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Incidence and risk factors for capecitabine-induced cardiotoxicity: a retrospective study of 452 consecutive patients with metastatic breast cancer

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ABSTRACT

Objectives: Case-reports of capecitabine cardiotoxicity resemble those seen with intravenous 5-fluorouracil with chest pain as the predominant manifestation, but few studies of capecitabine cardiotoxixcity are available. We aimed to determine the incidence of symptomatic cardiotoxicity from capecitabine in breast cancer patients and to identify risk factors.

Methods: We reviewed medical records of consecutive women with breast cancer treated with capecitabine (1000 mg/m^2 twice daily) from 2002 to 2012 at one institution.

Results: Twenty-two of 452 patients (4.9%) (95%CI: 2.9%-6.9%) had symptoms of cardiotoxicity (chest pain: n = 13, dyspnea: n = 9, palpitations: n = 2). Eleven patients had changes on ECG (atrial fibrillation: n = 5, ST deviations: n = 3, T-wave abnormalities: n= 2, and QTc prolongation: n = 1). Two patients (0.4%) sustained acute myocardial infarction. One patient (0.2%) developed cardiac arrest with lethal outcome. Four of six patients (66%) retreated with capecitabine had recurrent symptoms at retreatment. Cardiac comorbidity (p = 0.001), hypercholesterolemia (p = 0.005) and current smoking (p = 0.023) were risk factors for cardiotoxicity in univariate analyses and remained significant when adjusted for age. Patients with cardiac co-morbidity were 5.5 times (95% CI: 2.0-14.8) more likely to develop cardiotoxicity. In the subgroup of patients with apparently no cardiac co-morbidity the incidence of cardiotoxicity was lower (3.7 %) and hypercholesterolemia (p = 0.035) and current smoking (p = 0.020) were risk factors of cardiotoxicity.

Conclusion: The incidence of cardiotoxicity from capecitabine resembles that of intravenous 5-fluorouracil (≈5 %). Cardiac co-morbidity, hypercholesterolemia and current smoking were associated with development of cardiotoxicity.

Strengths and limitations of this study

- Our study is a large single center study including all patients with breast cancer treated with capecitabine over a 10-year period.
- The primary end-point, cardiotoxicity, is mainly diagnosed on the basis of subjective symptoms (chest pain, dyspnea, palpitations) which may cause information bias.
- In spite of a relatively large sample size, the number of events is low which limit the power of the logistic regression analyses used to analyse for risk factors.
- Our study is a retrospective clinical study. The patients' history records are incomplete with respect to information on risk factors for cardiovascular disease (hypertension, hypercholesterolemia, diabetes and smoking) and baseline electrocardiograms.

INTRODUCTION

Capecitabine is an oral pro-drug of 5-fluorouracil (5-FU) that is converted to 5-FU in a three-stage process involving several enzymes.[1] The last step is catalyzed by thymidine phosphorylase.[1] Many tissues throughout the body express thymidine phosphorylase, but some human carcinomas express this enzyme in higher concentrations than surrounding normal tissues.[1] This, in theory should increase the concentration of 5-FU at the tumour site and decrease the concentration of 5-FU in healthy tissues resulting in less side effects.[1]

Capecitabine is licensed for adjuvant treatment in patients with colon cancer stage III and for the treatment of metastatic colorectal cancer, metastatic breast cancer and advanced gastric cancer (combination therapy). The main side effects from capecitabine are hand-and-foot syndrome, diarrhea, stomatitis, fatigue, anorexia, nausea and vomiting, abdominal pain, myelosuppression, hyperbilirubinemia and cardiotoxicity.[2-5]

Case reports of cardiotoxicity after administration of capecitabine are similar to those seen with intravenous 5-FU treatment with chest pain as the predominant manifestatio.[6-14] Other less frequent clinical manifestations are arrhythmias, myocardial infarction, heart failure, cardiogenic shock and sudden death.[15] Cardiotoxicity from 5-FU occurs with an incidence of 0.55% to 19.9%.[15] There are few studies of capecitabine cardiotoxicity with incidences ranging from 3% to 35%.[16-20]

Patients with breast cancer may have increased risk for cardiotoxicity from capecitabine compared to other cancer patients because of treatment with other carditotoxic therapies such as radiotherapy of the chest, trastuzumab and anthracyclines. We aimed to study the pattern and incidence of cardiotoxicity in women with metastatic breast cancer treated with capecitabine and to identify potential risk factors for capecitabine-induced cardiotoxicity.

MATERIAL AND METHODS

Selection of patients

We included patients with metastatic breast cancer consecutively treated with capecitabine from first of January 2002 to 31. December 2012. Inclusion criteria were female, metastatic breast cancer and capecitabine treatment (+/- trastuzumab). The chemotherapy regimen was capecitabine 2000 mg/m² divided in two daily doses for 14 days followed by one week off.

Data collection

Approval from the Danish Data Protection Agency was obtained. We collected data from medical records on age, height, weight, body surface area, capecitabine dose, cardiotoxicity, cardiac comorbidity (previous acute myocardial infarction, ischemic heart disease, arrhythmias, heart failure or reduced ejection fraction), hypertension, smoking status, hypercholesterolemia, diabetes, electrocardiogram (ECG) (before treatment start and if symptoms), hemoglobin levels, creatinine levels and previous treatment with anthracyclines, trastuzumab, breast- and thoracic radiotherapy. Renal function (estimated glomerular filtrationrate, eGFR) was estimated by the Chronic Kidney Disease Epidemiology Collaboration equation for Caucasian women incorporating age and plasma creatinine concentrations. All the collected data were prospectively defined.

End point: cardiotoxicity

Cardiotoxicity was defined as significant symptoms of likely cardiac origin or changes on ECG that started during treatment with capecitabine and was not present before treatment start. Medical records were reviewed by one of the authors. Cases with suspected cardiotoxicity were further reviewed by one cardiologist (MVN) who made the final decision to classify the case as cardiotoxicity or not. All patients were followed to cessation of capecitabine. Endpoints were evaluated unblinded and the investigators had access to patients' medical record.

Statistics

Mann-Whitney U test was used to compare differences in age between patients with cardiotoxicity and patients without cardiotoxicity. Fisher's exact test and chi squared test were used to analyze differences in numeric variables between the two groups. Chi squared test for trend were used to analyze differences for ordinal variables. Possible risk factors for cardiotoxicity were tested using univariate binomial logistic regression and adjusted for age with multivariate binomial logistic regression. In order to test the robustness of the data and due to missing data, sensitivity analyses were performed for variables that tended to be significant or were significant in univariate analyses. Sensitivity analyses were performed both with multiple imputation and as worst- and best case scenarios for one variable at a time. Due to the low number of events (=22), the potential risk of over fitting of the statistical models and the risk of collinearity among the covariates, we only adjusted for age in the multivariate logistic regression analyses. C statistics were performed for variables that were significant in univariate logistic regression analyses. P-values below 0.05 were regarded significant. IBM SPSS software version 21 was used for all analysis.

RESULTS

Study population

A total of 452 consecutive women with metastatic breast cancer were eligible for analysis (Figure 1). Patient characteristics are listed in table 1. Median age was 63 years, 333 patients initially (74%) received 100% dose, while 84 patients (19%) were treated with 75% dose and 26 patients (6%) were treated with 50% dose, respectively. Totally, 242 patients (54%) had previously received treatment with anthracyclines, 54 patients (12%) were treated previously or concurrent with trastuzumab, while 132 patients (29%) had a history of left-sided breast irradiation. Radiotherapy was given approximately 3 months after the primary diagnosis of breast cancer and mean time from diagnosis to start of capecitabine was 4 years (0.9-58 years). Forty-two patients (9.3%) had cardiac

comorbidities prior to initiation of treatment. Of these 6 (1%) had a history of ischemic heart disease while 37 (8%) had other types of cardiac disease including atrial fibrillation (n = 13), supraventricular tachyarrhythmia (n = 3), pericardial exudatives (n = 3), epirubicin-induced cardiomyopathy (n = 3), atrio-ventricular blocks (n = 1), aortic valve disease (n = 1) and reduced left ventricular ejection fraction of unknown cause (n = 4).

Table 1: Patient characteristics

Characteristic	All patients (n = 452)	Patients with Cardiotoxicity (n = 22)	Patients without Cardiotoxicity (n = 430)	P-value[b]
	No. (%)	No. (%)	No. (%)	
Age (n = 452)				
Median	63	63	63	0.742
Range	28 – 88	36 – 82	28 – 88	
Capecitabine dose (n = 443)				
50%	26 (6)	1 (5)	25 (6)	0.636
75%	84 (19)	6 (27)	78 (18)	
100%	333 (74)	15 (68)	318 (74)	
IHD (incl. previous ACS) (n = 436)	6 (1)	0	6 (1)	1.000
Other cardiac diseases (n = 436)	37 (8)	7 (32)	30 (7)	0.000
ECG at treatment start[d] (n =				

161)				
Normal	132 (29)	9 (41)	123 (29)	0.454
Abnormal	29 (6)	3 (14)	26 (6)	
Hypertension (n = 442)	126 (28)	9 (41)	117 (27)	0.186
Hypercholesterolemia (n = 390)	53 (12)	7 (32)	46 (11)	0.002
Diabetes mellitus (n = 435)	21 (5)	1 (5)	20 (5)	1.000
Smoking status (n = 371)				
Current smoker	105 (23)	9 (41)	96 (22)	0.264
Former smoker	66 (15)	4 (18)	62 (14)	
Never smoked	200 (44)	5 (23)	195 (45)	
BMI (n = 382)				
Underweight (BMI <18.5)	21 (5)	3 (14)	18 (4)	0.337
Normal (BMI 18.5-24.9)	208 (46)	8 (36)	200 (47)	
Overweight (BMI 25.0-29.9)	109 (24)	9 (41)	100 (23)	
Obese (BMI > 29.9)	44 (10)	0	44 (10)	
Number of risk factors for IHD[c] (n = 452)				
0	131 (29)	3 (14)	128 (30)	0.005
1	182 (40)	8 (36)	174 (41)	
2	90 (20)	5 (23)	85 (20)	
3	32 (7)	3 (14)	29 (7)	

4	17 (4)	3 (14)	14 (3)	
5	0	0	0	
Previous treatment with anthracyclines (n = 444)	242 (54)	9 (41)	233 (54)	0.189
Previous or concurrent treatment with trastuzumab (n = 403)	54 (12)	3 (14)	51 (12)	0.752
Previous breast irradiation (n = 431)	275 (61)	8 (36)	267 (62)	0.052
Left side	132 (29)	5 (23)	127 (30)	0.218
Right side	115 (25)	1 (5)	114 (27)	
Bilateral	25 (6)	2 (9)	23 (5)	
Side unknown	3 (1)	0	3 (1)	
Previous thoracic irradiation (n = 407)	96 (21)	4 (18)	92 (21)	1.000
Anemia[a] (n = 437)	126 (28)	3 (14)	123 (29)	0.164
eGFR (n = 429)				
Low (< 60 mL/min/1.73m^2)	71 (17)	6 (27)	65 (15)	0.105
Normal (≥ 60 mL/min/1.73m^2)	358 (79)	13 (59)	345 (80)	

[a]defined according to the World Health Organization: Hgb < 7.4 mmol/L for non-pregnant females.

