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Post-operative serum CA19-9, YKL-40, CRP, and IL-6 in combination with CEA as prognostic markers for recurrence and survival in colorectal cancer.

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ABSTRACT

Background:

In colorectal cancer (CRC) patients, guidelines only recommend measurement of pre-operative carcinoembryonic antigen (CEA), although post-operative CEA may be more informative.

However, the sensitivity of both pre-operative and post-operative CEA in identifying relapse is limited. We studied whether CA19-9, YKL-40, C-reactive protein (CRP), and interleukin (IL)-6 add prognostic information combined with post-operative CEA.

Material and methods:

This post-hoc analysis included 147 radically resected stage II (n=38), III (n=91), and IV (n=18) CRC patients treated with adjuvant 5-fluorouracil (5-FU)-based therapy in the phase III LIPSYT study (ISRCTN98405441). We collected post-operative blood samples a median 48 days after surgery. We analysed relapses, sensitivity, positive predictive value (PPV), and disease-free (DFS) and overall survival (OS) by bootstrap, Kaplan-Meier, and adjusted Cox-models in the elevated vs. normal biomarker groups.

Results:

Elevated post-operative CEA associated with impaired DFS (HR 7.23; CI_{95%} 3.85-13.58), impaired OS (HR 7.16; CI_{95%} 3.76-13.63), and more relapses (HR 7.9; CI_{95%} 3.4-18.2); but sensitivity for CEA in finding relapses was only 31% (CI_{95%} 21-48%). Normal CEA combined with an elevated YKL-40 or elevated CRP showed more relapses (HR for YKL-40 2.13 [CI_{95%} 1.10-4.13], HR for CRP 3.14; [CI_{95%} 1.21-8.16]), impaired DFS (HR 2.18 [CI_{95%} 1.12-4.24] or 3.23 [CI_{95%} 1.34-7.82]), and impaired OS (2.33 [CI_{95%} 1.24-4.40] or 2.68 [CI_{95%} 1.12-6.44]). Elevated CEA combined with a concomitantly elevated CA19-9, YKL-40, CRP, or IL-6 showed a respective PPV of 100%, 90%, 100%, and 100%.

Conclusion:

In radically operated stage II to IV CRC patients who received adjuvant 5-FU-based, a post-operatively elevated CEA alone or in combination with CA19-9, YKL-40, CRP, or IL-6, or a normal CEA combined with an elevated YKL-40 or with an elevated CRP, may indicate patients at high risk of relapse.

INTRODUCTION

Treatment of choice for locoregional colorectal cancer (CRC) and resectable metastases is surgery with curative intent. Despite improvements achieved in surgery and in adjuvant chemotherapy, 30-50% of stage II-III patients and 50% with resected stage IV disease will develop a recurrence [1-4].

There is a need for biomarkers to determine patients' risk for recurrence; routine high-risk factors have proven unsatisfactory [1, 3, 4]. Several new multigene assays provide individualized prognostic information, and liquid biopsies are emerging as a method to detect minimal residual disease. Yet, methodologically, these are challenging technologies, and data to support their use in everyday clinical practice are insufficient [3-5].

Blood-based biomarkers are easy to sample and could be ideal for evaluating risk of cancer recurrence. CEA, carcinoembryonic antigen, is a tumour-associated glycoprotein, rather than a tumour-specific marker. For years, CEA has been the only widely recommended biomarker for CRC prognostics and follow-up [6, 7]. However, the sensitivity of pre-operative CEA in detecting CRC recurrence is only 50–80%, and the specificity 80% [8]. There are studies showing that, post-operative CEA is more informative than pre-operative CEA [9, 10]. New biomarkers are therefore essential to improve CEA's clinical utility.

