# Targeted therapy, molecular imaging and biomarkers in cancer treatment

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# Chapter 6

# Bleomycin-induced pulmonary changes on restaging CT scans in two thirds of testicular cancer patients: no correlation with fibrosis markers

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Submitted

*Previously presented at the ASCO Annual Meeting 2015*

**ABSTRACT**

Background: Metastatic testicular cancer has a favorable prognosis when treated with BEP (Bleomycin, Etoposide and cisPlatin) chemotherapy. Bleomycin-induced pneumonitis (BIP) is a well known side-effect which can be fatal. In this study, we investigated the prevalence of lesions suspect for bleomycin-induced pulmonary changes on restaging CT scans after treatment, and whether the fibrosis markers Transforming Growth Factor-beta1 (TGF-β1), Growth Differentiation Factor-15 (GDF-15), and hs-CRP were predictive of this.

Methods: Patients between 18 and 50 years of age with metastatic testicular cancer treated with BEP chemotherapy were included in this prospective non-randomized cohort study. Post chemotherapy restaging CT scans were analyzed for abnormalities that were suspect for bleomycin-induced pulmonary changes as judged by two independent radiologists. The radiographic abnormalities were graded as minor, moderate or severe. Plasma samples were collected before, during and after treatment and were quantified for TGF-β1, GDF-15 and hs-CRP.

Results: 66 patients treated with BEP chemotherapy were included. Forty-five (68%) showed signs of bleomycin-induced pulmonary changes on the restaging CT scan, 37 were classified to have minor and eight to have moderate abnormalities. No differences in TGF-β1, GDF-15, or hs-CRP plasma levels were found between these groups.

Conclusion: Bleomycin-induced pulmonary changes are very common on restaging CT scans after BEP chemotherapy for metastatic testicular cancer. TGF-β1, GDF15 and hs-CRP plasma levels before, during and after treatment are not different between patients with and without radiological signs of pulmonary bleomycin-induced toxicity and, therefore, not helpful as early fibrosis biomarkers to predict this.

## INTRODUCTION

Metastatic testicular cancer has a favorable prognosis with a 5 year overall survival over 90% when treated with the current standard of BEP (bleomycin, etoposide and cisplatin) chemotherapy. Bleomycin is considered to be an essential component of the regimen [1]. Although well tolerated, in roughly 10% of the patients treated with this regime, bleomycin-induced pulmonary toxicity (BIP) is observed, with clinical symptoms such as dry cough, dyspnea, crackles during auscultation, abnormalities on chest X-ray and fever [2]. BIP is predominantly a fibrotic lung disease and, although its pathogenesis is not resolved, the immune system appears to be involved [3,4].

BIP occurs during bleomycin treatment, but can also develop after a treatment free interval of weeks to months [5]. In 1-3% of the affected patients pulmonary toxicity is fatal [6]. The cumulative bleomycin dose is an important denominator of the risk of BIP, although a safe dose has not been established [7]. Known risk factors are smoking, impaired renal function and higher age, but there is neither a test to predict which patients will develop BIP, nor a therapy to prevent it [8,9]. Standard strategy in case of BIP signs during treatment is to stop bleomycin administration. The change in diffusion capacity (TLCO) is used in several centers to terminate bleomycin administration, although these changes appear not to be specifically caused by bleomycin [10].



In earlier reports, restaging CT scans after completion of BEP chemotherapy for testicular cancer are reported to show signs suggestive for bleomycin-induced pulmonary changes [11-13]. These radiologic changes may be a good surrogate endpoint for the susceptibility for BIP. However, since the bleomycin effect on CT scan is observed only after completion of treatment, an upfront or early biomarker that identifies patients likely to develop BIP would be preferable as these patients could be treated with a non bleomycin containing schedule. An early biomarker change would facilitate a premature halt of the weekly bleomycin administration.