[b]P-value for the statistical tests of differences between patients with cardiotoxicity and patients without cardiotoxicity.

[c]Risk factors for IHD includes: hypertension, hypercholesterolemia, diabetes mellitus, smoking, and BMI.

[d]All patients do routinely have ECG taken before treatment start and all ECGs are routinely seen by doctors, but not all ECGs were available in the medical records.

ACS = acute coronary syndrome; BMI = body mass index; ECG = electrocardiogram; eGFR = estimated glomerular filtration rate; IHD = ischemic heart disease.

Cardiotoxicity

Twenty-two cases of cardiotoxicity (4.9%) (95% Confidence interval (CI): 2.91%-6.89%) were identified from medical records (Supplementary table). The most common complaints were chest pain (13 patients) followed by dyspnea (9 patients) and palpitations (2 patients) (Figure 2). Eleven of these 22 patients (50%) had changes on ECG. Five patients had atrial fibrillation (1 paroxysmal and 4 new onset), while three patients had ST deviations and two patients developed negative or fluctuating T-waves. Of the 13 patients with chest pain two (0.4%) had elevated troponins and were classified as acute myocardial infarctions. One patient (0.2%) with dyspnea and progressing chestpain developed cardiac arrest with lethal outcome.

First occurrence of cardiotoxicity was in first cycle for 11 patients (50%), second cycle for four patients (18%), third cycle for 3 patients (14%) and fourth cycle for 1 patient (4.5%), while three patients (14%) had late occurrence of cardiotoxicity (8^{th}, 9^{th} and 12^{th} cycle).

Cardiac therapy was initiated in 10 of the 22 patients with cardiotoxicity (Supplementary table), while 6 were retreated with capecitabine. Three patients were retreated at the same dose intensity without initiation of cardiac therapy, two were retreated at the same dose intensity, but received cardiac therapy with verapamil, and one patient were treated at reduced dose. The three patients treated with full dose and no initiation of cardiac therapy all had recurrent symptoms at retreatment, while one patient treated with verapamil had recurrent symptoms. The other two patients, one

treated with verapamil and one treated at reduced dose intensity, did not have symptoms at retreatment.

Seven events of cardiotoxicity occurred in the sub-group of patients with cardiac co-morbidity (n = 42) (16.7 %), while 15 patients in the sub-group of patients with apparently no cardiac co-morbidity (n = 410) developed cardiotoxicity (3.7 %).

Risk factors for cardiotoxicity

In univariate logistic regression analyses cardiac comorbidity (p = 0.001), hypercholesterolemia (p = 0.005) and current smoking (p = 0.023) were risk factors for cardiotoxicity (Table 2) and they remained significant after adjustment for age with multivariate logistic regression. Patients with cardiac co-morbidity were 5.5 times (95% CI: 2.0-14.8) more likely to develop cardiotoxicity than patients without cardiac co-morbidity. In the subgroup of patients with apparently no cardiac co-morbidity hypercholesterolemia (p = 0.035) and current smoking (p = 0.020) were significant risk factors in univariate analyses and remained significant after adjustment for age (Table 2).

Sensitivity analyses were performed for variables significant in the univariate analyses. In the entire study group, hypercholesterolemia, cardiac co-morbidity and current smoking remained significant risk factors after multiple imputation of missing values (p = 0.008, p = 0.001 and p = 0.023, respectively) and after imputation with worst- and best-case scenarios (hypercholesterolemia p = 0.005 (best-case), p = 0.032 (worst-case); cardiac co-morbidity p = 0.007 (worst-case), p = 0.001 (best-case); current smoking p = 0.045 (worst-case), p = 0.032 (best-case)). In the subgroup of patients with apparently no cardiac co-morbidity current smoking remained significant after both multiple imputation analyses (p = 0.021) and worst- and best-case analyses (p = 0.025 and p = 0.042, respectively), while hypercholesterolemia was unaffected by multiple imputation (p = 0.043) but susceptible to worst- and best-case analyses. The p-value for the univariate regression analysis was significant (p = 0.035) when cases with missing data were imputed with best case scenario

(none having hypercholesterolemia), but became insignificant (p = 0.132) in worst case scenario (all having hypercholesterolemia).

Table 2: Univariate and bivariate logistic regression models of risk factors for cardiotoxicity. Bold P-values indicate a significant difference (P < 0.05).

Variable	Beta coefficient (95%-CI)	Odds ratio (95%-CI)	P-value	Beta coefficient (95%-CI)	Odds ratio (95%-CI)	P-value
	Univariate			Bivariate: Adjusted for age		
All patients (N = 452) (events = 22)						
Age[a]	0.01 (-0.03-0.04)	1.01 (0.97-1.05)	0.789	-	-	-
Hypercholesterolemia[b]	1.42 (0.43-2.40)	4.12 (1.54-11.00)	**0.005**	1.42 (0.38-2.46)	4.14 (1.46-11.75)	**0.008**
Hypertension[b]	0.58 (-0.29-1.46)	1.79 (0.75-4.31)	0.192	0.60 (-0.33-1.52)	1.82 (0.72-4.57)	0.206
Diabetes[b]	-0.07 (-2.12-1.99)	0.94 (0.12-7.32)	0.949	-0.11 (-2.17-1.96)	0.90 (0.11-7.13)	0.921
Smoking[b]						
Current smoker	1.30 (0.17-2.42)	3.66 (1.19-11.21)	**0.023**	1.30 (0.18-2.42)	3.66 (1.19-11.22)	**0.023**
Former smoker	0.92 (-0.42-2.27)	2.52 (0.66-9.66)	0.179	0.92 (-0.43-2.27)	2.51 (0.65-9.67)	0.181

BMI[a]	-0.05 (-0.16-0.06)	0.95 (0.85-1.06)	0.380	-0.05 (-0.16-0.07)	0.96 (0.85-1.07)	0.413
Previous irradiation of left breast[b]	-0.14 (-1.05-0.78)	0.87 (0.35-2.18)	0.763	-0.13 (-1.05-0.80)	0.88 (0.35-2.22)	0.785
"Cardiac co-morbidity"[b]	1.63 (0.67-2.59)	5.12 (1.96-13.39)	**0.001**	1.70 (0.70-2.69)	5.47 (2.02-14.80)	**0.001**
Anemia[b]	-0.86 (-2.12-0.39)	0.42 (0.12-1.47)	0.176	-0.86 (-2.12-0.39)	0.42 (0.12-1.47)	0.176
eGFR[a]	-0.01 (-0.03-0.02)	0.99 (0.97-1.02)	0.487	-0.01 (-0.03-0.02)	0.99 (0.97-1.02)	0.638
Previous anthracyclines[b]	-0.58 (-1.43-0.29)	0.56 (0.24-1.34)	0.194	-0.73 (-1.77-0.31)	0.48 (0.17-1.36)	0.171
Previous or concurrent trastuzumab[b]	0.08 (-1.17-1.34)	1.08 (0.31-3.80)	0.903	0.11 (-1.17-1.41)	1.12 (0.31-4.09)	0.864
Dose of capecitabine[a]	-0.01 (-0.03-0.02)	1.00 (0.97-1.02)	0.666	-0.01 (-0.03-0.02)	1.00 (0.97-1.02)	0.682
Previous thoracic irradiation[b]	0.08 (-1.08-1.24)	1.08 (0.34-3.44)	0.892	0.12 (-1.05-1.28)	1.12 (0.35-3.60)	0.847
Patients with no history of heart disease (n = 399) (events = 15)						
Age[a]	0.001 (-0.04-	1.00 (0.96-	0.94	-	-	-

	0.05)	1.05)	9			
Hypercholesterolemia[b]	1.32 (0.01-2.55)	3.75 (1.10-12.81)	**0.035**	1.34 (0.04-2.63)	3.81 (1.04-13.94)	**0.043**
Hypertension[b]	0.05 (-1.11-1.22)	1.05 (0.33-3.38)	0.929	0.04 (-1.17-1.25)	1.04 (0.31-3.51)	0.947
Diabetes[b]	0.48 (-1.61-2.57)	1.62 (0.20-13.10)	0.651	0.48 (-1.63-2.58)	1.61 (0.20-13.26)	0.658
Smoking[b]						
Current smoker	1.64 (0.26-3.01)	5.13 (1.30-20.33)	**0.020**	1.63 (0.25-3.00)	5.09 (1.28-20.18)	**0.021**
Former smoker	0.18 (-2.10-2.46)	1.20 (0.12-11.76)	0.876	0.19 (-2.10-2.47)	1.21 (0.12-11.85)	0.872
BMI[a]	-0.04 (-0.18-0.09)	0.96 (0.84-1.09)	0.530	-0.04 (-0.17-0.10)	0.96 (0.84-1.10)	0.578
Previous irradiation of left breast[b]	-0.41 (-1.56-0.76)	0.67 (0.21-2.13)	0.495	-0.41 (-1.56-0.77)	0.67 (0.21-2.15)	0.497
Anemia[b]	-1.62 (-3.51-0.43)	0.20 (0.03-1.54)	0.121	-1.62 (-3.51-0.43)	0.20 (0.03-1.54)	0.121
eGFR[a]	0.00 (-0.03-0.03)	1.00 (0.97-1.03)	0.876	0.01 (-0.03-0.05)	1.01 (0.97-1.05)	0.668
Previous anthracyclines[b]	0.67 (-0.39-1.72)	1.96 (0.68-5.60)	0.212	0.92 (-0.33-2.18)	2.52 (0.72-8.86)	0.150

Previous or concurrent trastuzumab[b]	0.42 (-0.87-1.72)	1.53 (0.42-5.60)	0.525	0.48 (-0.87-1.83)	1.62 (0.42-6.26)	0.484
Dose of capecitabine[a]	-0.01 (-0.04-0.02)	0.99 (0.96-1.02)	0.573	-0.01 (-0.04-0.02)	0.99 (0.96-1.02)	0.579
Previous thoracic irradiation[b]	0.35 (-1.02-1.72)	1.42 (0.36-5.59)	0.621	0.40 (-0.99-1.79)	1.48 (0.37-5.96)	0.577

[a]scale variable. [b]categorical variable. 95%-CI = 95% confidence interval; BMI = body mass index; "Cardiac co-morbidity" = all types of heart disease; eGFR = estimated glomerular filtration rate.

