Association between the inflammatory biomarker, C-reactive protein, and the response to radiochemotherapy in patients with esophageal cancer

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Abstract. Locally advanced esophageal cancer has been treated by a multimodality regimen encompassing combined radiochemotherapy (RCT). The tumor response to neoadjuvant RCT is a major determinant of further therapeutic strategies, whether surgery or a continuation of RCT, and therefore, also of the patient's overall prognosis. The present study included patients with histologically proven squamous cell esophageal carcinoma. The C-reactive protein (CRP) level was measured prior to and following the completion of neoadjuvant RCT. Only CRP measurements taken within 2 weeks of the start of RCT were analyzed. Further measurements were then taken at 6, 12, 18, 24, 30, 36 and 40 weeks following RCT. CRP levels were high prior to treatment; however, eventually decreased and normalized following the therapy. In univariate analysis, pre-therapeutic CRP levels had a significant influence on the response rate (P=0.033), whilst post-therapeutic CRP levels had no significant influence (P=0.383). Pre-therapeutic CRP levels, however, not post-therapeutic CRP levels were significantly correlated with the response rate (P=0.045 and P=0.444, respectively), and no association was observed between CRP levels and survival. This preliminary data indicated that the pre-therapeutic serum CRP level is a possible indicator of treatment response to RCT.

Introduction

Despite improvements in the clinical outcome of patients with esophageal cancer, their prognosis remains generally very poor. Surgery alone is the standard treatment for early-stage disease, however, patients with locally advanced disease undergo a multimodality regimen encompassing combined radiochemotherapy (RCT) and surgery (1,2). RCT is administered prior to a planned operation or is used as a definitive procedure if surgery is not possible due to co-morbidity, or because complete resection is not feasible for technical reasons. Whether or not surgery is possible is always decided by an interdisciplinary team.

The tumor response to neoadjuvant RCT is a major determinant of further therapeutic strategies, whether surgery or a continuation of RCT is used, and therefore, also the overall prognosis of the patient. A significant response (complete or subtotal tumor regression) has a major bearing on the prognosis. Therefore, one option is to measure response by imaging with computed tomography (CT) or positron emission tomography/CT (PET/CT) following the administration of a sufficient radiation dose (e.g., 45 Gy), and then decide how to proceed (3).

From a clinician's perspective, the evaluation of treatment response using CT imaging alone may be difficult in numerous cases owing to its relatively low sensitivity (4,5) and the irritating co-variants of PET/CT (6-8). This is a challenge to clinicians an, therefore, the identification of biomarkers that predict the response to RCT may assist with optimizing treatment for advanced esophageal carcinoma.

The association between inflammation and cancer is widely accepted as a reliable concept (9-11). However, the complex interaction between inflammatory cascades and cancer progression is not well understood and is currently being investigated (12-14). Chronic inflammatory processes affect all stages of tumor development, as well as therapy. Cancer-associated inflammation (15) or, as it has also been referred to, inflammation-induced cancer (11), encompasses a wide array of factors that coordinate the tumor-promoting and tumor-antagonizing effects of inflammation and enable cross-talk between cancer progression and inflammatory processes.

C-reactive protein (CRP) is a representative biomarker of an acute immunogenic inflammatory response to infection or tissue damage. In addition to its well-established pathophysiological functions, CRP has been shown to mediate the complex interactions between a tumor and its inflammatory microenvironment, which in turn can promote tumor growth (16-18). Furthermore, CRP expression is upregulated by inflammatory lipid sphingosine-1-phosphate, a bioactive

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sphingolipid metabolite involved in cancer-associated inflammation. Increased CRP levels can then activate extracellular signal-regulated kinase, which in turn leads to the transcriptional activation of matrix metaloproetinase-9, promoting tumor invasion and metastasis (14).

A large body of evidence has emerged over the last few years for a pivotal role of CRP in cancer progression and treatment response (19-27), and there is also level I evidence for CRP having prognostic value in a number of different cancer types (28-34).

CRP dynamics in the serum have been shown to correlate with progression and poor prognosis in patients with esophageal carcinoma (35-38), and the level of preoperative CRP is an independent prognostic factor in patients with potentially resectable esophageal carcinoma (39). However, the clinical significance of CRP levels in patients with unresectable tumors and those requiring induction or even definitive CRT has not been fully investigated with respect to treatment response and prognosis (40,41), and little data are available regarding the association between CRP and the response to RCT (42,43).

The present study investigated the possible association between CRP and the response to RCT, and that between CRP and prognosis, by measuring CRP dynamics in serum the during the induction of RCT for patients with clinical T3 or T4 esophageal squamous cell carcinoma, all of whom underwent subsequent esophagectomy. The present study assessed the potential of serum CRP as a biomarker for the prediction of response to RCT.