CA19-9, carbohydrate antigen 19-9, is a widely used marker in gastrointestinal cancers [11], but its prognostic value after resection of CRC is less established [6, 7]. CRC is associated with inflammation, which promotes carcinogenesis and tumour growth [12]. Therefore, inflammation-related biomarkers or mediators such as YKL-40 (also known as human cartilage glycoprotein-39 or chitinase-3-like-1 protein), c-reactive protein (CRP), and interleukin (IL)-6 have emerged as prognostic biomarkers. YKL-40 is a secreted protein produced by non-malignant cells such as macrophages and by cancer cells such as colon cancer cells [13]. YKL-40 promotes cancer proliferation and inflammatory cytokine production [14]. CRP is a non-specific acute-phase

reactant reflecting tissue damage, and is also a sensitive and stable marker of inflammation [15]. In cancer patients, including CRC, elevated serum CRP is associated with inflammation, cachexia, and poor prognosis both in metastatic and localized disease [16]. IL-6, mainly produced by monocytes and macrophages, among others, as well as by various tumour cells [17] stimulates YKL-40 production and angiogenesis; it is regarded as an important tumour-promoting factor in various human cancers, including CRC [18]. Our aim was to evaluate the prognostic value of post-operative CA19-9, YKL-40, CRP, and IL-6 in combination with CEA in radically resected stage II-IV CRC patients receiving adjuvant chemotherapy.

MATERIAL AND METHODS

The original LIPSYT trial was an open-label, prospective, randomised single-institution study in patients with radically resected CRC (<http://www.controlled-trials.com/ISRCTN98405441>). Patients included received treatment at the Department of Oncology of Helsinki University Hospital Finland) between November 1997 and August 2001. That study's primary aim was to assess treatment tolerability in a two-by-two factorial design; the secondary aim was to study biomarkers.

This is a post hoc analysis of the LIPSYT trial, with 150 patients, of which 3 never started treatment. Inclusion criteria were age 18 to 75 years, histologically confirmed radically resected stage II–IV CRC (radical metastasectomy of mostly liver metastases, n=18), WHO performance status 0–2 and adequate bone-marrow-, kidney-, and liver function. Exclusion criteria included history of invasive cancer other than CRC; metabolic, neurological, or psychiatric illness incompatible with chemotherapy; serious thromboembolic event currently under treatment; pregnancy, lactation, or absence of adequate contraception in fertile patients. The protocol was approved by an institutional review board and all study participants gave their signed informed consent. Adjuvant chemotherapy consisted of 5-FU and LV as bolus injection (Mayo regimen) or continuous infusion (simplified de Gramont regimen) according to randomization [19].

Assessment of biomarkers

CEA, YKL-40, CRP, IL-6, and CA19-9 were measured in serum samples collected post-operatively before adjuvant chemotherapy began. Median time from surgery to laboratory sampling and treatment initiation was 48 days (range 19-124) and more than 8 weeks in 40 (27%). The reason for treatment delay was referral-delay to oncology for 33 patients, and 7 patients being unfit to start oncologic treatments within 8 weeks.

CRP, CEA, and CA19-9 were determined by the routine laboratory of the university hospital with automatic analyzers as follows: CRP: immunoturbidimetric method, CEA and CA19-9: immunoenzymatic assay, Bayer Immuno 1 (CEA: 10/1998-10/2005; and CA19-9: 1/1998-1/2006), or immunochemiluminometric assay, Abbott Architect (CEA: 10/2005→ and CA19-9: 1/2006→). All measurements were performed by technicians blinded to study endpoints.

Blood samples for YKL-40 and IL-6 were collected in gel tubes and centrifuged within 2 hours; serum was stored at -20 °C until analysis. YKL-40 and IL-6 were determined in duplicate with commercially available enzyme-linked immunosorbent assays (ELISAs); YKL-40: MicroVue YKL-40 ELISA (Catalog #8020), Quidel Corporation, San Diego, CA, USA; and IL-6: Quantikine HS600B, R&D Systems, Abingdon, OX, UK; according to manufacturer's instructions. For YKL-40, the detection limit was 20 ng/ml and intra- and inter-assay coefficients of variation (CVs) of <5% and <6% [17]. For IL-6, detection limit was .01 pg/ml and intra- and inter-assay CVs of ≤8% and ≤11% [20].

An age-corrected percentile for YKL-40 was calculated according to the formula [17]: $\text{percentile} = 100 / (1 + (\text{YKL-40}^{-3}) * (1.062^{\text{age}}) * 5000)$. Cut-off values were: ROC corrected cut-off for age-corrected YKL-40 level as the 70.7th percentile of normal controls YKL-40 (see statistics), 4.5 pg/ml for IL-6 [20]; 10 mg/l for CRP; 5 µg/l for CEA; and 26 kU/l for CA19-9 according to clinical routine at HUSLab, Helsinki, Finland.

