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## Cardiotoxicity after anticancer treatment

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## THE ROLE OF SOLUBLE APOPTOTIC PROTEINS IN DETECTING ANTHRACYCLINE CARDIOTOXICITY

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**ABSTRACT****Aim**

To evaluate whether circulating soluble apoptosis-related protein levels change after anthracycline-containing chemotherapy and radiotherapy in relation to cardiac dysfunction or the applied treatment.

**Methods**

Circulating apoptotic proteins were determined with immunoassay in 40 breast cancer patients following surgery (T0), one month after epirubicin-based chemotherapy (T1) and one year later (T2). Chemotherapy, consisting of standard-dose (n=21) or high-dose myeloablative chemotherapy (n=19), preceded irradiation and tamoxifen. Circulating apoptotic proteins were compared with previous cardiac evaluations, including measurement of LVEF, B-type and N-terminal atrial natriuretic peptide (BNP and NT-ANP), diastolic function with echocardiography and a standard electrocardiogram.

**Results**

Soluble tumor necrosis factor receptor 1 (+ 30%), 2 (+ 43%) and Fas (+ 40%) were transiently increased at T1 compared to T0, whereas Fas ligand (- 64%) was transiently decreased, especially in the high-dose group. Apoptosis markers were not associated with cardiac dysfunction.

**Conclusions**

We observed interestingly significant, but transient changes in levels of soluble apoptotic proteins, particularly after high-dose chemotherapy. No relation was found between apoptosis-related proteins and standard markers of cardiotoxicity.

## INTRODUCTION

Late complications of anticancer treatment become increasingly relevant due to improved survival of cancer patients. Cardiovascular toxicity for instance, is a well known adverse effect of several chemotherapeutic agents, especially anthracyclines.<sup>1</sup> Anthracyclines can cause heart failure, which mostly occurs during the first year after treatment,<sup>2</sup> but can also take years to develop.<sup>3</sup> Asymptomatic cardiac dysfunction is considered to precede symptoms of heart failure. We have previously shown that left ventricular ejection fraction (LVEF) decreases during the first year following epirubicin-containing chemotherapy and chest wall irradiation, in patients treated for breast cancer who did not experience symptomatic cardiac dysfunction.<sup>4</sup> Unfortunately, LVEF measurement is relatively insensitive, since it detects only loss of systolic cardiac function. More sensitive methods, enabling earlier detection or even prediction of anthracycline-related cardiotoxicity, may allow clinicians to intervene before heart failure develops.

In patients with heart failure unrelated to anthracyclines, increased cardiomyocyte apoptosis has been reported<sup>5</sup> (For review see <sup>6</sup>). Plasma levels of several soluble (s) members of the tumor necrosis factor (TNF)-superfamily of apoptosis-related proteins (TNF $\alpha$ , TNF Receptor (TNF-R) 1 and 2, Fas and Fas Ligand) are increased in heart failure patients and correlate with New York Heart Association (NYHA) class,<sup>7,8</sup> and increased TNF-related apoptosis-inducing ligand (TRAIL) cDNA expression has been reported in peripheral blood mononuclear cells of heart failure patients.<sup>9</sup> Next to plasma markers of apoptosis, serum levels of the acute phase reactant high-sensitivity C-reactive protein (HS-CRP) are elevated in heart failure patients and positively associated with the NYHA class.<sup>10</sup>

Currently, it is unknown whether circulating sTNF-related apoptotic protein levels change during follow-up after anticancer treatment in relation to cardiac injury. We have reported that plasma sTNF-related apoptotic proteins are increased after a median follow-up of more than 6 years in patients who had received adjuvant epirubicin-containing chemotherapy and chest wall irradiation for breast cancer.<sup>11</sup> This increase was particularly prominent in patients who underwent high-dose chemotherapy with hematopoietic stem cell rescue. The question rises whether this rise in soluble apoptotic proteins is causative for or coinciding with (early) cardiotoxicity in this breast cancer population. Previously, we described that subclinical cardiotoxicity occurred during the first year after anthracycline-containing chemotherapy and chest wall irradiation in 40 patients treated with anthracycline-based chemotherapy for breast cancer.<sup>4</sup> In the current study, we extended the results of this prospective study by measuring plasma sTNF-related apoptotic protein levels in plasma samples from these patients.

The primary aim of the current prospective study was to evaluate whether circulating sTNF-related apoptotic protein levels change during the first year following the start of anthracycline-containing chemotherapy and radiation therapy

in relation to cardiac function. Secondly, we investigated the influence of the applied chemotherapy regimen on the circulating apoptosis-related protein levels.