Materials and methods

Patient selection and data acquisition. The present study included patients with histologically proven squamous cell esophageal carcinoma, predominantly located in middle or upper third of the esophagus, and staged II or III, according to the UICC classification (w). Patients with distant metastases were excluded.

The present study was approved by the responsible ethics committees and was performed in accordance with the Helsinki agreement. Each individual case was discussed at a multidisciplinary team meeting involving radiation oncologists, dedicated surgeons, medical oncologists, pathologists, and radiologists. Pre-therapeutic CRP levels were measured. The data were prospectively collected and stored in databases, from which the demographics, co-morbidity, tumor pathology, morbidity and mortality were subsequently retrieved. Case records were subsequently searched for any missing data. Survival data were obtained either from Cancer Registries or by direct contact with physicians.

Staging, neoadjuvant treatment and surgery. Pre-therapeutic staging included upper endoscopy with biopsy and endoscopic ultrasound, abdominal and thoracic CT and, in all cases, a PET/CT scan. Neoadjuvant RCT consisted of 5 cycles of carboplatin (AUC 2) and paclitaxel (45 mg/m^2) in combination with 45-50 Gy of radiotherapy using the volumetric modulated arc therapy technique. The treatment was delivered as an in-patient regimen. Surgery was performed 6 weeks following the completion of RCT.

RCT was delivered by the same radiation oncologists and the delineation of target volumes was consistently supervised by two experienced radiation oncologists, as well as the preparation of chemotherapy and lab work-up. Surgery was performed by the same three experienced surgeons, using an abdominal and transthoracic approach. The stomach was used as the conduit for reconstruction in all cases. The standard UICC classification (44) was used to evaluate pathology.

CRP measurement. The CRP level was measured prior to and following the completion of neoadjuvant RCT. Only CRP measurements taken within 2 weeks of the start of RCT were analyzed. Further measurements were then taken at 6, 12, 18, 24, 30, 36 and 40 weeks after RCT.

CRP measurements were performed in serum samples with an automated immunoturbidimetric analyzer (Cobas® Integra 800; Roche Diagnostics, Basel, Switzerland). Normal reference values of <10 and <8 mg/l were provided by the manufacturers, respectively.

Statistical analysis. Statistical analysis was performed using SPSS® (version 22) for Windows (IBM SPSS, Chigago, IL, USA). The data are expressed as either the mean ± standard deviation or the median with their 95% confidence intervals (CI). A comparison of data between the two patient groups was performed using χ^2 tests for categorical data and Mann-Whitney U tests for continuous data. Correlations between variables were tested for using the Pearson test, survival was calculated using the Kaplan-Meier method and differences between groups were evaluated using the log rank test. In order to determine the influence of different variables on the outcome, Cox regression analyses were performed. P<0.05 was considered to indicate a statistically significant difference.

Results

Response to RCT. All patient characteristics and tumor variables are summarized in Table I. All patients received RCT, which was delivered as a neoadjuvant therapy in 34 cases (73.91%) and as a definitive treatment in 12 cases (26.08%). A response was observed in 34 cases (73.91%), of which, 6 (17.6%) were a complete response and 28 (82.3%) were a partial remission. No response was reported in 12 cases (26%).

CRP levels during and following RCT, and the association with response to RCT. Table II shows pre- and post-RCT measurements of CRP. CRP levels were high prior to treatment, however, eventually decreased and normalized following therapy. In univariate analysis, pre-therapeutic CRP levels had a significant influence on the response rate (P=0.033), whilst post-therapeutic CRP levels had no significant influence (P=0.383).

CRP levels during and after RCT, and correlation with response to RCT. Pre-therapeutic CRP levels, however, not post-therapeutic CRP levels were significantly correlated with the response rate (P=0.045 and P=0.444, respectively), and no association was observed between CRP levels and survival. Fig. 1 summarizes the association between the levels of CRP and response to RCT.

Table I. Characteristics of the 46 patients undergoing radio-chemotherapy followed by surgery.

Variable	No. of patients
Mean age in years (range)	65.3 (51-81)
Gender	
Female	11 (23.9%)
Male	35 (76%)
Tumor location	
Upper	6 (13%)
Middle	38 (82.6%)
Lower	2 (4.3%)
Tumor depth	
T1	0 (0%)
T2	8 (17.3%)
T3	30 (65.2%)
T4	8 (17.3 %)
Nodal status	
N1	25 (54.3%)
N2	18 (39.1%)
N3	3 (17.3%)
Tumor stage	
IB	1 (2.1%)
II-III	45 (97.8%)
IV	0 (0%)

Table II. CRP measurement prior to and following radiochemotherapy (n=42 patients).

Measurement	Normal	High	Low
Pre-therapy			
CRP	22 (47.9%)	24 (52.2%)	0
Post-therapy			
CRP	26 (56.5%)	20 (43.5%)	0

Normal concentrations of CRP = <0.5 mg/dl. CRP, C-reactive protein.

