

Cardiac Energetics Before, During and After Anthracycline-based Chemotherapy in Breast Cancer Patients Using ^{31}P Magnetic Resonance Spectroscopy: A Pilot Study

Gillian Macnaught

The University of Edinburgh Centre for Cardiovascular Science

Olga Oikonomidou

Edinburgh Cancer Research Centre: Cancer Research UK Edinburgh Centre

Christopher T. Rodgers

University of Cambridge Wolfson Brain Imaging Centre

William Clarke

Oxford Radcliffe Hospitals NHS Trust: Oxford University Hospitals NHS Foundation Trust

Annette Cooper

The University of Edinburgh Centre for Cardiovascular Science

Heather McVicar

Edinburgh Cancer Research Centre: Cancer Research UK Edinburgh Centre

Larry Hayward

Edinburgh Cancer Research Centre: Cancer Research UK Edinburgh Centre

Saeed Mirsadraee

Brompton Hospital: Royal Brompton Hospital

Scott Semple

The University of Edinburgh Centre for Cardiovascular Science

Martin A Denvir (✉ martin.denvir@ed.ac.uk)

The University of Edinburgh

Short communication

Keywords: Cardiac Energetics, Breast Cancer, chemotherapy

DOI: <https://doi.org/10.21203/rs.3.rs-114644/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

PURPOSE

To explore the utility of phosphorus magnetic resonance spectroscopy (^{31}P MRS) in identifying anthracycline-induced cardiac toxicity in patients with breast cancer.

METHODS

20 patients with newly diagnosed breast cancer receiving anthracycline-based chemotherapy had cardiac magnetic resonance assessment of left ventricular ejection fraction (LVEF) and ^{31}P MRS to determine myocardial Phosphocreatine/Adenosine Triphosphate ratio (PCr/ATP) at three time points: pre, mid and end-chemotherapy. Plasma high sensitivity cardiac troponin-I (cTn-I) tests and electrocardiograms were also performed at these same time points.

RESULTS

PCr/ATP ratio did not change significantly between pre- and mid-chemo (2.16 ± 0.46 v 2.00 ± 0.56 , $p=0.80$) and pre- and end-chemo (2.16 ± 0.46 v 2.17 ± 0.86 , $p=0.99$). Mean LVEF reduced significantly by 5.1% between pre- and end-chemo (61.4 ± 4.4 vs 56.3 ± 8.1 %, $p=0.02$). Change in PCr/ATP ratios from pre- to end-chemo correlated inversely with changes in LVEF over the same period ($r=-0.65$, $p=0.006$). Plasma cTn-I increased progressively during chemotherapy from pre- to mid-chemo (1.35 ± 0.81 to 4.40 ± 2.64 ng/L; $p=0.01$) and from mid to end-chemo (4.40 ± 2.64 to 18.33 ± 13.23 ng/L; $p=0.001$).

CONCLUSIONS

In this small cohort pilot study, we did not observe a clear change in mean PCr/ATP values during chemotherapy despite evidence of increased plasma cardiac biomarkers and reduced LVEF. Future similar studies should be adequately powered to take account of patient drop-out and variable changes in PCr/ATP.

Introduction

Anthracyclines are widely used in the treatment of breast cancer and are well recognised to carry increased risk of cardiotoxicity [1-3]. This can occur as an early, acute manifestation or many years after treatment as late onset cardiomyopathy [4, 5]. It is increasingly apparent that there may be a chronic subclinical phase associated with low grade cardiac injury with no apparent clinical impact on the contractile function of the heart [6]. This period may remain latent for many years with the patient remaining asymptomatic and with apparently normal cardiac function. Identification of early and subtle cardiac dysfunction during this period could provide a window of opportunity for therapeutic intervention if those at risk or those experiencing this low-grade decline could be accurately identified. Recent studies have suggested that it might be possible to detect subclinical cardiac dysfunction using echocardiographic strain measurements of the left ventricle and that such changes might predict future risk of developing cardiac dysfunction [7,8]. Other studies have suggested that plasma levels of cardiac troponin could also provide a useful marker of early and ongoing cardiac injury following anthracycline treatment [9, 10].