## **PATIENTS AND METHODS**

Plasma samples from 40 breast cancer patients, who had participated in a cardiac side study of a nation wide randomized trial,<sup>12</sup> comparing anti-tumor efficacy of standard-dose chemotherapy to high-dose chemotherapy and hematopoietic stem cell rescue.<sup>4</sup> The study was approved by the medical ethical committee and all patients gave their written informed consent. Twenty one patients received standard-dose chemotherapy and 19 high-dose chemotherapy followed by hematopoietic stem cell rescue.

### **Treatment**

Patients were treated with standard-dose of 5 courses of fluorouracil (500 mg/m<sup>2</sup>), epirubicin (90 mg/m<sup>2</sup>) and cyclophosphamide (500 mg/m<sup>2</sup>) (FEC), or 4 cycles of FEC, followed by high-dose cyclophosphamide (1500 mg/m<sup>2</sup>), thiotepa (120 mg/m<sup>2</sup>) and carboplatin (400 mg/m<sup>2</sup>) daily for 4 days (4x FEC + CTC). Peripheral stem cell reinfusion took place 7 days after the start of CTC. Total epirubicin dose was 450 mg/m<sup>2</sup> for the standard-dose FEC-treated group and 360 mg/m<sup>2</sup> for the high-dose 4x FEC + CTC-treated group. Tamoxifen (40 mg/day, orally) was started thereafter in both treatment groups. Locoregional irradiation followed upon hematological recovery from the last chemotherapy cycle. Patients received 46-50 Gy in 23-25 fractions on their supraclavicular and axillary lymph nodes. Chest wall irradiation was performed with doses between 40 and 50 Gy in 20-25 fractions. In patients who underwent breast conserving treatment, a boost of 16-20 Gy in 8-10 fractions was applied to the tumor bed. Twenty-five patients received right sided chest wall irradiation and the remaining 15 underwent radiation therapy to the left side of the chest wall.

### **Cardiac evaluation**

Cardiac evaluation was performed as described previously.<sup>4</sup> Briefly, cardiac evaluations had been performed following surgery but before the start of chemotherapy (T0), one month after chemotherapy before chest wall irradiation (T1) and one year after the start of chemotherapy (T2). Next to a history and physical examination with special attention to signs and symptoms related to heart failure, the cardiac evaluations consisted of a standard electro- and echocardiogram, and plasma natriuretic peptide (N-terminal atrial and B-type natriuretic peptide (NT-ANP and BNP)) measurement. LVEF measurements were done at T0 and T2. None of the patients had pre-existent cardiac disease at the time of enrollment and symptomatic heart failure had not occurred during the one year follow-up period. Two patients had pre-existent hypertension for which they

received medical treatment consisting of an angiotensin converting enzyme inhibitor and a calcium blocker, respectively. Two patients did not complete the cardiac evaluation. Both patients participated in the cardiac evaluation study until T1. Table 1 summarizes the earlier published results of the cardiac evaluations.<sup>4</sup>

**Table 1.** Cardiac functional characteristics at the different time points.<sup>4</sup>

	<b>T0</b>	<b>T1</b>	<b>T2</b>	<b>P-value T0 vs T1</b>	<b>P-value T0 vs T2</b>	<b>P-value T1 vs T2</b>
<b>LVEF</b>	0.61 (0.47-0.76)	N/A	0.54 (0.42-0.67)	N/A	<b>&lt;0.001</b>	N/A
<b>LVEF decrease <math>\geq 0.10</math></b>	N/A	N/A	10	N/A	N/A	N/A
<b>LVEF &lt; 0.50</b>	0	N/A	6	N/A	N/A	N/A
<b>LVEF decrease <math>\geq 0.10</math> to value &lt; 0.50</b>	N/A	N/A	4	N/A	N/A	N/A
<b>LVESD (mm)</b>	45 (36-53)	45 (36-52)	46 (39-55)	0.480	0.850	0.793
<b>LVEDD (mm)</b>	29 (20-43)	30 (21-36)	28 (21-35)	0.404	0.545	0.429
<b>E/A ratio</b>	1.2 (0.8-1.8)	1.2 (0.5-2.1)	1.1 (0.7-1.8)	0.411	0.248	0.575
<b>Dt (ms)</b>	176 (95-263)	180 (75-275)	185 (120-296)	0.894	0.991	0.213
<b>IVRT (ms)</b>	80 (55-110)	80 (53-130)	80 (47-100)	0.387	0.721	0.405
<b>QTc (ms)</b>	405 (382-437)	417 (378-539)	421 (388-456)	<b>0.02</b>	<b>&lt;0.001</b>	0.079
<b>QTc &gt; 440 ms</b>	0	5	5	-	-	-
<b>BNP (pmol/L)</b>	1.7 (0.4-12.4)	3.0 (0.6-11.3)	4.5 (0.4-17.9)	<b>0.034</b>	<b>0.004</b>	0.071
<b>BNP &gt; 10 pmol/L</b>	1	3	4	-	-	-
<b>NT-ANP (pmol/L)</b>	231 (108-411)	283 (106-1007)	316 (168-594)	<b>0.005</b>	<b>&lt;0.001</b>	<b>0.001</b>
<b>NT-ANP &gt; 500 pmol/L</b>	0	1	3	-	-	-