Transforming growth factor-beta 1 (TGF-β1) is a cytokine involved in many physiological and pathological processes, including immune response, cell proliferation, angiogenesis, fibrosis and oncogenesis [14]. TGF-β1 plays an important role in development of BIP and fibrosis in animal models [15-17]. In patients treated with radiotherapy, or patients that underwent stem cell transplantation, a relationship between TGF-β1 levels and treatment-induced pulmonary toxicity was found [18-19]. Growth differentiation factor 15 (GDF-15) (also known as macrophage inhibitory cytokine 1 (MIC-1)) is a member of the TGF-β1 super family. GDF-15 levels are upregulated in many cancer types [20]. GDF-15 expression is induced during fibrosis development and correlates with lung function impairment in systemic sclerosis patients [21]. GDF-15 is a product of activated macrophages and hereby a role player in inflammatory processes [22]. In addition, we evaluated the role of the known inflammation marker high sensitive C-reactive protein (hs-CRP).

In this study, we investigated the prevalence of lesions suspect for bleomycin-induced pulmonary changes found on restaging CT scans after treatment with BEP chemotherapy in patients with metastatic testicular cancer, and whether fibrosis markers TGF-β1 and GDF-15 and the inflammation marker hs-CRP were predictive for these changes.

## MATERIAL AND METHODS

### Patients

We performed a prospective non-randomized biomarker cohort study for which eligibility criteria were: patients between 18 and 50 years of age with metastatic testicular cancer who were to be treated with BEP chemotherapy in the University Medical Center Groningen. Primary assessments were related to cardiovascular parameters. Exclusion criteria were a medical history of cardiovascular disease or a creatinine clearance < 60 mL/min. An additional analysis was performed on pulmonary parameters. Criteria for this analysis were a minimum of at least two bleomycin administrations (60 USP) and presence of a chest CT scan as part of the restaging investigations after chemotherapy. Patients were treated with a standard regimen of 3 or 4 three weekly BEP chemotherapy courses, depending on International Germ Cell Consensus Classification (IGCCC) prognosis group. During the first six days of each course, patients received daily anti-emetic therapy (dexamethasone and ondansetron). The local ethics committee approved the study. All patients gave written informed consent.

### CT scans

Post chemotherapy restaging CT scans were analyzed for abnormalities suspect for bleomycin-induced pulmonary changes as judged by two independent radiologists who were blinded for clinical phenotype. Patients were instructed to inhale during CT scans of the thorax. CT scanning was started 30 seconds after intravenous contrast injection (55 cc Lomeron 350). Scans were made in caudalcranial direction from the deepest costophrenic pleural recesses to above the thorax aperture. For reconstruction the following parameters were used: 3/1.5 mm on Sensation-16 and Symbia T16 and 2/1.5 mm on S-64, Definition and mCT (Kernel B40f).

Radiologic criteria for abnormalities on restaging CT scan that were regarded as bleomycin-induced were that these abnormalities were newly developed since start of BEP chemotherapy and could not readily be explained by other factors such as metastases or infection. When available, the next CT scan (follow-up scan), made as part of the routine follow-up after completion of treatment was assessed to judge whether abnormalities on the restaging CT scan, suspected to be the result of bleomycin-induced pulmonary changes, diminished or resolved. The type of abnormality was described and abnormalities were scored to be unifocal or multifocal with the location and number of lobes involved noted. Extension of radiographic abnormalities were graded as minor (only outer third of the lung involved), moderate (outer and middle third of the lung involved, but not extending across to the mediastinum) or severe abnormalities (whole width of the lung from periphery to mediastinum involved) according to Bellamy et al [11].

### Measurement of biomarkers

EDTA plasma samples were collected for all biomarkers before start of chemotherapy (day 1), for TGF-β1 on day 1 of every subsequent chemotherapy course, for GDF-15 and hs-CRP at day 8 of the third course and for all measured biomarkers at follow-up 4 weeks after the end

of chemotherapy. EDTA blood samples were centrifuged at 3000 g at 4°C for 10 minutes and plasma was stored at minus 20°C. In these samples total TGF-β1 levels were analyzed after activation with hydrochloric acid using a human TGF-β1 Quantikine ELISA-kit (R&D systems Abingdon, United Kingdom). GDF-15 levels were measured with the Human GDF-15 Quantikine ELISA-kit (R&D systems). Hs-CRP levels were analyzed using the nephelometer.

### Statistics

Differences in biomarker levels were investigated between patients with and without pulmonary changes on CT scan and in patients with and without clinical BIP. We also analyzed differences between the groups based on amount of changes on CT scan (no, minor, moderate or severe abnormalities). Biomarker levels were evaluated as absolute values, relative values (normalized to pre-chemotherapy levels) and absolute increases. Mann Whitney U tests and Kruskal Wallis tests were used to evaluate differences in biomarker levels between groups. Chi square and Fisher exact tests were used to analyze categorical data. A Spearman correlation test was used to test correlations. *P*-values ≤0.05 were considered significant. Statistics were calculated with IBM SPSS statistics 22. Graphs were made using GraphPad Prism version 5.00.