Left Ventricular Ejection Fraction (LVEF), assessed by a variety of imaging methods, is recognised as insensitive for the detection of early anthracycline-induced cardiomyopathy [11]. The widely used values for a clinically relevant decline in ejection fraction associated with clinical symptoms of heart failure, or an asymptomatic decrease in LVEF of 10% to less than 55% [10, 12] represent a relatively late manifestation of cardiac toxicity. In some cases, by the time a reduction in LVEF occurs the patient may have irreversible cardiac damage. As cancer survival rates increase there is a clear need not only to identify early markers of anthracycline myocardial toxicity but also to explore new techniques that could potentially identify baseline characteristics, pre-chemotherapy, that might predict the likelihood of developing subsequent cardiotoxicity. An accurate method of identifying, and possibly even predicting, early cardiac toxicity could aid oncologists in optimising chemotherapy treatment whilst limiting the short and long-term cardiotoxic effects of these agents.

This study explores the potential utility of phosphorus magnetic resonance spectroscopy (^{31}P -MRS) acquired using a clinical 3T MR system to identify early anthracycline-induced cardiac toxicity and baseline predisposition to cardiac injury. This technique measures the phosphocreatine/adenosine triphosphate concentration ratio (PCr/ATP), which reflects cardiac cellular energetics. The normal range of PCr/ATP is approximately 1.8 – 2.2, whereas decreased PCr/ATP is associated with a drop in available energy reserve in the heart, associated with many forms of heart failure [13-14].

Conventional cardiac investigations of electrocardiogram and ultra-high sensitivity plasma cardiac troponin-I (cTn-I) have also been applied. To our knowledge PCr/ATP has not previously been used sequentially to assess cardiac injury in breast cancer patients before, during and after chemotherapy.

Methods

Ethics statement

This study was approved by the South East Scotland Research Ethics Committee (14/SS/1041) and all participants gave full written informed consent.

Study population and recruitment

Twenty female breast cancer patients with no prior exposure to anthracyclines scheduled to receive anthracycline-based chemotherapy either in the adjuvant or the neoadjuvant setting were recruited from the Breast clinic at the South East Scotland Cancer Centre. The combination chemotherapy regimens were either 6 cycles of adjuvant FEC-80 (Fluorouracil, Epirubicin (80mg/m³), cyclophosphamide), or 3 cycles of FEC (Fluorouracil, Epirubicin (100mg/m³), cyclophosphamide) followed by 3 cycles of Docetaxel (FEC-T). Four patients had a history of hypertension : two treated with ramipril and two with bendroflumethiazide (table 1). None of the patients had coronary heart disease, diabetes, hypercholesterolaemia or heart failure at baseline.

The mean time between scans was 7 weeks (range 6-12 weeks) and was purposely flexible to allow for patient preference and intercurrent chemotherapy related illness including nausea, fatigue and general malaise.

High sensitivity cardiac Troponin-I (cTn-I) measurements

Blood samples were taken immediately prior to the 1st and 4th chemotherapy cycles and within 2 weeks of completion of the 6th chemotherapy cycle. Plasma Cardiac Tn-I was measured using a high sensitivity assay (ARCHITECT STAT Troponin I assay; Abbott Laboratories). This assay has a limit of detection of 1.2 ng/L, with a coefficient of variation of 23% at the limit of detection (1.2 ng/L) and less than 10% at 6 ng/L [15]. The upper reference limit, determined by the manufacturer as the 99th centile of samples from 4590 healthy individuals, is 16 ng/L in women [15]. As part of clinical follow-up, cTn-I was also measured within 3 months of completion of chemotherapy in patients where values increased above the normal range (0-16 ng/l) at any time point during chemotherapy.

Cardiac Magnetic Resonance Imaging (CMRI)

Participants attended the Edinburgh Imaging QMRI facility at three time points: pre-, mid- and end-chemotherapy. Mid-chemotherapy was defined as between cycles 3 and 4 and end-chemotherapy was within 27±19 days of completing cycle 6. On each occasion participants were positioned head first supine in a 3T Verio MR scanner (Siemens Healthineers, Erlangen, Germany) between anterior and posterior parts of an 8-element cardiac ^{31}P receive array coil (Rapid Biomedical, Rimpar, Germany).

