Evidence for Acute Vascular Toxicity of Cisplatin-based Chemotherapy in Patients with Germ Cell Tumour

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Abstract. *Background: Acute early vascular toxicity of chemotherapy for germ cell tumour (GCT) is poorly understood. To explore the pathogenesis of this complication we evaluated laboratory parameters associated with vascular disease. Patients and Methods: In 33 GCT patients the following parameters were investigated with routine laboratory methods before and after chemotherapy: von Willebrand factor antigen (vWF:AG), collagen binding capacity (vWF:CB), lipoprotein (a), homocysteine, plasminogen activator inhibitor I, total cholesterol, high density lipoprotein, low density lipoprotein, troponine I. Statistical evaluation involved descriptive analysis and the Wilcoxon signed rank test. Results: Levels of vWF:AG and vWF:CB increased significantly upon therapy (p=0.002). All other parameters remained unchanged. Upon late measurement, vWF:AG and vWF:CB were normalised. Conclusion: As von Willebrand factor is released from endothelial cells upon damage, we postulate that early vascular toxicity of chemotherapy is caused by direct damage of the vascular endothelium. Long-term vascular complications of chemotherapy appear to be different, pathogenetically.*

Today more than 95% of all patients with testicular germ cell tumors (GCTs) can effectively be cured (1). Cisplatin-based chemotherapy is the mainstay of successful management of disseminated disease (2, 3). However, the cost of cure is far from negligible. Patients are faced with a large number of acute adverse effects and late sequelae. Nausea and emesis, myelodepression, hearing loss, pulmonary fibrosis, renal impairment, and peripheral neuropathy represent the most frequent early complications (4). Late consequences of chemotherapy involve secondary malignancies, as well as metabolic and endocrinologic disorders (5, 6). Cardiovascular morbidity is another recognized late toxicity. In a recent Norwegian follow-up study on survivors of GCT, chemotherapy was found to be associated with hazard ratios of 2.3, 5.7, and 3.1, respectively, for developing atherosclerotic disease, coronary artery disease, and myocardial infarction, respectively, during the late course (7). Long-lasting degenerative processes initiated by chemotherapy are considered to be pathogenetic in these late cardiovascular events (8, 9). In addition to late cardiovascular morbidity, not-e-worthy is the accumulating awareness of early vascular toxicity occurring at the time of chemotherapy administration or immediately thereafter. Apparently, both the venous and the arterial compartments of the vascular system are involved in this type of complication (10). The association of venous thrombosis and pulmonary embolism with chemotherapy in GCT patients represents well-established clinical experience (11, 12). In spite of former views of authorities (13), cases with arterial occlusions, myocardial infarctions, and cerebral strokes have increasingly been reported in recent years (14-16). Accordingly, a German survey documented an incidence rate of 0.3% of serious arterial cardiovascular events occurring during the time of chemotherapy (17). Clearly, early vascular events can hardly be caused by chronic degenerative processes. To date, there is only very limited understanding of the pathogenesis of early vascular toxicity. Nuver *et al.* found a significant increase of von Willebrand factor secondary to chemotherapy (15). The authors hypothesized that this finding might indicate vascular damage occurring at the time of chemotherapy and that these primary changes might be of significance for the well-known cardiovascular complications in the long term.

We looked to von Willebrand factor and a number of other blood-borne parameters associated with vascular damage, coagulation disturbances and vascular degenerative processes,

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Table I. *Patient characteristics*.

Patients, total (n)	33
Age: median, range	37 years, 20-55 years
Clinical stage *: I (n)	14
II (n)	13
III (n)	6
Pure seminoma (n)	9
Nonseminoma (n)	24
Cycles applied: 2 (n)	17
3 (n)	12
4 (n)	4
Additional late examination (n)	11
Blood samples scheduled (n)	156
Blood samples drawn (n)	125

*Clinical stage according to Lugano classification. (n) Number of patients.

Table II. *Average Values for von Willebrand factor (vWF:AG) at different study points and comparison with starting value*.

Cycle	Patients examined (n)	vWF Q1 (%)	vWF median (%)	vWF Q3 (%)	*p*-value*
1 Starting value	33	74	101	131	-
Day 15	26	92	123	166	<0.0001
2 Day 1	31	121	176	270	<0.0001
Day 15	24	122	173	199	<0.0001
3 Day 1	16	142	201	271	<0.0001
Day 15	11	101	148	307	0.0020
Late measurement	11	79	92	104	0.7480

Q1 25% quantile; Q3 75% quantile; *for difference from starting value (signed rank Wilcoxon test).

respectively, in GCT patients receiving chemotherapy, and we compared the values obtained prior to chemotherapy with those found after completion of therapy. Our aim was to find any significant changes that might provide insight into the pathogenesis of vascular toxicity resulting from cisplatin-based chemotherapy.