Values are represented as median (range). T0 = after surgery, before chemotherapy; T1 = 1 month after chemotherapy, before radiation therapy; T2 = 1 year after the start of chemotherapy. Abbreviations: LVEF = left ventricular ejection fraction, LVESD = left ventricular end-systolic diameter, LVEDD = left ventricular end-diastolic diameter, E/A ratio = early peak flow velocity / atrial peak flow velocity – ratio, Dt = deceleration time, IVRT = isovolumic relaxation time, QTc = corrected QT time, BNP = B-type natriuretic peptide, NT-ANP = N-terminal atrial natriuretic peptide

### Plasma soluble apoptosis markers

Peripheral blood samples had been collected in 10 mL disposable tubes containing either 2-natrium-ethylenediamine tetra-acetic acid (EDTA), heparin or no additive. Tubes containing EDTA or heparin as an additive were placed immediately on ice. In tubes containing no additive, blood was allowed to clot at room temperature until centrifugation. Serum and plasma were separated within 30 min of collection by centrifuging at 4 °C, and stored at –80 °C until determination. Circulating levels of TNF $\alpha$ , sTNF-R1, sTNF-R2, sFas, sFas ligand and sTRAIL were determined

using commercially available ELISA kits (Quantikine; R&D systems, Minneapolis, MN, USA) following the manufacturer's instructions. TNF $\alpha$  was measured in EDTA plasma, sTNF-R1 and sTNF-R2 in heparin plasma. Serum was used for sFas, sFas ligand and sTRAIL.

### **Platinum**

Serum platinum levels were determined in the patients who received carboplatin. This analysis was performed using a highly sensitive assay in which high-pressure decomposition of plasma is followed by adsorptive voltametric determination of platinum, with a quantification limit of 6 pg/g.<sup>13</sup>

### **HS-CRP**

Serum HS-CRP was determined as a marker for inflammation and assayed with the BNII Nephelometer (Dade Behring, Brussels, Belgium). The lower detection limit was 0.16 mg/L (normal values 0.16-10 mg/L).

### **Statistics**

Quantitative variables were compared between two groups using an unpaired t test for normally distributed variables or a Mann-Whitney-U test for skewed distributed variables. Paired analysis was performed with a Wilcoxon signed ranks test. Normally distributed variables are reported as mean  $\pm$  SD, skewed distributed variables are reported as median and range. Correlations between variables were calculated using Pearson's correlation coefficient or Spearman rank sum test. All P values were two-sided and  $P < 0.05$  was considered statistically significant.

## **RESULTS**

### **Plasma soluble apoptosis markers**

Table 2 and figure 1 show plasma apoptosis marker levels at the different time points in the whole group. Between T0 and T2, a transient increase in median plasma levels of sTNF-R1, sTNF-R2 and sFas was observed at T1. Plasma sFas ligand concentrations were transiently decreased. At T2, sFas remained higher compared to pre-treatment levels. After the decrease at T1 compared to T0, plasma sTRAIL was raised from T1 to T2, to levels higher than pre-treatment values.

Serum levels of sTNF-R1 ( $R = 0.573$ ,  $P < 0.001$ ) and sTNF-R2 ( $R = 0.362$ ,  $P = 0.035$ ) correlated positively with serum HS-CRP levels at T1. One year after chemotherapy, a weak positive correlation was observed between serum HS-CRP and sTNF-R2 ( $R = 0.418$ ,  $P = 0.038$ ) and sFas ( $R = 0.450$ ,  $P = 0.024$ ).

