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PRELIMINARY REPORT

Does the Serum Metallothionein Level Reflect the Stage of Testicular Germ Cell Tumor?

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Increased levels of metallothionein (MT) have recently been found in the blood serum of men with newly diagnosed testicular germ cell tumors (TGCT). In light of previously published results, the aim of this study was to investigate the difference in serum MT levels among patients with different stages of TGCT and compare MT with commonly used markers (α-fetoprotein, β-human chorionic gonadotropin and lactate dehydrogenase). The concentration of total MT was determined in the serum of 25 men with TGCT (seminoma or non-seminoma) by differential pulse voltammetry. Serum samples were obtained prior to chemotherapy, after two cycles of chemotherapy and 1 year after chemotherapy. A statistically significant difference in MT levels in patients with different stages of TGCT was observed in the serum of patients with non-seminoma obtained before chemotherapy. Although not significant, an increase in serum MT levels commensurate with the disease stage increase was also observed in patients with seminomatous TGCT. The results indicate that, in combination with the existing markers, MT could be useful for the identification of the histological type of tumor and stage of the disease before biopsy diagnosis.

Key Words: Metallothionein, Non-seminoma, Seminoma, Serum marker, Testicular germ, Cell tumor.

Introduction

Testicular germ cell tumors (TGCT) are uncommon but particularly important malignancies as they tend to affect children and young men, representing the most common tumor in men aged 15−35 years. Incidence rates have been increasing over the past 50 years in many industrialized populations (1,2).

Using the histopathological criteria, TGCT are classified as seminoma and non-seminoma. In addition, the stage of tumor at diagnosis is used to determine the extent or spread of the disease and to help in the diagnosis, treatment and prognosis of testicular cancer (3). In clinical practice, there are three tumor markers commonly used in the diagnosis and prognosis of testicular cancer: α-fetoprotein (AFP), β-human chorionic gonadotropin (β-HCG) and lactate dehydrogenase (LDH) (3). Although crucial in the management of testicular cancer, these markers are not specific for testicular cancer and their values are elevated in only 51% of testicular cancer cases (4). It has also been observed that β-HCG and/or AFP are elevated at relapse in about 2/3 of patients with non-seminoma and in only 1/3 of those with seminoma, whereas LDH has limited sensitivity, specificity and positive predictive value and does not add to the early detection of relapse (5−7). Therefore, there is a need for additional, minimally invasive biomarkers to optimize individual treatments of cancer patients.

Metallothioneins (MT) are a group of low molecular mass, cysteine-rich proteins. Their enhanced synthesis in rapidly proliferating tissues suggests their crucial role in normal and neoplastic cell growth (8). Increased levels of MT in serum have been found in patients with lymphoid

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leukemia, lung carcinoma, thyroid carcinoma, head and neck cancer (9,10) and prostate cancer (11). In a recently published study, we found significantly higher levels of serum MT in patients with newly diagnosed TGCT compared to healthy volunteers (12). Elevated levels of serum MT in men with newly diagnosed TGCT make MT a candidate biomarker for testicular cancer development. In an ideal case, a tumor marker reflects the body's tumor burden and subsequently increases with progressive or recurrent disease, decreases with response to treatment, and normalizes with remission (13). Therefore, in the current study, we evaluated serum MT levels among patients with different stages of TGCT in the samples obtained before chemotherapy (I), after two cycles of chemotherapy (II) and 1 year after chemotherapy (III) and compared serum MT levels with those of commonly used tumor markers (AFP, β-HCG and LDH).

Subjects and Methods

Subjects

The study population consisted of 25 men (age: 18−49 years), non-smokers, with newly diagnosed TGCT ascertained by the Department of Oncology at the University Hospital Centre, Zagreb, Croatia. Based on histological reports, the patients were classified as having seminoma or non-seminoma. After orchiectomy, patients were treated with two, three or four cycles of standard chemotherapy, every 3 weeks with bleomycin, etoposide, cisplatin (bleomycin 30 units iv weekly on days 2, 9 and 16; etoposide 100 mg/m^2 iv on days 1−5; cisplatin 20 mg/m^2 iv on days 1−5) depending on the stage of the disease (1, 2 or 3). Venous blood was sampled from each patient before any type of treatment (I), after two cycles of chemotherapy (II) and 1 year after chemotherapy (III). Levels of serum tumor markers AFP, β-HCG and LDH were determined as part of routine biochemical tests used for differential diagnosis and surveillance of patients.

Before entering the study, all subjects were informed about the aim and experimental details and provided signed consent for voluntary participation. The study was performed in accordance with the ethical principles of the Declaration of Helsinki and approved by the institutional Ethics Committee.

Metallothionein Assay

MT was determined in heat-treated blood serum to enable the analysis of MTs as heat stable proteins. The concentration of total MT was determined by differential pulse voltammetry according to a modified Brdička procedure (14,15) as described in Tariba et al. (12).

