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Original Article

Changes of acute-phase protein levels in the serum of lung cancer patients following radiotherapy

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Abstract: Purpose: the assessment of serum level changes of C-reactive protein (CRP), ferritin (FER), and albumin (ALB) as inflammation markers in Non Small Cell Lung Cancer patients (stages IIIA – inoperable and stage IIIB) treated with radiotherapy. Significant findings: Normal pre-radiotherapy levels of CRP were found in 18 patients, of FER in 17, and of ALB in 22. Higher levels of CRP were found in 9 patients and of FER in 10. Lower ALB was found in 5 patients.Post-radiotherapy CRP levels were significantly higher (compared to the pre-radiotherapy levels) in 25 patients. The same was observed regarding FER in 18 patients whereas 12 patients had lower post-radiotherapy levels. The statistical analysis (non parametrical Wilcoxon test) revealed that these differences were statistically significant (p-value< 0.001). Conclusions: The levels of CRP, FER, and ALB are reliable and useful biomarkers correlated with the acute complication of lung parenchyma damage induced by radiotherapy.

Keywords: Acute phase proteins, lung cancer, radiotherapy

Introduction

Lung cancer is the most common cause of cancer-related death in USA. It is estimated that in total, 225,500 new cases (116,750 men and 105,770 women) with lung cancer (brochogenic and pulmonary) were diagnosed and 157,300 related deaths (86,200 men and 71,100 women) occurred in 2010. The non-small cell lung cancer is responsible for more than 85% of cases with lung cancer [1].

According to the NCCN v.2.2012 guidelines, the standard treatment for patients with good performance status and inoperable stage III is the combined chemoradiotherapy [2, 3]. The sequential treatment is preferred in frail patients who cannot tolerate well the concurrent treatment [4]. The aim of radiotherapy administration is the improvement of locoregional control and as a consequence the prolongation of the Disease Free Survival (DFS) and the Overall Survival (OS).

During radiotherapy, a series of proteins (acute phase proteins) are produced that induce inflammation and oxidative stress. They are produced in the liver and their concentration is related to the presence of an inflammation or a neoplasm. These proteins are CRP, ferritin, and albumin.

Serum albumin is an indicator of splanchnic protein function. In the presence of hypothrepsia or inflammation, its synthesis is suppressed.

As a part of the systemic inflammatory reaction to the tumor presence, cytokines that increase catabolism are released. The IL-6 and IL-Ib increase the hepatic production of acute phase proteins and inhibit the albumin production from the kupffer cells. TNF increases the capillary vessels permeability and thus albumin penetrates the blood vessel wall [5].

CRP is an acute reaction indicator and because of its rapid mobility it provides reliable information on the current inflammatory status. Its relation with cytokines and its possible functional role have given a significant dimension to its clinical use as a parameter of active inflammation [6]. Ferritin is a ferrum binding protein and in patients with lung cancer is elevated [7, 8]. The raised levels of ferritin are due to inflammation rather to increased ferrum concentration [9]. It can be detected in samples from the airways such as bronchoalveolar lavage (BAL), bronchial secretions, and in pleural effusion from metastatic cancer [10]. In an extensive research in the PubMed/MEDLINE no other study was found assessing the alteration of the levels of the above mentioned proteins in the serum of patients suffering from stage III lung cancer treated with external radiotherapy. The aim of the present study was to assess the serum levels of CRP, albumin, and ferritin before and 2 months following treatment.

Material and methods

Study design – inclusion criteria

The primary aim of the present prospective study was to assess the alteration of CRP, ferritin, and albumin levels in the peripheral blood of patients with lung cancer, before and 2 months following 3-Dimensional Conformal Radiotherapy (3DCRT). The study was approved by the Medical School Ethics committee of the Kapodistrian University of Athens. All patients were informed regarding their participation in the study. The inclusion criteria were: a) Zubrod performance status 0 to 2. b) Primary non-small cell lung cancer, stages IIIA (inoperable due to comorbidity) and IIIB. c) Absence of acute inflammation signs.

