Towards a more personalized approach in the treatment of esophageal cancer focusing on predictive factors in response to chemoradiation

Wang, Da

CHAPTER 6

Longitudinal analysis of cytokine expression during neoadjuvant chemo-radiotherapy and subsequent surgery in esophageal cancer patients

Dirk J. Bosch
Da Wang
Véronique E.M. Mul
Geke A.P. Hospers
Johannes G.M. Burgerhof
Michel M.R.F. Struys
John Th.M. Plukker

American Journal of Surgery 2016 Jul;212(1):89-95.

Abstract

Background

The purpose of this study was to provide more insight in the course of cytokine concentrations related to pathological response (pR) and complications after neoadjuvant chemoradiotherapy (NCRT) and esophagectomy in esophageal cancer patients.

Methods

Patients treated with NCRT followed by transthoracic esophagectomy (TTE; N=35) or TTE alone (N=8) were included. Eight different cytokine concentrations were determined during NCRT, esophagectomy and the first postoperative week.

Results

Platelet activating factor (PAF) before NCRT was associated with pR (p=0.011) and remained elevated in patients with a better response. Concentrations of intestinal fatty acid binding protein and angiopoietin 1 (Ang-1) were different between patients with and without NCRT. Decreased concentrations of Ang-1 on the third postoperative day were associated with postoperative complications (p=0.046).
Conclusion: In this observational study, elevated PAF concentrations before NCRT were associated with pR. NCRT is associated with low Ang-1 concentrations, while decreased Ang-1 concentrations were associated with postoperative complications.

Introduction

In esophageal cancer (EC) patients, neoadjuvant chemoradiotherapy (NCRT) followed by esophagectomy with curative intent is standard treatment of care. The beneficial effect of preoperative NCRT leads to an improved resectability due to the radio-sensitizing effect of chemotherapy and pathological complete responses (pR) of 15-30% (1,2).

Induced immune response by NCRT has been demonstrated to change the levels of several soluble mediators, including interleukin (IL) 6 and inflammatory lipid metabolites such as platelet activating factor (PAF), which might be related with both tumor response and tumor progression (3-5). Moreover, cytokine concentrations throughout all phases of the multimodal treatment might be correlated with complications caused by either NCRT or subsequent surgery (6).

Esophageal resection is generally associated with an enhanced expression and release of pro- and anti-inflammatory cytokines due to the surgical trauma and prolonged one-lung ventilation (OLV) (7,8). Patients with high systemic inflammation markers have an increased risk for postoperative complications (9). Although reported toxicity in the CROSS treated EC patients was acceptable during NCRT, the association of patient's immunologic response to multimodality treatment with early complications after subsequent surgery is not clear yet (1).

A better understanding of patient's immune response with improved view on pathophysiological mechanism would be helpful to assess patient's ongoing pR while undergoing NCRT as well as to potentially stratify patients who are at risk for surgery. To provide prognostic value for cytokines on the occurrence of pR and complications caused by either NCRT or subsequent surgery we conducted a longitudinal observational study at different time points during a multimodality treatment of EC patients according to the CROSS regiment.

Patients and Methods

Patients

Between 2011 and 2013, we prospectively included 51 consecutive patients with histologically prov-

en EC who underwent a transthoracic esophagectomy (TTE) with curative intent after approval by the multidisciplinary tumor board. Patient's characteristics are described in Table 1. Patients received routine clinical care without any extra study-based intervention. All patients, regardless of NCRT, had an identical preoperative workup and were seen by a dietitian. In the case of cardiopulmonary history, patients were referred to a cardiologist or pulmonologist for preoperative workup and postoperative recommendations were given. Patients with unforeseen early progression or discontinuation of neoadjuvant treatment and those treated with a transhiatal approach were excluded (N=8). Of the remaining 43 patients, 35 patients underwent NCRT (adenocarcinoma: N=32 and squamous cell carcinoma: N=3) and eight patients underwent transthoracic esophagectomy alone (adenocarcinoma: N=7 and squamous cell carcinoma: N=1). In total, 278 blood samples were collected during the entire treatment period, with 50 (15%) missing values hazardly divided over different time points. Approval by the institutional review board (METC 2010.374) was obtained and all patients provided written informed consent.