C Statistics: Predictors of cardiotoxicity

The ability of the variables, cardiac co-morbidity, hypercholesterolemia and current smoking, to discriminate between patients that will develop cardiotoxicity and those who will not was tested with c-statistics. In unselected patients the presence of cardiac co-morbidity was a poor predictor of cardiotoxicity (c = 0,617 (95%CI 0.483-0.754), p = 0.061), as was the presence of hypercholesterolemia (c = 0.662 (95%CI 0.478-0.766), p = 0.072) and smoking status (c = 0.651 (95%CI 0.523-0.779), p = 0.031). In the subgroup of patients with apparently no cardiac co-morbidity the presence of hypercholesterolemia (c = 0.601 (95%CI 0.427-0.775), p = 0.216) and smoking status (c = 0.691 (95%CI 0.526-0.857), p = 0.031) were poor factors to discriminate between patients who will develop cardiotoxicity and those who will not.

DISCUSSION

Incidence of cardiotoxicity

We observed an incidence of cardiotoxicity from capecitabine at ≈ 5% in women with metastatic breast cancer which is similar to incidences reported in previous studies of both men and women treated with capecitabine,[18-20] although some studies have reported lower[16] or higher incidences.[17] The difference in incidences in-between studies may be due to different risk profiles in the study populations. Moreover, prospective studies with regular cardiac assessments may detect more cardiotoxicity since they may identify asymptomatic patients and patients with mild symptoms. The incidence of cardiotoxicity in our study and other studies of capecitabine cardiotoxicity is within the range of incidences of cardiotoxicity in studies with 5-FU[15, 17, 20-22] and a large study found similar incidences for the two treatments.[20] However, a prospective study with 644 patients reported similar incidences of cardiotoxicity for capecitabine and continuous 5-FU infusion schedules, but a lower incidence for short (bolus) 5-FU infusion schedules.[18]

Pattern of cardiotoxicity

The most common event of cardiotoxicity was chest pain, which was the main symptom in 59% of the 22 cases. Most of the patients with chest pain had normal ECG and normal coronary enzymes and severe events, such as acute myocardial infarction and cardiac arrest with lethal outcome, was rare. This pattern is in concordance with other studies[15] and the sudden onset of chest pain and the rare occurrence of life-threatening complications correspond well to the theory of fluoropyrimidine induced vasospastic angina. Furthermore, the angiographically normal arteries reported in several case reports[6-8, 11] and the presence of silent ischemic episodes on Holter recordings[23] supports this theory.

Retreatment of patients with cardiotoxicity

Retreatment with capecitabine after occurrence of cardiotoxicity was attempted in 6 of 22 patients with little success. Four patients had recurrent cardiac symptoms, suggesting that retreatment should

be done with great precaution. However, a retrospective study of 668 patients[16] and a prospective study of 644 patients[18] treated with 5-FU or capecitabine reported a benefit from dose-reduction and initiation of anti-angina therapy that prevented symptoms at retreatment in 9 of 12 patients and 12 of 15 patients, respectively. Their findings suggest that retreatment at reduced dose and with appropriate anti-angina therapy are feasible, however close cardiac monitoring is crucial. Furthermore, nitroglycerin was effective to abolish symptoms of cardiotoxicity.[16] In contrast, a small and non-randomized study could not demonstrate an prophylactic effect of calcium channel blockers on occurrence of cardiotoxicity.[24] Larger studies with systematic, predefined strategies for dose reduction and initiation of anti-angina therapy are needed.

Risk factors for cardiotoxicity

Patients with cardiac comorbidity were at increased risk of cardiotoxicity, which is in accordance with four previous studies of 5-FU or capecitabine cardiotoxicity,[17, 21, 23, 25] while four other studies found no increased risk for patients with pre-existing heart disease.[19, 26-28] While heart disease may be a risk marker for cardiotoxicity, it is not a prerequisite for cardiotoxicity to occur. The patient who sustained cardiac arrest and died in our study had no cardiac co-morbidities and no risk factors for ischemic heart disease. Likewise, severe cardiotoxicity has been reported for several patients without cardiac co-morbidity.[11, 29-32] Also, we found that a history of heart disease was a poor predictor of cardiotoxicity.

In the present study hypercholesterolemia and current smoking were risk factors for cardiotoxicity both in the entire study group and in the subgroup of patients with no apparent cardiac co-morbidity. Similarly, a larger prospective study of 644 patients without cardiac co-morbidities reported that smoking was associated with ECG changes in bivariate analyses.[18] However, they found no association between ECG changes and hyperlipidaemia.[18] While Kosmas et al.[18] prospectively measured cholesterol and triglyceride levels in blood; our study is based on medical

recordings. Thus incomplete data with risk of information bias is a limitation of the present study. Both studies have a low number of events limiting the statistical power and multiple testing increases the risk of false positive results.

Previous treatment with potentially cardiotoxic therapy with anthracyclines or previous or current treatment with trastuzumab did not increase the risk of cardiotoxicity. However, two of three patients with epirubicin-induced cardiomyopathy developed cardiotoxicity during treatment with capecitabine suggesting that the degree of pre-existing heart damage may be relevant. Patients with previous left-sided breast irradiation were not at increased risk of cardiotoxicity in our study. Similarly, a large prospective study of 5-FU cardiotoxicity found that previous breast irradiation was not a risk factor.[21] The lack of association between other cardiotoxic therapies and capecitabine-induced cardiotoxicity suggests that the mechanisms behind these cardiotoxicities are different. Moreover, radiation-induced cardiovascular disease may be a late event.[33] In our study the mean time from chest irradiation to capecitabine start was 4 years.

Methodological considerations

The predominant manifestation of cardiotoxicity from fluoropyrimidines is chest pain followed by other subjective symptoms. Only approximately 50% of the patients have objective signs (mostly ECG changes) of cardiotoxicity. Thus, the event (cardiotoxicity) is based mainly on subjective symptoms and is therefore affected by the patient's own perception and reaction to the symptoms and the physician's assessment of the patient's symptoms. Both patients and physicians may pay more attention to cardiac symptoms if the patient has heart disease before treatment start. These factors may explain some of the difference in incidence in-between studies. Research in new and sensitive cardiac biomarkers like e.g. copeptin to detect myocardial ischemia is needed.[34]

In spite of a relatively large sample size, the number of events is low (22 events) which cause statistical difficulties. Due to the limited number of events, the statistical power is low and the risk

of type II statistical errors increases. With respect to the logistic regression analyses, the low number of events limits the number of covariates allowed in the model. The low number of events and multiple testing (increasing the risk of false positive results) are weaknesses of most studies analysing risk factors and it makes the conclusions that can be drawn from these studies less valid. Like most other large studies on this subject our study is retrospective which may results in incomplete data. Missing data affects power and if not missing at random, they may cause bias. We dealt with the missing data by sensitivity analyses with worst and best case scenarios and with multiple imputation. The sensitivity analyses showed that missing data had little influence on our study results.

A major limitation is that baseline ECGs was only preserved for 36% of the patients. Among the 11 patients with ECG changes during capecitabine treatment, 2 had missing baseline ECGs.

Conclusion

The incidence of cardiotoxicity from capecitabine of ≈ 5% is close to that of 5-FU with incidences of 1%-5% in larger studies. Our study results suggest that cardiac co-morbidity and current smoking are risk factors for cardiotoxicity. Whether smoking cessation can prevent some cases of cardiotoxicity deserves further investigations. All the identified risk factors were poor predictors of cardiotoxicity and initial optimal cardiac treatment, information about the risk and follow-up if symptoms present are the options today.

Contributors:

AP: Data collection, data processing, statistics, writing of the paper and incorporation of input from the other authors. NS and KV: Conception and design of the study, data collection and review of the manuscript. MVN: Review of cases with suspected cardiotoxicity and review of the manuscript.

FOL: Conception and design of the study and review of the manuscript. MS: Interpretation of study results and statistics and review of the manuscript. DN: Conception and design of the study, data collection, data interpretation, input to the manuscript and review of the manuscript.

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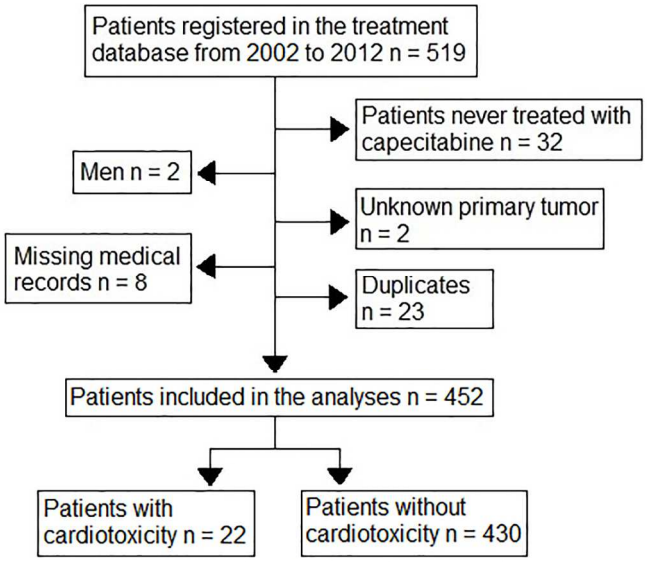

Figure 1: Flow diagram

179x135mm (300 x 300 DPI)

