SUPPLEMENTARY MATERIAL

SUPPLEMENTARY METHODS

Postcycletwo venous sampling was performed 24±4 hours after completion of the anthracycline infusion, and the followup echocardiogram was performed within 72 hours of blood sampling. Serum was allowed to clot for 30 minutes and centrifuged at 1,500 × g for 10 minutes at 4°C; EDTA plasma was centrifuged under the same conditions. Aliquots were stored at −80°C and thawed once for analysis.

The troponin change threshold of 0.02 ng/mL (20 ng/L) was a prespecified absolute delta from baseline and was not the assay limit of detection or the laboratory upper reference limit. The NTproBNP threshold was a prespecified absolute increase of 100 pg/mL. Assays, platforms, analytical ranges, and qualitycontrol specifications are summarized below. Because several baseline TNFα values were close to the lower bound of the manufacturer's stated measuring range, TNFα analyses were treated as exploratory and interpreted cautiously; TNFα did not contribute to the composite classification or regression models.

Supplementary Table S1: Laboratory Assay Platforms and Analytical Characteristics

Analyte/measure	Specimen	Manufacturer and assay	Platform/method	Analytical sensitivity/range	Precision/QC
Cardiac troponin I	Serum	Abbott STAT High Sensitive Troponin-I	ARCHITECT i2000SR; chemiluminescent microparticle immunoassay	LoD 1.2 ng/L; 10% CV concentration 4.7 ng/L; analytical range 0–50,000 ng/L	Two-level internal QC each run; CV <5%
NT-proBNP	Serum	Roche Elecsys proBNP II	cobas e 411; electrochemiluminescence immunoassay	LoD 5 pg/mL; measuring range 5–35,000 pg/mL	Two-level internal QC each run; CV <5%
CK-MB activity	Serum	Roche CK-MB Gen.2	cobas c 311; kinetic UV/immunoinhibition	LoQ 3 U/L; measuring range 3–200 U/L	Daily calibration/QC; CV <5%
LDH	Serum	Roche LDHI2	cobas c 311; kinetic UV	Measuring range 10–1,000 U/L	Daily calibration/QC; CV <3%
CRP	Serum	Roche CRP Gen.3	cobas c 311; particle-enhanced immunoturbidimetry	LoD 0.3 mg/L; measuring range 0.6–350 mg/L	Daily calibration/QC; CV <5%
IL-6	Serum	Elabscience E-EL-H6156	Sandwich ELISA; 450 nm	Sensitivity 0.94 pg/mL; range 1.56–100 pg/mL	Duplicate wells; intra/inter-assay CV <10%
TNF-α	Serum	Elabscience E-EL-H0109	Sandwich ELISA; 450 nm	Sensitivity 4.69 pg/mL; range 7.81–500 pg/mL	Duplicate wells; intra/inter-assay CV <10%
MDA	Plasma	Cayman TBARS Assay Kit, item 10009055	Colorimetric thiobarbituric-acid-reactive substances assay	Standard curve 0–50 µmol/L	Duplicate analysis; CV <10%
SOD	Plasma	Cayman Superoxide Dismutase Assay Kit, item 706002	Tetrazolium-salt colorimetric assay	Working standard 0.025–0.25 U/mL after dilution	Duplicate analysis; CV <10%
GPx	Plasma	Cayman Glutathione Peroxidase Assay Kit, item 703102	Coupled enzymatic assay at 340 nm	Activity determined from NADPH oxidation rate	Duplicate analysis; CV <10%
Catalase	Plasma	Cayman Catalase Assay Kit, item 707002	Colorimetric formaldehyde method	Standard curve 5–75 µmol/L formaldehyde	Duplicate analysis; CV <10%
Reduced GSH	Plasma	Cayman Glutathione Assay Kit, item 703002	Enzymatic recycling/colorimetric assay	Standard curve 0–16 µmol/L	Duplicate analysis; CV <10%

CK-MB, creatine kinase-MB; CRP, C-reactive protein; CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; GPx, glutathione peroxidase; GSH, glutathione; IL-6, interleukin-6; LDH, lactate dehydrogenase; LoD, limit of detection; LoQ, limit of quantification; MDA, malondialdehyde; NT-proBNP, N-terminal pro-B-type natriuretic peptide; QC, quality control; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-α.

ECHOCARDIOGRAPHY AND ADJUDICATION DETAILS

Echocardiography used a Philips EPIQ 7C system, and LVEF was calculated by the biplane Simpson method. The same cardiologist interpreted baseline and post-cycle-two studies while unaware of biomarker results. The clinical CTRCD criterion was an absolute LVEF decline ≥10 percentage points to <50%, a new LVEF <40%, or new heart-failure symptoms with cardiologist-confirmed anthracycline-associated dysfunction. Biomarker-defined myocardial injury required an absolute troponin I increase ≥0.02 ng/mL and/or NT-proBNP increase ≥100 pg/mL without symptoms or clinical CTRCD. Two investigators adjudicated classifications independently, with disagreement resolved by consensus with the treating cardiologist.

SOURCE DATA FOR FIGURE 1

Supplementary Table S2: Summary Data and Standardized Change-Score Differences Underlying Figure 1

Outcome	No composite mean	No composite SD	Composite mean	Composite SD	Hedges g	95% CI
Troponin increase	0.0056	0.0051	0.0232	0.0115	2.35	1.91 to 2.80
NT-proBNP increase	40.4	33.7	171.4	67.1	2.87	2.38 to 3.35
CK-MB increase	1.8	2.2	8.8	4.1	2.44	1.99 to 2.89
LDH increase	23.3	25.6	54.7	37	1.07	0.69 to 1.45
LVEF decline	2.3	2.1	9.1	3.7	2.56	2.10 to 3.02
MDA increase	0.37	0.28	1.25	0.51	2.44	1.99 to 2.89
SOD decline	4.4	3.8	12.2	5.3	1.82	1.41 to 2.23
GPx decline	2.6	3.1	8.1	4.2	1.59	1.19 to 1.99
Catalase decline	3	4.3	10.5	4.3	1.74	1.33 to 2.14
GSH decline	0.41	0.36	1.44	0.66	2.21	1.78 to 2.65
CRP increase	2.36	2.24	6.96	4.11	1.59	1.19 to 1.99
IL-6 increase	1.5	1.31	5.28	2.64	2.11	1.68 to 2.54
TNF-α increase	1.21	1.34	4.86	2.07	2.30	1.86 to 2.75

Hedges g was calculated from the two group means, standard deviations, and sample sizes (n=108 and n=42). Positive values indicate a larger adverse change in the composite group. These estimates are descriptive and unadjusted.