Study design

The primary objective of this observational study included concentrations of pro- and anti-inflammatory cytokines throughout all phases of multimodal treatment. We included (interleukins (IL) 1β, IL-6, IL-8, IL-10, and tumor necrosis factor alpha (TNF-α)) to compare immune response between patients with and without NCRT. In an attempt to monitor vascular permeability, an important determinant of systemic inflammatory response syndrome (SIRS), we included two cytokines reflecting the endothelial function (angiopoietin 1 (Ang-1) and platelet activating factor (PAF)). Since NCRT might influence intestinal integrity, we also measured the intestinal fatty acid binding protein (I-FABP). All selected cytokines are associated with inflammatory response, but with different pathophysiological mechanism.
EDTA-serum samples were obtained at eight predetermined time (P) points and were identical for all patients (Figure 1); before NCRT (P1), 7 days later (P2), end of NCRT/before surgery (P3), during two lung ventilation (P4), during one lung ventilation (P5), at the first (P6), third (P7), and seventh (P8) postoperative day. By choosing these time points, we provided a complete overview of immune responses on the most important moments at different interventions during EC treatment. Cytokine concentrations were determined by means of sandwich ELISA (Enzyme-Linked Immunosorbent As-

say) based on capture and biotin-labelled detection antibodies. D Streptavidin-HRP and OPD substrate were used to quantify the amount of cytokines. Samples were diluted 1:1 in 0.1% BSA/PBS buffer, except for PAF, which was diluted 1:1000 in 0.1% BSA/PBS buffer. All cytokines were analyzed on the same moment in a specialized laboratory.

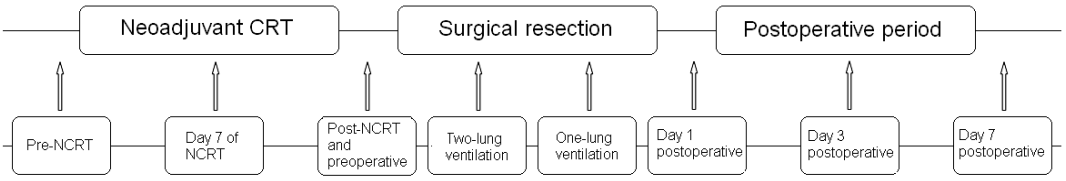

Figure 1: Timeline of sampling throughout different phases of treatment.

Neoadjuvant chemoradiotherapy

All included patients received the full NCRT doses, without any delay. NCRT consisted of Carboplatin (AUC 2) and paclitaxel 50 mg/m2 weekly with concurrent radiotherapy of 41.4 Gy in daily fractions of 1.8 Gy, five times per week according to the CROSS scheme (Chemoradiotherapy for Oesophageal Cancer Followed by Surgery Study (1). All included patients had clinical tumor stages cT1N1-3 or cT2-T4aN0-3 without distant metastases (M0), according to the 7th TNM AJCC edition. Surgical resection was planned 4-8 weeks after NCRT.

Surgery

All patients underwent a TTE with two-field lymphadenectomy (mediastinal and upper abdomen) by two experienced surgeons. All patients had a double lumen intubation for selective one-lung ventilation, which was based on pressure-controlled ventilation with low tidal volumes (6-8 ml/kg) and low PEEP (5-8 cm H2O).

Definitions of outcome

The primary endpoints consisted of cytokine levels throughout the whole multimodality treatment. The secondary endpoints were correlated to cytokine concentrations and included: complications caused by NCRT. All complications were scored prospectively before the surgical procedure according to the National Cancer Institute common toxicity criteria for adverse events (CTCAE version 4.03). Pathologic response after NCRT, which was classified according to the Mandard classification varying from major

(Mandard 1), moderate (Mandard 2 and 3) to minor (Mandard 4 and 5) pathological response. Complications after esophagectomy were scored prospectively according to Clavien-Dindo classification.