## RESULTS

### Patients

Between May 2006 and June 2012, 78 patients were included in the biomarker study. Two patients withdrew consent during the study. In total, five patients were excluded from analysis because data were incomplete due to missed measurements. Five patients received ≤ 2 BEP-courses. Data of 66 patients was analyzed (Figure 1, Table 1). Five of these 66 patients did not receive all initially planned bleomycin administrations because of clinical signs of BIP (n = 3), development of bleomycin skin toxicity (n = 1) and development of a pulmonary embolism (n = 1).

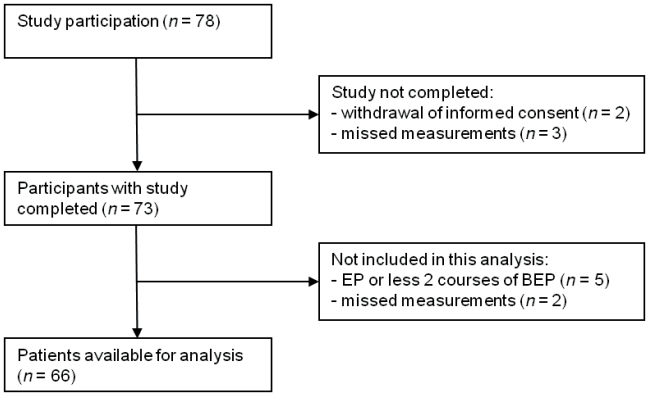


**Figure 1.** CONSORT diagram.

*Table 1: Patient characteristics.*

| | | Median (range) | Number (%) |
|---|---|---|---|
| Total number of patients | | | 66 |
| Age (years) | | 31 (19-46) | |
| Diagnosis | | | |
| | Non-seminoma | | 54 (82%) |
| | Seminoma | | 12 (18%) |
| IGCCC prognosis group | | | |
| | Good | | 55 (83%) |
| | Intermediate | | 10 (15%) |
| | Poor | | 1 (2%) |
| Cumulative administered bleomycin dose (USP) | | | |
| | 150 | | 1 (2%) |
| | 180 | | 1 (2%) |
| | 240 | | 2 (3%) |
| | 270 | | 52 (79%) |
| | 330 | | 1 (2%) |
| | 360 | | 9 (14%) |
| Pretreatment creatinine clearance (mL/min) | | 77 (53-107) | |
| Pulmonary metastases | | | |
| | Yes | | 7 (11%) |
| | No | | 59 (89%) |
| Smoking | | | |
| | Yes | | 29 (44%) |
| | No | | 26 (39%) |
| | Ex | | 10 (15%) |
| | Unknown | | 1 (2%) |

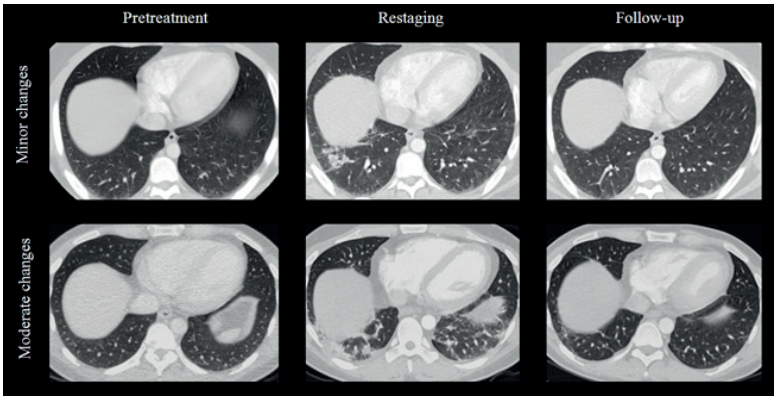


**Figure 2.** Representative examples of minor graded (upper panel) and moderate graded (lower panel) bleomycin-induced pulmonary changes; pretreatment, restaging and follow-up CT scan (left to right).

