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Baseline Proteomic Biomarkers for Predicting Chemotherapy-Induced Cardiotoxicity in Breast Cancer Patients

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Abstract

Treatment with the anthracycline doxorubicin (DOX) is associated with cumulative dose-dependent cardiotoxicity in a subset of cancer patients, limiting its use in those patients. However, there are no qualified clinical biomarkers to predict cardiotoxicity. In this study, 83 breast cancer patients were enrolled and treated with a combination of DOX (60 mg/m²) and cyclophosphamide (600 mg/m²). Thirty-nine patients were randomly selected for biomarker discovery, in which nine patients experienced treatment-related cardiotoxicity after completion of chemotherapy. The remaining 44 patients were assigned to the biomarker-validation cohort, in which ten patients experienced cardiotoxicity. SOMAscan analysis of plasma samples before treatment for the biomarker-discovery cohort identified 48 proteins with differential baseline levels between the patients with and without cardiotoxicity. Olink proteomic analysis of the validation cohort confirmed 6 proteins, of which baseline levels of biglycan, carbonic anhydrase 6, cadherin-5, CD109, and thrombospondin-4 were higher, and the level of cystatin-F was lower, in patients with cardiotoxicity. A logistic regression analysis indicated that these 6 proteins and higher baseline levels of left ventricular ejection fraction (LVEF) were associated with an increased risk of cardiotoxicity. A model using partial least squares discriminant analysis of these proteins in combination with baseline LVEF predicted cardiotoxicity with a sensitivity of 80%, specificity of 88%, and overall accuracy of prediction of 86%. These biomarkers and the predictive model could provide new tools for identifying cancer patients at high risk of DOX-induced cardiotoxicity. A multi-center qualification of these biomarkers is underway.

Introduction

Anthracycline (AC)-based chemotherapy (e.g., DOX) is one of the most effective and commonly used treatments for a wide range of cancers; however, a serious adverse side-effect of anthracycline treatment is cumulative dose-dependent cardiotoxicity, which may manifest as subclinical heart disease, cardiomyopathy, left ventricular dysfunction (LVD), congestive heart failure (CHF), etc., as described in the black box warning for DOX. Although potential predictors for increased risk of DOX-induced cardiotoxicity in breast cancer patients were recently described, there are currently no clinically validated biomarkers for the prediction of cardiotoxicity caused by DOX treatment. Conventional blood-based biomarkers, such as cardiac troponin T (cTnT) and I (cTnI), are limited to the identification of nascent drug-induced cardiotoxicity. Imaging tests (e.g., echocardiography) are the most practical monitoring tools for assessment of left ventricular ejection fraction (LVEF); however, imaging tools are costly and LVEF has not been qualified as a biomarker for early detection of cardiotoxicity. Sensitive imaging measurements, such as systolic longitudinal and segment myocardial strains, are promising monitoring approaches, but clinical validation is still needed. Therefore, development of new predictive biomarkers of cardiotoxicity prior to the occurrence of overt cardiac tissue damage and dysfunction would be extremely valuable for the prevention of permanent damage and/or identification of patients at high risk for cardiac damage.

Materials and Methods

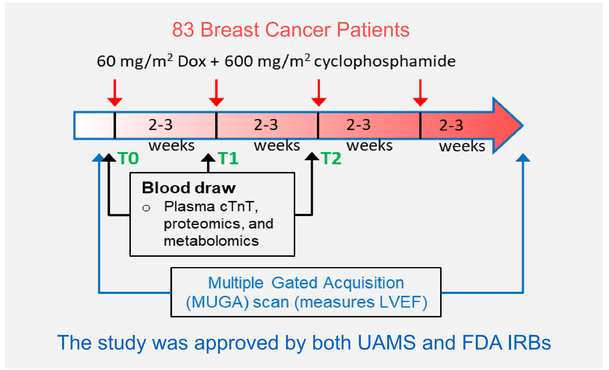

Figure 1. DOX treatment, blood sample collection, and cardiac function monitoring for breast cancer patients. LVEF, left ventricular ejection fraction.