Statistical Analysis

Statistical analyses were performed using Statistica 12 for Windows (StatSoft Inc., Tulsa, OK). In order to normalize the distribution of the MT concentration data, a logarithmic transformation was applied. One-way analysis of variance (one-way ANOVA) was used to evaluate the difference in serum MT levels between patients with different stages of TGCT (stage 1, 2 or 3). Spearman's rank correlation (r, p) was calculated for the associations between MT and AFP, β-HCG and LDH levels. Data were considered statistically significant at $p < 0.05$.

Results and Discussion

The discovery and validation of additional markers in combination with current markers is important for improving the diagnosis and management of testicular cancer. Although AFP, β-HCG and LDH are the most commonly used serum markers for the management of TGCT, they are not very sensitive and specific because their values can also be increased in the presence of other tumors (16,17). AFP is expressed in 50−70% of non-seminomatous TGCTs, whereas in patients with pure

Table 1. Concentrations of metallothionein (MT) and levels of serum tumor markers (AFP, β-HCG and LDH) (median and range) in patients with seminoma and non-seminoma before chemotherapy (I), after two cycles of chemotherapy (II) and 1 year after chemotherapy (III) Q1

	MT (mg/mL)	AFP (μg/L)	β-HCG (IU/L)	LDH (U/L)
All patients (n = 25)				
I	0.55 (0.27−1.21)	5.0 (1.40−19,032.0)	1.20 (0.0−14,101.0)	201.0 (96.0−1,726.0)
II	0.64 (0.25−1.08)	3.41 (1.0−81.0)	0.0 (0.0−16.0)	179.0 (127.0−250.0)
III	0.26 (0.18−0.74)	3.0 (0.74−12.0)	0.0 (0.0−1.20)	167.0 (119.0−219.0)
Seminoma (n = 11)				
I	0.67 (0.27−1.21)	2.70 (1.40−11.0)	0.0 (0.0−11.0)	203.0 (96.0−665.0)
II	0.65 (0.25−0.94)	3.75 (1.0−9.0)	0.0	179.0 (127.0−236.0)
III	0.26 (0.20−0.70)	3.10 (1.20−12.0)	0.0	168.0 (138.0−190.0)
Non-seminoma (n = 14)				
I	0.48 (0.30−1.16)	7.0 (2.0−19,032.0)	2.60 (0.0−14,101.0)	201.0 (120.0−1,726.0)
II	0.59 (0.25−1.08)	3.31 (2.0−81.0)	0.0 (0.0−16.0)	178.5 (138.0−250.0)
III	0.26 (0.18−0.74)	2.86 (0.74−5.0)	0.0 (0.0−1.20)	166.0 (119.0−219.0)

seminoma an elevated AFP raises suspicion of the presence of non-seminomatous elements. Elevated serum β-HCG levels are typically present in both seminomas and non-seminomas, whereas LDH is a less specific marker but has an independent prognostic value in men with advanced testicular cancer (4,18). The concentration of MT found in our study and the levels of commonly used serum tumor markers (AFP, β-HCG and LDH) in patients with seminoma and non-seminoma obtained before chemotherapy (I), after two cycles of chemotherapy (II) and 1 year after chemotherapy (III) are presented in Table 1. The levels of AFP, β-HCG and LDH in the serum of our subjects obtained 1 year after chemotherapy were found to be normal in both types of TGCT (according to the American Joint Committee on Cancer, 2010).

The values obtained for MT in TGCT patients (before and after therapy) were significantly higher than those measured in the serum of healthy volunteers (median and range 0.412 (0.230−0.687) mg/mL) (published in Tariba et al. 2015). In patients with germ cell tumors, the level of commonly used tumor markers usually reflects the stage of the disease (3,19). Figure 1 indicates an increase in the serum MT concentration commensurate with cancer stage increase (stage 1, 2 or 3), when all patients (Figure 1A), patients with seminoma (Figure 1B) and patients with non-seminoma (Figure 1C) were considered. According to the results of one-way ANOVA, there is a statistically significant difference in serum MT levels across different stages of TGCT in the samples of patients with non-seminoma obtained before chemotherapy. In the available literature there are no similar results for comparison. The existing data on MT levels in testicular cancer patients come from the studies that investigated the levels of MT using immunohistochemical staining in TGCT specimens obtained after radical orchiectomy. The most recent results of a meta-analytical approach, which comprises the data from these studies for the evaluation of immunohistochemically determined MT as a cancer biomarker, found no association between MT staining and tumor stage in TGCT (20).