Statistics

Results were presented as frequencies with percentages, means or medians. To determine the effect of different interventions on cytokine concentrations, changes after NCRT (P1 vs. P3), during surgery (P3 vs. P6) and in the postoperative course (P6 vs. P8) were analyzed with a paired sample t-test or Wilcoxon signed rank test. Cytokine concentrations on P1, P2, and P3 were related to complications after NCRT and pathological response defined by major, moderate and minor response (Mandard classification) by using a Kruskal-Wallis test. Furthermore, all time points were assessed whether they were associated with postoperative complications according to the Clavien-Dindo classification by a Kruskal-Wallis test. Differences in cytokine concentrations between patients with or without NCRT were analyzed by independent sample t-test or Mann-Whitney test. For statistical analyses we used the Statistical Package for Social Sciences: SPSS version 20.0.0 (SPSS Inc., Chicago, IL USA).

Table 1: Patient characteristics

Variable	N=43 (%)
Age in years (median)	66.0
Sex (M/F)	35/8
NCRT (Y/N)	35/8
Histology	
Adenocarcinoma	39 (90.7)
Squamous CC	4 (9.3)
cTNM classification	
T1	3 (7.0)
T2	6 (14.0)
T3	34 (79.0)
N0	11 (25.6)
N1	16 (37.2)
N2	15 (35.9)
N3	1 (2.3)
M0	42 (97.7)
M1a	1 (2.3)
ASA classification	
ASA I	5 (11.6)
ASA II	29 (67.4)
ASA III	9 (20.9)
Surgery	
TTE right sided	25 (58.1)
TTE left sided	18 (41.9)
Complications after neoadjuvant CRT	
No complications	18 (51.4)
Minor complications	15 (42.9)
Moderate complications	2 (5.7)
Complications after surgery	
No	12 (27.9)
Clavien Dindo I	1 (2.3)
Clavien Dindo II	21 (48.8)
Clavien Dindo III	0 (0.0)
Clavien Dindo IV	5 (11.6)
Clavien Dindo V	4 (9.3)
Postoperative course	
Reoperation	3 (7.0)
OR-time including anesthetic time (mean)	8.46 hrs
ICU-stay (median)	1.0 day
Hospital stay (median)	14.0 days

ASA: American Society of Anesthesiologists.

Results

More than half of this cohort had no complications caused by NCRT (51.4%), while fifteen patients (42.9%) suffered from mild complications and two patients (5.7%) had moderate complications (both with thromboembolic disease) after NCRT. Almost three-quarters of this cohort had one or more post-operative complications (72.1%; Table 1) ranked according to the Clavien-Dindo classification, with pneumonia (44.2%) and arrhythmias (32.6%) as most frequently observed.

Cytokine concentrations during NCRT

Enhanced I-FABP concentrations were observed after NCRT (36.1 pg/ml vs. 352.2 pg/ml; $p<0.001$, Table 2). However, despite of this massive increase, concentrations of I-FABP were not correlated to complications after NCRT. Instead concentrations of TNF-α on P3 (end of NCRT) were related to complications caused by NCRT (without complications 33.5 pg/ml vs. 86.5 pg/ml with mild complications and 71.8 pg/ml with moderate complications; $p=0.019$). None of the remaining preoperative cytokine concentrations were significantly different or associated with complications after NCRT or subsequent surgery.

Table 2: Paired sample analysis in cytokine concentrations on different time points (mean)

	Pre-NCRT	Post-NCRT	p-value
Ang-1 (ng/ml)	3.4	3.9	0.101
I-FABP (pg/ml)	36.1	352.2	**0.001**
IL-1β (pg/ml)	14.5	13.9	0.702
IL-6 (pg/ml)	20.3	15.5	0.687
IL-8 (pg/ml)	7.7	12.0	0.055
IL-10 (pg/ml)	32.8	46.0	0.555
PAF (ug/ml)	4.8	5.1	0.219
TNF-**α** (pg/ml)	62.8	58.6	0.989
	Preoperative	Postoperative	p-value
Ang-1 (ng/ml)	4.6	5.1	0.561
I-FABP (pg/ml)	309.4	50.3	**0.001**
IL-1β (pg/ml)	16.6	14.5	0.654
IL-6 (pg/ml)	23.3	452.9	**0.001**
IL-8 (pg/ml)	11.3	7.0	0.262
IL-10 (pg/ml)	46.5	61.6	**0.002**
PAF (ug/ml)	5.3	4.7	**0.011**
TNF-**α** (pg/ml)	62.7	64.3	0.539
	1st postoperative Day	7th postoperative day	p-value
Ang-1 (ng/ml)	5.0	4.9	0.860
I-FABP (pg/ml)	51.1	33.0	0.626
IL-1β (pg/ml)	26.4	23.3	**0.035**
IL-6 (pg/ml)	344.3	169.5	**0.003**
IL-8 (pg/ml)	10.4	8.4	0.308
IL-10 (pg/ml)	60.2	35.7	**0.012**
PAF (ug/ml)	4.6	5.3	**0.029**
TNF-**α** (pg/ml)	75.9	51.2	**0.039**