Statistics

Clinicopathological parameters and tumour-marker values are presented as frequencies or medians with range for nonparametric distributions. The chi-squared test served for comparisons between categorical variables, and the Mann-Whitney or Kruskal-Wallis for non-normally distributed continuous variables.

Time-dependent receiver-operating characteristic (ROC) curves were constructed for DFS at 5 years of follow-up, and area under the curve (AUC) values determined (timeROC package in R, <https://cran.r-project.org/web/packages/timeROC/v0.3> by Paul Blanche). The cut-off value for YKL-40 (70.7th) was obtained by maximizing Youden's index at 5 years of follow-up, giving sensitivity and specificity equal weight.

Descriptive survival analysis was performed with the Kaplan–Meier estimator, and the log-rank test served to compare groups. Disease-free survival (DFS) was defined as a composite endpoint of recurrence or death from any cause with censoring at the last date of follow-up at 10+ years from post-operative sampling. The endpoint for overall survival (OS) was death from any cause with censoring at the last date of follow-up. Median follow-up time for patients alive was 11.9 (range 8.9-12.7) years. Unadjusted and adjusted hazard ratios (HRs) and 95% confidence intervals (CI_{95%}) were estimated with the Cox regression proportional hazard model. Firth's penalized maximum likelihood bias correction method for Cox regression was used, if mentioned.

Adjustment was for age, sex, inflammatory disease, and stage in univariate analysis and further for CEA, CA19-9, YKL-40, CRP, and IL-6 in multivariate models. Inflammatory disease was a history of autoimmune disease such as rheumatoid arthritis, iritis, psoriasis, ulcerative colitis, coeliac disease, and thyroiditis in 14 patients. None had active ulcerative colitis. For the multivariate model, all biomarkers with background variables were included (into the model). Relapse events at 7 years were defined as CRC recurrence (distant or local), new CRC, or death from CRC, but new non-CRC cancer or deaths from other causes were not events. Specificity, sensitivity, and positive predictive value (PPV) for a biomarker being elevated vs. normal were calculated for relapse at 7 years by using non-colorectal deaths as competing events. The 95% bias-corrected accelerated CIs (BCa) were obtained by a bootstrapping method with 1000 replications (boot package in R [21]). The Clopper-Pearson confidence interval was calculated for PPV in subgroup analysis. Relapse was analysed with the Cox proportional hazards regression, with other deaths as competing risks, and

adjusted for age (median cut off), sex, inflammatory disease, and TNM stage. Subgroup analyses are, however, unadjusted.

The statistical significance level was set at 5%; all tests were two-sided. Statistical analyses were done with SPSS version 24.0 (IBM SPSS Statistics, version 22.0 for Mac; SPSS, Inc., Chicago, IL, USA), R version 3.6.1 (Foundation for Statistical Computing, Vienna, Austria), SAS for Windows (v9.4, SAS institute Inc., Cary, NC, USA) and STATA/MP (v.15.1, StataCorp LLC, College Station, TX, USA).

RESULTS

Baseline characteristics and biomarker levels

The 147 patients were of median 60 years (range 31-76) and most presented with a locoregional disease (88% stage II-III, Table 1). After 10 years, 77 (52%) were still alive. Relapse was detectable in 65 (44%), with no new relapses after 6.3 years. Cause of death was metastatic CRC (mCRC) in 84% (58/70), cardiovascular in 10% (7/70), second cancer in 3% (2/70), and other cause in 3% (2/70). Levels of CEA, CA19-9, YKL-40, or CRP, according to primary location (right colon vs. left colon vs. rectum), showed no differences, but in patients with rectal cancer (Table 1), IL-6 was higher.

CEA

Elevated CEA showed an increased hazard of relapse; the HR adjusted for background variables was 7.91 (CI_{95%} 3.43-18.24, other deaths as competing risk; Table 2). For CEA elevated vs. normal, the specificity was 97% (CI_{95%} 91-100), sensitivity 31% (CI_{95%} 21-48), and PPV 89%, CI_{95%} (65-99%; Table 2).