Pre-treatment workup

Thorough history was taken from all participating patients followed by clinical examination. The absence of systemic inflammatory, infectious, or rheumatic disease was then confirmed. Complete hematologic, biochemical, and radiological testing was followed. The latter included chest and upper abdominal (with contrast) CT-scan and brain MRI with iv gadolinium administration. The above examinations were part of the disease staging in order to exclude distant metastases and active inflammation. The diagnosis of the malignancy was confirmed with cytology or histopathology after taking a sample from the primary malignancy.

Radiotherapy simulation

The patients were submitted to virtual simulation, in supine position, using the wingboard special immobilizing system. This way, the patients' hands were resting securely above and behind the head in order the tattoos that align the chest to be delineated and the radiation physist to be able to use oblique fields to minimize the radiation dose in the surrounding sensitive normal tissues.

Radiotherapy planning

For radiotherapy planning, CT-scan was performed in order to cover the anatomical area extending from the 6^{th} cervical vertebra to the mid abdomen. This was conducted with and without contrast (due to its effect to the tissue heterogeinity requiring correction). The Ct-scan slices had a thickness of 5 mm and the data were transferred to the Prosoma® Treatment Planning System via DICOM network.

Organs at risk – Target volume delineation

This planning was performed using the CT slices derived from the Prosoma Treatment Planning System simulation. When atelectasis was present or in case of allergy PET scan was performed. The tumor targets were defined according to the ICRU reports 50, 62 [11]. As macroscopically visible tumor target (Gross tumor volume – GTV) defined the primary tumor, the affected lymph node with maximum diameter greater than 1 cm (in the short axis), the increased metabolic activity in PET scan, and the presence of cancer cells in the mediastinoscopy. The clinical tumor target (clinical target volume - CTV) is related to the microscopic spread of the disease and it is usually considered as 1-1.5 cm around GTV. The geometric tumor target (planning target volume - PTV) is the margin of set up error covering the inaccuracies of everyday positioning and those derived from the internal organs movement around the Internal Target Volume and it is usually considered as 0.5-1.5 cm around CTV. The Organs at Risk (OARs) as well as the tolerance doses, were determined according to the ICRU Report 62. In detail: Spinal cord (Max Dose point) ≤ 50

Gy; Lung $V_{20} \leq$ 30-35%; Heart $V_{60} \leq$ 30%, Mean Dose ≤ 35 Gy; Esophagus Mean Dose ≤ 34 Gy, Max ≤ 105 % of prescription dose; Brachial plexus ≤ 66 Gy.

Dose description and Plan evaluation

In all cases the radiotherapy technique used was the 3-Dimensional Conformal Radiotherapy (3DCRT). Due to the fact that the optimal radiotherapy dose has not yet been defined and it is still under investigation, it was decided to administer a biologically equivalent dose equal to 60 Gy in 13 daily photon radiation sessions according to a hypofractionated schedule from Monday to Friday. The 95% of the PTV had to be covered by the 95%-110% of the total dose. The treatment was performed with linear accelerator, and energies of 6 to 15 MV were used in order to optimize the dose distribution. In case of air gap, the energy of 6 MV was preferred, while in large mediastinal lymph node masses or in tumors attached to the thoracic wall a higher energy was usually preferred to improve the dose distribution.

Chemotherapy was also administered to the patients studied according to the current NCCN® (National Comprehensive Cancer Network) guidelines. The treatment planning was performed using the Eclipse™ treatment planning system (Varian Medical Systems, United States) that includes the Pencil Beam algorithm to calculate the radiation dose. The evaluation of the treatment planning was performed using the distribution of the isodose curves and the Dose Volume Histogramms (DVHs).

Measurement of acute phase serum proteins

Peripheral blood sample was taken before the beginning of radiation therapy and 2 months following its completion. The samples were centrifuged and serum CRP and ferritin were measured using nephelometric method (Beckman Coulter, Image Immunochemistry System, U.S.A.), while albumin was measured using photometric method.

Follow-up

The patients' follow-up at 2 months' intervals included: medical history, clinical examination, haemotological and biochemical testing, thoracic and upper abdominal CT-scan, with contrast. At the first 2-month follow-up interval, the values of CRP, albumin, and ferritin were measured as well.