Cytokine concentrations related to pathological response

Only PAF concentrations obtained before NCRT were statistically associated with the Mandard classification on the resected specimen (p=0.011). Nevertheless, throughout all time points during NCRT, patients with high or elevated concentrations of PAF showed better pathological responses (Table 3). Other cytokines could not be related to pathological response.

Table 3: Pathologic response according to the Mandard classification and PAF concentrations during NCRT.

	Pre-NCRT	Day 7 NCRT	Post-NCRT
Major Response (N=4)	7,43	5,65	9,07
Moderate Response (N=19)	4,72	4,67	5,44
Minor Response (N=12)	4,1	4,52	4,82

Cytokine concentrations in patients with or without NCRT

Preoperative concentrations of Ang-1 and I-FABP were significantly different in patients who underwent NCRT before esophagectomy (Table 4). Decreased concentrations of Ang-1 were also observed in all the subsequent postoperative measurements, but only significantly lower after the third postoperative day (p=0.047). Although not significant, mean concentrations of the majority of pro-inflammatory cytokines throughout the pre- and postoperative course were elevated in patients with NCRT.

Table 4: Preoperative and postoperative cytokine concentrations in EC patients with or without NCRT (mean)

	Preoperative			1st postoperative day		
	Without NCRT	With NCRT	p-value	Without NCRT	With NCRT	p-value
Ang-1 (ng/ml)	6.5	4.2	**0.027**	7.5	4.4	0.179
I-FABP (pg/ml)	74.9	362.7	**0.025**	1.7	58.5	0.069
IL-1β (pg/ml)	19.9	15.0	0.055	15.2	27.2	0.667
IL-6 (pg/ml)	22.6	23.8	0.473	330.0	498.9	0.496
IL-8 (pg/ml)	7.0	11.7	0.903	7.9	9.7	0.609
IL-10 (pg/ml)	32.3	48.6	0.519	59.6	65.7	0.604
PAF (ug/ml)	5.6	5.2	0.714	5.1	4.6	0.739
TNF-**α** (pg/ml)	62.2	62.5	0.230	56.2	77.0	0.775
	3th postoperative day			7th postoperative day		
	Without NCRT	With NCRT	p-value	Without NCRT	With NCRT	p-value
Ang-1 (ng/ml)	5.5	3.6	**0.047**	6.8	4.4	0.078
I-FABP (pg/ml)	2.3	4.5	0.911	23.0	36.3	0.596
IL-1β (pg/ml)	13.8	23.5	0.706	12.8	24.8	0.744
IL-6 (pg/ml)	78.4	103.6	0.960	28.2	199.2	0.252
IL-8 (pg/ml)	4.7	6.5	0.380	2.3	9.2	0.391
IL-10 (pg/ml)	56.8	43.1	0.514	24.1	37.4	0.454
PAF (ug/ml)	6.5	4.3	0.319	5.4	5.2	0.707
TNF-**α** (pg/ml)	64.3	63.5	0.269	50.4	50.5	0.999

Cytokine concentrations during surgery

A total of 43 patients underwent surgical resection with curative intent. Surgical insult was responsible for large variations in most cytokines. Significantly elevated concentrations after surgery (P3 vs. P6) were observed for IL-6 and IL-10, while PAF and I-FABP were significantly decreased after surgery (Table 2). There were no differences in cytokine concentrations between the NCRT and surgery only group and none of the examined cytokines were correlated to postoperative complications.