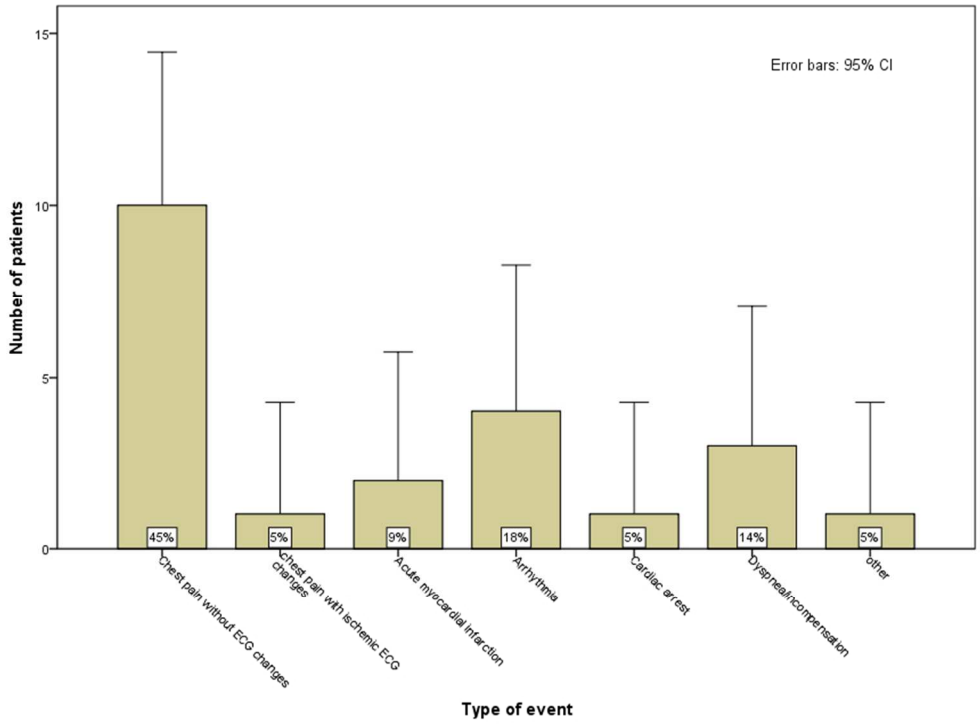

Figure 2: The distribution of the different manifestations of cardiotoxicity
Explaining text: Proportion of patients with chest pain without ECG changes (n = 10), chest pain with ECG changes but not acute myocardial infarction (n = 1), acute myocardial infarction (n = 2), arrhythmia (n = 4), cardiac arrest (n = 1), dyspnea/incompensation (n = 3) and other (n = 1, with QT prolongation and right bundle branch block). Patients with more than one of the manifestations (n = 2) are classified according to their primary complaint. The percentages showed on each bar reflect the distribution of symptoms among the 22 patients with cardiotoxicity.

232x186mm (96 x 96 DPI)

Supplementary table: Details about patients with cardiotoxicity and initiated therapy

Case	Location of metastases	Pre-existing cardiovascular disease	Cycle of onset	Dose intensity at onset	Type of event	Description	ECG changes	Raised cardiac biomarkers?	Cardiac therapy	Subsequent treatment with capecitabine, dose	Symptoms at retreatment
1	Liver, lung, abdomen	No	1	100%	Chest pain	Oppressive chest pain	-	-	No	Yes, 100%	Yes, oppressive chest pain
2	Right lung, pleura, mediastinal lymph nodes, adrenal glands, abdominal carcinosis, columna and pelvis	Atrial fibrillation, NYHA 3, moderate pulmonary hypertension, LVEF = 53% after 1. cycle, hypertension	2	75%	Incompensation, dyspnea	Dyspnea and peripheral edema	ST-depressions in V5-V6	-	-	No	-
3	Liver	No	1	100%	Chest pain	Exertional chest pain radiating to the neck	No changes from pretreatment ECG (inverted T-waves in V1-V2)	-	-	No	-
4	Liver, bone Th3 + Th7, lymph nodes in left axilla	No	2	75%	Cardiac arrest	Chest pain and progressing dyspnea, cardiac arrest, death	Asystole	-	Adrenalin and amiodaron	-	-
5	Mediastinal lymph nodes,	Hypertension, hyperlipidem	1	75%	QTc prolongation, right	Asymptomatic	QTc prolongation, right	-	-	No	-

	thyroid gland, bone Th1 + Th5	ia			bundle branch block		bundle branch block				
6	Neck, sternal and left axillary lymph nodes	No	1	100%	Chest pain	Intermittent, retrosternal, oppressive chest pain radiating to both arms, LVEF 60% at echocardiography	Normal	No	No	No	-
7	Mediastinal lymph nodes	No	4	75%	Chest pain	Chest pain, dyspnea and malaise	Normal	No	No	No	-
8	Pleura, bone	No	1	100%	Chest pain	Compressive chest pain	No changes, ST-depression in V2-V4	-	Metoprolol and Isosorbidm ononitrate	No	-
9	Pleura	No	1	100%	Arrhythmia	Dyspnea	Atrial fibrillation/ atrial flutter, 170 bpm.	-	Adenosine, metoprolol and amiodaron	No	-
10	Liver, lung, bone	No	2	75%	Chest pain, Arrhythmia	Chest pain and palpitations	Atrial fibrillation, 119 bpm	-	Verapamil	Yes, 75%	Palpitations, dyspnea, atrial fibrillation
11	Liver, lungs, neck and right supraclavicular lymph nodes	SVT, previous pulmonary embolism, hyperlipidemia	1	100%	AMI	Oppressive chest pain radiating to right side, dyspnea. Effect of nitroglycerin iv. Normal coronary angiography and normal	Initially ST-elevation in V1-V3, AVF and V6. Negative T-waves in	Marginal raised TnI (52 ng/l) and raised CK-MB (4.4 µg/l)	Nitroglycerin iv, Acetylsalicylic Acid and Magnesium Hydroxide, clopidogrel,	No	-

						echocardiography.	lead II-III		fondaparinux		
12	Lungs, mediastinal lymph nodes	Hypertension, diabetes, hyperlipidemia	3	50%	Dyspnea	Dyspnea and light chest pain	Normal	No	No	Yes, 50%	No
13	Bone	Epirubicin-induced cardiomyopathy	9	100%	Chest pain, arrhythmia	Compressive chest pain	Atrial fibrillation and non-sustained VT	-	-	No	-
14	Bone, skin, liver and left axillary lymph nodes	Hypertension, NYHA 2-3 Echo: LVEF 50-55%, mitral valve insufficiency, discrete pulmonary, hypertension	3	75%	Incompensatio, dyspnea	Progressing dyspnea, NYHA 3, peripheral edema, cardiomegaly Echo: LVEF unchanged, moderate to severe pulmonary hypertension, tricuspid insufficiency	Bundle branch block	-	Furosemide and potassium-chloride	No	-
15	Neck and infraclavicular lymph nodes	Atrial fibrillation, COPD	3	100%	Arrhythmia, pulmonary congestion	Dyspnea, dizziness, malaise X-ray: pulmonary congestion and pericardial exudate	Atrial fibrillation 117 bpm	-	Digoxin, Centyl Mite with potassium-chloride	No	-
16	Brain	No	8	100%	Chest pain	Oppressive chest pain radiating to the neck	Normal	-	-	Yes, 100%	Recurrent episodes with chestpain

17	Mediastinal lymph nodes	No	2	100%	Chest pain	Oppressive chestpain radiating to neck and arm, dyspnea	Sinus rhythm, supraventricular extra systoles, no ST-deviations	No	Verapamil	Yes, 75%	No
18	Sternum and sacroiliac bone	Hyperlipidemia	1	100%	Chest pain	Compressive chest pain radiating to the back and both arms. Echo: normal	Normal	-	No	No	-
19	CNS, liver, right axillary lymph nodes	Hypertension, hyperlipidemia	12	75%	AMI	Chest pain radiating to head, neck and jaw. LVEF = 40 %.	Normal	Raised troponins (400 ng/l), CK-MB unknown	Acetylsalicylic Acid with magnesium hydroxide (magnyl), clopidogrel, fondaparinux, metoprolol, ACE-inhibitor, Statin	No	-
20	Pelvis bone, right axillary lymph nodes	Hypertension, hyperlipidemia	1	100%	Chest pain	Oppressive chest pain Echo: normal	-	-	-	No	-
21	Lung, bone	Epirubicin-induced cardiomyopathy, LVEF = 34-40%	1	100%	Arrhythmia	Dizziness and palpitations.	Telemetry: Atrial flutter 150 bpm, neg. T-waves in I and AVL	-	Metoprolol, ACE-inhibitor	No	-

22	Pelvic and columnar bone	Atrial fibrillation, hypertension, hyperlipidemia	1	100%	Chest pain	Chest pain	Fluctuating negative T-waves in lead II, III, aVF and V1-V5	-	No	Yes, 100%	Episodes with pain located to jaw and chest

COPD = chronic obstructive pulmonary disease, bpm = beats per minute, NYHA = New York Heart association functional classification, LVEF = left ventricular function, TnI = cardiac troponin I, SVT = supraventricular tachycardia, LMWH = low molecule weight heparin, CK-MB = creatine kinase MB, CNS = central nerve system

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Not relevant
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5
Bias	9	Describe any efforts to address potential sources of bias	-
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	6
		(*d*) If applicable, explain how loss to follow-up was addressed	Not applicable
		(*e*) Describe any sensitivity analyses	6
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6 + Figure 1
		(b) Give reasons for non-participation at each stage	Figure 1
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6-7
		(b) Indicate number of participants with missing data for each variable of interest	Table 1
		(c) Summarise follow-up time (eg, average and total amount)	-
Outcome data	15*	Report numbers of outcome events or summary measures over time	10
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10
		(*b*) Report category boundaries when continuous variables were categorized	Not applicable
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11
Discussion			
Key results	18	Summarise key results with reference to study objectives	16-18
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16-19
Generalisability	21	Discuss the generalisability (external validity) of the study results	16-19
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	20

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Incidence and risk factors for capecitabine-induced symptomatic cardiotoxicity: a retrospective study of 452 consecutive patients with metastatic breast cancer

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Incidence and risk factors for capecitabine-induced symptomatic cardiotoxicity: a retrospective study of 452 consecutive patients with metastatic breast cancer

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Word count: 3840 incl. tables and statements but excl. references

ABSTRACT

Objectives: Case-reports of capecitabine cardiotoxicity resemble those seen with intravenous 5-fluorouracil with chest pain as the predominant manifestation, but few studies of capecitabine cardiotoxixcity are available. We aimed to determine the incidence of symptomatic cardiotoxicity from capecitabine in breast cancer patients and to identify risk factors.