Patients and Methods

Patients and study design. Thirty-three consecutive patients with histologically proven GCT underwent chemotherapy with the cisplatin-etoposide-bleomycin protocol (PEB) according to European guidelines (3). Clinical details of the patients are provided in Table I. Blood samples for study were drawn after overnight fasting at days 1 and 15 during each cycle of therapy. Eleven patients were examined once again several months (3 to 35 months) after completion of the chemotherapy. All patients had given informed consent. The study was approved by an ethical committee. Twenty percent of the scheduled blood samples could not be taken for various clinical reasons.

Laboratory methods. The following parameters were investigated: von Willebrand factor antigen (vWF:AG), collagen-binding capacity (vWF:CB), alpha lipoprotein, homocysteine, plasminogen activator inhibitor I (PAI-I), total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and troponine I. All laboratory tests were performed with standard methods. VWF:AG and vWF:CB were measured by enzyme-linked immunosorbent assay, serum lipids by enzymatic colorimetry, alpha lipoprotein by nephelometry, homocysteine by chemiluminiscence-microimmuno-assay, PAI-I by bio-immunoassay, and troponine I by microparticle immunoassay.

Data processing and statistical analysis. All laboratory values were initially stored using a commercially available databank system (Microsoft Excel, version XP) and later transferred into SASv9.2 (SAS Institute Inc., Cary, North Carolina, USA) for statistical evaluation. The analysis involved tabulation of data and descriptive statistical analysis. Box-whisker plots were established to show median values of the entire group of patients during the course of therapy. Individual value plots (spaghetti plots) were established to illustrate the change of values in individual patients. The signed rank Wilcoxon test was used to compare pre-therapeutic values with those obtained after completion of therapy (18). A *p*-value of less than 0.05 was defined as being significant.

Results

No significant changes were recorded with respect to the following parameters: homocysteine, alpha lipoprotein, PAI-I, total cholesterol, HDL, LDL, and troponine I. A significant increase was found regarding vWF:AG and vWF:CB when starting values were compared to values obtained during cycle 3 (Table II, Figures 1 and 2). As only four patients completed four cycles, and as half of the scheduled blood samples of these cases were incomplete, values relating to cycle 4 were omitted from further analysis. With regard to cycle 3, 2 out of 16 scheduled measurements at day 1 were missed, while 7 did not take place at the end of that cycle. Hence, if starting values are compared to values of cycle 3, then there is a strongly significant increase of levels of vWF:AG.

Measurements of collagen-binding capacity (vWF:CB) and vWF:AG are dependent on multimeric size of the vWF molecule, but both measures are strongly interrelated biologically (19). Accordingly, both of these parameters revealed almost identical measures in all of the examinations of this study. For reasons of brevity, only measurements regarding vWF:AG are presented herein. Importantly, none of the late measurements of vWF:AG and vWF:CB or any of the other parameters were significantly different from their starting values.

Discussion

The central finding of this study is the increase of vWF during the course of cisplatin-based chemotherapy and the subsequent normalization of this value within several months.

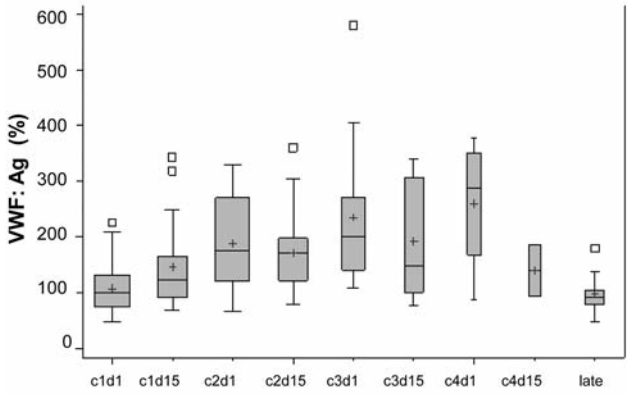

Figure 1. *Box-whisker plot of values of von Willebrand factorF at various times of chemotherapy. Boxes represent the quartiles and median values. The whiskers are defined by means of the extreme values, followed by the outlying observations which are marked by a square. The "+" is the arithmetic mean. Box size varies with number of patients examined. Overall there is a significant increase of vWF during chemotherapy if compared with the starting value. Values of cycle 4 are disregarded for further analysis because the patient number was too small. C, cycle number; d, day number.*