^{31}P -MRS

The ^{31}P MRS protocol used for this study has been described in detail elsewhere [16]. In brief the total MR protocol acquisition time including positioning, set-up and acquisition of cine MR imaging to calculate ejection fraction and MRS was 60 minutes. ^{31}P MR spectra were acquired over 30 minutes using a 3D UTE-CSI pulse sequence (TR/TE = 1000/~0.6ms, FOV = $350 \times 350 \times 350 \text{mm}^3$, $22 \times 22 \times 10$ CSI matrix). A flip angle (α) of 30° was applied to the mid-septum of the myocardium. Voxels were carefully planned so that one full voxel was aligned with the mid-septum. Figure 1 shows an example of the planning of the voxel positioning. While spectra are acquired from each voxel, that acquired from the mid-septum voxel (denoted by the red square in Figure 1) is the spectrum used for analysis. The ^{31}P MRS acquisition was not ECG gated.

Analysis of the ^{31}P spectra was carried out using a custom Matlab implementation of AMARES [17] and was analysed by two independent observers blinded to the time-point of analysis. This analysis quantifies the amount of phosphocreatine (PCr) and adenosine triphosphate (ATP) present in the cardiac spectrum as a concentration ratio ("PCr/ATP"). The PCr/ATP ratio was corrected for saturation effects and blood contamination. PCr/ATP ratios were calculated for each of the three time-points.

Left Ventricular Ejection Fraction

A series of cardiac cine images were acquired from base to apex in the short axis plane using the system's integrated body coil (TrueFISP sequence: TR/TE=85.8/1.45ms, $\alpha=50^\circ$, FOV = $400 \times 338 \text{mm}^2$, matrix = 256×205 , Grappa acceleration factor=3, slice thickness=8mm). Two independent operators subsequently calculated the left ventricular ejection fraction (LVEF) from these images using QMass® software (Medis, Leiden, The Netherlands). This was carried out for images acquired at each time point.

We used the definition of the Cardiac Review and Evaluation Committee (CREC) supervising Trastuzumab trials which defined cardiotoxicity as a decrease in LVEF of 5 points to less than 55% *with* accompanying signs or symptoms of heart failure, or a decline of 10 points to less than 55% *without* heart failure signs or symptoms [10, 12].

Electrocardiograms

A 12-lead ECG was acquired at each of the three time points immediately prior to each MRI scan. These were reported by a Cardiologist and any abnormalities grouped by ST segment change, left ventricular hypertrophy and changes in the QT interval.

Healthy volunteers

Thirteen healthy volunteers (48.8 ± 11.2 years, range 31-60) with no background medical conditions and on no medications underwent a single MRI imaging protocol and a single ^{31}P MRS as described for patients. PCr/ATP ratios and LVEF were calculated for each volunteer. Scanning was repeated for 3 of these normal subjects to assess repeatability of measurements. The maximum difference between repeated PCr/ATP measurements and repeated LVEF measurements for volunteers defined the cut-off above which a real change could be assumed to occur in patients between pre- to mid- chemotherapy, mid- to end-chemotherapy and pre- to end-chemotherapy. Volunteers did not undergo ECG or plasma troponin measurements and did not undergo sequential repeat MR scans.

Statistical analysis

All statistical analysis was carried out using Minitab 17 Statistical Software (Minitab 17 Statistical Software (2010), State College, PA: Minitab, Inc). Data were analysed using paired Student's t-tests or one-way ANOVA as appropriate. Associations between various parameters were assessed using Pearson's Coefficient. Significance was accepted at $p < 0.05$.

The mean PCr/ATP ratio for age-matched female healthy controls was 1.75 ± 0.59 (range 1.11 to 2.18, $n=13$). In 3 healthy volunteers with repeat measurements of PCr/ATP the difference in values between scans were 0.58, 0.53 and 0.20. This was consistent with other published work suggesting that a change in PCr/ATP of 0.5, with 95% confidence, could be detected with

a sample size of 9 patients using a similar protocol as that used in patients with heart failure [18]. However, since we could not accurately predict the change in PCr/ATP associated with the anthracycline regimes used in our patients we recognised that, taking drop-out into account, we might not be adequately powered to detect a smaller change in PCr/ATP even with the proposed 20 patients agreed with our ethical review board.

Results

Patient characteristics

All participants were women with median age 51 (range 31-67 years) and median body mass index 28 (range 23-36). 6 patients (30%) received FEC80, fourteen (70%) received FEC-T chemotherapy and eighteen (90%) received radiotherapy. Two patients treated with the FEC-T received Trastuzumab from the mid-chemo time point. Ten patients had left sided tumours and one had left and right sided tumours. Nine participants were post-menopausal.