Methods: We reviewed medical records of consecutive women with breast cancer treated with capecitabine (1000 mg/m^2 twice daily) from 2002 to 2012 at one institution.

Results: Twenty-two of 452 patients (4.9%) (95%CI: 2.9%-6.9%) had symptoms of cardiotoxicity (chest pain: n = 13, dyspnea: n = 9, palpitations: n = 2). Eleven patients had changes on ECG (atrial fibrillation: n = 5, ST deviations: n = 3, T-wave abnormalities: n= 2, and QTc prolongation: n = 1). Two patients (0.4%) sustained acute myocardial infarction. One patient (0.2%) developed cardiac arrest with lethal outcome. Four of six patients (66%) retreated with capecitabine had recurrent symptoms at retreatment. Cardiac comorbidity (p = 0.001), hypercholesterolemia (p = 0.005) and current smoking (p = 0.023) were risk factors for cardiotoxicity in univariate analyses and remained significant when adjusted for age. Patients with cardiac co-morbidity were 5.5 times (95% CI: 2.0-14.8) more likely to develop cardiotoxicity. In the subgroup of patients with apparently no cardiac co-morbidity the incidence of cardiotoxicity was lower (3.7 %) and hypercholesterolemia (p = 0.035) and current smoking (p = 0.020) were risk factors of cardiotoxicity.

Conclusion: The incidence of cardiotoxicity from capecitabine resembles that of intravenous 5-fluorouracil (≈5 %). Cardiac co-morbidity, hypercholesterolemia and current smoking were associated with development of cardiotoxicity.

Strengths and limitations of this study

- Our study is a large single center study including all patients with breast cancer treated with capecitabine over a 10-year period.
- The primary end-point, cardiotoxicity, is mainly diagnosed on the basis of subjective symptoms (chest pain, dyspnea, palpitations) which may cause information bias.
- In spite of a relatively large sample size, the number of events is low which limit the power of the logistic regression analyses used to analyse for risk factors.
- Our study is a retrospective clinical study. The patients' history records are incomplete with respect to information on risk factors for cardiovascular disease (hypertension, hypercholesterolemia, diabetes and smoking) and baseline electrocardiograms.

INTRODUCTION

Capecitabine is an oral pro-drug of 5-fluorouracil (5-FU) that is converted to 5-FU in a three-stage process involving several enzymes.[1] The last step is catalyzed by thymidine phosphorylase.[1] Many tissues throughout the body express thymidine phosphorylase, but some human carcinomas express this enzyme in higher concentrations than surrounding normal tissues.[1] This, in theory should increase the concentration of 5-FU at the tumour site and decrease the concentration of 5-FU in healthy tissues resulting in less side effects.[1]

Capecitabine is licensed for adjuvant treatment in patients with colon cancer stage III and for the treatment of metastatic colorectal cancer, metastatic breast cancer and advanced gastric cancer (combination therapy). The main side effects from capecitabine are hand-and-foot syndrome, diarrhea, stomatitis, fatigue, anorexia, nausea and vomiting, abdominal pain, myelosuppression, hyperbilirubinemia and cardiotoxicity.[2-5]

Case reports of cardiotoxicity after administration of capecitabine are similar to those seen with intravenous 5-FU treatment with chest pain as the predominant manifestatio.[6-14] Other less frequent clinical manifestations are arrhythmias, myocardial infarction, heart failure, cardiogenic shock and sudden death.[15-19] Cardiotoxicity from 5-FU occurs with an incidence of 0.55% to 19.9%.[15] There are few studies of capecitabine cardiotoxicity with incidences ranging from 3% to 35%.[20-24]

We aimed to study the pattern and incidence of symptomatic cardiotoxicity in women with metastatic breast cancer treated with capecitabine and to identify potential risk factors for capecitabine-induced cardiotoxicity.

MATERIAL AND METHODS

Selection of patients

We included patients with metastatic breast cancer consecutively treated with capecitabine from first of January 2002 to 31. December 2012. Inclusion criteria were female, metastatic breast cancer and capecitabine treatment (+/- trastuzumab). The chemotherapy regimen was capecitabine 2000 mg/m² divided in two daily doses for 14 days followed by one week off.

Data collection

Approval from the Danish Data Protection Agency was obtained. We collected data from medical records on age, height, weight, body surface area, capecitabine dose, cardiotoxicity, cardiac comorbidity (a history of previous acute myocardial infarction, ischemic heart disease, arrhythmias, heart failure or reduced ejection fraction), risk factors for ischemic heart disease (a history of hypertension or intake of antihypertensive drugs, smoking status, a history of hypercholesterolemia or intake of lipid lowering drugs, a history of diabetes or intake of antidiabetics), electrocardiogram (ECG) (before treatment start and if symptoms), hemoglobin levels, creatinine levels and previous treatment with anthracyclines, trastuzumab, breast- and thoracic radiotherapy. Renal function (estimated glomerular filtrationrate, eGFR) was estimated by the Chronic Kidney Disease Epidemiology Collaboration equation for Caucasian women incorporating age and plasma creatinine concentrations. All the collected data were prospectively selected.

Registration of symptoms and identification of cases with cardiotoxicity

Before start of chemotherapy and before each cycle adverse events were scored according to NCI/CTCAE V. 3.0. ECGs were performed before first cycle of capecitabine and at clinical suspicion of cardiotoxicity. Medical records were reviewed by one of the authors. Cardiotoxicity was defined as significant symptoms of likely cardiac origin (chest pain or acute myocardial infarction (confirmed with elevation in troponins over the cut-off point), palpitations, dyspnea of likely cardiac origin and incompensation) and/or changes on ECG that started during treatment with

capecitabine and was not present before treatment start. Cases with suspected cardiotoxicity were further reviewed by one cardiologist (MVN) who made the final decision to classify the case as cardiotoxicity or not. All patients were followed to cessation of capecitabine. Endpoints were evaluated unblinded and the investigators had access to patients' medical record.

Statistics

Mann-Whitney U test was used to compare differences in age between patients with cardiotoxicity and patients without cardiotoxicity. Fisher's exact test and chi squared test were used to analyze differences in numeric variables between the two groups. Chi squared test for trend were used to analyze differences for ordinal variables. Possible risk factors for cardiotoxicity were tested using univariate binomial logistic regression and adjusted for age with multivariate binomial logistic regression. In order to test the robustness of the data and due to missing data, sensitivity analyses were performed for variables that tended to be significant or were significant in univariate analyses. Sensitivity analyses were performed both with multiple imputation and as worst- and best case scenarios for one variable at a time. Due to the low number of events (=22), the potential risk of over fitting of the statistical models and the risk of collinearity among the covariates, we only adjusted for age in the multivariate logistic regression analyses. C statistics were performed for variables that were significant in univariate logistic regression analyses. P-values below 0.05 were regarded significant. IBM SPSS software version 21 was used for all analysis.

RESULTS

Study population

A total of 452 consecutive women with metastatic breast cancer were eligible for analysis (Figure 1). Patient characteristics are listed in table 1. Median age was 63 years, 333 patients initially (74%) received 100% dose, while 84 patients (19%) were treated with 75% dose and 26 patients (6%)

were treated with 50% dose, respectively. Totally, 242 patients (54%) had previously received treatment with anthracyclines, 54 patients (12%) were treated previously or concurrent with trastuzumab, while 132 patients (29%) had a history of left-sided breast irradiation. Radiotherapy was given approximately 3 months after the primary diagnosis of breast cancer and mean time from diagnosis to start of capecitabine was 4 years (0.9-58 years). Forty-two patients (9.3%) had cardiac comorbidities prior to initiation of treatment. Of these 6 (1%) had a history of ischemic heart disease while 37 (8%) had other types of cardiac disease including atrial fibrillation (n = 13), supraventricular tachyarrhythmia (n = 3), pericardial exudatives (n = 3), epirubicin-induced cardiomyopathy (n = 3), atrio-ventricular blocks (n = 1), aortic valve disease (n = 1) and reduced left ventricular ejection fraction of unknown cause (n = 4).

Table 1: Patient characteristics

Characteristic	All patients (n = 452)	Patients with Cardiotoxicity (n = 22)	Patients without Cardiotoxicity (n = 430)	P-value[b]
	No. (%)	No. (%)	No. (%)	
Age (n = 452)				
Median	63	63	63	0.742
Range	28 – 88	36 – 82	28 – 88	
Capecitabine dose (n = 443)				
50%	26 (6)	1 (5)	25 (6)	0.636
75%	84 (19)	6 (27)	78 (18)	
100%	333 (74)	15 (68)	318 (74)	

IHD (incl. previous ACS) (n = 436)	6 (1)	0	6 (1)	1.000
Other cardiac diseases (n = 436)	37 (8)	7 (32)	30 (7)	0.000
ECG at treatment start[d] (n = 161)				
Normal	132 (29)	9 (41)	123 (29)	0.454
Abnormal	29 (6)	3 (14)	26 (6)	
Hypertension (n = 442)	126 (28)	9 (41)	117 (27)	0.186
Hypercholesterolemia (n = 390)	53 (12)	7 (32)	46 (11)	0.002
Diabetes mellitus (n = 435)	21 (5)	1 (5)	20 (5)	1.000
Smoking status (n = 371)				
Current smoker	105 (23)	9 (41)	96 (22)	0.264
Former smoker	66 (15)	4 (18)	62 (14)	
Never smoked	200 (44)	5 (23)	195 (45)	
BMI (n = 382)				
Underweight (BMI <18.5)	21 (5)	3 (14)	18 (4)	0.337
Normal (BMI 18.5-24.9)	208 (46)	8 (36)	200 (47)	
Overweight (BMI 25.0-29.9)	109 (24)	9 (41)	100 (23)	
Obese (BMI > 29.9)	44 (10)	0	44 (10)	
Number of risk factors for				

IHD[c] (n = 452)				
0	131 (29)	3 (14)	128 (30)	0.005
1	182 (40)	8 (36)	174 (41)	
2	90 (20)	5 (23)	85 (20)	
3	32 (7)	3 (14)	29 (7)	
4	17 (4)	3 (14)	14 (3)	
5	0	0	0	
Previous treatment with anthracyclines (n = 444)	242 (54)	9 (41)	233 (54)	0.189
Previous or concurrent treatment with trastuzumab (n = 403)	54 (12)	3 (14)	51 (12)	0.752
Previous breast irradiation (n = 431)	275 (61)	8 (36)	267 (62)	0.052
Left side	132 (29)	5 (23)	127 (30)	0.218
Right side	115 (25)	1 (5)	114 (27)	
Bilateral	25 (6)	2 (9)	23 (5)	
Side unknown	3 (1)	0	3 (1)	
Previous thoracic irradiation (n = 407)	96 (21)	4 (18)	92 (21)	1.000
Anemia[a] (n = 437)	126 (28)	3 (14)	123 (29)	0.164
eGFR (n = 429)				
Low (< 60 mL/min/1.73m^2)	71 (17)	6 (27)	65 (15)	0.105

Normal (≥ 60 mL/min/1.73m^2)	358 (79)	13 (59)	345 (80)	

[a]defined according to the World Health Organization: Hgb < 7.4 mmol/L for non-pregnant females.

