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ORIGINAL RESEARCH

Candidate Plasma Biomarkers to Detect Anthracycline-Related Cardiomyopathy in Childhood Cancer Survivors: A Case Control Study in the Dutch Childhood Cancer Survivor Study

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BACKGROUND: Plasma biomarkers may aid in the detection of anthracycline-related cardiomyopathy (ACMP). However, the currently available biomarkers have limited diagnostic value in long-term childhood cancer survivors. This study sought to identify diagnostic plasma biomarkers for ACMP in childhood cancer survivors.

METHODS AND RESULTS: We measured 275 plasma proteins in 28 ACMP cases with left ventricular ejection fraction <45%, 29 anthracycline-treated controls with left ventricular ejection fraction ≥53% matched on sex, time after cancer, and anthracycline dose, and 29 patients with genetically determined dilated cardiomyopathy with left ventricular ejection fraction <45%. Multivariable linear regression was used to identify differentially expressed proteins. Elastic net model, including clinical characteristics, was used to assess discrimination of proteins diagnostic for ACMP. NT-proBNP (N-terminal pro-B-type natriuretic peptide) and the inflammatory markers CCL19 (C-C motif chemokine ligands 19) and CCL20, PSPD (pulmonary surfactant protein-D), and PTN (pleiotrophin) were significantly upregulated in ACMP compared with controls. An elastic net model selected 45 proteins, including NT-proBNP, CCL19, CCL20 and PSPD, but not PTN, that discriminated ACMP cases from controls with an area under the receiver operating characteristic curve (AUC) of 0.78. This model was not superior to a model including NT-proBNP and clinical characteristics (AUC=0.75; P=0.766). However, when excluding 8 ACMP cases with heart failure, the full model was superior to that including only NT-proBNP and clinical characteristics (AUC=0.75 versus AUC=0.50; P=0.022). The same 45 proteins also showed good discrimination between dilated cardiomyopathy and controls (AUC=0.89), underscoring their association with cardiomyopathy.

CONCLUSIONS: We identified 3 specific inflammatory proteins as candidate plasma biomarkers for ACMP in long-term childhood cancer survivors and demonstrated protein overlap with dilated cardiomyopathy.

Key Words: anthracycline-related cardiomyopathy ■ biomarkers ■ cancer therapy–related cardiac dysfunction ■ cardio-oncology ■ chemokine ligands ■ childhood cancer survivors

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CLINICAL PERSPECTIVE

What Is New?

- Candidate inflammatory biomarkers for diagnosing anthracycline cardiomyopathy in childhood cancer survivors were identified.
- These biomarkers were also dysregulated in patients with genetically determined dilated cardiomyopathy.

What Are the Clinical Implications?

- As findings were exploratory, the diagnostic value of the identified plasma biomarkers should be confirmed in a larger cohort.

Nonstandard Abbreviations and Acronyms

ACMP	anthracycline-related cardiomyopathy
CCL19	C-C-motif chemokine ligand 19
CCL20	C-C-motif chemokine ligand 20
CCS	childhood cancer survivor
DCM	dilated cardiomyopathy
PSPD	pulmonary surfactant protein D

Childhood cancer survivors (CCSs) treated with anthracyclines, mitoxantrone, and/or chest-directed radiotherapy are at high risk for heart failure, with 11.6% developing heart failure within 40 years from cancer diagnosis.[1] Because of the high risk of heart failure and the potential benefits of early detection and treatment of cardiac dysfunction, life-long echocardiographic surveillance is currently recommended.[2]

Blood biomarkers with a high sensitivity and sufficient specificity could be useful as a time-efficient and cost-effective triage test, where survivors with a normal biomarker level can safely be deferred from further workup with an echocardiogram.[3] Blood biomarkers could also be used in addition to an echocardiogram to improve its diagnostic accuracy or for prognostic reasons. Up until now, NT-proBNP (N-terminal pro-B-type natriuretic peptide) and troponins have been studied but lack sufficient sensitivity to detect asymptomatic left ventricular (LV) dysfunction in long-term CCSs and are therefore not recommended for surveillance purposes.[2,4] Few studies have used plasma proteomics to identify additional biomarkers that might improve detection of anthracycline-related cardiomyopathy (ACMP), some of which were in patients with pediatric cancer in the short-term phase[5] and others that assessed the value of natriuretic peptides, cardiac troponin T, soluble suppression of tumorigenicity-2, and galectin-3 carnitine, in long-term CCSs.[6,7] However, most of the studies using larger-scale proteomic analyses have been conducted in adult patients with cancer during or shortly after anthracycline treatment.[8–10]

In this discovery case-control study in the DCCSS LATER 2 CARD (Dutch Childhood Cancer Survivor Study, LATER cohort, part 2, cardiology), we sought to identify candidate plasma proteins that would be able to discriminate ACMP cases from anthracycline-treated controls with normal LV function, using a large biomarker panel consisting of markers for ventricular wall stress, oxidative stress, inflammation, cellular adhesion, apoptosis, and extracellular matrix remodeling. To further support the hypothesis that the selected markers are associated with cardiomyopathy and not with a systemic effect of anthracyclines in those sensitive to them, we compared plasma levels of the proteins that we identified in ACMP with plasma levels in patients with genetically determined dilated cardiomyopathy (DCM).

METHODS

The data that support the findings of this study are available from the corresponding author on reasonable request.

Study Design and Study Participants

We conducted a cross-sectional case–control study as part of the DCCSS LATER 2 CARD. The design of this cohort study has been published.[11] In short, DCCSS LATER 2 CARD is a multicenter study in 5-year CCSs diagnosed with a malignancy before the age of 18 years and between January 1, 1963, and December 31, 2001, who were treated with (potentially) cardiotoxic cancer treatments. Participants visited the outpatient clinic between February 2016 and February 2020 for questionnaires, physical examination, blood sampling, electrocardiography, and echocardiography. For primary analysis of this biomarker case-control study, we included CCSs treated with anthracyclines or mitoxantrone, with or without concomitant chest-directed radiotherapy. CCSs with congenital heart disease were excluded. The first 30 ACMP cases (defined as a LV ejection fraction [LVEF] <45%) included in the DCCSS LATER 2 CARD were selected and matched with 30 anthracycline-treated controls without ACMP (defined as LVEF ≥53% without grade ≥2 diastolic dysfunction or valvular disease) (Figure 1). We chose to include these first 30 ACMP cases (1) because the inclusion for the DCCSS LATER 2 CARD was still ongoing at time of this case-control study and (2) to ensure a random selection of ACMP cases. Controls were propensity score matched to ACMP cases, where the propensity score was estimated with logistic regression of