In univariate analysis adjusted for baseline characteristics, elevated CEA associated with impaired DFS (HR 7.23, CI_{95%} 3.85-13.58; 10-year DFS rate 6% for elevated CEA vs. 63% for normal CEA; Table 3, Figure 1A) and impaired OS (HR 7.16, CI_{95%} 3.76-13.63); 10-year OS rate 6% for elevated CEA vs. 68% for normal CEA; Table 3, Figure 1B).

In multivariate analysis, elevated CEA associated with impaired DFS (HR 8.63, CI_{95%} 3.82-19.50) and impaired OS (HR 10.17, CI_{95%} 4.35-23.79; Table 3).

CA19-9

Elevated CA19-9 levels vs. normal had an adjusted HR of 2.08 (95% CI .87-4.98) for relapse, using other deaths as competing events. Elevated vs. normal CA19-9 showed sensitivity of 16% (CI_{95%} 8-27%), specificity 89% (CI_{95%} 79-95%), and PPV 53% (CI_{95%} 27-78%, Table 2). No association appeared between elevated CA19-9 and DFS or OS (Table 3), which was true also for the subgroup with elevated CA19-9 and normal CEA.

All five patients with concomitantly elevated CEA and CA19-9 relapsed (PPV 100%, CI_{95%} 48-100%, the Clopper–Pearson interval), had a numerically shorter DFS (.41 vs. .76 years, HR 3.16 CI_{95%} .82-14.0, with Firth’s bias correction) and significantly shorter OS (1.8 vs. 2.9 years, HR 4.46; CI_{95%} 1.1-20.0, Firth’s bias correction), compared to patients with either elevated or both normal.

YKL-40

Elevated YKL-40 showed an increased hazard of relapse; HR adjusted for background variables being 1.73 (CI_{95%} 1.02-2.93, other deaths as competing risk). The sensitivity for finding relapses was 56% (CI_{95%} 43-69), specificity 69% (CI_{95%} 58-79), and PPV 57% (CI_{95%} 45-69%; Table 2). In univariate analysis, elevated YKL-40 vs. normal associated with impaired DFS (HR 1.84, CI_{95%} 1.14-2.96, 10-year DFS rate 37% vs. 59%) and impaired OS (HR 1.97, CI_{95%} 1.20-3.23, 10-year OS rate 43% vs. 64%; Table 3). In adjusted multivariate analysis, elevated YKL-40 associated with impaired OS (HR 2.24, CI_{95%} 1.23-4.05), but not with impaired DFS (HR 1.58, CI_{95%} .88-2.86; Table 3).

In the subgroup with normal CEA, elevated YKL-40 had an unadjusted HR of 2.13 (95% CI 1.10-4.13) for relapse, impaired DFS (HR 2.30, CI_{95%} 1.27-4.16; Figure 2A), and OS (HR 2.40, CI_{95%} 1.28-4.52; Figure 2B) compared with normal YKL-40. Patients with normal CEA and elevated

YKL-40 had a 10-year DFS rate of 49% vs. 71% in patient with both normal, and a 10-year OS rate of 55% vs. 77%.

In the small subgroup with concomitant elevation of both YKL-40 and CEA, nine of ten patients without competing deaths had a relapse (PPV 90%, CI_{95%} 56-100%, Clopper–Pearson interval; Table 2), numerically shorter DFS (.68 vs. .76 years, HR .82, CI_{95%} .31-2.15) and numerically shorter OS (1.5 vs. 2.6 years, HR 1.32, CI_{95%} .51-3.44) than did patients with either elevated or both normal.

CRP

Elevated CRP levels vs. normal levels had an adjusted HR of 1.95 (CI_{95%} .97-3.94) for relapse using other deaths as competing events. Elevated CRP showed sensitivity of 20% for finding relapses (CI_{95%} 12-30%), specificity of 96% (CI_{95%} 89-99%) and PPV of 77% (CI_{95%} 44-93%; Table 2). In univariate analysis, elevated vs. normal CRP associated with impaired DFS (HR 2.31, CI_{95%} 1.21-4.39; 10-year DFS rate 18% vs. 53%) and impaired OS (HR 2.40, CI_{95%} 1.20-4.80; 10-year OS rate 24% vs. 59%). In adjusted multivariate analysis, elevated CRP associated with impaired DFS (HR 2.53; CI_{95%} 1.10-5.81), but not with OS (HR 2.49; CI_{95%} .95-6.51; Table 3).