513774-L-bw-wang
Processed on: 19-9-2017

Cytokine concentrations in the postoperative period

Many of the deviating concentrations after surgical resection returned to normal in the first postoperative week. Concentrations of IL-1β, IL-6, IL-10, and TNF-α decreased significantly between P6 and P8, while PAF concentrations were rising (Table 2). Ang-1 concentrations on P7 were correlated to postoperative complications according to the Clavien-Dindo classification (p=0.046). Comparing patients with or without complications, concentrations of Ang-1 were consequently decreased in patients with postoperative complications (Figure 2).

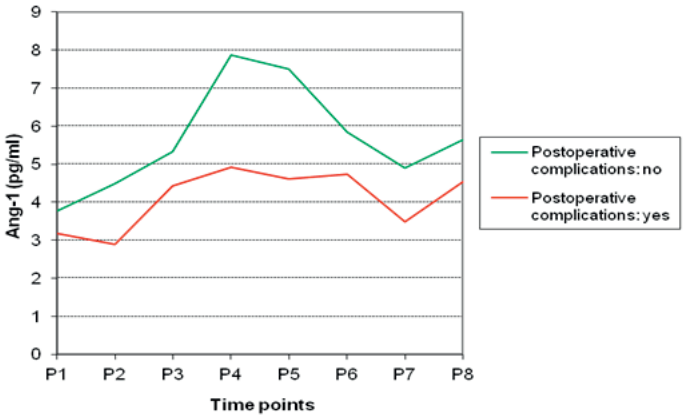

Figure 2: Ang-1 concentrations (mean) related to postoperative complications throughout all phases during multimodal treatment of EC patients.

Discussion

Both neoadjuvant chemoradiotherapy (NCRT) and transthoracic esophagectomy (TTE) were responsible for fluctuations in cytokine concentrations in current study. During NCRT we observed rapidly increased concentrations of I-FABP, suggesting intestinal damage. Besides I-FABP, also Ang-1 concentrations showed to be different between patients with and without NCRT. These decreased concentrations of Ang-1 seemed to be related to postoperative complications. Finally, we observed that elevated PAF concentrations were associated with a better pathological response to NCRT.

Systemic effects of both chemotherapy and radiotherapy induce pro-inflammatory responses through IL-1β, IL-6, IL-8, and TNF-α within few hours after exposure (4, 10, 11, 12). With present data, we were not able to confirm these statements; instead we observed significant elevated concentrations of

I-FABP after NCRT. In a study of Derikx et al, a similar increase of I-FABP was associated with early damage of the mucosal cell integrity and related to chemotherapy-induced gastrointestinal (GI) mucositis. Moreover, a rapid decrease in the postoperative phase even suggests that the esophagus seem to be the source of elevated I-FABP concentrations. However despite this plausible theory, elevated I-FABP concentrations after NCRT had no clinical relevance in our study population.

Predicting pathological response (pR) before, during or after NRCT would be of great interest and would add to the possibility of individualizing treatment strategies. These decisions however should be based on objective and reliable measurements. In the current study, concentrations of PAF before NCRT were significant associated with the pR assessment according to the Mandard classification. High concentrations of PAF were associated with better pathological response. In comparison with PAF concentrations in healthy volunteers (3,50 pg/ml), we observed concentrations that were twice as high in patients with major pR. PAF is produced by various tissues and cell types in response to different stimuli, including oxidative stress(13, 14). Both chemo – and radiotherapy are potent pro-oxidative stressors and lead to apoptotic or necrotic cells. Furthermore, PAF is known to activate several pathways such as nuclear factor-kappa B (NF-κB) and is responsible for augmentation of chemotherapy-induced effects by releasing cytokines (5, 13). To date, there are no remarkable useful biomarkers available related to the degree of tumor response, although some pro-inflammatory cytokines seems to plays an active role (15, 16, 17). We suggest that the value of PAF as a possible marker of tumor-necrosis deserves further evaluation in a large prospective cohort.