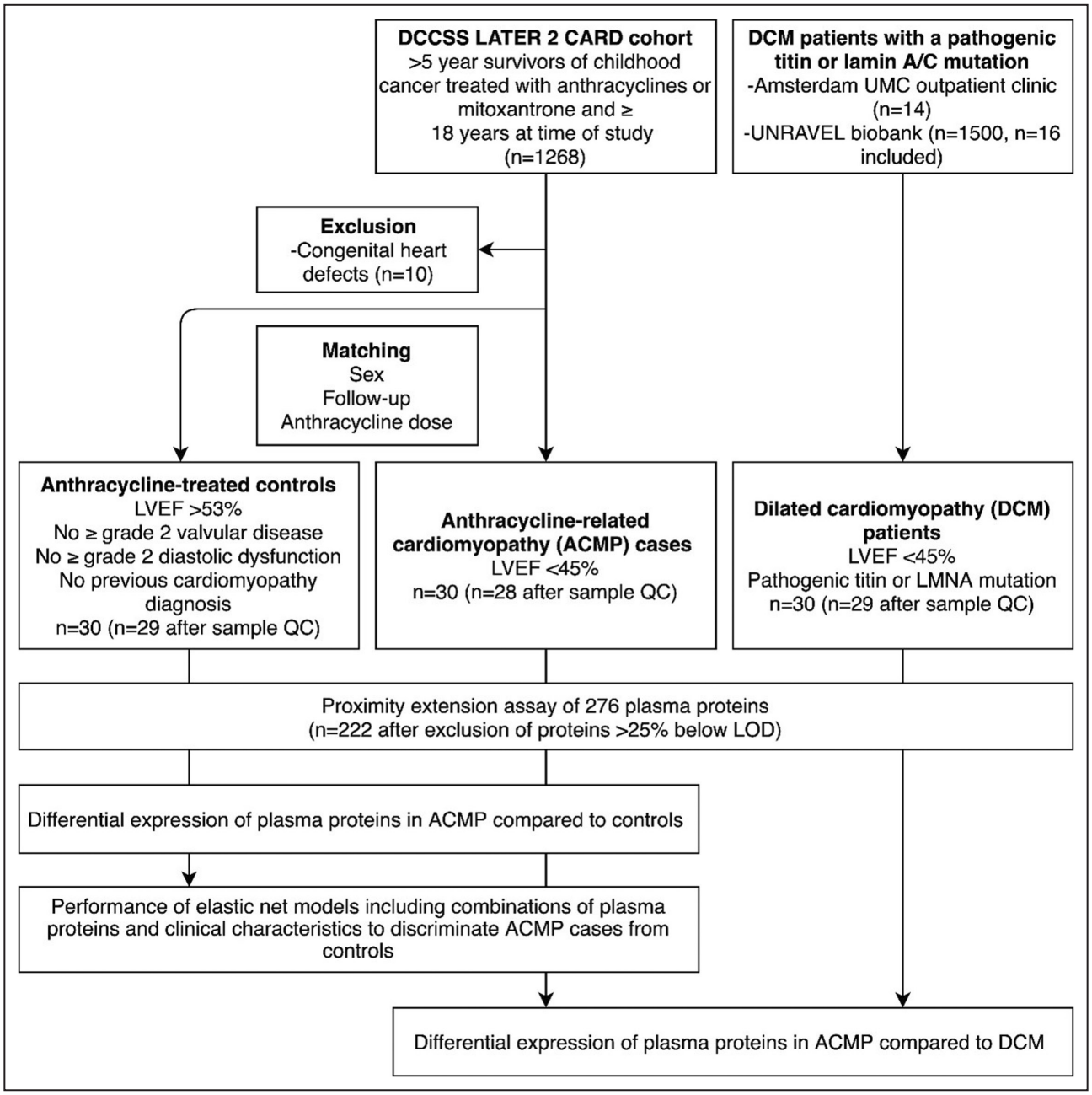

Figure 1. Study design of the LATER CARD biomarker case-control study.
ACMP indicates anthracycline-related cardiomyopathy; DCCSS LATER 2 CARD, Dutch Childhood Cancer Survivor Study, LATER cohort, part 2, cardiology; DCM, dilated cardiomyopathy; LMNA, lamin A/C; LOD, limit of detection; LVEF, left ventricular ejection fraction; QC, quality control; and UMC, University Medical Center.

case status on the covariates sex, time since cancer diagnosis, and cumulative anthracycline/mitoxantrone dose (calculated as doxorubicin equivalents).[12] For secondary analysis, we included patients with DCM with LVEF <45% and a pathogenic titin truncating variant or a lamin A/C mutation from the Amsterdam University Medical Center and the UNRAVEL database at the University Medical Center Utrecht in the Netherlands.[13] These patients with DCM were included to test whether the plasma proteins selected to be discriminative for ACMP would also be discriminative for DCM.

Ethical Approval

The investigation conforms with the principles outlined in the Declaration of Helsinki. The LATER CARD study was approved by the medical ethics board of all participating centers and included blood biobanking for future analysis. The medical ethics board of

the Amsterdam University Medical Center and the University Medical Center Utrecht approved the biobanking of blood samples from patients with DCM. UNRAVEL follows the code of conduct and the use of data in health research and has been approved by the biobank board of the medical ethics committee of the University Medical Center Utrecht.[13] Informed consent was obtained from all participants.

Data Collection

Patient and cancer treatment characteristics were obtained from the central database of the LATER study (ACMP cases and controls) and from medical records (patients with DCM). Cumulative anthracycline dose was calculated as the doxorubicin equivalent dose.[12] Cardiac medication use, heart failure symptoms, and modifiable cardiovascular risk factors were obtained from questionnaires (CCSs) and medical records (patients with DCM). In ACMP cases and controls, self-reported heart failure and cardiovascular risk factors were considered present if patients reported the use of medications for the condition. All participants underwent a physical examination at time of blood sampling to obtain body mass index and blood pressure. Fasting citrate blood samples were obtained from participants within 6 months from the qualifying echocardiogram (86% of samples were obtained at the same day). Samples were centrifuged at 3000*g* for 10 minutes, stored within 1 hour at −80 °C, and shipped on dry ice to the central biobank. In ACMP cases and controls, echocardiographic parameters, including biplane LVEF, were measured by a core laboratory blinded for clinical characteristics.[14] In patients with DCM, echocardiographic parameters were obtained from medical records.

Plasma Protein Measurements

Plasma levels of 276 proteins were measured with a proximity extension assay in 3 μL of citrate plasma per patient using the Cardiovascular III, Organ Damage, and Inflammation panels from Olink Proteomics (Uppsala, Sweden). We chose these 3 panels because of their known association with cardiovascular disease, apoptosis, inflammation, and remodeling. Panel validation data can be found at Olink.com. The proximity extension assay is based on pairs of antibodies that are linked to proximity probes. On binding of the antibody pair to their target protein, the probes are brought in proximity and are extended by a DNA polymerase that can subsequently be detected with real-time polymerase chain reaction. Protein levels are expressed as normalized protein expression values, which are relative units expressed on the log2 scale, where a 1-unit higher normalized protein expression value represents a doubling of protein concentration. Study groups were randomly distributed over the plate. Samples that did not pass Olink quality control (>0.3 normalized protein expression median deviation from the internal control) were excluded. Protein levels below the linear limit of detection were replaced with the estimated normalized protein expression value at the nonlinear part of the calibration curve if <25% was below limit of detection. Proteins with ≥25% below limit of detection were excluded (n=54 proteins). These 54 proteins were not exclusively expressed in one of the study groups. Two polymerase chain reaction readout failures were median imputed (macrophage-capping protein in 1 ACMP case and transmembrane serine protease 15 in 1 DCM case).

Statistical Analysis

Descriptive Statistics

Continuous variables were checked for normality by visual inspection using histograms and are presented as mean±SD for normally distributed variables and as median with range for skewed variables. Categorical variables are presented as numbers and percentages. Continuous variables were compared with the *t* test or Wilcoxon signed-rank test, where appropriate. Categorical variables were compared with the χ^2 test or the Fisher exact test (when expected counts were <5). All analyses were conducted in R version 3.6.1.

Primary Analysis: ACMP Cases Compared With Anthracycline-Treated Controls

Differential expression of plasma proteins in ACMP cases compared with controls was tested with multivariable linear regression models, estimating log2 fold changes. Models were adjusted for sex, time since cancer diagnosis, anthracycline/mitoxantrone dose, and chest-directed radiotherapy dose. *P* values were corrected for multiple testing with the *q* value, which can be interpreted as a false discovery rate.[15] A *q* value <0.1 was considered statistically significant. In sensitivity analyses, models were adjusted for NT-proBNP levels and were restricted to ACMP cases without self-reported heart failure.