In the subgroup with normal CEA and elevated CRP, unadjusted HR for relapse was 3.14 (CI_{95%} 1.21-8.16). Patients with normal CEA and elevated CRP had an impaired DFS (HR 3.54, CI_{95%} 1.57-8.02; Figure 2C) and an impaired OS (HR 3.10, CI_{95%} 1.29-7.45; Figure 2D) compared with patients with normal CEA and normal CRP. Patients with normal CEA and elevated CRP had a 10-year DFS rate of 30% vs. 65% with normal CEA and normal CRP, and a 10-year OS rate of 40% vs. 71%.

All four patients with concomitant elevation of CEA and CRP had a relapse (PPV 100%, CI_{95%} 40-100%; Table 2), numerically shorter DFS (.39 vs. .76 years, HR 3.13, CI_{95%} .90-10.90), and

numerically shorter OS (1.8 vs. 2.6 years, HR 1.52, CI_{95%} .48-4.77) than did patients with either elevated or both normal.

IL-6

Elevated IL-6 predicted increased hazard of relapse; HR adjusted for background variables was 2.12 (CI_{95%} 1.11-4.03, other deaths as competing risk). Sensitivity for elevated IL-6 in finding relapses was 28% (CI_{95%} 17-40%), specificity 91% (CI_{95%} 84-97%) and PPV 75% (CI_{95%} 53-90%; Table 2). In univariate analysis, elevated IL-6 associated with impaired OS (HR 1.99, CI_{95%} 1.05-3.76; 10-year OS rate for elevated IL-6 vs. normal IL-6 was 33% vs. 58%) but not with impaired DFS (HR 1.77, CI_{95%} .95-3.30, 10-year DFS rate for elevated IL-6 vs. normal IL-6 being 25% vs. 53%; Table 3). In multivariate analysis, IL-6 was not an independent biomarker of DFS or OS (Table 3).

Normal CEA and elevated IL-6 did not associate with impaired DFS (HR 1.36, CI_{95%} .53-3.51) or impaired OS (HR 1.30, CI_{95%} .48-3.51).

All six patients with concomitantly elevated CEA and IL-6 relapsed (PPV 100%, CI_{95%} 54-100%) and, had a numerically shorter DFS (.41 vs. .76, HR 2.16, CI_{95%} .75-6.16) and OS (1.1 vs. 2.6, HR 1.96, CI_{95%} .71-5.44) than did patients with either elevated or both normal.

DISCUSSION

The present study demonstrates that post-operatively elevated serum CEA, YKL-40, CRP, and IL-6 predict recurrence and impaired survival, but in adjusted multivariate analysis only CEA remained significantly associated with both impaired DFS and impaired OS. In patients with normal CEA and elevated YKL-40 or CRP, their DFS and OS were impaired. If elevated CEA was combined with elevated CA19-9, YKL-40, CRP, or IL-6, the risk of CRC relapse was very high (90-100%) and the OS short. If validated, these concomitantly elevated biomarkers may prove useful for identifying patients suitable for a more intensive follow-up to earlier identify resectable metastases, and in identifying low-risk patients who may need adjuvant chemotherapy or high-risk who need addition of oxaliplatin.

According to guidelines, CEA is the only biomarker that should be routinely measured in patients with CRC [6, 7]. Elevated preoperative CEA has been shown to be an important prognostic factor in addition to TNM stage and other known prognostic factors [22, 23]. However, both the sensitivity and specificity of CEA are limited, and a notable part of high-risk patients can remain undetected if only CEA is measured [8, 24, 25]. In the Cochrane review, CEA was insufficiently sensitive, even with low thresholds (<2.5) and they concluded that a rise in CEA never occurs in up to 20% of patients with a true recurrence (false negatives) and thus multiple modalities are recommended [26]. Postoperative CEA is better than preoperative in some studies [9, 10]. There exists a clear need for more precise combination of biomarkers.

