Supplementary Materials

A fibrosis biomarker early predicts cardiotoxicity due to anthracycline-based breast cancer chemotherapy

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Table S1. Clinical characteristics in BC patients at baseline, after completion of ACC therapy (post-ACC), and 3 months after ACC (3m-post-ACC) (CUN cohort).

	Baseline	Post-ACC	3m-post-ACC
Renal function parameter			
eGFR, mL/min/1.73m2	95.3 ± 16.2	103 ± 16.8*	101 ± 18.7*
Liver function parameters			
AST, IU/L	18.0 (15.0-20.0)	19.0 (15.0-25.0)*	20.0 (17.0-25.0)*
ALT, IU/L	13.0 (11.0-18.0)	20.0 (15.0-36.0)*	18.0 (14.0-27.5)*†
GGT, IU/L	17.0 (13.0-26.0)	24.0 (17.0-35.0)*	19.0 (14.0-28.0)†
ALP, IU/L	60.0 (50.0-72.0)	67.0 (55.0-78.0)*	63.0 (51.0-76.0)†
Echocardiographic parameters			
GLS, %	-20.6±2.0	-20.5±2.4	-19.6±2.5*†
LVEF (3D), %	63.8±5.3	63.3±4.5	62.3±5.9
Biomarkers‡			
Cardiomyocyte stress/damage			
NTproBNP, pg/mL	41.4 (24.4-64.1)	71.5 (42.9-117)*	57.1 (35.2-96.2)*
hs-TnT, ng/L	3.0 (3.0-4.5)	8.1 (4.0-11.1)*	8.0 (4.9-11.9)*
Myocardial fibrosis			
PICP, ng/mL	72.0 (59.5-86.5)	61.8 (51.1-74.0)	87.6 (69.2-119)*†
CITP:MMP-1 ratio	1.5 (1.2-3.0)	2.1 (1.2-3.6)	2.0 (1.3-3.8)

BC means breast cancer; ACC, anthracycline-based cancer chemotherapy; eGFR, estimated glomerular filtration rate; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyltransferase; ALP, alkaline phosphatase; 3D, three-dimensional; LVEF, left ventricular ejection fraction; NT-proBNP, amino-terminal pro-brain natriuretic peptide; hs-TnT, high sensitivity troponin T; PICP, procollagen type I C-terminal propeptide; CITP, collagen type I C-terminal telopeptide; MMP-1, matrix metalloproteinase-1. Quantitative variables are expressed as mean±SD or as median (IQR) and categorical variables as number (percentage). *P<0.05 vs basal, †P<0.05 vs post-ACC. ‡P values in the biomarker analyses were adjusted according to the Benjamini and Hochberg multiple-test correction (5% FDR)

Table S2. Differences in echocardiographic parameters and in biomarkers after completion of ACC therapy (post-ACC) and 3 months after ACC in BC patients with presence versus those with absence of subclinical left ventricular dysfunction (LVD) at 3 months after ACC (CUN cohort).

	Difference vs absence of subclinical LVD						P for interaction
	Post-ACC			3m-post-ACC			
	Difference	95% CI	P value	Difference	95% CI	P value	
Echocardiographic parameters							
GLS, %	0.82	0.09 to 1.55	0.028	4.34	3.45 to 5.24	<0.001	<0.001
LVEF (3D), %	0.57	-1.61 to 2.76	0.61	-5.53	-8.68 to -2.38	0.001	<0.001
Biomarkers (log2)							
Cardiomyocyte stress/damage							
NTproBNP, pg/mL	0.63	0.19 to 1.07	0.005	0.51	-0.05 to 1.07	0.08	0.25
hs-TnT, ng/L	-0.03	-0.59 to 0.53	0.91	0.08	-0.34 to 0.51	0.70	0.89
Myocardial fibrosis							
PICP, ng/mL	-0.01	-0.21 to 0.20	0.93	0.70	0.46 to 0.95	<0.001*	<0.001*
CITP:MMP-1 ratio	-0.09	-0.57 to 0.38	0.71	0.05	-0.53 to 0.63	0.88	0.60

Abbreviations as in table S1. *Significant after Benjamini and Hochberg multiple-test correction (5% FDR) in the biomarker analyses

Table S3. Echocardiographic parameters and biomarkers in BC patients at baseline, after completion of ACC therapy (post-ACC), 3 months post-ACC (3m-post-ACC) and 12 months post-ACC (12m-post-ACC).