Elastic net logistic regression was used to identify a combination of plasma proteins best discriminating ACMP cases from controls. The elastic net simultaneously performs variable selection and shrinkage of coefficients of a large number of predictors and is relatively robust to collinear predictors.[16] Predictors entered in the elastic net were all plasma proteins and the clinical characteristics sex, age at cancer diagnosis, time since cancer diagnosis, anthracycline/mitoxantrone dose, and chest-directed radiotherapy dose. NT-proBNP was not subjected to selection and coefficient shrinkage, as we aimed to find proteins independent of NT-proBNP.

Predictors were standardized to have a mean of 0 and an SD of 1. We used a nested cross-validation strategy to test performance of the elastic net on data not seen during training of the model. Matched case-control pairs were divided into a training set and test set with 10×10-fold cross-validation. The elastic net parameters (α and λ) were optimized on the training set with 5-fold cross-validation, and the parameter combination that was within 1 SE from the optimal area under the receiver operating characteristic curve (AUC) was chosen. Median model performance over the cross-validation folds was evaluated on the test set with the AUC, and with sensitivity and specificity at the threshold maximizing the sum of sensitivity and specificity. Proteins selected in ≥40% of the cross-validation folds were considered important. Performance of the elastic net model, including all proteins and clinical characteristics, was compared with an elastic net model including only NT-proBNP and clinical characteristics. AUCs were compared, and 95% CIs were calculated with the Wilcoxon signed-rank test. In additional analysis in asymptomatic CCSs, elastic net models were also fitted in ACMP cases without heart failure and their matched controls only.

Secondary Analysis: ACMP Cases Compared With Patients With DCM

Differential expression of plasma proteins in ACMP cases compared with patients with DCM was tested with multivariable linear regression models, adjusted for sex, age at blood sample, and LVEF. A *q* value <0.1 was considered statistically significant. The group of proteins discriminating ACMP cases from controls was tested for their ability to also discriminate patients with DCM from controls, with the elastic net using the same modeling steps as described for the primary analysis.

RESULTS

Patient Characteristics

After exclusion of 4 samples that did not pass quality control, we included 28 ACMP cases, 29 matched anthracycline-treated controls, and 29 patients with DCM in this study (Figure 1). Characteristics of the participants are outlined in Table 1. ACMP cases and controls were successfully matched with respect to sex (46.4% and 48.3% men, respectively; *P*=1.0), time since cancer diagnosis (median, 25.4 and 29.4 years, respectively; *P*=0.107), and cumulative anthracycline dose (median, 360.0 and 300.0 mg/m^2, respectively; *P*=0.626). Compared with ACMP cases, patients with DCM were older (median, 37.6 and 56.0 years, respectively; *P*<0.001) and were more frequently men (46.4% and 82.8%; *P*=0.006). Mean LVEF in ACMP cases was 40.6±5.8%, versus 58.1±3.2% in controls, and was lowest in patients with DMC (37.0±7.5%). Cardiac medications were used by all of the patients with DCM, by 16 (57.1%) of the ACMP cases, and by 3 (10.3%) of the controls. Heart failure was reported by 8 (28.6%) of the ACMP cases and 22 (75.9%) of the patients with DCM. Hypertension, diabetes, and dyslipidemia were reported by a minority of CCSs and patients with DCM. Characteristics of ACMP cases without heart failure compared with matched controls are presented in Tables S1 through S3.

Primary Analysis: ACMP Cases Compared With Anthracycline-Treated Controls

Differential Expression of Plasma Proteins in ACMP Cases Compared With Controls

In multivariable linear regression analyses, adjusted for sex, time since cancer diagnosis, anthracycline dose, and chest-directed radiotherapy dose, plasma levels of NT-proBNP, C-C motif chemokine 19 (CCL19), pleiotrophin, C-C motif chemokine 20 (CCL20), and PSPD (pulmonary surfactant protein D) were significantly higher in ACMP cases compared with controls (*q* value <0.1; Figure 2 and Table S2). When we additionally adjusted for NT-proBNP levels, CCL19, CCL20, PSPD, and pleiotrophin remained significantly upregulated (Table S3). When we performed the analysis in 20 ACMP cases without heart failure (reflecting a surveillance population) and their matched controls, NT-proBNP was not significantly upregulated (*P*=0.231), whereas the other 4 proteins remained significantly upregulated (Table S3). Biomarkers that have previously been shown to have diagnostic or prognostic value in patients with heart failure, including soluble suppression of tumorigenicity-2, galectin-3, troponin I, tumor necrosis factor, interleukin-6, osteopontin, and tumor necrosis factor receptor superfamily member 6, were not differentially expressed in ACMP cases compared with controls (all *q* values >0.1; Table S2).

Discriminative Plasma Proteins Identified With Elastic Net

The elastic net model trained on all proteins and clinical characteristics selected 45 proteins in >40% of the elastic net cross-validation folds, which indicates they are potentially important in discriminating ACMP cases from controls (Figure 2 and Table S2). Next to NT-proBNP, which was not subjected to selection, this panel mainly consisted of inflammatory markers, such as CCL19, CCL20, CCL25 (C-C-motif chemokine ligand 25), and PSPD, and adhesion molecules, such as chitinase 3 like 1, P selectin, Ephrin type-B receptor 4, and intracellular adhesion molecule 2. All proteins that were significantly upregulated in the multivariable linear regression analysis were also selected by the elastic net, except for

Table 1. Characteristics of the Patients With ACMP, Anthracycline-Treated Controls, and Patients With DCM

Characteristic	Controls (n=29)	Patients with ACMP (n=28)	Patients with DCM (n=29)	*P* value for ACMP-controls	*P* value for DCM-ACMP
Male sex	14 (48.3)	13 (46.4)	24 (82.8)	1	0.006
Age at cancer diagnosis, y	7.97 (4.03–11.82)	8.30 (3.52– 13.11)	NA	0.936	NA
Age at blood sampling, y	43.30 (34.71–46.97)	37.63 (30.26–45.30)	56.00 (39.00–64.00)	0.271	<0.001
Time since cancer diagnosis, y	29.44 (24.13–32.33)	25.35 (18.85–30.21)	NA	0.107	NA
Primary cancer diagnosis			NA	0.671	
Leukemias	8 (27.6)	5 (17.9)	NA		
Lymphomas	11 (37.9)	10 (35.7)	NA		
Neuroblastoma	0 (0.0)	1 (3.6)	NA		
Renal tumors	3 (10.3)	2 (7.1)	NA		
Bone tumors	3 (10.3)	7 (25.0)	NA		
Soft tissue sarcomas	3 (10.3)	3 (10.7)	NA		
Germ cell tumors	1 (3.4)	0 (0.0)	NA		
Anthracyclines	27 (93.1)	23 (82.1)	NA	0.253	NA
Anthracycline cumulative dose, mg/m^2*	300.00 (216.00–400.00)	360.00 (169.00–462.50)	NA	0.626	NA
Mitoxantrone	7 (24.1)	7 (25.0)	NA	1	NA
Mitoxantrone dose, mg/m^2	50.00 (40.00–102.00)	120.00 (50.00–121.00)	NA	0.299	NA
Chest RT	2 (20.0)	3 (20.0)	NA	0.670	NA
Chest RT cumulative dose, Gy	20.00 (20.00–20.00)	25.00 (19.50–37.50)	NA	0.554	NA
DCM-causing mutation				NA	NA
Titin	NA	NA	23 (79.3)	NA	NA
Lamin A/C	NA	NA	6 (21.7)	NA	NA
Heart failure	0 (0.0)	8 (28.6)	22 (75.9)	0.006	0.001
Cardiac medication(s)	3 (10.3)	16 (57.1)	29 (100)	0.001	<0.001
Hypercholesterolemia	1 (3.4)	2 (7.1)	9 (31.0)	0.611	0.051
Diabetes	0 (0.0)	0 (0.0)	2 (6.9)	NA	0.491
Hypertension	1 (3.4)	2 (7.1)	6 (20.7)	0.611	0.253
Systolic blood pressure, mmHg	125.5 (16.1)	117.1 (19.8)	114.9 (18.4)	0.086	0.669
Diastolic blood pressure, mmHg	78.8 (10.1)	72.9 (15.5)	72.7 (13.3)	0.093	0.962
Heart rate, bpm	69.2 (14.4)	71.2 (12.6)	69.3 (8.3)	0.582	0.509
BMI, kg/m^2	25.1 (4.6)	25.0 (4.9)	26.3 (4.2)	0.926	0.301
Biplane LVEF, %	58.1 (3.2)	40.6 (5.8)	37.0 (7.5)	<0.001	0.045
LVIDd, cm	4.6 (0.6)	5.2 (0.7)	6.2 (0.8)	0.003	<0.001