Both in the preoperative and postoperative phase, Ang-1 concentrations were different between patients with and without NCRT. Moreover, Ang-1 was also associated with postoperative complications. Although Ang-1 concentrations were not significantly different after NCRT, extensive apoptosis of vascular endothelial cells affects endothelial function, including promotion of vessel maturation through angiogenesis (18). The endothelial barrier, as an important regulator of homeostasis, seems to play a crucial role in the pathogenesis of (infectious) complications. Angiopoietin (1 and 2) as a growth factor is mainly restricted to endothelial regulation of fluid, electrolyte and protein transport. Disturbance of homeostasis due to increased microvascular permeability is related to the Ang/Tie pathway. Angiopoietin specifically binds to the endothelial receptor tyrosine kinase Tie-2. Ang-1 mediated Tie2 signaling will lead to the maintenance of cellular integrity and quiescence of the endothelial barrier by covering

vessels with periendothelial cells, whereas Ang-2 mediated Tie2 signaling will lead to the removal of these cells (19-21). A decreased concentration of Ang-1 is by various authors related to infectious complications (19, 20, 22). NCRT might negatively interact with the endothelial function of Ang-1 whereas surgery is associated with inflammatory response resulting in increased vascular permeability and inflammation.

However, there are also some limitations in this study. Firstly, there were some missing values (15%), which were spread hazardly over the time points so we do not expect any significant impact on the results. Secondly, in this observational study with a rather small study population (N=43), only eight patients who underwent esophagectomy alone were included. However, in this prospective study all consecutive patients were included according to common practice. Despite a small sample size, we believe that this data contribute to our knowledge regarding differences in immunological response between patients with or without NCRT. Nevertheless, present data of this potential novel concept of cytokine evaluation, in which we included a total of 278 blood samples, gives a reliable impression of cytokine alterations throughout a multimodal treatment in EC patients. A possible confounding effect may exist due to the diversity of this population containing both adenocarcinomas and squamous cell carcinoma with a different underlying etiology of the disease. However, the effect on neoadjuvant chemoradiation according to the CROSS regiment were significant in both groups. With respect to our small study population, we should keep in mind that other possible confounding factors, including medical history, age, smoking and nutritional status may also play a role.

In conclusion, cytokine alterations throughout a multimodality treatment in esophageal cancer patients could be of additional value in clinical practice. PAF concentrations before neoadjuvant treatment might be related to pathological response whereas low Ang-1 concentrations are potentially important in the development of postoperative complications. NCRT might be the cause of decreased concentrations of Ang-1, since this was more frequently observed in patients who underwent NCRT. As potentially confounding factors might interfere with our results, larger prospective studies are necessary to confirm our conclusions.

References

[1] van Hagen P, Hulshof MC, van Lanschot JJ, Steyerberg EW, van Berge Henegouwen MI, Wijnhoven BP et. al. Preoperative chemoradiotherapy for esophageal or junctional cancer. N Engl J Med, 2012; 366:2074-2084.

[2] Berger AC, Farma J, Scott WJ, Freedman G, Weiner L, Cheng JD et. al. Complete response to neoadjuvant chemoradiotherapy in esophageal carcinoma is associated with significantly improved survival. J Clin Oncol, 2005; 23:4330-4337.

[3] Makuuchi Y, Honda K, Osaka Y, Kato K, Kojima T, Daiko H et. al. Soluble interleukin-6 receptor is a serum biomarker for the response of esophageal carcinoma to neoadjuvant chemoradiotherapy. Cancer Sci, 2013; 104:1045-1051.

[4] Debucquoy A, Goethals L, Geboes K, Roels S, Mc Bride WH, Haustermans K. Molecular responses of rectal cancer to preoperative chemoradiation. Radiother Oncol, 2006; 80:172-177.

[5] Darst M, Al-Hassani M, Li T, Yi Q, Travers JM, Lewis DA et. al. Augmentation of chemotherapy-induced cytokine production by expression of the platelet-activating factor receptor in a human epithelial carcinoma cell line. J Immunol, 2004; 172:6330-6335.

[6] Hart JP, Broadwater G, Rabbani Z, Moeller BJ, Clough R, Huang D et. al. Cytokine profiling for prediction of symptomatic radiation-induced lung injury. Int J Radiat Oncol Biol Phys, 2005; 63:1448-1454.

[7] van Sandick JW, Gisbertz SS, ten Berge IJ, Boermeester MA, van der Pouw Kraan TC, Out TA et. al. Immune responses and prediction of major infection in patients undergoing transhiatal or transthoracic esophagectomy for cancer. Ann Surg, 2003; 237:35-43.