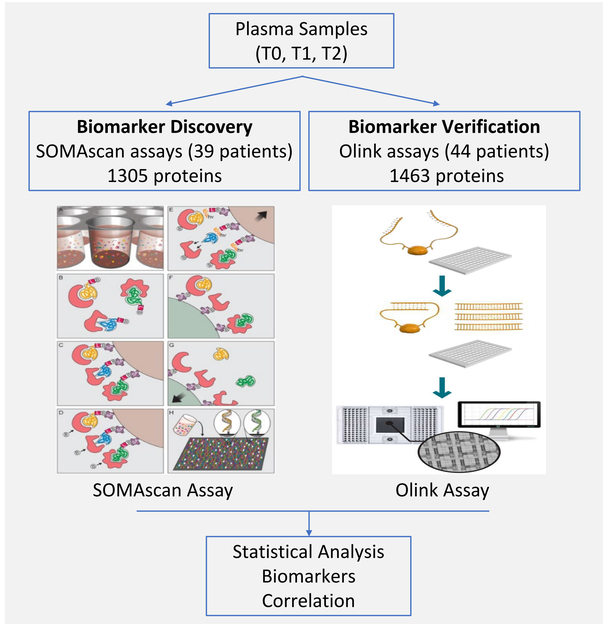

Figure 2. Flow chart showing proteomics approaches for biomarker discovery using SOMAscan assays and verification using Olink assays. *T*-test was performed to find significantly changed proteins between the normal and abnormal (cardiotoxicity) groups. Statistical analyses were performed using the software R.

Results and Discussion

Table 1. Characteristics of breast cancer patients stratified by cardiotoxicity status after doxorubicin-based chemotherapy

Characteristics	Overall (N=83)	Normal after DOX (N=64)	Cardiotoxicity after DOX (N=19)	*P*-value
Age (years)	52.0±11.4	52.1±10.0	51.8±15.8	0.9341
BMI (kg/m2)	32.0±7.5	31.7±7.6	33.1±7.4	0.4879
Baseline LVEF (%)	63.8±6.7	62.6±6.6	67.7±5.5	0.0015
LVEF Reduction (%)	-3.5±8.6	0.2±6.1	-14.7±4.0	3.20E-16
ER+/PR+, HER2-	57 (68.7%)	42 (65.6%)	15 (78.9%)	0.2716
ER-/PR-/HER2-	26 (31.3%)	22 (34.4%)	4 (21.1%)	0.3995
Hypertension	38 (45.8%)	29 (45.3%)	9 (47.4%)	0.8745
Diabetes	13 (15.7%)	9 (14.1%)	4 (21.1%)	0.4821
Vitamin D deficiency	21 (25.3%)	13 (20.3%)	8 (42.1%)	0.0550

Table 2. Biomarker candidates before DOX treatment (T0)

Protein Biomarker	Discovery		Validation	
	CT/N	*P*-value	CT/N	*P*-vlue
Biglycan	1.34	0.0325	2.77	0.0022
Cadherin-5	1.29	0.0416	1.28	0.0155
Carbonic anhydrase 6 (CA6)	1.41	0.0358	1.43	0.0242
CD109	1.77	0.0218	1.23	0.0366
Cystatin-F	0.60	0.0344	0.48	0.0327
Thrombospondin-4 (TSP4)	1.44	0.0096	1.50	0.0013

CT, cardiotoxicity, decreased LVEF after chemotherapy; N, normal, remained normal LVEF after chemotherapy.

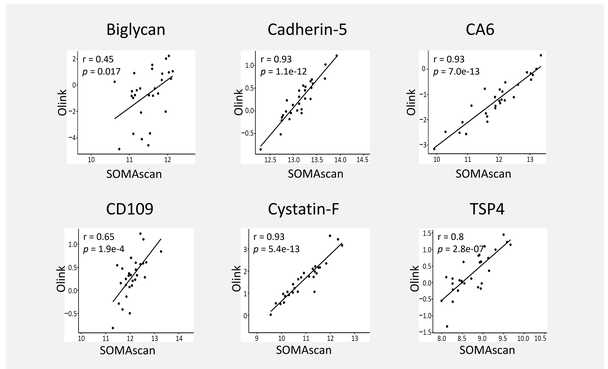

Figure 3. Correlation between SOMAscan and Olink assays for the biomarker candidates measured in the same set of samples (n=28).

Table 3. Association of baseline protein levels with cardiotoxicity (T0) as analyzed by logistic regression for the validation cohort

	Univariate Analysis		Multivariable Analysis[b]	
Protein	OR (95% CI)[a]	*P*-value	OR (95% CI)	*P*-value
Biglycan	4.84 (1.58-14.81)	0.0058	10.85 (1.74-67.84)	0.0108
Cadherin-5	26.71 (1.84-387.84)	0.0161	12.88 (0.77-215.97)	0.0757
CA6	15.25 (0.84-276.89)	0.0655	5.51 (0.26-118.62)	0.2760
CD109	8.07 (1.04-62.50)	0.0457	18.55 (1.08- 320.00)	0.0444
Cystatin-F	0.46 (0.21-1.03)	0.0599	0.45 (0.18-1.14)	0.0915
TSP4	16.28 (2.13-124.62)	0.0072	68.81 (3.59-1290.60)	0.0049

[a]OR, odds ratio; CI, confidence interval. [b]Multivariable logistic regression analysis was adjusted for baseline LVEF, triple negative, vitamin D deficiency, and hypertension.