Categorical values are presented as number (percentage). Continuous values are presented as median (interquartile range). ACMP indicates anthracycline-related cardiomyopathy; BMI, body mass index; bpm, beats per minute; Chest RT, chest-directed radiotherapy; DCM, dilated cardiomyopathy; LVEF, left ventricular ejection fraction; LVIDd, left ventricular end-diastolic diameter; and NA, not applicable.

*Doxorubicin equivalents (daunorubicin*0.6+epirubicin*0.8+idarubicin*3).

pleiotrophin, which was selected in 18% of the folds, suggesting pleiotrophin does not contribute much to the discrimination when combined with other proteins.

Performance of the Elastic Net in Discriminating ACMP Cases From Controls

The elastic net model trained on all proteins and clinical characteristics had a cross-validated AUC of 0.78, a sensitivity of 87%, and a specificity of 78% (Table 2). Discrimination of this model was slightly but not significantly higher compared with an elastic net model trained on NT-proBNP and clinical characteristics only (AUC=0.75; *P*=0.766). To better reflect a surveillance population of asymptomatic CCSs, we repeated the analysis in 20 ACMP cases without self-reported heart failure and their matched controls (n=21). In this analysis, discrimination of the elastic net model trained on all proteins and clinical characteristics retained its discriminative value better compared with the elastic net trained on

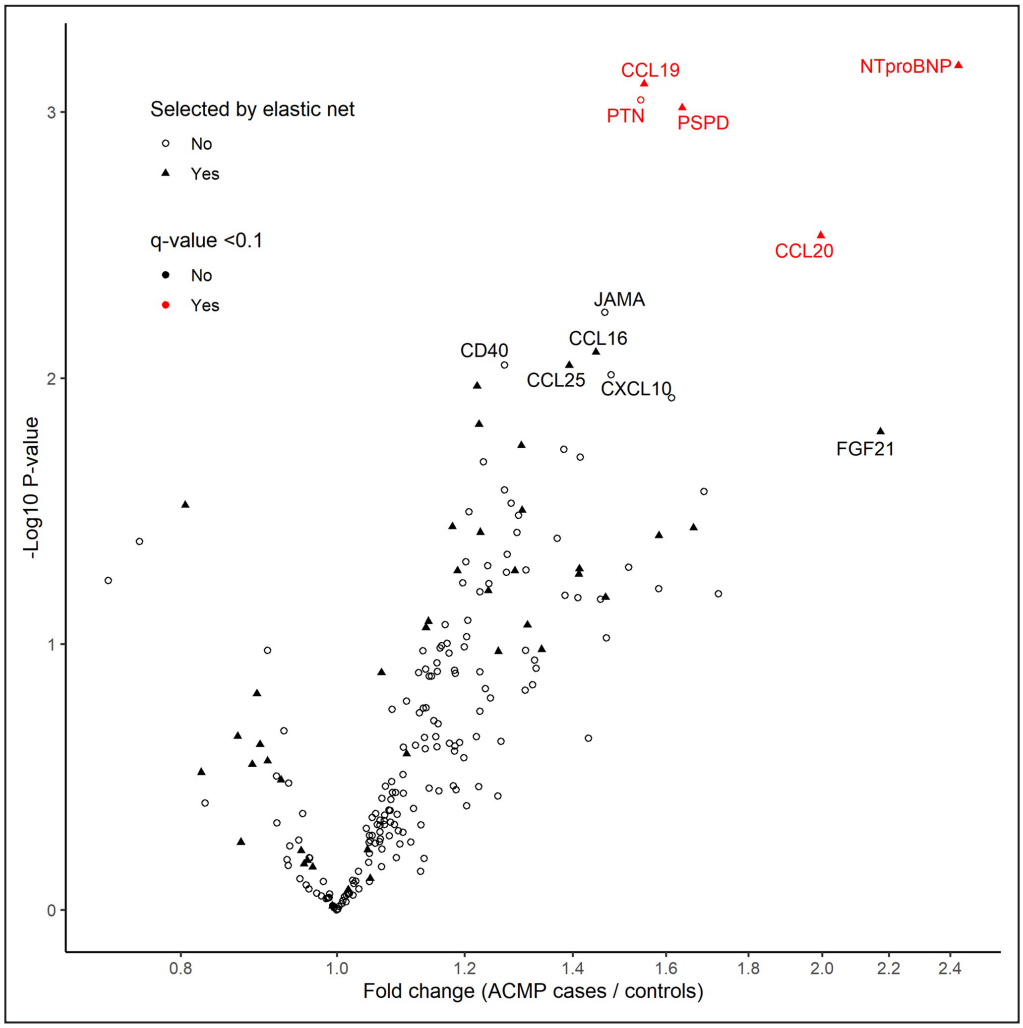

Figure 2. Volcano plot, showing fold changes (*x* axis) and *P* values (*y* axis) of 222 plasma proteins in anthracycline-related cardiomyopathy (ACMP) compared with matched anthracycline-treated controls.

Fold changes and *P* values were estimated with multivariable linear regression analysis, adjusted for sex, time since cancer diagnosis, anthracycline dose, and chest-directed radiotherapy dose. Significantly upregulated proteins (*q* value <0.1) are shown in red. Proteins selected by the elastic net in >40% of the cross-validation folds are shown as a triangle. CCL indicates C-C motif chemokine ligand; CD40, cluster of differentiation 40; CXCL10, C-X-C motif chemokine ligand 10; FGF21, fibroblast growth factor 21; JAMA, junctional adhesion molecule A; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PSPD, pulmonary surfactant protein D; and PTN, pleiotrophin.

NT-proBNP and clinical characteristics only (AUC=0.75 versus AUC=0.50; *P*=0.022) (Table 2). More important, CCL19, CCL20, and PSPD were also selected by the elastic net in >40% of the cross-validation folds in this analysis in ACMP cases without heart failure.

Secondary Analysis: Plasma Protein Expression in ACMP Cases Compared With Patients With DCM

In multivariable linear regression analyses adjusted for sex, age, and LVEF, none of the 5 upregulated proteins in ACMP cases compared with controls (NT-proBNP, CCL19, CCL20, PSPD, and pleiotrophin) were differentially expressed in ACMP cases compared with patients with genetically determined DCM (Table S2). Similar results were obtained when not adjusting the multivariable linear regression analysis for differences in LVEF between ACMP and DCM (NT-proBNP, CCL19, CCL20, PSPD, and pleiotrophin all had *P*>0.05). In the elastic net model, the 45 discriminative proteins for ACMP, including NT-proBNP, were also highly discriminative for DCM compared with controls (elastic net AUC=0.89), and the AUC remained high when excluding NT-proBNP from this protein panel (elastic net AUC=0.86).