[b]P-value for the statistical tests of differences between patients with cardiotoxicity and patients without cardiotoxicity.

[c]Risk factors for IHD includes: hypertension, hypercholesterolemia, diabetes mellitus, smoking, and BMI.

[d]All patients do routinely have ECG taken before treatment start and all ECGs are routinely seen by doctors, but not all ECGs were available in the medical records.

ACS = acute coronary syndrome; BMI = body mass index; ECG = electrocardiogram; eGFR = estimated glomerular filtration rate; IHD = ischemic heart disease.

Cardiotoxicity

Twenty-two cases of symptomatic cardiotoxicity (4.9%) (95% Confidence interval (CI): 2.91%-6.89%) were identified from medical records (Supplementary table). The most common complaints were chest pain (13 patients) followed by dyspnea (9 patients) and palpitations (2 patients) (Figure 2). Eleven of these 22 patients (50%) had changes on ECG. Five patients had atrial fibrillation (1 paroxysmal and 4 new onset), while three patients had ST deviations and two patients developed negative or fluctuating T-waves. Of the 13 patients with chest pain two (0.4%) had elevated troponins and were classified as acute myocardial infarctions. One patient (0.2%) with dyspnea and progressing chestpain developed cardiac arrest with lethal outcome.

First occurrence of cardiotoxicity was in first cycle for 11 patients (50%), second cycle for four patients (18%), third cycle for 3 patients (14%) and fourth cycle for 1 patient (4.5%), while three patients (14%) had late occurrence of cardiotoxicity (8th, 9th and 12th cycle).

Cardiac therapy was initiated in 10 of the 22 patients with cardiotoxicity (Supplementary table), while 6 were retreated with capecitabine. Three patients were retreated at the same dose intensity without initiation of cardiac therapy, two were retreated at the same dose intensity, but received cardiac therapy with verapamil, and one patient were treated at reduced dose. The three patients treated with full dose and no initiation of cardiac therapy all had recurrent symptoms at retreatment, while one patient treated with verapamil had recurrent symptoms. The other two patients, one treated with verapamil and one treated at reduced dose intensity, did not have symptoms at retreatment.

Seven events of cardiotoxicity occurred in the sub-group of patients with cardiac co-morbidity (n = 42) (16.7 %), while 15 patients in the sub-group of patients with apparently no cardiac co-morbidity (n = 410) developed cardiotoxicity (3.7 %).

Risk factors for symptomatic cardiotoxicity

In univariate logistic regression analyses cardiac comorbidity (p = 0.001), hypercholesterolemia (p = 0.005) and current smoking (p = 0.023) were risk factors for cardiotoxicity (Table 2) and they remained significant after adjustment for age with multivariate logistic regression. Patients with cardiac co-morbidity were 5.5 times (95% CI: 2.0-14.8) more likely to develop cardiotoxicity than patients without cardiac co-morbidity. In the subgroup of patients with apparently no cardiac co-morbidity hypercholesterolemia (p = 0.035) and current smoking (p = 0.020) were significant risk factors in univariate analyses and remained significant after adjustment for age (Table 2).

Sensitivity analyses were performed for variables significant in the univariate analyses. In the entire study group, hypercholesterolemia, cardiac co-morbidity and current smoking remained significant risk factors after multiple imputation of missing values (p = 0.008, p = 0.001 and p = 0.023, respectively) and after imputation with worst- and best-case scenarios (hypercholesterolemia p = 0.005 (best-case), p = 0.032 (worst-case); cardiac co-morbidity p = 0.007 (worst-case), p = 0.001

(best-case); current smoking p = 0.045 (worst-case), p = 0.032 (best-case)). In the subgroup of patients with apparently no cardiac co-morbidity current smoking remained significant after both multiple imputation analyses (p = 0.021) and worst- and best-case analyses (p = 0.025 and p = 0.042, respectively), while hypercholesterolemia was unaffected by multiple imputation (p = 0.043) but susceptible to worst- and best-case analyses. The p-value for the univariate regression analysis was significant (p = 0.035) when cases with missing data were imputed with best case scenario (none having hypercholesterolemia), but became insignificant (p = 0.132) in worst case scenario (all having hypercholesterolemia).

Table 2: Univariate and bivariate logistic regression models of risk factors for symptomatic cardiotoxicity. Bold P-values indicate a significant difference (P < 0.05).

Variable	Beta coefficient (95%-CI)	Odds ratio (95%-CI)	P-value	Beta coefficient (95%-CI)	Odds ratio (95%-CI)	P-value
	Univariate			Bivariate: Adjusted for age		
All patients (N = 452) (events = 22)						
Age[a]	0.01 (-0.03-0.04)	1.01 (0.97-1.05)	0.789	-	-	-
Hypercholesterolemia[b]	1.42 (0.43-2.40)	4.12 (1.54-11.00)	**0.005**	1.42 (0.38-2.46)	4.14 (1.46-11.75)	**0.008**
Hypertension[b]	0.58 (-0.29-1.46)	1.79 (0.75-4.31)	0.192	0.60 (-0.33-1.52)	1.82 (0.72-4.57)	0.206
Diabetes[b]	-0.07 (-2.12-	0.94 (0.12-	0.94	-0.11 (-2.17-	0.90 (0.11-	0.92

	1.99)	7.32)	9	1.96)	7.13)	1
Smoking[b] Current smoker Former smoker	1.30 (0.17-2.42) 0.92 (-0.42-2.27)	3.66 (1.19-11.21) 2.52 (0.66-9.66)	**0.023** 0.179	1.30 (0.18-2.42) 0.92 (-0.43-2.27)	3.66 (1.19-11.22) 2.51 (0.65-9.67)	**0.023** 0.181
BMI[a]	-0.05 (-0.16-0.06)	0.95 (0.85-1.06)	0.380	-0.05 (-0.16-0.07)	0.96 (0.85-1.07)	0.413
Previous irradiation of left breast[b]	-0.14 (-1.05-0.78)	0.87 (0.35-2.18)	0.763	-0.13 (-1.05-0.80)	0.88 (0.35-2.22)	0.785
"Cardiac co-morbidity"[b]	1.63 (0.67-2.59)	5.12 (1.96-13.39)	**0.001**	1.70 (0.70-2.69)	5.47 (2.02-14.80)	**0.001**
Anemia[b]	-0.86 (-2.12-0.39)	0.42 (0.12-1.47)	0.176	-0.86 (-2.12-0.39)	0.42 (0.12-1.47)	0.176
eGFR[a]	-0.01 (-0.03-0.02)	0.99 (0.97-1.02)	0.487	-0.01 (-0.03-0.02)	0.99 (0.97-1.02)	0.638
Previous anthracyclines[b]	-0.58 (-1.43-0.29)	0.56 (0.24-1.34)	0.194	-0.73 (-1.77-0.31)	0.48 (0.17-1.36)	0.171
Previous or concurrent trastuzumab[b]	0.08 (-1.17-1.34)	1.08 (0.31-3.80)	0.903	0.11 (-1.17-1.41)	1.12 (0.31-4.09)	0.864
Dose of capecitabine[a]	-0.01 (-0.03-0.02)	1.00 (0.97-1.02)	0.666	-0.01 (-0.03-0.02)	1.00 (0.97-1.02)	0.682
Previous thoracic	0.08 (-1.08-	1.08 (0.34-	0.89	0.12 (-1.05-	1.12 (0.35-	0.84

irradiation[b]	1.24)	3.44)	2	1.28)	3.60)	7
Patients with no history of heart disease (n = 399) (events = 15)						
Age[a]	0.001 (-0.04-0.05)	1.00 (0.96-1.05)	0.949	-	-	-
Hypercholesterolemia[b]	1.32 (0.01-2.55)	3.75 (1.10-12.81)	**0.035**	1.34 (0.04-2.63)	3.81 (1.04-13.94)	**0.043**
Hypertension[b]	0.05 (-1.11-1.22)	1.05 (0.33-3.38)	0.929	0.04 (-1.17-1.25)	1.04 (0.31-3.51)	0.947
Diabetes[b]	0.48 (-1.61-2.57)	1.62 (0.20-13.10)	0.651	0.48 (-1.63-2.58)	1.61 (0.20-13.26)	0.658
Smoking[b] Current smoker Former smoker	 1.64 (0.26-3.01) 0.18 (-2.10-2.46)	 5.13 (1.30-20.33) 1.20 (0.12-11.76)	 **0.020** 0.876	 1.63 (0.25-3.00) 0.19 (-2.10-2.47)	 5.09 (1.28-20.18) 1.21 (0.12-11.85)	 **0.021** 0.872
BMI[a]	-0.04 (-0.18-0.09)	0.96 (0.84-1.09)	0.530	-0.04 (-0.17-0.10)	0.96 (0.84-1.10)	0.578
Previous irradiation of left breast[b]	-0.41 (-1.56-0.76)	0.67 (0.21-2.13)	0.495	-0.41 (-1.56-0.77)	0.67 (0.21-2.15)	0.497

Anemia[b]	-1.62 (-3.51-0.43)	0.20 (0.03-1.54)	0.121	-1.62 (-3.51-0.43)	0.20 (0.03-1.54)	0.121
eGFR[a]	0.00 (-0.03-0.03)	1.00 (0.97-1.03)	0.876	0.01 (-0.03-0.05)	1.01 (0.97-1.05)	0.668
Previous anthracyclines[b]	0.67 (-0.39-1.72)	1.96 (0.68-5.60)	0.212	0.92 (-0.33-2.18)	2.52 (0.72-8.86)	0.150
Previous or concurrent trastuzumab[b]	0.42 (-0.87-1.72)	1.53 (0.42-5.60)	0.525	0.48 (-0.87-1.83)	1.62 (0.42-6.26)	0.484
Dose of capecitabine[a]	-0.01 (-0.04-0.02)	0.99 (0.96-1.02)	0.573	-0.01 (-0.04-0.02)	0.99 (0.96-1.02)	0.579
Previous thoracic irradiation[b]	0.35 (-1.02-1.72)	1.42 (0.36-5.59)	0.621	0.40 (-0.99-1.79)	1.48 (0.37-5.96)	0.577

[a]scale variable. [b]categorical variable. 95%-CI = 95% confidence interval; BMI = body mass index; ”Cardiac co-morbidity” = all types of heart disease; eGFR = estimated glomerular filtration rate.