CA19-9 is a biomarker widely used in gastrointestinal cancers including CRC; an elevated level has been a negative prognostic marker in mCRC [27] and in localized CRC [28-30]. Our patients with both post-operatively elevated CEA and CA19-9 relapsed and had short DFS and OS, but CA19-9 alone was not an independent predictor of relapse and survival in CRC. Some findings are contrary to ours [30, 31], but findings in line with ours imply that CA19-9 has potential value in combination

with elevated CEA [29, 32, 33]. More convincing data are essential before adoption of CA19-9 surveillance into clinical practice.

Chronic inflammation plays an important role in colorectal carcinogenesis and is associated with poor prognosis [12], but data concerning YKL-40 as a prognostic post-operative biomarker in CRC after radical surgery are limited [34]. YKL-40 has been elevated in CRC patients and more sensitive than CEA and CA19-9, especially in localized CRC [35-37]. High levels of pre-treatment YKL-40 are associated with short OS in chemotherapy for mCRC [38], and in patients undergoing liver resection [39]. We demonstrate that in radically resected CRC patients, high YKL-40 is a predictor of impaired OS. Furthermore, patients with normal CEA but with elevated YKL-40 had impaired DFS (10-year DFS rate estimate 49 vs. 71%) and OS (10-year OS rate 55 vs. 77%). Our findings are in line with those of Cintin et al. [34] showing post-operatively elevated YKL-40 to be an independent predictor of higher recurrence rates and impaired survival in stage II-IV disease. Our patients with concomitantly elevated CEA and YKL-40 had a 90% recurrence rate. As YKL-40 predicts survival regardless of CEA level, it may add additional information to CEA measurement and improve risk stratification.

Discordant prognostic significance, especially for pre-operative CRP, is evident in one comprehensive review [40]. We showed that elevated post-operative CRP associated with impaired DFS and OS in univariate analysis, in line with earlier findings [41, 42]. Our patients with normal CEA and elevated CRP had significantly worse 10-year DFS rates (30 vs. 65%) and OS rates (40 vs. 71%) than patients with both markers normal. In fact, all with post-operatively elevated CEA and CRP relapsed. Accordingly, CRP is a strong predictor of poor survival, a complementary one to CEA.

Data concerning IL-6 as a prognostic biomarker in CRC are ambiguous according to one review, and in most studies presented IL-6 was not an independent prognostic biomarker [43]. Our results are in accordance with these findings as IL-6 was prognostic only in univariate analysis for OS, but

not in multivariate analysis. In a study with stage I-III CRC patients, those with high pre-operative IL-6, had significantly shorter DFS [44], which is in line with our univariate analysis. All our patients with concomitantly elevated CEA and IL-6 recurred.

Post-operatively elevated CEA is prognostic for relapse [9, 10], in line with our findings, and identifies patients in need for more intensive adjuvant therapy [45, 46]. Stage II colon cancer patients with elevated post-operative CEA benefit from adjuvant therapy vs. surgery alone, or from more intensified therapy with addition of oxaliplatin [46, 47].

Among the strengths of our study are that all patients underwent high-quality surgery followed by long-term monitoring. All received adjuvant therapy with 6 months of 5-FU, although without oxaliplatin. A further strength is the follow-up routine with clinical examination, routine laboratory measurements, including tumour markers, and radiology for 10 years within the prospective study at a single institution. Limitations are the small sample sizes in subgroup analyses, resulting in limited statistical power. Secondly, as routine CEA, CA19-9, and CRP were not systematically analysed pre-operatively, we therefore could not study persistently elevated markers post-operatively vs. markers that post-operatively normalized. Another limitation is that some sampling of biomarkers and initiation of adjuvant therapy was delayed because of delayed referrals, perhaps impacting results of efficacy of adjuvant therapy but will probably not have had an influence on the prognostic effect of the post-operative markers. These patients were treated in 1997-2001 with 5-FU-based and the results should be validated in patients treated with oxaliplatin-based chemotherapy.

In conclusion, we showed that post-operatively elevated CEA alone or in combination with postoperatively elevated YKL-40, CRP, CA19-9 or IL-6, associate with high recurrence rates in radically resected stage II-IV CRC patients. In addition, postoperative normal CEA in combination with elevated YKL-40 or CRP may indicate patient's high risk of relapse.