Table 2. Cross-Validated Performance Measures of Elastic Net Models, Including Clinical Characteristics and Plasma Proteins, to Discriminate ACMP Cases From Anthracycline-Treated Controls

Performance measure	NT-proBNP+clinical characteristics*	All proteins+clinical characteristics*	Wilcoxon test *P* value
Main analysis in all participants (n=57)			
AUC (95% CI)	0.75 (0.71–0.80)	0.78 (0.72–0.83)	0.766
Sensitivity (95% CI)	0.86 (0.82–0.90)	0.87 (0.83–0.91)	…
Specificity (95% CI)	0.78 (0.82–0.90)	0.78 (0.72–0.84)	…
Analysis in asymptomatic cases without heart failure (n=41)			
AUC (95% CI)	0.50 (0.50–0.62)	0.75 (0.63–0.75)	0.022
Sensitivity (95% CI)	0.89 (0.84–0.93)	0.90 (0.86–0.94)	…
Specificity (95% CI)	0.61 (0.54–0.68)	0.69 (0.62–0.76)	…

Performance measures are reported for elastic net models fitted in all participants and in asymptomatic cases without heart failure. ACMP indicates anthracycline-related cardiomyopathy; AUC, area under the receiver operating characteristic curve; and NT-proBNP, N-terminal pro-B-type natriuretic peptide.

*Clinical characteristics included sex, age at cancer diagnosis, time since cancer diagnosis, anthracycline/mitoxantrone dose (doxorubicin equivalents), and chest-directed radiotherapy dose. Sensitivities and specificities are reported at the threshold maximizing the sum of sensitivity and specificity.

DISCUSSION

In this discovery case-control study, we identified 3 inflammatory proteins, CCL19, CCL20, and PSPD, as candidate plasma biomarkers for detection of ACMP in long-term CCSs, independently of clinical characteristics, such as anthracycline dose, independently of NT-proBNP levels, and independently of the presence of heart failure. Supporting the role of these proteins in detecting ACMP is their similarly increased presence in patients with genetically determined DCM. As our sample population is small, we regard the results as a promising finding that awaits confirmation in a larger cohort.

Previous studies in survivors of breast cancer treated with anthracyclines have also reported an association of inflammatory biomarkers with decreased LV function.[10,17] In one of the studies, at a mean of 11±5.5 years after treatment with anthracyclines and/or radiotherapy, 11 plasma proteins related to cardiovascular disease were associated with decreasing LVEFs that were still in the normal range (median LVEF, 58%; interquartile range, 55%–60%).[10] We confirm upregulation of one of these proteins, the inflammatory adipokine and chemokine retinoic acid receptor responder 2, which, in our study, showed an association with ACMP in CCSs that did not surpass the multiple testing threshold in our study (Table S2). In another study in patients with breast cancer with more severely depressed LVEF (ie, ≤40%; n=5) compared with anthracycline-treated controls (n=10), a transcriptomics analysis demonstrated differential expression in genes related to lymphocyte activation and B-cell receptor signaling,[17] which is interesting in relation to our study because CCL19 and CCL20 are chemotactic for T and B cells. In accordance with previous studies in CCSs and breast cancer survivors, we show that galectin-3, soluble suppression of tumorigenicity-2, interleukin-6, tumor necrosis factor, and troponin I are not differentially expressed in ACMP cases compared with controls.[4,6,10] This finding is interesting because these biomarkers have been shown to be predictive of heart failure in the general population but may be related to other causes of heart failure.[18–20]

Inclusion of a third group of patients with genetically determined DCM in secondary analysis allowed us to study potential overlap in biomarker profile in ACMP compared with DCM. Interestingly, we did not find significant differences between ACMP cases and patients with DCM in plasma levels of the proteins upregulated in ACMP and most proteins identified with elastic net. This overlap in upregulation strengthens the hypothesis that these proteins are associated with cardiomyopathy and do not reflect a systemic sensitivity for anthracyclines.

Despite the association of CCL19, CCL20, and PSPD with cardiomyopathy in our study, the cellular source(s) contributing to the elevated plasma levels remain uncertain. CCL19 and CCL20 are chemokines secreted by immune cells and cardiac fibroblasts in the heart under the influence of proinflammatory cytokines, but also by peripheral immune cells residing in lymph nodes.[21] PSPD is an innate immune pattern recognition collection expressed in the myocardium, but also in the lung and the vascular endothelium.[22] In addition, previous studies in patients with heart failure have demonstrated discrepancies between plasma and myocardial protein levels of other inflammatory proteins, such as galectin-3, growth differentiation factor 15, tumor necrosis factor, and interleukin-6.[23,24] It is therefore likely that the elevated plasma levels found in our study in ACMP and DCM are to a large extent produced by extracardiac sources, such as peripheral immune cells or vascular endothelial cells.

As for clinical utility, it is promising that the biomarker panel had a high sensitivity, while maintaining sufficient specificity to limit false positives, even in those patients in whom NT-proBNP could not discriminate between

ACMP and controls. However, clinical utility of the identified plasma biomarker levels for the diagnosis of LV dysfunction may be better assessed in larger cohorts and will be the subject of ongoing research.

Limitations

One may question the generalizability of our results to a surveillance population because we defined cardiomyopathy as an LVEF <45% and because 8 patients already had symptoms of heart failure. However, the LVEF thresholds of <45% for ACMP and ≥53% for controls made it possible to make a clear distinction between ACMP and controls, which was of importance in this discovery study. We also replicated the results in ACMP cases without heart failure. The patients with DCM were not matched to the patients with ACMP. However, we adjusted the analyses for differences in sex, age, and LVEF. This study should be seen as exploratory, with the purpose to select promising biomarker candidates to further study for their diagnostic value to detect asymptomatic cardiomyopathy in the DCCSS LATER 2 CARD cohort.[11]

CONCLUSIONS

We identified the chemokine ligands CCL19 and CCL20 and the innate immune system marker PSPD as candidate diagnostic plasma biomarkers for anthracycline-related cardiomyopathy in long-term CCSs. By demonstrating overlap in expression of these biomarkers with those found in patients with genetically determined DCM, the hypothesis is strengthened that these protein markers are related to cardiac dysfunction. Further exploration and validation of the findings in a larger cohort are still needed.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Tables S1–S3

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SUPPLEMENTAL MATERIAL

Table S1. Patient characteristics of ACMP cases without heart failure and matched controls.

Characteristic	Controls (n=21)	ACMP cases (n=20)
Male sex	8 (38.1)	11 (55.0)
Age at cancer diagnosis	7.39 [5.32, 10.40]	8.54 [3.10, 12.83]
Age at blood sampling	39.52 [32.81, 46.97]	34.48 [30.00, 43.41]
Time since cancer diagnosis	28.09 [22.22, 33.21]	21.44 [16.21, 27.06]
Anthracyclines	19 (90.5)	15 (75.0)
Anthracyclines cumulative dose*, mg/m^2	288.60 [186.00, 400.00]	234.00 [72.00, 472.50]
Mitoxantrone	7 (33.3)	7 (35.0)
Mitoxantrone dose, mg/m^2	50.00 [40.00, 102.00]	120.00 [50.00, 121.00]
Chest RT	2 (9.5)	1 (5.0)
Chest RT cumulative dose, Gray	20.00 [20.00, 20.00]	50.00 [50.00, 50.00]
Cardiac medication(s)	2 (9.5)	8 (40.0)
Hypercholesterolemia	1 (4.8)	1 (5.0)
Diabetes	0 (0.0)	0 (0.0)
Hypertension	0 (0.0)	1 (5.0)
Systolic blood pressure, mmHg	124.86 (15.92)	121.95 (21.07)
Diastolic blood pressure, mmHg	77.95 (10.26)	75.85 (16.85)
Heart rate, bpm	69.66 (14.93)	74.54 (12.31)
BMI, kg/m^2	26.36 (4.59)	25.59 (5.24)
Biplane LVEF, %	57.66 (2.42)	41.28 (3.28)
LVIDd, cm	4.59 (0.61)	5.11 (0.68)

Categorical values are presented as number (%). Continuous values are presented as mean ± standard deviation (sd) or as median [inter-quartile range]. *Doxorubicin equivalents (Daunorubicin*0.6 + epirubicin*0.8 + idarubicin*3). BMI=Body mass index, LVEF=left ventricular ejection fraction, LVIDd=left ventricular end diastolic diameter, chest RT=chest-directed radiotherapy.