**Table 2.** Circulating apoptosis-related proteins at the different time points

	T0	T1	T2	P-value T0 vs. T1	P-value T0 vs. T2	P-value T1 vs. T2
TNF $\alpha$ (pg/mL)	1.5 (0.2-38.4)	1.5 (0.4-45.8)	1.4 (0.4-9.0)	0.385	0.458	0.085
sTNF-R1 (ng/mL)	1.0 (0.7-1.6)	1.3 (0.8-4.6)	1.1 (0.7-2.0)	<b>&lt;0.001</b>	0.492	<b>&lt;0.001</b>
sTNF-R2 (ng/mL)	2.1 (1.3-3.3)	3.0 (1.6-8.1)	2.2 (1.4-4.1)	<b>&lt;0.001</b>	0.050	<b>&lt;0.001</b>
sFas (ng/mL)	8.2 (4.2-16.4)	11.5 (6.5-26.0)	9.7 (6.1-35.0)	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.005</b>
sFas ligand (pg/mL)	71 (38-137)	33 (8-157)	65 (40-132)	<b>&lt;0.001</b>	0.411	<b>&lt;0.001</b>
sTRAIL (pg/mL)	70 (48-118)	65 (16-144)	80 (33-161)	0.470	<b>0.026</b>	<b>0.014</b>

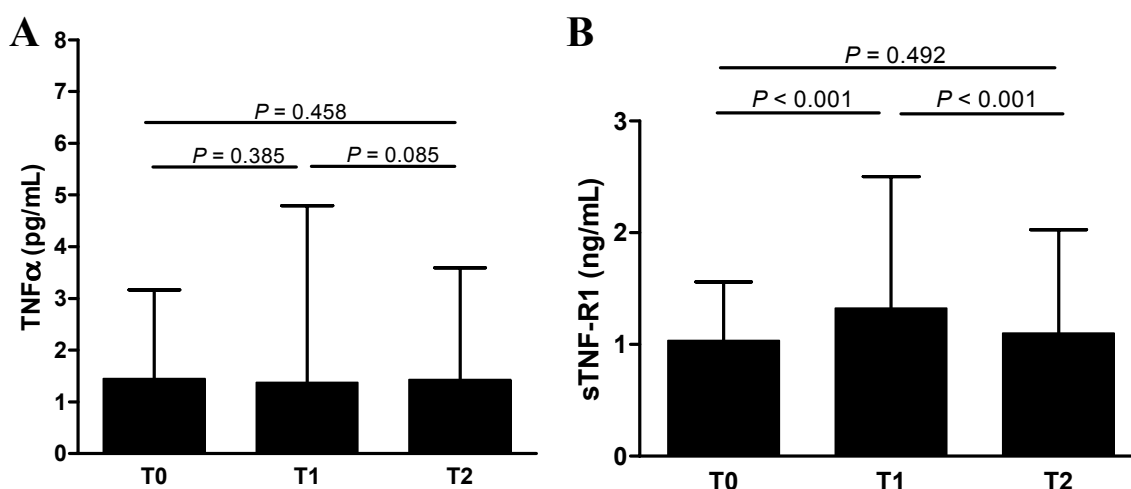
Values are presented as median (range). T0 = after surgery, before chemotherapy; T1 = 1 month after chemotherapy, before radiation therapy; T2 = 1 year after the start of chemotherapy. s = soluble, TNF $\alpha$  = tumor necrosis factor  $\alpha$ , TNF-R1 = TNF receptor 1, TNF-R2 = TNF receptor 2, TRAIL = TNF-related apoptosis-inducing ligand.

### Plasma soluble apoptosis markers and cardiac dysfunction

Plasma apoptosis marker levels and serum HS-CRP did not differ between the patients with a decrease in LVEF of more than 0.10 from pre-treatment values or a total LVEF value less than 0.50 one year after the start of chemotherapy. Furthermore, the changes in plasma apoptosis marker and serum HS-CRP levels between the different time points were not associated with a change in LVEF during the study period.