C Statistics: Predictors of symptomatic cardiotoxicity

The ability of the variables, cardiac co-morbidity, hypercholesterolemia and current smoking, to discriminate between patients that will develop symptomatic cardiotoxicity and those who will not was tested with c-statistics. In unselected patients the presence of cardiac co-morbidity was a poor predictor of symptomatic cardiotoxicity (c = 0,617 (95%CI 0.483-0.754), p = 0.061), as was the presence of hypercholesterolemia (c = 0.662 (95%CI 0.478-0.766), p = 0.072) and smoking status (c = 0.651 (95%CI 0.523-0.779), p = 0.031). In the subgroup of patients with apparently no cardiac co-morbidity the presence of hypercholesterolemia (c = 0.601 (95%CI 0.427-0.775), p = 0.216) and

smoking status (c = 0.691 (95%CI 0.526-0.857), p = 0.031) were poor factors to discriminate between patients who will develop cardiotoxicity and those who will not.

DISCUSSION

Incidence of symptomatic cardiotoxicity

We observed an incidence of symptomatic cardiotoxicity from capecitabine at ≈ 5% in women with metastatic breast cancer which is similar to incidences reported in previous studies of both men and women treated with capecitabine,[22-24] although some studies have reported lower[20] or higher incidences.[21] The difference in incidences in-between studies may be due to different risk profiles in the study populations. Moreover, prospective studies with regular cardiac assessments may detect more cardiotoxicity since they may identify asymptomatic patients and patients with mild symptoms.

The incidence of symptomatic cardiotoxicity in our study and other studies of capecitabine cardiotoxicity is within the range of incidences of symptomatic cardiotoxicity in studies with 5-FU[15, 21, 24-26] and a large study found similar incidences for the two treatments.[24] However, a prospective study with 644 patients reported similar incidences of cardiotoxicity for capecitabine and continuous 5-FU infusion schedules, but a lower incidence for short (bolus) 5-FU infusion schedules.[22]

Pattern of symptomatic cardiotoxicity

The most common event of symptomatic cardiotoxicity was chest pain, which was the main symptom in 59% of the 22 cases. Most of the patients with chest pain had normal ECG and normal coronary enzymes and severe events, such as acute myocardial infarction and cardiac arrest with lethal outcome, was rare. This pattern is in concordance with other studies[15] and the sudden onset of

chest pain and the rare occurrence of life-threatening complications correspond well to the theory of fluoropyrimidine induced vasospastic angina. Furthermore, the angiographically normal arteries reported in several case reports[6-8, 11] and the presence of silent ischemic episodes on Holter recordings[27] supports this theory.

Retreatment of patients with symptomatic cardiotoxicity

Retreatment with capecitabine after occurrence of cardiotoxicity was attempted in 6 of 22 patients with little success. Four patients had recurrent cardiac symptoms, suggesting that retreatment should be done with great precaution. However, a retrospective study of 668 patients[20] and a prospective study of 644 patients[22] treated with 5-FU or capecitabine reported a benefit from dose-reduction and initiation of anti-angina therapy that prevented symptoms at retreatment in 9 of 12 patients and 12 of 15 patients, respectively. Their findings suggest that retreatment at reduced dose and with appropriate anti-angina therapy are feasible, however close cardiac monitoring is crucial. Furthermore, nitro-glycerine was effective to abolish symptoms of cardiotoxicity.[20] In contrast, a small and non-randomized study could not demonstrate an prophylactic effect of calcium channel blockers on occurrence of cardiotoxicity.[28] Larger studies with systematic, predefined strategies for dose reduction and initiation of anti-angina therapy are needed.

Risk factors for symptomatic cardiotoxicity

Patients with cardiac comorbidity were at increased risk of symptomatic cardiotoxicity, which is in accordance with four previous studies of 5-FU or capecitabine cardiotoxicity,[21, 25, 27, 29] while other studies found no increased risk for patients with pre-existing heart disease.[23, 30-33] While heart disease may be a risk marker for cardiotoxicity, it is not a prerequisite for cardiotoxicity to occur. The patient who sustained cardiac arrest and died in our study had no cardiac co-morbidities and no risk factors for ischemic heart disease. Likewise, severe cardiotoxicity has been reported for several

patients without cardiac co-morbidity.[11, 34-37] Also, we found that a history of heart disease was a poor predictor of cardiotoxicity.

In the present study hypercholesterolemia and current smoking were risk factors for symptomatic cardiotoxicity both in the entire study group and in the subgroup of patients with no apparent cardiac co-morbidity. Similarly, a larger prospective study of 644 patients without cardiac co-morbidities reported that smoking was associated with ECG changes in bivariate analyses.[22] However, they found no association between ECG changes and hyperlipidaemia.[22] While Kosmas et al.[22] prospectively measured cholesterol and triglyceride levels in blood; our study is based on medical recordings. Thus incomplete data with risk of information bias is a limitation of the present study. Both studies have a low number of events limiting the statistical power and multiple testing increases the risk of false positive results.

Previous treatment with potentially cardiotoxic therapy with anthracyclines or previous or current treatment with trastuzumab did not increase the risk of symptomatic cardiotoxicity. However, two of three patients with epirubicin-induced cardiomyopathy developed symptomatic cardiotoxicity during treatment with capecitabine suggesting that the degree of pre-existing heart damage may be relevant. Patients with previous left-sided breast irradiation were not at increased risk of cardiotoxicity in our study. Similarly, a large prospective study of 5-FU cardiotoxicity found that previous breast irradiation was not a risk factor.[25] The lack of association between other cardiotoxic therapies and capecitabine-induced cardiotoxicity suggests that the mechanisms behind these cardiotoxicities are different. Moreover, radiation-induced cardiovascular disease may be a late event.[38] In our study the mean time from chest irradiation to capecitabine start was 4 years.

Methodological considerations

The predominant manifestation of symptomatic cardiotoxicity from fluoropyrimidines is chest pain followed by other subjective symptoms and ECG changes or other objective signs of cardiotoxicity

are not always present. In our study, only approximately 50% of the patients had objective signs (mostly ECG changes) of cardiotoxicity. Thus, the event (cardiotoxicity) was based mainly on subjective symptoms and is therefore affected by the patient's own perception and reaction to the symptoms and the physician's assessment of the patient's symptoms. Both patients and physicians may pay more attention to cardiac symptoms if the patient has heart disease before treatment start. These factors may explain some of the difference in incidence in-between studies. Research in new and sensitive cardiac biomarkers like e.g. copeptin to detect myocardial ischemia is needed.[39]

In spite of a relatively large sample size, the number of events is low (22 events) leading to wide confidence intervals, low statistical power and increased risk of type II statistical errors. With respect to the logistic regression analyses, the low number of events limits the number of covariates allowed in the model. The low number of events and multiple testing (increasing the risk of false positive results) are weaknesses of most studies analysing risk factors and it makes the conclusions that can be drawn from these studies less valid.

Like most other large studies on this subject our study is retrospective which may results in underestimation of the incidence of cardiotoxicity due to incomplete data and overlooking patients with mild symptoms and asymptomatic patients . Missing data affects power and if not missing at random, they may cause bias. We dealt with the missing data by sensitivity analyses with worst and best case scenarios and with multiple imputation. The sensitivity analyses showed that missing data had little influence on our study results.

A major limitation is that baseline ECGs was only preserved for 36% of the patients. Among the 11 patients with ECG changes during capecitabine treatment, 2 had missing baseline ECGs.

Conclusion

The incidence of symptomatic cardiotoxicity from capecitabine of ≈ 5% is close to that of 5-FU with incidences of 1%-5% in larger studies. Our study results suggest that cardiac co-morbidity and

current smoking are risk factors for symptomatic cardiotoxicity. Whether smoking cessation can prevent some cases of symptomatic cardiotoxicity deserves further investigations. All the identified risk factors were poor predictors of symptomatic cardiotoxicity and initial optimal cardiac treatment, information about the risk and follow-up if symptoms present are the options today.

Contributors:

AP: Data collection, data processing, statistics, writing of the paper and incorporation of input from the other authors. NS and KV: Conception and design of the study, data collection and review of the manuscript. MVN: Review of cases with suspected cardiotoxicity and review of the manuscript. FOL: Conception and design of the study and review of the manuscript. MS: Interpretation of study results and statistics and review of the manuscript. DN: Conception and design of the study, data collection, data interpretation, input to the manuscript and review of the manuscript.