Table S2. Results of the multivariable linear regression analyses comparing plasma levels of 221 proteins between anthracycline-related cardiomyopathy (ACMP) cases and anthracycline-treated controls and between ACMP cases and dilated cardiomyopathy patients (DCM). Models were adjusted for sex, time since cancer diagnosis, anthracycline and chest-directed radiotherapy dose.

		ACMP - controls				ACMP - DCM		
Protein	**Uniprot ID**	**Fold change**	**p-value**	**q-value**	**Selected by elastic net**	**Fold change**	**p-value**	**q-value**
NT-proBNP	NA	2.429	0.001	0.036	Forced	1.711	0.054	0.125
CCL19	Q99731	1.550	0.001	0.036	Yes	1.254	0.140	0.239
PTN	P21246	1.543	0.001	0.036	No	1.345	0.079	0.159
PSP-D	P35247	1.637	0.001	0.036	Yes	1.345	0.060	0.130
CCL20	P78556	1.995	0.003	0.086	Yes	1.562	0.105	0.195
JAM-A	Q9Y624	1.466	0.006	0.139	No	1.290	0.162	0.260
CCL16	O15467	1.448	0.008	0.144	Yes	1.427	0.014	0.057
CD40	P25942	1.270	0.009	0.144	No	1.121	0.326	0.433
CCL25	O15444	1.393	0.009	0.144	Yes	1.666	0.001	0.013
CXCL10	P02778	1.479	0.010	0.144	No	1.182	0.386	0.481
RARRES2	Q99969	1.222	0.011	0.144	Yes	1.376	0.000	0.005
CXCL9	Q07325	1.613	0.012	0.146	No	1.336	0.152	0.252
IL-6RA	P08887	1.225	0.015	0.168	Yes	1.190	0.045	0.112
FGF-21	Q9NSA1	2.173	0.016	0.168	Yes	3.666	0.001	0.017
SELP	P16109	1.301	0.018	0.169	Yes	1.352	0.024	0.074
CXCL11	O14625	1.383	0.018	0.169	No	1.230	0.213	0.313
GDF-15	Q99988	1.415	0.020	0.169	No	1.254	0.193	0.297
VEGFA	P15692	1.233	0.021	0.169	No	1.188	0.071	0.150
NUCB2	P80303	1.270	0.026	0.189	No	1.411	0.004	0.024
CHIT1	Q13231	1.689	0.027	0.189	No	1.329	0.114	0.205
CXCL6	P80162	1.282	0.030	0.189	No	1.247	0.074	0.155
CA14	Q9ULX7	0.805	0.030	0.189	Yes	1.528	0.000	0.002
IL-17A	Q16552	1.303	0.031	0.189	Yes	0.979	0.894	0.911
ENTPD2	Q9Y5L3	1.207	0.032	0.189	No	1.127	0.166	0.261
TNFRSF9	Q07011	1.296	0.033	0.189	No	1.428	0.007	0.038
PON2	Q15165	1.179	0.036	0.189	Yes	1.273	0.013	0.057
SIRT2	Q8IXJ6	1.664	0.037	0.189	Yes	1.307	0.325	0.433
NOS3	P29474	1.227	0.038	0.189	Yes	0.974	0.840	0.876
STX8	Q9UNK0	1.293	0.038	0.189	No	1.137	0.382	0.481
AIFM1	O95831	1.584	0.039	0.189	Yes	1.154	0.584	0.672
CSTB	P04080	1.370	0.040	0.189	No	1.284	0.120	0.211
PON3	Q15166	0.754	0.041	0.189	No	1.165	0.250	0.360
IL8	P10145	1.275	0.046	0.189	No	1.285	0.060	0.130
MCP-1	P13500	1.202	0.049	0.189	No	1.330	0.005	0.027
t-PA	P00750	1.240	0.051	0.189	No	1.547	0.001	0.015
FABP4	P15090	1.517	0.051	0.189	No	1.194	0.415	0.503
MMP-1	P03956	1.414	0.052	0.189	Yes	1.223	0.351	0.453
CPB1	P15086	1.310	0.053	0.189	No	1.037	0.825	0.864

SERPINA9	Q86WD7	1.289	0.053	0.189	Yes	1.205	0.329	0.435
CTSD	P07339	1.188	0.053	0.189	Yes	1.345	0.003	0.021
IL-12B	P29460	1.274	0.054	0.189	No	1.027	0.869	0.897
EPO	P01588	1.413	0.055	0.189	Yes	1.176	0.437	0.516
CCL24	O00175	0.721	0.058	0.189	No	1.122	0.603	0.690
HGF	P14210	1.197	0.059	0.189	No	1.400	0.002	0.021
IGFBP-7	Q16270	1.242	0.059	0.189	No	1.560	0.001	0.017
IL6	P05231	1.583	0.062	0.189	No	1.555	0.091	0.174
MEPE	Q9NQ76	1.241	0.063	0.189	Yes	1.355	0.019	0.065
NT-3	P20783	1.226	0.064	0.189	No	1.145	0.246	0.357
4E-BP1	Q13541	1.724	0.065	0.189	No	1.088	0.799	0.841
vWF	P04275	1.385	0.066	0.189	No	1.385	0.047	0.112
CASP-3	P42574	1.467	0.067	0.189	Yes	1.261	0.343	0.445
STAMBP	O95630	1.411	0.067	0.189	No	1.262	0.258	0.368
YES1	P07947	1.457	0.068	0.189	No	0.899	0.652	0.717
CDCP1	Q9H5V8	1.205	0.081	0.220	No	1.602	0.000	0.005
LRP1	Q07954	1.140	0.082	0.220	Yes	1.224	0.021	0.068
TNF	P01375	1.167	0.084	0.220	No	1.143	0.163	0.260
MVK	Q03426	1.313	0.085	0.220	Yes	1.087	0.633	0.709
Flt3L	P49771	1.136	0.087	0.221	Yes	1.275	0.014	0.057
BAMBI	Q13145	1.203	0.094	0.228	No	1.258	0.114	0.205
MAP4K5	Q9Y4K4	1.469	0.095	0.228	No	0.894	0.650	0.717
U-PAR	Q03405	1.170	0.099	0.228	No	1.338	0.004	0.026
GP6	Q9HCN6	1.161	0.102	0.228	No	1.105	0.372	0.471
MAEA	Q7L5Y9	1.199	0.103	0.228	No	1.093	0.636	0.709
MCP-1	P13500	1.159	0.103	0.228	No	1.310	0.006	0.032
ST1A1	P50225	1.340	0.105	0.228	Yes	1.188	0.430	0.514
PTPRJ	Q12913	1.309	0.105	0.228	No	1.375	0.075	0.156
DNER	Q8NFT8	0.906	0.106	0.228	No	1.132	0.106	0.195
uPA	P00749	1.131	0.106	0.228	No	1.358	0.000	0.005
TRANCE	O14788	1.259	0.107	0.228	Yes	1.238	0.146	0.248
PAI	P05121	1.326	0.115	0.238	No	1.794	0.003	0.021
PECAM-1	P16284	1.154	0.118	0.238	No	1.227	0.064	0.137
SCGB3A2	Q96PL1	1.329	0.123	0.238	No	1.291	0.195	0.298
LAP TGF-beta-1	P01137	1.135	0.124	0.238	No	1.351	0.002	0.018
IL2-RA	P01589	1.183	0.125	0.238	No	1.231	0.100	0.188
PRKRA	O75569	1.154	0.127	0.238	No	1.141	0.200	0.302
CCL15	Q16663	1.226	0.127	0.238	No	1.313	0.079	0.159
PD-L1	Q9NZQ7	1.124	0.128	0.238	No	1.192	0.063	0.136
RARRES1	P49788	1.066	0.128	0.238	Yes	1.064	0.211	0.313
PI3	P19957	1.184	0.129	0.238	No	1.321	0.017	0.062
TNFRSF14	Q92956	1.145	0.132	0.238	No	1.290	0.011	0.049
TIGAR	Q9NQ88	1.141	0.132	0.238	No	1.146	0.336	0.441
AXIN1	O15169	1.322	0.142	0.253	No	0.993	0.973	0.973
CASP-8	Q14790	1.236	0.147	0.259	No	1.979	0.000	0.002