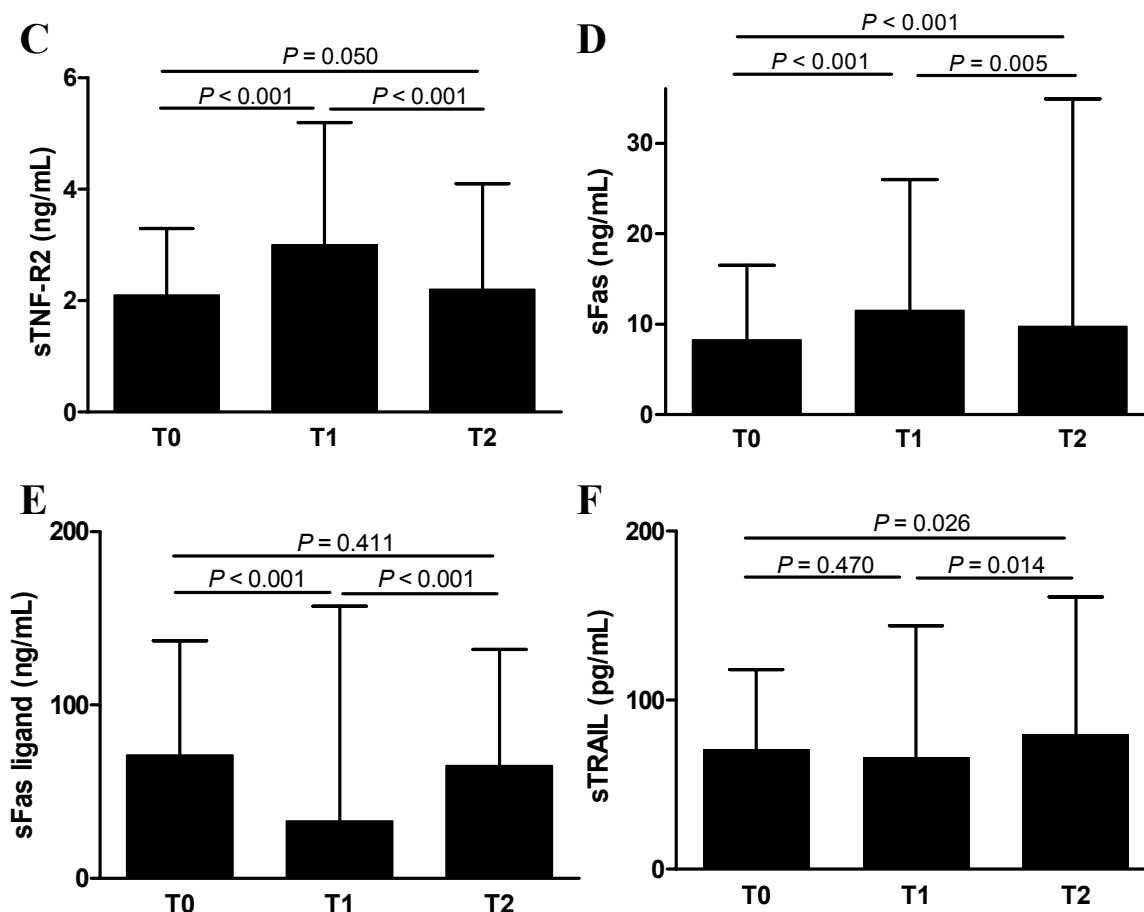
No associations were observed between the plasma apoptosis marker concentrations and echocardiographic functional parameters or the QTc time. At T1, serum HS-CRP levels were correlated positively with the corrected QT time (QTc) ( $R = 0.622$ ,  $P < 0.001$ ).

In the patients with increased plasma natriuretic peptide levels (NT-ANP > 500 pmol/L; BNP > 10 pmol/L) at T2, circulating apoptosis marker values were not different from patients with natriuretic peptide levels below the upper limit of normal. No correlations between natriuretic peptide plasma concentrations and plasma apoptosis marker levels or HS-CRP were observed.