All participants attended their pre-chemotherapy MRI scan while 5 were unable to attend the mid-chemotherapy scan due to inter-current illness and 1 was unable to attend the final end-chemotherapy MRI scan.

Cardiac Magnetic Resonance Imaging (CMR)

The mean LVEF for healthy volunteers was $63.6 \pm 4.5\%$ with range 57.6% to 74.3%. Differences in repeated measurements of LVEF for 3 volunteers were 0.5%, 0.6% and -0.5%, indicating a very high degree of repeatability.

LVEF was calculated for all 20 participants prior to chemotherapy, 15 at mid-chemotherapy and 19 at end-chemotherapy.

The mean (\pm standard deviation) LVEF at pre, mid and end-chemotherapy was $61.6 \pm 4.4\%$, $60.5 \pm 5.2\%$ and $56.3 \pm 8.1\%$ respectively (Figure 2). There was a significant decrease in LVEF between pre- and end-chemotherapy ($p=0.02$). There was no significant difference in LVEF between patients receiving FEC80 and those receiving FEC-T at mid (57.7 ± 5.6 vs $61.9 \pm 4.7\%$, respectively, $p=0.14$) or end-chemo time-points (55.9 ± 3.9 vs $56.5 \pm 9.6\%$, respectively, $p=0.94$).

In total, 6 patients experienced a significant decrease in LVEF according to agreed criteria [10]. 3 symptomatic patients (with breathlessness) experienced a 5% decrease in LVEF from pre- to mid-chemo. Two of these patients had no further change in LVEF from mid- to end-chemo and one had a significant increase. Three additional patients experienced a decrease of 10% in LVEF from mid- to end-chemo. One patient, receiving FEC-T, became symptomatic during treatment with associated ankle swelling and breathlessness associated with a 30% fall in LVEF between pre- and end-chemo to 30.5%. This patient responded rapidly to low dose diuretics and fluid restriction and was assessed by the cardiology team with full recovery of cardiac function within 2 weeks.

Cardiac energetics assessed by ^{31}P -MRS

The mean PCr/ATP ratio for 13 age-matched female healthy volunteers was 1.94 ± 0.43 (range 1.11 to 2.18). For 3 volunteers with repeat measurements of PCr/ATP the differences in values between scans were 0.58, 0.53 and 0.20. This is consistent with other published work at 3T showing that a change in PCr/ATP of 0.5, with 95% confidence, would require a sample size of 9 patients using a similar protocol to ours [18].

^{31}P MR spectra were successfully acquired for 19 breast cancer patients pre-chemotherapy, 11 at mid-chemotherapy and 17 end-chemotherapy. Missing scans were due to inter-current illness or technical issues for 1 participant at the pre-chemotherapy scan, 4 mid-chemotherapy and 2 end-chemotherapy. An example patient ^{31}P spectrum is shown in Figure 3.

For the whole cohort there was no significant difference in mean values for PCr/ATP ratio comparing pre-, mid- and end-chemotherapy time-points. However, there was a significant negative correlation between the change in PCr/ATP from pre- to mid- chemotherapy and the subsequent change in PCr/ATP from mid- to end chemotherapy ($r=-0.68$, $p=0.04$). The mean change in the PCr/ATP ratio at mid- and at end- chemotherapy relative to the mean baseline value is plotted in Figure 4.

Plasma High sensitivity cardiac Troponin-I

Plasma cardiac cTn-I levels (ng/L) were low (<5ng/L) in all patients at baseline prior to starting chemotherapy and increased progressively by mid-chemo with a further rise at the time of the last chemo cycle (figure 4). The threshold cTn-I level for identifying acute coronary syndrome (ACS) in women in our centre is 16ng/L and is based on the 99th upper centile of the normal range for a large sample of patients [15]. In our study, while all participants showed a small but significant rise in mean cTn-I value between baseline and mid-chemo (1.35 ± 0.81 to 4.40 ± 2.64 ng/L, $p=0.0001$) none of these values exceeded the ACS threshold (16ng/L). By end-chemo there was a further significant increase in mean plasma cTn-I (4.40 ± 2.64 to 14.84 ± 8.73 ng/L, $p=0.0001$) with six cTn-I values above the ACS threshold. These 6 patients, and a further 3 patients with borderline normal cTn-I levels at end-chemo, were invited back 2-3 months after end of chemotherapy for longer term follow-up with repeat blood cTn-I. In all cases cTn-I had returned to normal level (mean \pm SD, 1.55 ± 0.88 ng/L).