**Bleomycin-induced pulmonary changes on CT scan**

Pretreatment staging CT scans from chest and abdomen were made median 22 (range 1 - 78) days before start of chemotherapy. Restaging CT scans were made median 21 (range 5 - 112) days after the last course of chemotherapy. The first CT scan during follow-up was made median 175 (range 70 - 588) days after the restaging CT scan. In 45 out of 66 (68%) analyzed patients radiological signs suspect for bleomycin induced pulmonary changes were seen on the restaging scan. Of these, 37 were classified to have minor and eight to have moderate abnormalities (Figure 2). None were qualified as severe abnormalities. Most abnormalities were multifocal and found in the basal parts of the lungs.

There was no relation between the cumulative dose of bleomycin and development of lesions suspect for bleomycin toxicity on restaging CT scan. Renal function and smoking frequency were not different between groups (Table 2). On available follow-up scans of patients with suspected bleomycin-induced pulmonary changes (38/45; 84%), these changes diminished in 14 patients and disappeared in 24 patients. No differences were found in patient characteristics between these two groups (Table 3).

In three patients with clinical signs of BIP for which administration of bleomycin was halted, the restaging CT scan showed moderate, minor and no abnormalities respectively.



**Biomarker plasma levels**

Table 4 shows biomarker plasma levels of all patients at clinical relevant time points. Median plasma levels before start of chemotherapy were 4788 (range 550 - 24369) pg/mL for TGF-β1, 392 (range 187 - 1935) pg/mL for GDF-15 and 1.3 (range 0.2 - 50.4) mg/L for hs-CRP. No correlation was found between TGF-β1, GDF-15, hs-CRP and tumor marker levels (AFP, β-HCG and LDH) before start of treatment in patients with elevated tumor markers (data not shown).

No significant differences in absolute or relative levels of TGF-β1, GDF-15 and hs-CRP at the measured time points were found between patients with no, minor or moderate radiological signs of pulmonary bleomycin toxicity. In addition, there were also no significant differences found in patients with no or minor versus moderate pulmonary bleomycin toxicity (Table 4). However, patients who developed pulmonary abnormalities on CT scan (either minor or moderate) more frequently had an TGF-β1 increase between day one of the first course BEP (before start of chemotherapy) and day one of the second course (86 vs. 60%, p=0.047). Also, GDF-15 levels at the 8$^{th}$ day of the third course were higher in patients with moderate changes on CT scan than in patients with no or minor changes on CT scan, although this difference was not statistically significant (7246 pg/mL vs. 5222 pg/mL, p=0.087). All groups showed the same pattern of hs-CRP decrease during chemotherapy and an increase afterwards. Patients with clinical signs of BIP had no different biomarker levels compared to other patients (Figure 3). In patients with pulmonary abnormalities on follow-up scans that diminished but not disappeared (n=14) TGF-β1, GDF-15 and hs-CRP levels were not different compared to the patient group in which pulmonary abnormalities disappeared (n=24) (Table 3).

*Table 2: Characteristics of patients with and without signs of bleomycin-induced pulmonary changes on CT scan.*

| | Without changes(n = 21) | | | Minor changes (n = 37) | | | Moderate changes (n = 8) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Median | Range | N (%) | Median | Range | N (%) | Median | Range | N (%) | P-value* |
| Age (yrs) | 32 | 19-45 | | 31 | 20-45 | | 28 | 22-46 | | 0.768 |
| Weight pre-chemotherapy (kg) | 88 | 70-130 | | 88 | 62-139 | | 87 | 65-106 | | 0.852 |
| Bleomycin cumulative dose (USP) | 270 | 180-360 | | 270 | 150-360 | | 270 | 240-360 | | 0.231 |
| Serum creatinine pre-chemotherapy (mmol/L) | 79 | 63-107 | | 75 | 52-105 | | 82 | 64-89 | | 0.549 |
| Serum creatinine 4 weeks after last course of chemotherapy (mmol/L) | 82 | 57-104 | | 78 | 59-96 | | 82 | 68-90 | | 0.630 |
| Smoking | | | 8/21 (38%) | | | 15/36 (42%) | | | 3/8 (38%) | 0.878 |

*: P-value based on comparison of patients with moderate bleomycin-induced pulmonary changes on CT scan versus patients with no or minor changes (Mann-Whitney U tests)