**Figure 1.** Bar plots of (A) TNF $\alpha$  (pg/mL), (B) sTNF-R1 (ng/mL),





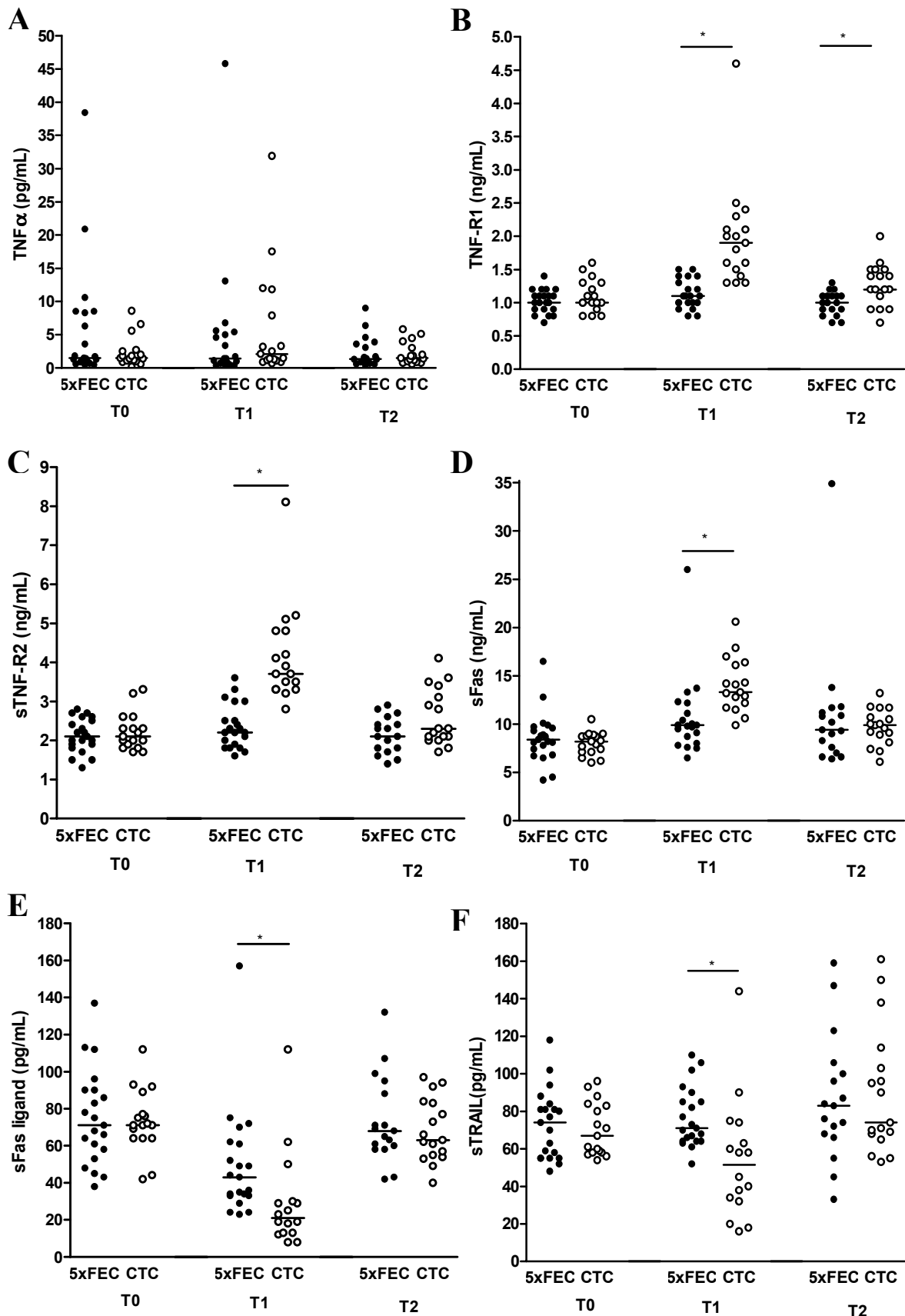
**Figure 1.** Bar plots of (C) sTNF-R2 (ng/mL), (D) sFas (ng/mL), (E) sFas ligand (pg/mL), (F) sTRAIL (pg/mL). Bars represent median values, error bars represent range (except for TNF $\alpha$ ; interquartile range).

### Plasma soluble apoptosis marker and treatment

Figure 2 represent plasma apoptosis markers levels according to the applied treatment regimen. Before the start of treatment, apoptosis marker levels did not differ between the treatment groups. At T1 however, sTNF-R1, sTNF-R2 and sFas plasma levels were markedly higher in the patients who had received high-dose chemotherapy. Plasma sFas and sTRAIL levels were lower in the high-dose treated group. One year after the start of chemotherapy, only a higher median sTNF-R1 plasma level was detected in high-dose group.

No differences were observed between patients who had received left sided compared to right sided chest wall irradiation with regard to the circulating apoptosis marker concentrations.

Serum HS-CRP levels did not differ between the treatment groups at any of the time points.



**Figure 2.** Dot plots of (A) TNF $\alpha$  (pg/mL), (B) sTNF-R1 (ng/mL), (C) sTNF-R2 (ng/mL), (D) sFas (ng/mL), (E) sFas ligand (pg/mL), (F) sTRAIL (pg/mL) according to chemotherapy regimen. Lines represent median values. \* P < 0.01

**Platinum**

In samples of the patients treated with the high-dose carboplatin-containing regimen, serum platinum was analyzed at T2, to evaluate whether persisting circulating platinum could explain for the higher plasma apoptosis marker levels observed in this subgroup of patients. Circulating platinum levels were available for 16 of the 19 patients in the high-dose group and was median 4091 (range 2027-7022) pg/g plasma. Serum platinum correlated positively with plasma sFas levels ( $R = 0.684$ ,  $P = 0.003$ ) and with sTNF-R1 ( $R = 0.558$ ,  $P = 0.025$ ) at T2. Serum platinum levels were not associated with serum HS-CRP levels.