IL10	P22301	1.309	0.149	0.260	No	1.423	0.105	0.195
TWEAK	O43508	0.892	0.154	0.265	Yes	1.045	0.609	0.693
LAT2	Q9GZY6	1.245	0.159	0.271	No	0.874	0.437	0.516
CD244	Q9BZW8	1.104	0.164	0.275	No	1.238	0.009	0.044
PTK7	Q13308	1.136	0.174	0.286	No	1.248	0.056	0.129
TNFSF13B	Q9Y275	1.131	0.174	0.286	No	1.235	0.015	0.058
CSF-1	P09603	1.082	0.176	0.286	No	1.146	0.047	0.112
INPPL1	O15357	1.227	0.179	0.288	No	1.082	0.656	0.717
CCL11	P51671	1.125	0.181	0.288	No	1.207	0.080	0.159
FOXO1	Q12778	1.149	0.194	0.305	No	1.105	0.415	0.503
TOP2B	Q02880	1.155	0.199	0.310	No	1.297	0.026	0.078
EGFR	P00533	0.927	0.212	0.326	No	1.175	0.003	0.021
TNFRSF10C	O14798	0.868	0.222	0.331	Yes	1.126	0.310	0.420
PLIN1	O60240	1.220	0.223	0.331	No	1.266	0.165	0.261
FABP9	Q0Z7S8	1.152	0.223	0.331	No	1.214	0.150	0.250
CD163	Q86VB7	1.133	0.224	0.331	No	1.456	0.004	0.025
PVALB	P20472	1.432	0.226	0.331	No	1.178	0.615	0.697
BANK1	Q8NDB2	1.264	0.232	0.331	No	1.023	0.912	0.922
KIM1	Q96D42	1.191	0.234	0.331	No	1.587	0.020	0.065
BID	P55957	1.174	0.236	0.331	No	1.235	0.116	0.208
CX3CL1	P78423	0.896	0.239	0.331	Yes	1.134	0.262	0.371
KLK6	Q92876	1.119	0.240	0.331	No	1.391	0.002	0.018
SLAMF1	Q13291	1.184	0.242	0.331	No	1.400	0.021	0.068
MB	P02144	1.154	0.243	0.331	No	1.237	0.162	0.260
FAS	P25445	1.099	0.244	0.331	No	1.334	0.002	0.018
TNF-R1	P19438	1.134	0.248	0.333	No	1.413	0.005	0.027
CPA1	P15085	1.183	0.253	0.337	No	1.171	0.393	0.485
TFPI	P10646	1.105	0.259	0.342	Yes	1.434	0.000	0.009
CAPG	P40121	1.198	0.268	0.350	No	1.741	0.001	0.013
SCF	P21583	0.906	0.275	0.357	Yes	1.168	0.191	0.296
TR	P02786	0.886	0.284	0.365	Yes	1.225	0.083	0.161
FOSB	P53539	0.824	0.304	0.387	Yes	1.071	0.646	0.717
ST2	Q01638	1.099	0.310	0.391	No	1.595	0.000	0.003
CNTN1	Q12860	0.917	0.314	0.393	No	1.244	0.011	0.050
CA12	O43570	0.923	0.324	0.403	Yes	1.238	0.020	0.065
GRN	P28799	1.081	0.329	0.405	No	1.304	0.002	0.021
HPGDS	O60760	0.933	0.333	0.407	No	1.145	0.096	0.182
IL-17C	Q9P0M4	1.181	0.341	0.409	No	0.809	0.341	0.445
CD5	P06127	1.071	0.342	0.409	No	1.169	0.079	0.159
TFF3	Q07654	1.224	0.343	0.409	No	1.068	0.750	0.800
OPN	P10451	1.141	0.349	0.412	No	1.353	0.060	0.130
NPPC	P23582	1.186	0.353	0.414	No	1.185	0.456	0.535
CXCL1	P09341	1.157	0.356	0.414	No	0.854	0.427	0.512
IL-18BP	O95998	1.083	0.361	0.414	No	1.360	0.003	0.021
CALR	P27797	1.087	0.362	0.414	No	0.891	0.310	0.420