Statistical analysis

The values of CRP, albumin, and ferritin were expressed as means ± standard deviations. The statistical comparisons of the above values before and 2 months after the completion of radiotherapy were performed using the Wilcoxon rank non parametric test. Statistical significance was accepted at the $p<0.05$ level.

Results

Patients and tumor characteristics

In the time period from 2/2010 to 5/2011 we prospectively followed up 27 patients. The median age was 69 years (range: 53-88 years). Twenty three were men and 4 women. The confirmed diagnosis according to the protocol was adenocarcinoma in 16 patients and squamous cell carcinoma in 11 patients. From the initial 31 patients who had met the inclusion criteria of the present study, 4 were excluded due to disease progress in the radiology examination and their data was not included in the study. A special database was created in which all clinical and histological data were inserted (analyzed in detail in **Table 1**). The values considered as normal were the following: CRP (< 5 mg/dL), Ferritin (30-400 ng/ml ♂, 13-150 ng/ml ♀), and Albumin (3.6-5.5 g/dL). Before radiotherapy, 18 patients were found within normal limits with regard to CRP. The respective numbers of patients were 17 with regard to ferritin and 22 with regard to albumin (**Table 2**). High levels of CRP were found in 9 patients and of ferritin in 10 patients. In contrast, low levels of albumin were found in 5 patients. Following radiotherapy, higher levels than the pre-radiotherapy ones were found in 25 patients with regard to CRP and in 18 patients with regard to ferritin. On the contrary, lower levels of albumin were observed in 12 patients. Statistical analysis revealed that all the above differences were statistically significant (p-value<0.001) (**Table 3**).

Discussion

The values of CRP, ferritin, and albumin are reliable and easily obtainable bio-indicators related

Table 1. Demographics and histology type of all patients included in the study.

Case No	Gender	Stage	Histology type	Age
1	Male	IIIB	ADENOCARCINOMA	65
2	Male	IIIA	SQUAMOUS CELL CARCINOMA	73
3	Male	IIIB	SQUAMOUS CELL CARCINOMA	64
4	Male	IIIA	SQUAMOUS CELL CARCINOMA	58
5	Male	IIIA	SQUAMOUS CELL CARCINOMA	61
6	Male	IIIB	SQUAMOUS CELL CARCINOMA	72
7	Male	IIIB	ADENOCARCINOMA	82
8	Male	IIIB	ADENOCARCINOMA	88
9	Male	IIIB	ADENOCARCINOMA	75
10	Male	IIIB	SQUAMOUS CELL CARCINOMA	82
11	Male	IIIB	ADENOCARCINOMA	79
12	Male	IIIA	ADENOCARCINOMA	53
13	Female	IIIB	ADENOCARCINOMA	67
14	Male	IIIA	ADENOCARCINOMA	55
15	Male	IIIA	ADENOCARCINOMA	62
16	Female	IIIB	SQUAMOUS CELL CARCINOMA	67
17	Female	IIIA	SQUAMOUS CELL CARCINOMA	65
18	Female	IIIB	ADENOCARCINOMA	60
19	Male	IIIB	ADENOCARCINOMA	74
20	Male	IIIB	ADENOCARCINOMA	88
21	Male	IIIB	ADENOCARCINOMA	78
22	Male	IIIB	ADENOCARCINOMA	55
23	Male	IIIA	SQUAMOUS CELL CARCINOMA	79
24	Male	IIIB	SQUAMOUS CELL CARCINOMA	73
25	Male	IIIB	SQUAMOUS CELL CARCINOMA	75
26	Male	IIIA	ADENOCARCINOMA	72
27	Male	IIIB	ADENOCARCINOMA	76