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Table 1 Patient characteristics and postoperative biomarker levels

		All patients		Primary location						
				Right colon		Left colon		Rectum		
		n =	147	n=	41	n=	46	n=	60	p
Sex	Male	75	51%	17	41%	24	52%	34	57%	.318
	Female	72	49%	24	59%	22	48%	26	43%	
Age	Median (range)	60	31-76	60.9	35-74	61.4	31-74	59.2	37-76	.378
Inflammatory disease*	No	133	91%	37	90%	44	96%	52	87%	.295
	Yes	14	9%	4	10%	2	4%	8	13%	
Chemotherapy regimen	5FU+LV bolusinj.	75	51%	20	49%	23	50%	32	53%	.891
	5FU+LV cont. inf.	72	49%	21	51%	23	50%	28	47%	
Radiotherapy	No	96	65%	41	100%	46	100%	9	15%	
	Yes	51	35%	0	0%	0	0%	51	85%	
TNM stage	IIA-B	38	26%	9	22%	11	24%	18	30%	.749
	IIIA-C	91	62%	25	61%	30	65%	36	60%	
	IV	18	12%	7	17%	5	11%	6	10%	
Relapse site	No	82	56%	22	54%	27	59%	33	55%	.969
	Local	13	9%	4	10%	3	7%	6	10%	
	Distal	52	35%	15	37%	16	35%	21	35%	
CEA ¹ , ug/l	Median (range)	1.9	<1-305	1.7	<1-89	2.0	<1-102	1.9	<1-305	.239
CA19-9 ² kU/l	Median (range)	6	<5-2003	5	<5-2003	<5	<5-67	7	<5-934	.150
YKL-40 ³ ng/ml	Median (range)	65	20-1524	65	20-242	55	20-1524	77	20-504	.451
CRP ⁴ , mg/l	Median (range)	<5	1-174	<5	4-174	<5	1-23	<5	1-35	.155
IL-6 ⁵ , pg/ml	Median (range)	2.3	.38-36	2.0	.38-36	2.2	.62-13.8	2.8	.46-20.1	.019

*Inflammatory diseases adjusted for: autoimmune diseases as rheumatoid arthritis, iritis, psoriasis, ulcerative colitis, coeliac disease and thyroiditis

5FU+LV = 5-fluorouracil and leucovorin with randomization to bolus injection (Mayo regimen) or continuous infused (de Gramont regimen).

¹ n=119, ² n=146, ³ n=130, ⁴ n=144, ⁵ n=143

Table 2. Number of patients with no relapse, vs. relapse with or without CRC death and other competing non-CRC deaths, assessed at 7 years. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for all 147 patients and subgroups, 101 with normal and 18 with elevated CEA.

	Marker	Cut point	All	No relapse	Relapse	Other CRC deaths	NPV and PPV		Sensitivity and Specificity	
All	CEA	≤5	114	73	35	6	NPV	64 %	Specificity	97 %
		>5	18	2	16	0	PPV	89 %	Sensitivity	31 %
	CA19-9	≤26	113	62	46	5	NPV	55 %	Specificity	89 %
		>26	17	8	9	0	PPV	53 %	Sensitivity	16 %
	YKL-40	≤70.7	81	51	28	2	NPV	63 %	Specificity	69 %
		>70.7	63	23	36	4	PPV	57 %	Sensitivity	56 %
	CRP	≤10	129	72	52	5	NPV	56 %	Specificity	96 %
		>10	17	3	13	1	PPV	77 %	Sensitivity	20 %
	IL-6	≤4.5	119	66	47	6	NPV	56 %	Specificity	92 %
		>4.5	24	6	18	0	PPV	75 %	Sensitivity	28 %
CEA ≤5	CA19-9	≤26	95	60	30	5	NPV	63 %	Specificity	88 %
		>26	11	8	3	0	PPV	27 %	Sensitivity	9 %
	YKL-40	≤70.7	68	50	16	2	NPV	73 %	Specificity	69 %
		>70.7	45	22	19	4	PPV	42 %	Sensitivity	54 %
	CRP	≤10	103	69	29	5	NPV	67 %	Specificity	96 %
		>10	10	3	6	1	PPV	60 %	Sensitivity	17 %
	IL-6	≤4.5	98	64	28	6	NPV	65 %	Specificity	91 %
		>4.5	13	6	7	0	PPV	54 %	Sensitivity	20 %
CEA >5	CA19-9	≤26	8	1	7	0	NPV	13 %	Specificity	100 %
		>26	5	0	5	0	PPV	100 %	Sensitivity	88 %
	YKL-40	≤70.7	8	1	7	0	NPV	13 %	Specificity	50 %
		>70.7	10	1	9	0	PPV	90 %	Sensitivity	56 %
	CRP	≤10	14	2	12	0	NPV	14 %	Specificity	100 %
		>10	4	0	4	0	PPV	100 %	Sensitivity	25 %
	IL-6	≤4.5	12	2	10	0	NPV	17 %	Specificity	100 %
		>4.5	6	0	6	0	PPV	100 %	Sensitivity	38 %