CTSZ	Q9UBR2	1.100	0.364	0.414	No	1.326	0.016	0.060
IFN-gamma	P01579	1.258	0.373	0.420	No	1.997	0.004	0.025
PCSK9	Q8NBP7	1.066	0.380	0.425	No	1.395	0.000	0.003
EGFL7	Q9UHF1	1.080	0.384	0.427	No	1.113	0.304	0.416
Ep-CAM	P16422	0.828	0.396	0.437	No	1.728	0.018	0.064
AMN	Q9BXJ7	1.203	0.405	0.443	No	1.095	0.496	0.576
SELE	P16581	1.115	0.415	0.449	No	1.400	0.036	0.098
CXCL16	Q9H2A7	1.077	0.420	0.449	No	1.338	0.003	0.021
LTBR	P36941	1.079	0.422	0.449	No	1.194	0.121	0.211
AP-N	P15144	1.078	0.423	0.449	No	1.250	0.018	0.064
ITGB2	P05107	1.057	0.434	0.454	No	1.255	0.007	0.038
PLXDC1	Q8IUK5	0.952	0.434	0.454	No	1.079	0.371	0.471
PDGF subunit A	P04085	1.089	0.437	0.454	No	1.070	0.625	0.704
PLC	P98160	1.071	0.440	0.454	No	1.221	0.040	0.104
LIF-R	P42702	1.051	0.448	0.460	No	1.270	0.003	0.021
IL-10RB	Q08334	1.064	0.459	0.463	No	1.339	0.001	0.017
ADA	P00813	1.070	0.460	0.463	No	0.985	0.879	0.900
TNF-R2	P20333	1.079	0.467	0.463	No	1.290	0.036	0.098
MCP-4	Q99616	0.918	0.470	0.463	No	1.116	0.398	0.489
Gal-4	P56470	1.085	0.477	0.463	No	1.318	0.037	0.099
FGR	P09769	1.071	0.477	0.463	No	0.751	0.009	0.044
PGF	P49763	1.059	0.477	0.463	No	1.217	0.035	0.098
CCL3	P10147	1.127	0.478	0.463	No	1.457	0.008	0.040
CDH5	P33151	1.063	0.480	0.463	No	1.160	0.080	0.159
GALNT10	Q86SR1	1.043	0.492	0.472	No	1.202	0.014	0.057
MCP-2	P80075	1.092	0.503	0.479	No	1.360	0.048	0.112
SHPS-1	P78324	1.063	0.509	0.480	No	1.373	0.001	0.017
DSG4	Q86SJ6	1.099	0.509	0.480	No	1.199	0.157	0.256
TGF-alpha	P01135	1.047	0.523	0.486	No	1.065	0.390	0.484
BLM hydrolase	Q13867	1.052	0.524	0.486	No	1.119	0.198	0.302
PGLYRP1	O75594	1.078	0.526	0.486	No	1.323	0.032	0.092
CLEC1A	Q8NC01	1.064	0.540	0.493	No	1.279	0.029	0.086
MPO	P05164	0.947	0.546	0.493	No	1.148	0.131	0.226
ENAH	Q8N8S7	1.048	0.547	0.493	No	1.089	0.292	0.403
CD6	P30203	1.063	0.552	0.493	No	1.165	0.167	0.261
NCF2	P19878	1.111	0.554	0.493	No	0.930	0.713	0.764
IGFBP-1	P08833	0.872	0.556	0.493	Yes	2.391	0.001	0.017
IL-1RT1	P14778	1.047	0.557	0.493	No	1.307	0.001	0.017
CNTN2	Q02246	1.056	0.561	0.493	No	1.313	0.008	0.041
EN-RAGE	P80511	1.094	0.565	0.494	No	1.447	0.059	0.130
MMP-3	P08254	0.935	0.573	0.498	No	1.381	0.019	0.065
IL18	Q14116	1.067	0.590	0.510	No	1.321	0.054	0.125
ICAM-2	P13598	1.045	0.593	0.510	Yes	1.272	0.003	0.023
Notch 3	Q9UM47	0.950	0.599	0.511	Yes	1.293	0.018	0.065
Gal-3	P17931	1.048	0.612	0.520	No	1.167	0.081	0.160

ALDH3A1	P30838	1.089	0.635	0.531	No	1.297	0.137	0.235
CCL28	Q9NRJ3	0.962	0.636	0.531	No	1.118	0.312	0.420
IL-1RT2	P27930	0.962	0.637	0.531	No	1.263	0.004	0.025
CXCL5	P42830	1.132	0.639	0.531	No	0.915	0.776	0.821
MMP-9	P14780	0.931	0.646	0.534	No	1.142	0.421	0.507
RETN	Q9HD89	0.959	0.651	0.535	Yes	1.155	0.205	0.307
TNFSF14	O43557	1.046	0.661	0.539	No	1.236	0.039	0.102
CST5	P28325	0.954	0.671	0.545	Yes	1.076	0.580	0.671
OSM	P13725	0.933	0.680	0.549	No	1.228	0.276	0.388
FGF-19	O95750	1.066	0.687	0.551	No	0.651	0.031	0.089
MMP-2	P08253	0.966	0.689	0.551	Yes	1.255	0.014	0.057
IL-18R1	Q13478	1.031	0.715	0.566	No	1.110	0.255	0.365
TNNI3	P19429	1.127	0.716	0.566	No	0.890	0.755	0.802
IGFBP-2	P18065	1.049	0.761	0.596	Yes	1.547	0.016	0.060
TMPRSS15	P98073	0.948	0.762	0.596	No	1.024	0.913	0.922
PDCD1	Q15116	1.023	0.772	0.599	No	1.062	0.659	0.717
COL1A1	P02452	1.027	0.777	0.599	No	1.266	0.027	0.080
CCL4	P13236	1.048	0.780	0.599	No	1.516	0.002	0.021
PDGFC	Q9NRA1	0.981	0.782	0.599	No	1.081	0.280	0.391
CD93	Q9NPY3	1.024	0.794	0.605	No	1.279	0.010	0.046
CRH	P06850	0.957	0.804	0.610	No	0.657	0.059	0.130
CD8A	P01732	1.032	0.832	0.625	No	1.138	0.481	0.562
CALCA	P01258	0.961	0.833	0.625	No	1.222	0.367	0.470
EPHB4	P54760	1.016	0.840	0.627	Yes	1.256	0.010	0.047
CCL23	P55773	1.018	0.863	0.634	No	1.153	0.218	0.319
AGR2	O95994	0.971	0.865	0.634	No	1.268	0.207	0.309
ST3GAL1	Q11201	1.017	0.866	0.634	No	1.136	0.282	0.391
TRAIL	P50591	0.989	0.870	0.634	No	0.989	0.874	0.898
TNFB	P01374	1.016	0.871	0.634	No	1.046	0.688	0.742
OPG	O00300	1.013	0.878	0.634	No	1.267	0.020	0.065
DLK-1	P80370	1.023	0.880	0.634	No	1.497	0.026	0.078
AZU1	P20160	0.978	0.885	0.635	No	1.247	0.155	0.254
ALCAM	Q13740	1.011	0.891	0.635	No	1.284	0.001	0.017
TIMP4	Q99727	0.989	0.896	0.635	No	0.962	0.680	0.736
TR-AP	P13686	0.988	0.901	0.635	No	1.177	0.088	0.170
IL-17RA	Q96F46	0.987	0.903	0.635	No	1.272	0.041	0.106
NBN	O60934	0.985	0.907	0.635	No	1.113	0.384	0.481
AXL	P30530	1.008	0.921	0.642	No	1.226	0.035	0.098
LDL receptor	P01130	1.013	0.932	0.647	No	1.438	0.039	0.102
PRTN3	P24158	1.007	0.948	0.655	No	1.225	0.047	0.112
MMP-10	P09238	0.994	0.964	0.661	No	1.258	0.120	0.211
CHI3L1	P36222	0.994	0.966	0.661	Yes	1.468	0.031	0.089
ENTPD6	O75354	1.003	0.970	0.661	No	1.112	0.147	0.248
IL7	P13232	0.996	0.978	0.663	No	1.019	0.862	0.894
TLT-2	Q5T2D2	1.001	0.990	0.664	No	1.211	0.045	0.112

RASSF2	P50749	1.001	0.992	0.664	No	1.007	0.954	0.958
DPP6	P42658	1.000	0.998	0.664	No	1.213	0.046	0.112
OPG	O00300	1.000	0.998	0.664	No	1.312	0.008	0.040

Table S3. Sensitivity analyses comparing plasma protein expression of ACMP cases with controls.

	Adjusted for NT-proBNP[1]		Analysis in 20 ACMP cases without heart failure[2]	
Protein	**Fold change**	**p-value**	**Fold change**	**p-value**
NT-proBNP	-	-	1.24	0.231
CCL19	1.45	0.010	1.61	0.002
CCL20	1.72	0.037	1.64	0.025
PSPD	1.52	0.012	1.88	<0.001
PTN	1.30	0.047	1.35	0.033

1: Linear regression model adjusted for sex, time since cancer diagnosis, anthracycline dose, chest-directed radiotherapy dose and NT-proBNP. 2: Linear regression in 20 asymptomatic cases compared to their matched controls, adjusted for sex, time since cancer diagnosis, anthracycline dose, chest-directed radiotherapy dose. CCL19=C-C motif chemokine ligand 19, CCL20=C-C motif chemokine ligand 20, NT-proBNP=N-terminal pro-B-type natriuretic peptide, PSPD=Pulmonary surfactant protein D, PTN=Pleiotrophin.