*Table 3: Characteristics of patients with follow-up CT scans and bleomycin induced pulmonary changes.*

| | | Patients in whom bleomycin induced changes disappeared (n = 24) | | | Patients in whom bleomycin induced changes diminished (n = 14) | | | P-value* |
|---|---|---|---|---|---|---|---|---|
| | T | Median | Range | N (%) | Median | Range | N (%) | |
| Age (yrs) | | 28 | 20-43 | | 30 | 26-45 | | 0.215 |
| Weight pre-chemotherapy (kg) | | 88 | 62-134 | | 92 | 72-139 | | 0.150 |
| Bleomycin cumulative dose (USP) | | 270 | 150-360 | | 270 | 270-360 | | 0.075 |
| Serum creatinine pre-chemotherapy (mmol/L) | | 75 | 62-105 | | 77 | 52-93 | | 0.565 |
| Serum creatinine 4 weeks after last course of chemotherapy (mmol/L) | | 76 | 62-91 | | 76 | 59-96 | | 0.693 |
| Smoking | | | | 10/24 (42%) | | | 7/13 (54%) | 0.484 |
| Moderate changes on CT scan | | | | 2/24 (8%) | | | 4/14 (29%) | 0.103 |
| TGF-β1 (pg/mL) | 1.1 | 4616 | 550-16140 | | 5447 | 2861-15842 | | 0.348 |
| | 2.1 | 7813 | 909-30130 | | 10364 | 4156-25887 | | 0.256 |
| | 3.1 | 9818 | 3582-32057 | | 9533 | 3329-14513 | | 0.687 |
| | 4.1 | 3546 | 1768-18911 | | 6602 | 3664-14108 | | 0.327 |
| | C | 4916 | 595-17636 | | 5909 | 3212-14207 | | 0.315 |
| GDF-15 (pg/mL) | 1.1 | 396 | 199-1935 | | 403 | 187-691 | | 0.882 |
| | 3.8 | 5235 | 2475-12013 | | 5721 | 4130-10761 | | 0.366 |
| | C | 697 | 299-1486 | | 1066 | 421-2601 | | 0.297 |
| hs-CRP (mg/L) | 1.1 | 1.7 | 0.3-50.4 | | 1.2 | 0.2-36.1 | | 0.405 |
| | 3.8 | 0.5 | 0.2-132 | | 0.5 | 0.2-2.1 | | 0.682 |
| | C | 2.3 | 0.3-29.1 | | 3.0 | 0.3-26.7 | | 0.445 |

*: Mann-Whitney U tests. T= time point: course number.day number; C = 4 weeks after last course of chemotherapy.

*Table 4: Biomarker levels in relation to presence of bleomycin-induced pulmonary changes on CT scan.*

| | | | Whole group (n = 66) | | Without changes (n = 21) | | Minor changes (n = 37) | | Moderate changes (n = 8) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Biomarker | T | N (%) | Median | Range | Median | Range | Median | Range | Median | Range | P-value* |
| TGF-β1 | 1.1 | 66 (100%) | 4788 | 550-24369 | 4306 | 1345-24369 | 5002 | 550-16140 | 5268 | 2052-13361 | 0.582 |
| (pg/mL) | 2.1 | 63 (95%) | 9570 | 909-42015 | 8940 | 1380-42015 | 8915 | 909-30130 | 10803 | 4156-23849 | 0.397 |
| | 3.1 | 59 (89%) | 7889 | 1484-49897 | 6201 | 2378-49897 | 8522 | 1484-32057 | 8512 | 3878-20372 | 0.765 |
| | 4.1 | 12 (18%) | 6774 | 1768-18911 | 6774 | 6232-7316 | 3912 | 1768-14108 | 13977 | 9043-18911 | 0.121 |
| | C | 64 (97%) | 5220 | 595-18839 | 5357 | 1622-18839 | 5041 | 595-17636 | 5116 | 3223-14207 | 0.527 |
| GDF-15 | 1.1 | 63 (95%) | 392 | 187-1935 | 388 | 198-721 | 366 | 199-1935 | 413 | 187-1876 | 0.397 |
| (pg/mL) | 3.8 | 58 (88%) | 5342 | 1439-18959 | 5209 | 1439-14400 | 5235 | 2475-12013 | 7246 | 4130-18959 | 0.087 |
| | C | 62 (94%) | 880 | 299-2601 | 808 | 350-2023 | 840 | 299-1610 | 1089 | 442-2601 | 0.073 |
| hs-CRP | 1.1 | 66 (100%) | 1.3 | 0.2-50.4 | 1.9 | 0.2-18.5 | 1.2 | 0.2-50.4 | 2.1 | 0.3-39.6 | 0.813 |
| (mg/L) | 3.8 | 61 (92%) | 0.5 | 0.2-132.0 | 0.5 | 0.2-25.7 | 0.5 | 0.2-132.0 | 0.7 | 0.2-7.2 | 0.626 |
| | C | 65 (98%) | 2.2 | 0.3-29.1 | 2.7 | 0.4-15.3 | 2.2 | 0.3-29.1 | 1.9 | 0.3-9.3 | 0.546 |