to the acute adverse event of lung parenchyma damage caused by radiotherapy. The present study suggests that the values of CRP and ferritin are statistically significantly elevated in the immediate post-radiotherapy interval compared to the pre-radiotherapy values ($p<0.001$). The albumin values were found statistically significantly lower ($p<0.001$). The combination of the increased values of CRP and ferritin and hypoalbuminemia may be attributed to several factors: firstly, to the reactive response (tissue stress) due to the existence of cancer cells that may activate the acute phase proteins production; secondly, to the concomitant administration of chemotherapy drugs and finally, to the patients' malnutrition (hypothrepsia) that reduce their tolerance to toxicity deriving from the treatment and their compliance [12]. According to McMillan et al hypoalbuminenia is a consequence of the systemic inflammatory reaction and the malnutrition (hypothrepsia) of oncologic patients and this is supported by the Glasgow Prognostic score (GPS) that evaluates the high CRP values with the low albumin values [13]. In the study of Schumacher et al, the increased CRP levels have been attributed to reduced T-lymphocytes reaction against the cancer cells [14]. The patients of the present study did not have lymphocytopenia that would indicate local or systemic inflammation. While bacterial inflammations are related to significantly elevated CRP values (>10 mg/dL) within 8-12 hours, the persistent increased CRP concentration, although not in very high levels (<10 mg/dL) has been reported in cancer patients [15]. In the present study no patient had initial CRP value

Table 2. Values of CRP, Ferritin, and Albumin before and after radiotherapy

CRP preRT	CRP postRT	Ferritin pre RT	Ferritin post RT	Albumin pre RT	Albumin post RT
3,91	8,3	80	264	4,1	3,6
2,96	7,77	93	232	4,5	4
1,39	4,56	102	254	5,1	4,7
6,1	9,4	409	603	3,5	3,2
3,48	5,28	320	623	5,2	4
1,83	7,3	206	523	4,9	3,9
2,26	4,9	229	456	5,1	3,8
7,23	11,4	520	678	3,4	3
2,97	5,01	256	345	3,6	3,2
1,01	6,5	308	469	4,7	4,4
5,7	8,1	432	567	3,7	3,2
2,51	5,8	107	378	5,5	5
3,01	7,3	187	327	3,8	3
3,4	5,2	345	476	3,7	3,3
7,8	11,2	543	675	3,3	3,1
6,1	12,1	456	689	3,9	3,6
4,8	8,3	339	567	4	3,5
3,7	5,8	123	234	5,2	5
2,8	7,3	324	499	4,6	4
1,37	6,7	287	356	5,3	4,9
3,9	8,6	136	321	5,2	4,1
4,6	7,9	401	662	4,7	4,3
6,9	12,3	458	638	3,2	3
7,2	9,7	467	642	3,6	3,4
4,4	7,6	321	543	4,7	4,3
7,8	9,9	567	765	3,7	3,4
9,3	14,1	578	654	3,3	3,1

Table 3. Statistical analysis of the differences between the values pre- and post-radiotherapy

CRP_pre	CRP_post	P
4,4 (2,2)	8,1 (2,5)	<0.001
Ferritin_pre	Ferritin_post	P
318,3 (154,8)	497,8 (161,5)	<0.001
Albumin_pre	Albumin_post	P
4,3 (0,7)	3,7 (0,6)	<0.001

higher than 10 mg/dL. Two months following radiotherapy, 5 out of 27 patients surpassed the specific value of 10 mg/dL (one case reached the value of 14.1 mg/dL). Forrest et al have correlated the systemic inflammatory reaction with negative prognosis in patients with inoperable non-small cell lung cancer [16]. The prognostic value of the specific serum proteins has been assessed in several studies of various neoplasms, most of which related to esophagus and stomach cancer [17-20]. Wang et al found in a recent study that the elevated CRP values and hypoalbuminemia in patients with esophagus cancer submitted to radiotherapy are poor prognostic factors [21]. The role of acute phase proteins was also explored in patients with colorectal cancer by McMillan et al who found that the presence of systemic inflammatory reaction before surgical excision was a negative indicator [22]. The prognostic value of the presence of inflammatory reaction has also been investigated in other neoplasms such as hepatocellular cancer [23], ovarian epithelial cancer [24], malignant histiocytoma [25], metastatic breast cancer [26], metastatic renal cancer [27], and inoperable pancreatic cancer [28]. All these

studies had similar outcomes.

In the present study, the disease stage was the same in all patients and the tumor load comparable. The tissue reaction against the tumor and radiotherapy is expected to be similar as well. These are some of the strengths of the present study. However, the conclusions of the present study with regard to the prognostic value of the investigated proteins are weakened by the small number of patients and the relatively short follow-up.

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