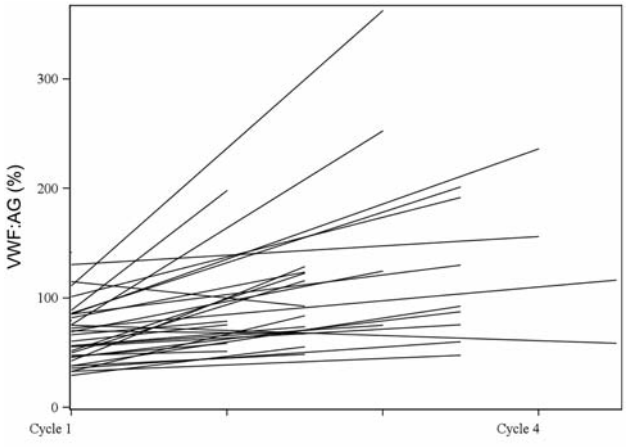

Figure 2. *Spaghetti plot showing individual values for all patients examined. Each of the lines denotes one individual patient. For each of the patients, only the starting value and the very last value obtained is given. Almost all of the patients demonstrate some increase of vWF during chemotherapy.*

Von Willebrand factor is released from endothelial cells at the time of vascular wall damage. The physiological function of this factor is to mediate repair mechanisms for endothelial injuries, mainly via adhesion and aggregation of platelets (20-22). Thus, the increase of vWF:AG during chemotherapy probably does indicate endothelial cell damage. It is rather tempting to speculate that some endothelial cells are rendered apoptotic along with cancer cells secondary to chemotherapy. Consequently, platelet adhesion and coagulation processes are initiated. Occasionally, vascular obstruction may occur at these repair sites because coagulation processes may take place in a hyperactive mode.

Enhanced clotting ability is a well-known general experience in cancer patients (23, 24) and this knowledge goes back to Trousseau, who noted this phenomenon as early as in 1865 (25). Cisplatin-triggered hypercoagulation, which has been documented experimentally (26-28), might also contribute to the formation of vascular occlusions in chemotherapy patients. Clinically, there is in fact sufficient evidence for the occurrence of acute vascular toxicity during chemotherapy of patients with GCT (29). Accordingly, myocardial infarction, cerebral stroke, and peripheral arterial occlusion have been reported repeatedly in patients with GCT (10, 17, 30, 31, 32) and in patients with other malignancies (33-35). As the vast majority of patients with GCT are young and otherwise healthy, and as these problems occur at the time of chemotherapy, these vascular problems are not likely caused by the known degenerative long-term effects of systemic therapy. Instead, they must be initiated by direct toxic effects of chemotherapy to vascular walls. The increase of vWF, as shown herein, would suggest endothelial damage secondary to chemotherapy with consecutive hyperactive coagulation to be the clue to the pathogenesis of this acute type of vascular toxicity. This hypothesis is substantiated by the absence of any change of those parameters associated with chronic atherosclerotic disease, *i.e.* homocysteine, alpha lipoprotein and cholesterol with subfractions.

Our results nearly mirror the report of Nuver *et al*., who were the first to observe the increase of vWF in patients with GCT during chemotherapy (15). Earlier, Licciardello *et al*. had noted increase of vWF in sporadic patients with head and neck cancer during cisplatin-based chemotherapy (36).

In supplementing the study of Nuver *et al*., the present investigation additionally analysed vWF:AG levels during the late course. Of note, without any exception, increased vWF levels returned to pre-chemotherapy levels within several months. This finding would be compatible with the conclusion that the vascular damage initiated by chemotherapy were no longer present. Moreover, the return of vWF:AG values to normal after some months would only weakly endorse the hypothesis made by Nuver *et al*., that these early vascular changes during systemic therapy may translate into chronic vascular disease (15, 37). Based on the present study, it appears much more probable that early vascular toxicity of chemotherapy is pathogenetically distinctly different from the well-known chronic vascular disease.

Certainly, the present investigation has several methodological limitations. The total number of patients (n=33)

examined obviously represents a small sample size and, thus, clinical significance of the results might still be debatable. The study is hampered by missing values for about 20% of cases, specifically in the third and fourth cycle of chemotherapy. On the other hand, the individual value plots (Figure 2) clearly demonstrate that vWF:AG levels do actually increase in all but two of the patients. Thus, there is an unequivocal trend of vWF:AG increase during chemotherapy. It appears quite improbable that the missing values would substantially influence the overall result. vWF:AG plasma levels are known to behave like acute-phase reactants, particularly in infectious clinical situations (38). We did not correlate the vWF:AG levels with other acute-phase proteins *e.g.* C-reactive protein. However, clinically, none of the patients examined had any inflammatory disease at the time of blood sampling. With regard to statistical evaluation, the significant increase of vWF:AG might conceivably be influenced by multiple testing. However, this concern appears rather theoretical because the total number of nine parameters tested is still not very high. Furthermore, the parameters tested are quite different from each other, both chemically and biologically.

Overall, we feel that in spite of the limitations outlined here, our study may provide valuable information to enhance the understanding of acute vascular toxicity of cisplatin-based chemotherapy. Clinically, this type of complication requires the attention of physicians caring for GCT patients.

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