*: P-value based on comparison of patients with moderate bleomycin-induced pulmonary changes on CT scan versus patients with no or minor changes (Mann-Whitney U tests).

T = time point course number.day number; C = 4 weeks after last course of chemotherapy

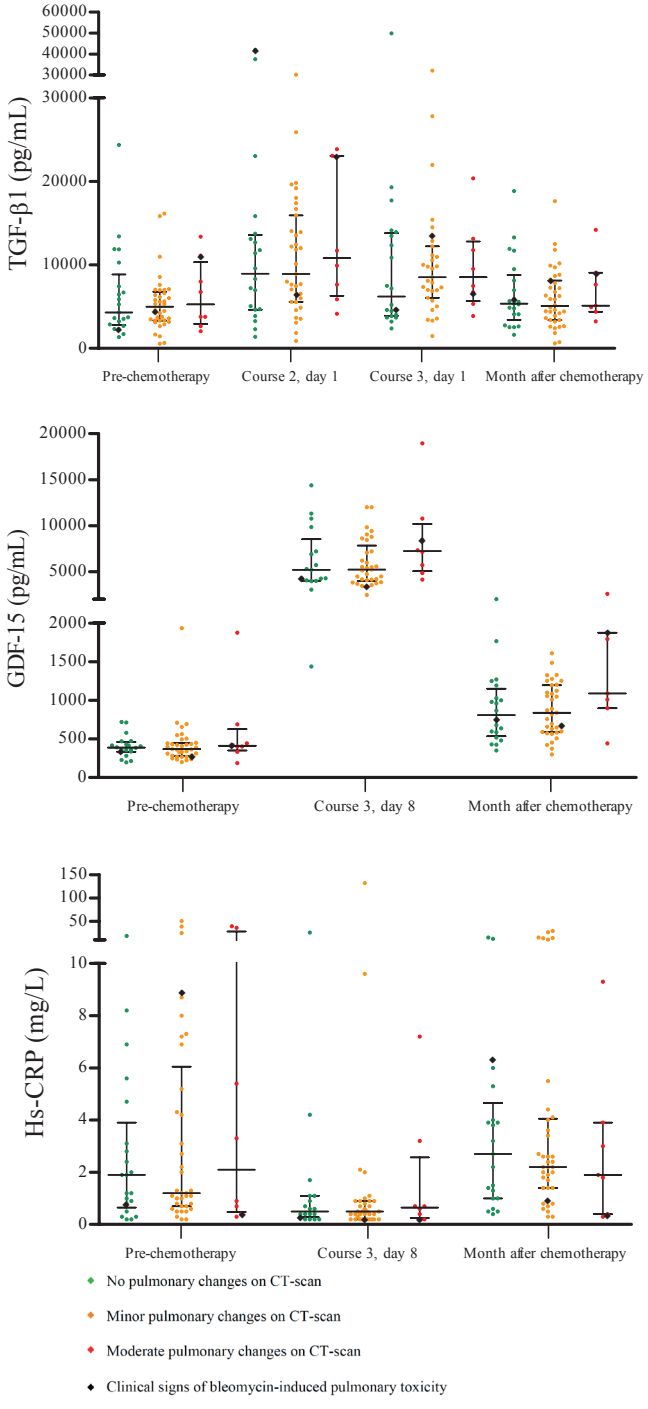


**Figure 3.** Plasma levels of TGF-β1 (upper graph), GDF-15 (middle graph) and hs-CRP (lower graph) during the chemotherapy courses.