Discussion

The present study an association between the response of squamous cell esophageal carcinoma to neoadjuvant RCT and the pre-therapeutic levels of CRP. A significant response to treatment, either complete or subtotal tumor regression, is a key indicator of prognosis. However, it is difficult to evaluate response by CT imaging alone due to its relatively low sensitivity (4,5), and clinical evaluation remains challenging. An inflammatory biomarker such as CRP, which is easy to measure and reflects the response to treatment, can help guide clinical decision making.

Guillem and Triboulet (35) revealed that patients with CRP levels >6 mg/l more frequently failed to respond to RCT (P=0.035), tended to have a shorter overall survival (P=0.061),
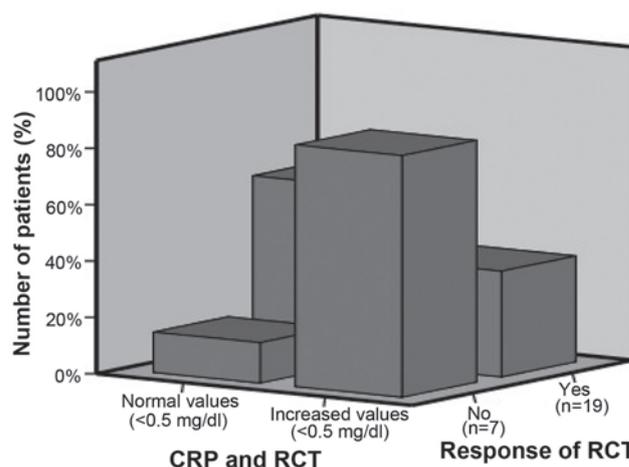
Figure 1. Association between CRP and the response to RCT. CRP, C-reactive protein; RCT, radiochemotherapy.

and had a significantly shorter disease-free survival (P=0.016). The authors concluded that the pretreatment measurement of serum CRP levels in esophageal cancer patients can be used in routine practice as an indicator of prognosis. This was the first attempt to associated CRP serum levels to neoadjuvant RCT response. A subsequent study demonstrated that, amongst patients with cancer of the gastroesophageal junction, the pre-therapeutic CRP level was a prognostic indicator, in agreement with the results of our pilot study. A multivariate analysis revealed that only the positive to total lymph node ratio [hazard ratio (HR), 2.02; 95% CI, 1.44-2.84; P<0.001] and preoperative CRP concentration (HR, 3.53; 95% CI, 1.88-6.64; P<0.001) were independent predictors of cancer-specific survival. Patients with no evidence of a preoperative systemic inflammatory response (CRP ≤10 mg/l) had a median survival of 79 months compared with 19 months for those with an elevated systemic inflammatory response (P<0.001). Together with these findings, the present study indicated that CRP is a potentially predictive cancer biomarker (36). The same research team subsequently reported a possible association between CRP and Albumin levels, and the response of cancer to treatment. Another attempt to associated inflammation with cancer was the development of the inflammation-based prognostic score (37), however, the usefulness of this score has not been confirmed, and was not supported by the present data.

Motoyama et al (38) revealed evidence for an association between CRP polymorphisms and CRP levels following esophagectomy for thoracic esophageal cancer. The authors demonstrated that, 12 h following surgery, CRP levels were significantly higher in patients harboring the CRP 1,059 G/C genotype (0.0266), and that this difference remained for 36 h after surgery (217±63 vs. 140±51 mg/l; P=0.0020). Logistic regression models revealed that patients harboring the CRP 1,059 G/G genotype had a significantly higher likelihood of a post-surgery increase in serum CRP (38).

Zingg et al (43) analyzed data for 70 patients exhibiting normal CRP levels, and 20 patients with raised CRP. The groups revealed no difference with respect to in descriptive, co-morbidities, white cell counts, pathological data or morbidity. In-hospital mortality was more frequent in the raised CRP group (3 vs. 1 patient; P=0.048), and Kaplan-Meier survival analysis

revealed a significant survival advantage for patients with a normal CRP levels compared with those with raised CRP levels (median survival, 65.4 vs. 18.7 months; log rank test, P=0.027). The Cox regression analysis identified three independent prognostic factors for survival: UICC stage (IIB/III vs. I/IIA; HR, 3.48; P=0.007), extent of resection (HR, 6.33; P=0.002) and CRP levels (raised vs. normal; HR, 5.07; P=0.001). The authors concluded that pre-therapeutic CRP levels are an independent prognostic marker for survival following neoadjuvant treatment in patients with esophageal cancer and may be of value in the re-staging process following neoadjuvant treatment (43). This is in agreement with the findings reported in the present study.

In conclusion, the present preliminary data indicated that the pre-therapeutic serum CRP level is a possible indicator of treatment response, however, this finding requires confirmation in a prospective trial with a larger patient number.

Acknowledgements

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