[8] Michelet P, D'Journo XB, Roch A, Doddoli C, Marin V, Papazian L et. al Protective ventilation influences systemic inflammation after esophagectomy: A randomized controlled study. Anesthesiology, 2006; 105:911-919.

[9] Christou NV, Tellado-Rodriguez J, Chartrand L, Giannas B, Kapadia B, Meakins J et. al. Estimating mortality risk in preoperative patients using immunologic, nutritional, and acute-phase response variables. Ann Surg, 1989; 210:69-77.

[10] Wichmann MW, Meyer G, Adam M, Hochtlen-Vollmar W, Angele MK, Schalhorn A et. al. Detrimental immunologic effects of preoperative chemoradiotherapy in advanced rectal cancer. Dis Colon Rectum, 2003; 46:875-887.

[11] Westerterp M, Boermeester MA, Omloo JM, Hulshof MC, Vervenne WL, Lutter R et. al. Differential responses of cellular immunity in patients undergoing neoadjuvant therapy followed by surgery for carcinoma of the oesophagus. Cancer Immunol Immunother, 2008; 57:1837-1847.

[12] Sadahiro S, Suzuki T, Maeda Y, Tanaka A, Kamijo A, Murayama C et. al. Effects of preoperative immunochemoradiotherapy and chemoradiotherapy on immune responses in patients with rectal adenocarcinoma. Anticancer Res, 2010; 30:993-999.

[13] de Oliveira SI, Andrade LN, Onuchic AC, Nonogaki S, Fernandes PD, Pinheiro MC et. al. Platelet-activating factor receptor (PAF-R)-dependent pathways control tumour growth and tumour response to chemotherapy. BMC Cancer, 2010; 10:200-2407-10-200.

[14] de Oliveira SI, Fernandes PD, Amarante Mendes JG, Jancar S. Phagocytosis of apoptotic and necrotic thymocytes is inhibited by PAF-receptor antagonists and affects LPS-induced COX-2 expression in murine macrophages. Prostaglandins Other Lipid Mediat, 2006; 80:62-73.

[15] Suzuki Y, Mimura K, Yoshimoto Y, Watanabe M, Ohkubo Y, Izawa S et. al. Immunogenic tumor cell death induced by chemoradiotherapy in patients with esophageal squamous cell carcinoma. Cancer Res, 2012; 72:3967-3976.

[16] Druzgal CH, Chen Z, Yeh NT, Thomas GR, Ondrey FG, Duffey DC et. al. A pilot study of longitudinal serum cytokine and angiogenesis factor levels as markers of therapeutic response and survival in patients with head and neck squamous cell carcinoma. Head Neck, 2005; 27:771-784.

[17] Heikkila K, Ebrahim S, Lawlor DA. Systematic review of the association between circulating interleukin-6 (IL-6) and cancer. Eur J Cancer, 2008; 44:937-945.

[18] Cho CH, Kammerer RA, Lee HJ, Yasunaga K, Kim KT, Choi HH et. al.. Designed angiopoietin-1 variant, COMP-Ang1, protects against radiation-induced endothelial cell apoptosis. Proc Natl Acad Sci U S A, 2004; 101:5553-5558.

[19] Ricciuto DR, dos Santos CC, Hawkes M, Toltl LJ, Conroy AL, Rajwans N et. al. Angiopoietin-1 and angiopoietin-2 as clinically informative prognostic biomarkers of morbidity and mortality in severe sepsis. Crit Care Med, 2011; 39:702-710.

[20] van Meurs M, Kumpers P, Ligtenberg JJ, Meertens JH, Molema G, Zijlstra JG. Bench-to-bedside review: Angiopoietin signalling in critical illness - a future target? Crit Care, 2009; 13:207.

[21] Hegeman MA, Hennus MP, van Meurs M, Cobelens PM, Kavelaars A, Jansen NJ et. al . Angiopoietin-1 treatment reduces inflammation but does not prevent ventilator-induced lung injury. PLoS One, 2010; 5:e15653.

[22] David S, van Meurs M, Kumpers P. Does low angiopoietin-1 predict adverse outcome in sepsis? Crit Care, 2010; 14:180.