## DISCUSSION

In this study, all restaging CT scans of patients with disseminated testicular cancer treated with BEP chemotherapy for signs of bleomycin-induced pulmonary toxicity were evaluated. In 68% of these patients radiological abnormalities on the restaging CT scan interpreted as signs of bleomycin-induced pulmonary changes were observed. Clinical presentation suspect for BIP during treatment, which led to premature termination of bleomycin administration, was seen in only 5% of patients. This discrepancy between clinical presentation of BIP and radiological signs of bleomycin-induced toxicity on CT scans is striking. Earlier studies reported a much higher incidence of abnormalities on restaging CT scans as well, compared to the 10% of the patients who classically develop clinical signs of bleomycin-induced pulmonary toxicity [11-13]. However, these radiological abnormalities are not very well documented and not reported in more recent reports of clinical trials in which bleomycin is a component of the combination regimen for metastatic testicular cancer [23]. No high-resolution CT scans were made in any of the patients precluding statements on the presence of interstitial pneumonitis.

The clinical relevance of radiological signs of bleomycin-induced pulmonary abnormalities on restaging CT scans remains unclear. Most of these abnormalities on CT scans were without accompanying symptoms and resolved spontaneously based on subsequent CT scans several months later during follow-up. We questioned whether post treatment radiological findings were accompanied by biochemical signs of active fibrosis and whether these could be used as early markers of the toxic effect of bleomycin on the lung.

TGF-β1 is a cytokine involved in many processes in the body and plays a pivotal role in the development of lung fibrosis [24]. Various preclinical studies showed a critical role for TGF-β1 in the BIP development. In the current study, we did not find a difference in TGF-β1 levels between patients with and without radiological signs of bleomycin-induced pulmonary changes. However, patients who developed bleomycin-induced pulmonary changes on CT scan more often showed an increase of TGF-β1 levels from pre chemotherapy to day one of the second course. This may indicate involvement of the TGF-β1 pathway in development of pulmonary abnormalities due to bleomycin administration.

The TGF-β1 plasma levels found in our patients at baseline had a broad range. Plasma TGF-β1 levels in healthy volunteers also show a threefold difference with a broad range in other studies [25,26]. Measured TGF-β1 levels in our patient group may also be tumor derived rather than selectively the result of bleomycin-induced pulmonary changes [27]. However, no correlation between known tumor markers in testicular cancer and TGF-β1 levels before treatment was found. An interesting approach to circumvent this would be to assess TGF-β1 levels during bronchoalveolar lavage (BAL) to assess only lung TGF-β1 levels [28]. We collected no platelet poor plasma, while TFG-β1 might have been released from platelets during collection and analyses of the samples [29]. This is a limitation of our study, but does not preclude comparison of TGF-β1 levels within our patient group.

We did not find differences in GDF-15 levels in patients with versus patients without bleomycin-induced pulmonary changes on CT scan. Although the increase of GDF-15 levels seemed

to be larger in patients with moderate pulmonary changes on CT scans, this was not significant. The number of patients with moderate changes (n = 8) might have been too small to detect significant differences. In addition, GDF-15 is also known as apoptosis marker [22]. Therefore, its distinctive value during cancer therapy is probably limited. Nevertheless, it could be worth examining GDF-15 levels in a larger patient group with a moderate degree of pulmonary changes on CT scans after BEP chemotherapy.

Hs-CRP could be an easily accessible biomarker for subclinical inflammation, but levels did not differ in patients with compared to patients without bleomycin-induced pulmonary changes. This concurred with steroid administration as anti-emetic drugs during chemotherapy. Therefore, hs-CRP is probably not an usable biomarker for bleomycin-induced pulmonary changes during chemotherapy.

In conclusion, pulmonary radiological abnormalities suspect for bleomycin induced changes are very common on restaging CT scans after BEP chemotherapy for metastatic testicular cancer, occurring in two third (68%) of the patients. Most of these radiological abnormalities appear to resolve on follow-up CT scans. TGF-β1, GDF-15 and hs-CRP plasma levels before and during treatment were not significantly different between patients with and without radiological signs of pulmonary bleomycin-induced toxicity and therefore do not seem helpful as early predictive biomarkers.



**ACKNOWLEDGEMENTS**

Nynke Zwart and Gerrie Steursma are acknowledged for their technical and administrative support.

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