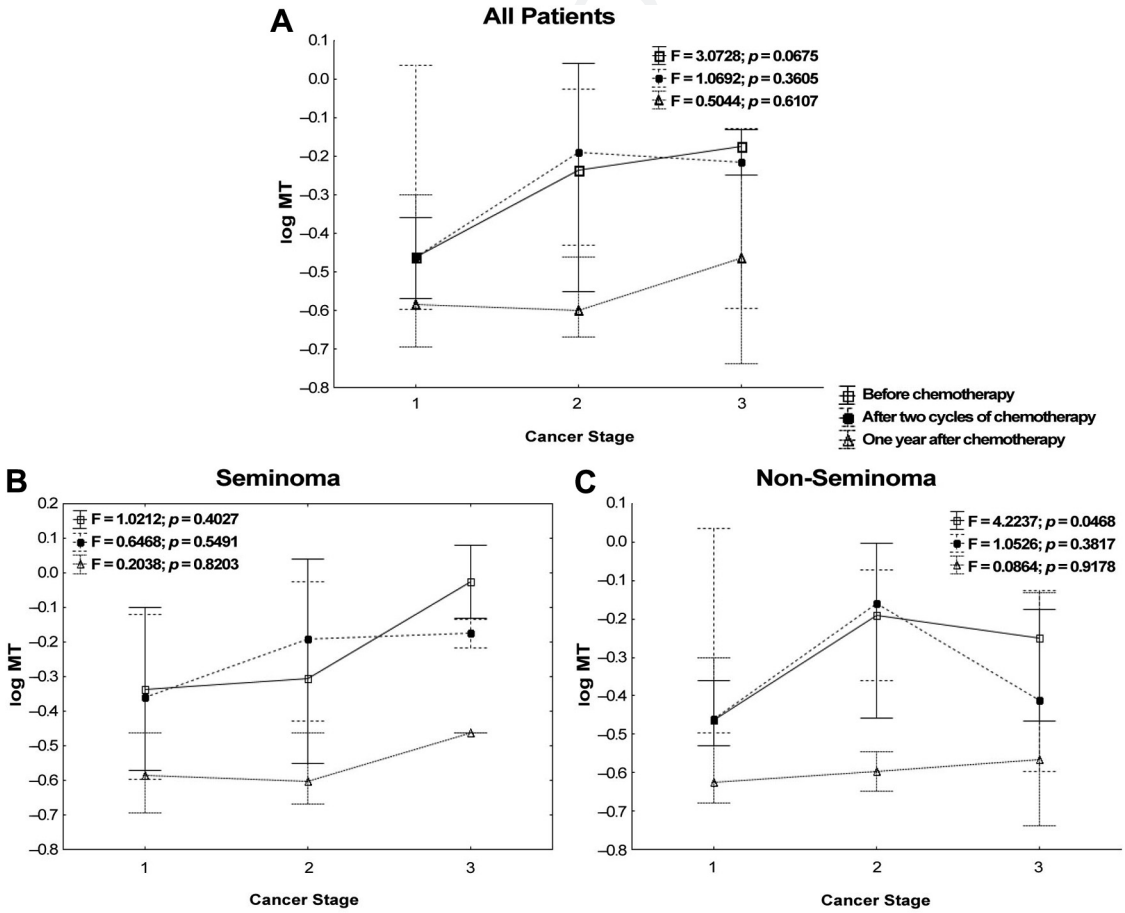

Figure 1. Concentrations of metallothionein (MT) (log transformed) in serum of patients with different stage of testicular germ cell tumor (stage 1, 2 or 3) obtained before chemotherapy, after two cycles of chemotherapy and 1 year after chemotherapy. (A) All patients. (B) Patients with seminoma. (C) Patients with non-seminoma. Full dot, square and triangle represent mean values, whereas vertical bars denote 0.95 confidence intervals. Data were considered statistically significant at $p < 0.05$.

We found a significant positive correlation between MT and AFP levels in all patients ($r = 0.594$, $p < 0.04$) and in seminoma patients ($r = 0.886$, $p < 0.02$) only in the samples obtained after two cycles of chemotherapy, whereas no significant correlation was found between these parameters before chemotherapy or 1 year after chemotherapy. No significant correlation was found between MT levels and β-HCG and LDH. The lack of significant correlation between the levels of MT and serum tumor markers in newly diagnosed TGCT patients suggests that the diagnostic value of MT is independent from that of commonly used testicular cancer markers.

Conclusion

Our results indicate the potential of serum MT as an additional marker that could provide helpful information for adequate management and treatment of TGCT, including early diagnosis, determination of histological type and stage of the disease. Nevertheless, based on the limited results, we cannot unambiguously assert that serum MT is a suitable marker for TGCT. The low number of patients may be considered a limiting factor for the power of the study. In particular, the lack of statistical significance between groups might be attributed to the inclusion of a limited number of patients. Additional studies including a larger number of subjects as well as patients with disease relapse are needed to further elucidate the significance of MT as a testicular cancer marker.

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Conflict of interest: The authors declare no conflicts of interest.

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