotte Verberne is the first author. She constructed the idea for this review while working on a colorectal cancer follow-up study. She performed the literature search, reviewed all articles on eligibility, constructed the tables and wrote the manuscript. Charlotte works as a surgical resident.

Helma de Jong is the first author (together with Charlotte Verberne). She performed the same literature search independently and reviewed all found articles, hereby including the eligible articles. In this way an independent literature review was performed. Hereby she helped writing the manuscript and reviewed the manuscript, with special attention to the tedious tables. Helma works as a clinical chemistry resident.

Irene Grossmann reviewed the literature on the available evidence on clinical effectiveness of CEA as tumor marker in the follow-up after curative treatment of colorectal cancer and overviewed the whole manuscript on accuracy and English language.

Geertruida de Bock works as a Professor in Epidemiology and managed the process of independent literature search and inter-reviewer correlations and gave supervision on the whole manuscript.

Theo Wiggers is Professor in Oncological Surgery and closely involved in follow-up studies for colorectal cancer. He is the supervisor for all surgical-medical topics in this manuscript.

Ido Kema is Professor in Clinical Chemistry and gave feedback on the whole manuscript, especially on the parts of study quality, future perspectives and the whole Discussion section.

Anneke Muller-Kobold is the last author. She has a PhD and works as clinical chemist in the University Hospital of Groningen. She gave direction to the whole process of writing the manuscript and punctually reviewed all chapters of the review.

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