Table 4. Validation of biomarkers for predicting cardiotoxicity using models of partial least squares discriminant analysis

Multivariable Model	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Biglycan	80.0 (44.4-97.5)	61.8 (43.6-77.8)	38.1 (18.1-61.6)	91.3 (72.0-98.9)	65.9 (50.1-79.5)
Cadherin-5	60.0 (26.2-87.8)	76.5 (58.8-89.3)	42.9 (17.7-71.1)	86.7 (69.3-96.2)	72.7 (57.2-85.0)
CA6	70.0 (34.8-93.3)	73.5 (55.6-87.1)	43.8 (19.8-70.1)	89.3 (71.8-97.7)	72.7 (57.2-85.0)
CD109	30.0 (6.7-65.2)	88.2 (72.5-96.7)	42.9 (9.9-81.6)	81.1 (64.8-92.0)	75.0 (59.7-86.8)
Cystatin-F	80.0 (44.4-97.5)	58.8 (40.7-75.4)	36.4 (17.2-59.3)	90.9 (70.8-98.9)	63.6 (47.8-77.6)
TSP4	60.0 (26.2-87.8)	85.3 (68.9-95.0)	54.5 (23.4-83.3)	87.9 (71.8-96.6)	79.5 (64.7-90.2)
Baseline LVEF	50.0 (18.7-81.3)	79.4 (62.1-91.3)	41.7 (15.2-72.3)	84.4 (67.2-94.7)	72.7 (57.2-85.0)
Biglycan, CA6, CD109, TSP4	80.0 (44.4-97.5)	73.5 (55.6-87.1)	47.1 (23.0-72.2)	92.6 (75.7-99.1)	75.0 (59.7-86.8)
Biglycan, CA6, CD109, TSP4, baseline LVEF	90.0 (55.5-99.7)	88.2 (72.5-96.7)	69.2 (38.6-90.9)	96.8 (83.3-99.9)	88.6 (75.4-96.2)
Biglycan, CA6, CD109, TSP4, Cadherin-5	70.0 (34.8-93.3)	73.5 (55.6-87.1)	43.8 (19.8-70.1)	89.3 (71.8-97.7)	72.7 (57.2-85.0)
Biglycan, CA6, CD109, TSP4, Cadherin-5, baseline LVEF	70.0 (34.8-93.3)	82.4 (65.5-93.2)	53.8 (25.1-80.8)	90.3 (74.2-98.0)	79.5 (64.7-90.2)
Biglycan, CA6, CD109, TSP4, Cystatin-F	90.0 (55.5-99.7)	76.5 (58.8-89.3)	52.9 (27.8-77.0)	96.3 (81.0-99.9)	79.5 (64.7-90.2)
Biglycan, CA6, CD109, TSP4, Cystatin-F, baseline LVEF	80.0 (44.4-97.5)	88.2 (72.5-96.7)	66.7 (34.9-90.1)	93.8 (79.2-99.2)	86.4 (72.6-94.8)
All proteins	90.0 (55.5-99.7)	76.5 (58.8-89.3)	52.9 (27.8-77.0)	96.3 (81.0-99.9)	79.5 (64.7-90.2)
All Proteins, baseline LVEF	80.0 (44.4-97.5)	88.2 (72.5-96.7)	66.7 (34.9-90.1)	93.8 (79.2-99.2)	86.4 (72.6-94.8)

Values in brackets are 95% confidence intervals (CI).

Conclusion

- Forty-eight proteins with differential baseline levels before initiation of DOX-based therapy were identified using the SOMAscan assay from 39 breast cancer patients.
- Olink proteomic analysis of the validation cohort confirmed 6 proteins associated with an increased odds of cardiotoxicity, including higher levels of biglycan, carbonic anhydrase 6, cadherin-5, CD109, and thrombospondin-4, as well as lower levels of cystatin-F.
- A predictive model using partial least squares discriminant analysis for these proteins and in combination with baseline LVEF was able to predict cardiotoxicity with a sensitivity of 80%, specificity of 88%, and 86% of overall accuracy of prediction.
- Data from T1 and T2 are being analyzed.

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