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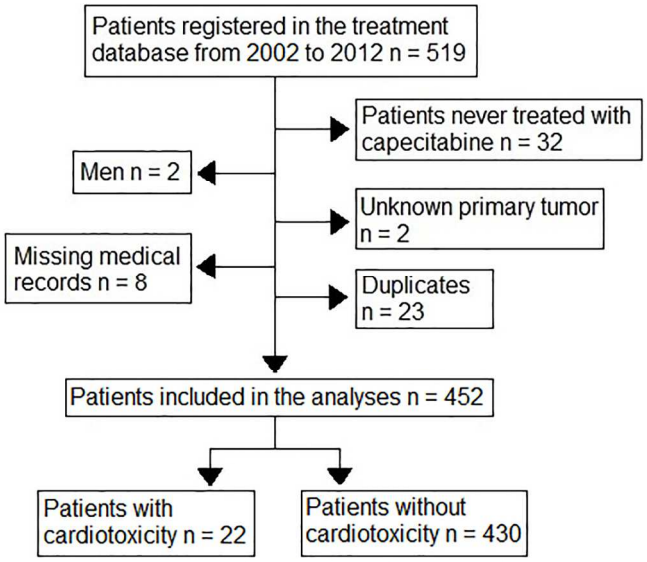

Figure 1: Flow diagram

179x135mm (300 x 300 DPI)

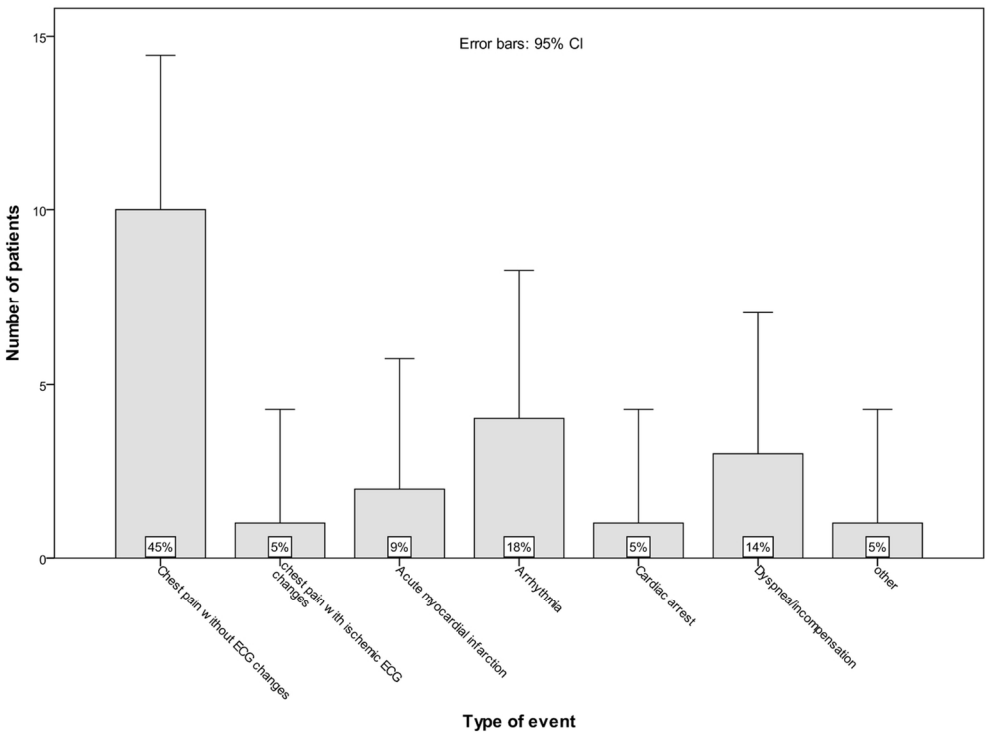

Figure 2: The distribution of the different manifestations of cardiotoxicity.

The percentages showed on each bar reflect the distribution of symptoms among the 22 patients with cardiotoxicity. Proportion of patients with chest pain without ECG changes (n = 10), chest pain with ECG changes but not acute myocardial infarction (n = 1), acute myocardial infarction (n = 2), arrhythmia (n = 4), cardiac arrest (n = 1), dyspnea/incompensation (n = 3) and other (n = 1, with QT prolongation and right bundle branch block). Patients with more than one of the manifestations (n = 2) are classified according to their primary complaint.

119x94mm (300 x 300 DPI)

Supplementary table: Details about patients with cardiotoxicity and initiated therapy

Case	Location of metastases	Pre-existing cardiovascular disease	Cycle of onset	Dose intensity at onset	Type of event	Description	ECG changes	Raised cardiac biomarkers?	Cardiac therapy	Subsequent treatment with capecitabine, dose	Symptoms at retreatment
1	Liver, lung, abdomen	No	1	100%	Chest pain	Oppressive chest pain	-	-	No	Yes, 100%	Yes, oppressive chest pain
2	Right lung, pleura, mediastinal lymph nodes, adrenal glands, abdominal carcinosis, columna and pelvis	Atrial fibrillation, NYHA 3, moderate pulmonary hypertension, LVEF = 53% after 1. cycle, hypertension	2	75%	Incompensation, dyspnea	Dyspnea and peripheral edema	ST-depressions in V5-V6	-	-	No	-
3	Liver	No	1	100%	Chest pain	Exertional chest pain radiating to the neck	No changes from pretreatment ECG (inverted T-waves in V1-V2)	-	-	No	-
4	Liver, bone Th3 + Th7, lymph nodes in left axilla	No	2	75%	Cardiac arrest	Chest pain and progressing dyspnea, cardiac arrest, death	Asystole	-	Adrenalin and amiodaron	-	-
5	Mediastinal lymph nodes,	Hypertension, hyperlipidem	1	75%	QTc prolongation, right	Asymptomatic	QTc prolongation, right	-	-	No	-

	thyroid gland, bone Th1 + Th5	ia			bundle branch block		bundle branch block				
6	Neck, sternal and left axillary lymph nodes	No	1	100%	Chest pain	Intermittent, retrosternal, oppressive chest pain radiating to both arms, LVEF 60% at echocardiography	Normal	No	No	No	-
7	Mediastinal lymph nodes	No	4	75%	Chest pain	Chest pain, dyspnea and malaise	Normal	No	No	No	-
8	Pleura, bone	No	1	100%	Chest pain	Compressive chest pain	No changes, ST-depression in V2-V4	-	Metoprolol and Isosorbidm ononitrate	No	-
9	Pleura	No	1	100%	Arrhythmia	Dyspnea	Atrial fibrillation/ atrial flutter, 170 bpm.	-	Adenosine, metoprolol and amiodaron	No	-
10	Liver, lung, bone	No	2	75%	Chest pain, Arrhythmia	Chest pain and palpitations	Atrial fibrillation, 119 bpm	-	Verapamil	Yes, 75%	Palpitations, dyspnea, atrial fibrillation
11	Liver, lungs, neck and right supraclavicular lymph nodes	SVT, previous pulmonary embolism, hyperlipidemia	1	100%	AMI	Oppressive chest pain radiating to right side, dyspnea. Effect of nitroglycerin iv. Normal coronary angiography and normal	Initially ST-elevation in V1-V3, AVF and V6. Negative T-waves in	Marginal raised TnI (52 ng/l) and raised CK-MB (4.4 µg/l)	Nitroglycerin iv, Acetylsalicylic Acid and Magnesium Hydroxide, clopidogrel,	No	-

						echocardiography.	lead II-III		fondaparinux		
12	Lungs, mediastinal lymph nodes	Hypertension, diabetes, hyperlipidemia	3	50%	Dyspnea	Dyspnea and light chest pain	Normal	No	No	Yes, 50%	No
13	Bone	Epirubicin-induced cardiomyopathy	9	100%	Chest pain, arrhythmia	Compressive chest pain	Atrial fibrillation and non-sustained VT	-	-	No	-
14	Bone, skin, liver and left axillary lymph nodes	Hypertension, NYHA 2-3 Echo: LVEF 50-55%, mitral valve insufficiency, discrete pulmonary, hypertension	3	75%	Incompensatio, dyspnea	Progressing dyspnea, NYHA 3, peripheral edema, cardiomegaly Echo: LVEF unchanged, moderate to severe pulmonary hypertension, tricuspid insufficiency	Bundle branch block	-	Furosemide and potassium-chloride	No	-
15	Neck and infraclavicular lymph nodes	Atrial fibrillation, COPD	3	100%	Arrhythmia, pulmonary congestion	Dyspnea, dizziness, malaise X-ray: pulmonary congestion and pericardial exudate	Atrial fibrillation 117 bpm	-	Digoxin, Centyl Mite with potassium-chloride	No	-
16	Brain	No	8	100%	Chest pain	Oppressive chest pain radiating to the neck	Normal	-	-	Yes, 100%	Recurrent episodes with chestpain

17	Mediastinal lymph nodes	No	2	100%	Chest pain	Oppressive chestpain radiating to neck and arm, dyspnea	Sinus rhythm, supraventricular extra systoles, no ST-deviations	No	Verapamil	Yes, 75%	No
18	Sternum and sacroiliac bone	Hyperlipidemia	1	100%	Chest pain	Compressive chest pain radiating to the back and both arms. Echo: normal	Normal	-	No	No	-
19	CNS, liver, right axillary lymph nodes	Hypertension, hyperlipidemia	12	75%	AMI	Chest pain radiating to head, neck and jaw. LVEF = 40 %.	Normal	Raised troponins (400 ng/l), CK-MB unknown	Acetylsalicylic Acid with magnesium hydroxide (magnyl), clopidogrel, fondaparinux, metoprolol, ACE-inhibitor, Statin	No	-
20	Pelvis bone, right axillary lymph nodes	Hypertension, hyperlipidemia	1	100%	Chest pain	Oppressive chest pain Echo: normal	-	-	-	No	-
21	Lung, bone	Epirubicin-induced cardiomyopathy, LVEF = 34-40%	1	100%	Arrhythmia	Dizziness and palpitations.	Telemetry: Atrial flutter 150 bpm, neg. T-waves in I and AVL	-	Metoprolol, ACE-inhibitor	No	-

22	Pelvic and columnar bone	Atrial fibrillation, hypertension, hyperlipidemia	1	100%	Chest pain	Chest pain	Fluctuating negative T-waves in lead II, III, aVF and V1-V5	-	No	Yes, 100%	Episodes with pain located to jaw and chest

COPD = chronic obstructive pulmonary disease, bpm = beats per minute, NYHA = New York Heart association functional classification, LVEF = left ventricular function, TnI = cardiac troponin I, SVT = supraventricular tachycardia, LMWH = low molecule weight heparin, CK-MB = creatine kinase MB, CNS = central nerve system

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Not relevant
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5
Bias	9	Describe any efforts to address potential sources of bias	-
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	6
		(*d*) If applicable, explain how loss to follow-up was addressed	Not applicable
		(*e*) Describe any sensitivity analyses	6
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6 + Figure 1
		(b) Give reasons for non-participation at each stage	Figure 1
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6-7
		(b) Indicate number of participants with missing data for each variable of interest	Table 1
		(c) Summarise follow-up time (eg, average and total amount)	-
Outcome data	15*	Report numbers of outcome events or summary measures over time	10
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10
		(*b*) Report category boundaries when continuous variables were categorized	Not applicable
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11
Discussion			
Key results	18	Summarise key results with reference to study objectives	16-18
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16-19
Generalisability	21	Discuss the generalisability (external validity) of the study results	16-19
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	20

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.