**DISCUSSION**

The main finding of the present prospective study is a transient change in soluble levels of several of the TNF-related apoptotic proteins, particularly a rise in sFas and a decrease in sFas ligand, following adjuvant antineoplastic treatment for early stage, high-risk breast cancer. The transient changes in serum soluble apoptosis marker levels were not associated with the development of cardiac dysfunction, since no relation with symptomatic cardiac dysfunction, decreased LVEF or increased natriuretic peptide plasma levels was observed.

Anthracyclines are well known to induce cardiac toxicity in a dose dependent manner.<sup>14</sup> Both chemotherapy regimens applied in this study contained a relatively low dose of epirubicin of either 450 mg/m<sup>2</sup> or 360 mg/m<sup>2</sup>. This may be an explanation for the fact that none of the patients developed symptoms of cardiac failure during the first year after the start of treatment. However, an asymptomatic decrease in LVEF to a value below 0.50 was observed in 15% of the patients. This indicates that even low doses of anthracyclines have detrimental effects on the heart, which may ultimately lead to the development of heart failure. We observed no differences in plasma apoptosis marker levels between patients with a LVEF decline more than 0.10 or to an absolute value less than 0.50, and patients with a normal LVEF. At one year, apoptosis marker levels were not different in patients with plasma natriuretic peptide concentrations above the upper limit of normal. As a consequence, the value of circulating apoptosis marker measurement for the early detection of cardiac dysfunction appears limited.

In patients with heart failure due to coronary artery disease and non-ischemic causes, increased serum HS-CRP levels have been reported.<sup>10</sup> Although HS-CRP serum levels were not associated with other parameters representative of cardiac function, we observed a positive association of serum HS-CRP levels with the QTc at T1.

Currently, only limited data with regard to circulating soluble apoptosis marker levels during and after adjuvant antineoplastic treatment are available. In several types of (active) malignant diseases, plasma levels of TNF-related apoptotic proteins are raised. Compared to healthy controls, breast cancer patients have

higher TNF $\alpha$  plasma concentrations before treatment, and a more advanced TNM stage is positively associated with higher TNF $\alpha$  levels.<sup>15</sup> A small study among 17 breast cancer patients showed that pre-treatment serum sTNF-R levels are higher in patients than in healthy controls.<sup>16</sup> Plasma sFas concentrations were also elevated in 162 primary and 71 recurrent breast cancer patients, compared to controls.<sup>17</sup> Additionally, plasma sFas levels are higher in breast cancer patients before surgery, than in healthy subjects.<sup>18</sup> In our study population, the baseline (T0) measurement was performed after surgery. For evaluating the effects of anticancer treatment on circulating levels of the apoptotic proteins, obtaining pre-treatment samples would have been required. However, as is indicated above, patients with active breast cancer, thus prior to surgery, have higher plasma apoptotic protein levels as compared to controls. Our study was carried out after surgery. Therefore, the changes in circulating apoptotic protein levels can not be attributed to the presence of active malignancy. In addition, there were no signs of cancer recurrence at the different time points of blood sampling.

Previously, we described that plasma apoptosis markers are elevated in long-term disease-free breast cancer survivors, after a median follow-up duration of more than 6 years after the start of chemotherapy, especially after high-dose chemotherapy.<sup>11</sup> Compared to healthy controls, higher plasma TNF $\alpha$ , sFas, sFas ligand and sTRAIL levels were observed in the population of long-term disease-free breast cancer survivors. In particular, patients who had received 4 cycles of FEC followed by myeloablation with high-dose CTC, had higher sFas ligand and sTRAIL plasma concentration at a median follow-up of 6 years, compared to the patients treated with 5 cycles of FEC. Remarkably, in the current study, we found a transient increase in sFas, but a transient decrease in sFas ligand plasma levels, at one month after chemotherapy. Plasma sFas levels tend to increase with advancing age.<sup>18</sup> This may partly explain the higher circulating apoptotic protein levels, which we observed in our population of long-term disease-free breast cancer survivors.<sup>11</sup> Alternatively, the initial change in circulating apoptosis marker levels, observed in the current study, may be indicative of the generalized injury induced by the antineoplastic treatment, and the high-dose chemotherapy regimen in particular. The convalescence of the circulating apoptotic proteins one year after the start of chemotherapy, may point to a recovery of the body after the induced damage. It can be hypothesized that the increase in circulating apoptotic proteins after longer follow-up may be the result of an autonomically progressive process, which is initiated by the initial injury induced by the anticancer treatment. As a result, these patients might be at increased risk of developing late sequelae related to the anticancer treatment. In addition, patients with increased circulating apoptotic proteins after antineoplastic treatment may be more susceptible to chemotherapy-related complications, for instance when they are re-treated with cytostatic agents. The origin of the elevated sTNF-related apoptotic proteins remains to be determined. Next to the inflamed heart,<sup>19</sup> several extracardiac sources of the circulating apoptotic proteins, such as peripheral skeletal muscles<sup>20</sup> and activation