	Baseline	Post-ACC	3m-post-ACC	12m-post-ACC
CUN cohort				
Echocardiographic parameters				
GLS, %	-20.6±2.0	-20.7±2.4	-19.7±2.1*†	-19.5±2.8*†
LVEF (3D), %	63.3±4.9	63.4±4.4	62.7±5.4	61.5±7.3
Biomarkers&				
Cardiomyocyte stress/damage				
NTproBNP, pg/mL	39.0 (24.1-64.2)	68.4 (42.9-117)*	54.9 (34.2-95.5)*	46.0 (21.0-78.5)
hs-TnT, ng/L	3.0 (3.0-4.5)	8.6 (4.0-12.0)*	7.5 (4.3-10.0)*	4.6 (3.0-6.4)*†‡
Myocardial fibrosis				
PICP, ng/mL	66.6 (59.0-87.5)	64.6 (51.9-74.3)	85.1 (69.8-115)*†	90.7 (70.8-117)*†
HULAFE cohort				
Echocardiographic parameters				
GLS, %	-16.5±1.9	-15.5±2.5*	-15.2±2.0*	-15.7±2.6
LVEF (2D), %	66.9±5.8	65.0±5.8	62.5±5.5*	61.6±6.3*
Biomarkers&				
Cardiomyocyte stress/damage				
NT-proBNP, pg/mL	60.6 (29.5-99.5)		74.2 (40.7-120)*	94.1 (55.0-157)*†
hs-TnT, ng/L	6.9 (3.0-10.6)		12.6 (8.9-19.2)*	9.3 (7.1-11.4)*†
Myocardial fibrosis				
PICP, ng/mL	73.3 (57.8-99.4)		87.7 (66.8-113)*	93.3 (80.4-123)*

Abbreviations as in table S1. Quantitative variables are expressed as mean±SD or as median (IQR) and categorical variables as number (percentage) *P<0.05 vs basal, †P<0.05 vs post-ACC, ‡P<0.05 vs 3m-post-ACC. &P values in the biomarker analyses were adjusted according to the Benjamini and Hochberg multiple-test correction (5% FDR).

Table S4. Baseline clinical characteristics of BC patients categorized according to the absence or presence of cardiotoxicity at 12 months after completion of ACC therapy (CUN cohort).

	Cardiotoxicity		P value
	No (n=55)	Yes (n=10)	
Oncologic parameters, n (%)			
Breast cancer side			
Left	31 (56.4)	6 (60.0)	
Right	23 (41.8)	4 (40.0)	0.90
Bilateral	1 (1.8)	0 (0.0)	
Ki67, %	40.7 ± 25.2	42.5 ± 27.4	0.84
HER2 positive, n (%)	14 (25.5)	3 (30.0)	0.76
Other treatments, n(%)			
Carboplatin	17 (30.9)	2 (20.0)	0.49
Anti-HER2			
Trastuzumab	9 (16.4)	0 (0.0)	0.10
Trastuzumab+Pertuzumab	5 (9.1)	3 (30.0)	
Radiotherapy, n(%)	46 (83.6)	9 (90.0)	0.61
Surgery before ACC, n (%)	18 (32.7)	2 (20.0)	0.71
Liver function parameters			
AST, IU/L	17.5 (14.0-20.0)	17.5 (13.8-20.3)	0.69
ALT, IU/L	13.0 (11.5-18.5)	12.0 (9.5-14.0)	0.78
GGT, IU/L	17.0 (13.0-27.0)	17.0 (14.8-42.3)	0.33
ALP, IU/L	61.0 (51.0-75.0)	53.0 (38.5-66.0)	0.10
Biomarkers			
CITP:MMP1 ratio	1.5 (1.2-3.3)	2.0 (1.2-3.2)	0.85

Abbreviations as in table S1. Quantitative variables are expressed as mean±SD or median (inter-quartile range) and categorical variables as number (percentage).