NPV = negative predictive value, PPV= positive predictive value

Table 3 Univariate and multivariate analysis for DFS and OS.

Unadjusted	DFS				OS			
	HR	CI _{95%} for HR	P-value		HR	CI _{95%} for HR	P-value	
Age ≤60 (n=72) vs. >60	.77	.47	1.25	.284	1.96	1.20	3.19	.007
Sex: Female (n=72) vs. Male	.81	.50	1.32	.404	.86	.54	1.39	.541
Tumour location								
Right (n=41)	1				1			
Left (n=46)	.88	.47	1.67	.703	.87	.46	1.63	.661
Rectal (n=60)	.95	.53	1.71	.869	1.03	.58	1.82	.931
Inflammatory disease (n=14)	1.53	.73	3.20	.263	1.10	.50	2.40	.815
Tumour stage								
Stage II (n=38)	1				1			
Stage III (n=91)	2.41	1.17	4.94	.017	1.45	.78	2.70	.245
Stage IV (n=18)	5.82	2.48	13.65	<.001	3.91	1.83	8.37	<.001
Chemo: LV5FU2 (n=72)	.76	.47	1.24	.274	.73	.45	1.18	.201
Adjusted*								
CEA ¹ elevated vs. normal	7.23	3.85	13.58	<.001	7.16	3.76	13.63	<.001
CA19-9 ² elevated vs. normal	1.98	.99	3.97	.054	1.94	.93	4.04	.076
YKL-40 ³ elevated vs. normal	1.84	1.14	2.96	.013	1.97	1.20	3.23	.007
CRP ⁴ elevated vs. normal	2.31	1.21	4.39	.011	2.40	1.20	4.80	.013
IL-6 ⁵ elevated vs. normal	1.77	.95	3.30	.071	1.99	1.05	3.76	.035
Multivariate model**								
(n=104)								
Age ≤60 vs. >60 years	1.36	.76	2.45	.300	1.82	.96	3.43	.065
Sex: Female vs. Male	.83	.47	1.48	.534	.76	.41	1.41	.389
Inflammatory disease	.54	.18	1.62	.272	.23	.06	.85	.028
Tumour stage								
TNM stage II	1				1			
TNM stage III	1.72	.80	3.69	.167	1.54	.68	3.47	.301
TNM stage IV	2.65	1.03	6.84	.043	2.47	.87	6.97	.088
CEA ¹ elevated vs. normal	8.63	3.82	19.50	<.001	1.17	4.35	23.79	<.001
CA19-9 ² elevated vs. normal	1.16	.51	2.64	.731	1.06	.45	2.51	.897
YKL-40 ³ elevated vs. normal	1.58	.88	2.86	.126	2.24	1.23	4.05	.008
CRP ⁴ elevated vs. normal	2.53	1.10	5.81	.028	2.49	.95	6.51	.063
IL-6 ⁴ elevated vs. normal	1.17	.50	2.73	.713	1.14	.48	2.71	.772

* adjusted for age, sex, inflammatory disease and stage.

** Variables included CEA, CA 19-9, YKL-40, CRP and IL-6; age, sex, inflammatory disease, stage.

¹ n=119, ² n=146, ³ n=130, ⁴ n=144, ⁵ n=143

Figure legends

Figure 1. Disease-free survival (DFS, panel A) and overall survival (OS) (panel B) in post-operatively elevated vs. normal CEA.

Figure 2. The subgroup with normal post-operative CEA and elevated YKL-40 with disease free survival (DFS) in panel A and overall survival (OS) in panel B and the subgroup with normal postoperative CEA and elevated CRP with DFS in panel C and OS in panel D.