of the immune system have been described.<sup>21</sup> The increased serum apoptosis marker levels may indicate an augmented apoptotic state. Hypothetically, the increase in plasma apoptosis marker levels may be the result of an increase in membrane-expressed apoptotic proteins. Elevated plasma concentrations of the soluble forms of these proteins may be the result of subsequent cleavage and shedding of membrane-bound proteins. Alternatively, an increase in the rate of proteolytic cleavage, leading to reduced membrane-expressed protein, might also account for the rise in plasma apoptosis marker levels. The circulating apoptosis-related receptors are viewed as anti-apoptotic,<sup>22,23</sup> while the ligands are considered to be pro-apoptotic.<sup>24-26</sup> The transient increase and decrease in plasma sFas and plasma sFas ligand, respectively, might suggest a natural protection or compensation mechanism against the external noxe, induced by the antineoplastic treatment. The exact association between the basal apoptotic rate and the current findings of transient change in plasma apoptosis-related protein levels remains unclear and warrants further investigation.

The high-dose chemotherapy regimen contained carboplatin. Previously, we showed that platinum can reside in the circulation long after treatment and is associated with long-term sequelae of chemotherapy.<sup>27,28</sup> In the current study, we evaluated the influence of circulating platinum retention on the plasma apoptosis marker levels and observed that, one year after the start of chemotherapy, circulating platinum was positively associated with plasma sTNF-R1 and sFas levels. This might be interpreted as a continuous external noxe for the apoptotic rate.

The transient increase in circulating apoptosis markers at one month after chemotherapy was especially pronounced in the patients treated with high-dose chemotherapy followed by hematopoietic stem cell rescue, compared to women treated with standard-dose chemotherapy. Data with regard to serum markers for apoptosis following high-dose chemotherapy during and after adjuvant treatment for setting is scarce. In 22 patients who received autologous peripheral blood stem cell transplantation for malignant non-Hodgkin's lymphoma or lung cancer, a transient increase in TNF $\alpha$  plasma levels was observed at one week after stem cell transplantation.<sup>29</sup>

Several pathophysiological mechanisms may contribute to the difference in apoptosis marker levels in high-dose compared to standard-dose treated patients. For instance, elevated circulating TNF $\alpha$ , sTNF-R, sFas, sFas ligand and sTRAIL levels have been reported in inflammatory conditions.<sup>30-32</sup> In this light, the increased circulating TNF-related apoptotic proteins may reflect an acute phase reaction as part of the neutropenic period induced by the high-dose regimen. We observed a positive association between HS-CRP serum levels and serum levels of the sTNF-Rs at T1, and sTNF-R1 and sFas one year after chemotherapy. In our study however, plasma apoptosis markers were measured after the neutropenic period. It can also be imagined that the higher circulating apoptosis marker levels in patients treated with high-dose chemotherapy are indicative of more tissue

and/or cellular injury induced by the myeloablative regimen in comparison to the standard-dose chemotherapy.

We analyzed six subtypes of TNF-related cytokines in order to find a relationship of these proteins with the development of cardiotoxicity induced by anticancer treatment. A possible limitation of our work is that multiple comparisons were performed, which may have increased the type I ( $\alpha$ ) error. Furthermore, the size of the study population was small.

In summary, we observed that plasma apoptotic receptor levels, particularly sFas, were increased and that plasma sFas ligand was decreased, one month after completion of epirubicin-containing chemotherapy, compared to after surgery, in patients receiving adjuvant breast cancer treatment consisting of chemotherapy and chest wall irradiation. These changes were more pronounced in the patients who received high-dose chemotherapy, and may be explained by the presence of platinum in the circulation. We observed no association between circulating apoptotic protein levels and the existence of cardiac dysfunction. Our findings suggest that the patients treated with myeloablative chemotherapy sustained more severe cellular damage, compared to the standard-dose-treated patients, although the precise mechanisms underlying the rise in plasma apoptotic proteins remain to be determined.

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