The specific type of chemotherapy regime had no impact on cTn-I levels. At mid-chemotherapy cTn-I was not significantly higher in patients receiving FEC-T compared to FEC80 (5.0 ± 2.9 vs 3.0 ± 0.89 ng/L, respectively, $p=0.12$) or at end-chemo in patients receiving FEC80 compared to those receiving FEC-T (18.3 ± 12.0 ng/l vs 13.2 ± 6.7 ng/L, respectively, $p=0.24$). As expected, the side of the cancer had no impact on cTn-I levels at any of the time points studied (end-chemo: left 15.1 ± 8.1 vs right 15.5 ± 10.1 , $p=0.92$).

Electrocardiogram (ECG)

There were no significant changes in PR interval or QRS duration during chemotherapy. There was a small but significant increase in the corrected QT interval (QTc) from 422 ± 15 to 438 ± 13 ms ($p=0.0001$) with most of this increase occurring between baseline and mid-chemo. Overall, no patient showed an increase of QTc of >60 ms or a QTc above 500 ms.

Exploratory analysis of possible association between LVEF, PCr/ATP, QT interval and cardiac troponin-I

A significant negative correlation was found between the change in PCr/ATP ratio and the change in LVEF from pre-chemo to end-chemo ($r=-0.65$, $p=0.006$, figure 5). A significant negative correlation was also found between the change in PCr/ATP ratio from pre- to end- chemotherapy and the change in LVEF from mid- to end-chemotherapy ($r=-0.77$, $p=0.002$). There was no apparent association between cTn-I and LVEF or PCr/ATP any of the time points. There was also no association found between changes in the corrected QT interval in the electrocardiogram and changes in cTn-I or changes in the QT interval and changes in LVEF. There was no apparent effect of trastuzumab treatment on cTn-I levels.

In total, 6 patients displayed cTn-I values above the normal range at the end-chemo timepoint. When examining each of these patients on an individual basis, no clear relationship was observed between cTn-I and baseline clinical data, including pre-existing hypertension or smoking history, LVEF or PCr/ATP ratio.

Discussion

This study has explored the applicability of PCr/ATP ratio, measured using ³¹P MRS, to detect early anthracyclines-induced cardiac injury in a pilot cohort. The widely accepted cellular mechanism underlying anthracycline-induced cardiac toxicity is based on generation of reactive oxygen species (ROS) resulting from mitochondrial damage associated with elevated intracellular iron [19]. Such damage to mitochondria is likely to cause early perturbations in high energy phosphates within cardiomyocytes and hence is the rationale for assessing ³¹P-MRS in this clinical setting. In addition, cardiotoxicity associated with breast cancer treatment is categorised as either type I, associated with irreversible cardiomyocyte death, or type II due to reversible cardiomyocyte dysfunction [9]. Impaired cardiac energetics could represent a possible underlying mechanism for type II toxicity.

We compared PCr/ATP ratio with other markers of cardiotoxicity including high sensitivity plasma cardiac Tn-I, ECG parameters and cardiac magnetic resonance imaging assessment of left ventricular ejection fraction (LVEF). While these

conventional factors suggested a progressive cardiotoxic effect of chemotherapy there was no clear signal in the PCr/ATP ratio data . The possible reasons for this are discussed.

Cardioprotection during chemotherapy has been demonstrated using ACE inhibitors [23], statins [24], angiotensin receptor antagonists [25], but not metoprolol [25]. Dexrazoxane has been shown to reduce cardiac troponin release during anthracycline based chemotherapy [26]. Only 2 patients in our study received ACE inhibitors for management of high blood pressure and hence background drug therapy is unlikely to have impacted our findings. Allopurinol [27] and metformin [28] have also been shown to affect cardiac energetics and these possibly merit further study using ³¹P-MRS to assess their mechanism of action during chemotherapy.

Patient specific factors such as age and physical fitness are also known to affect PCr/ATP ratio. Jakovljevic et al [29] found that PCr/ATP ratio was significantly reduced in older compared to younger women. They also found that older women who are physically active maintained high PCr/ATP ratios similar to younger sedentary women. Genetic factors are also known to play a part in susceptibility to cardiotoxicity in response to chemotherapy [30]. Therefore, physical activity, pre-treatment levels of physical fitness and genetics could provide intrinsic protection during chemotherapy, potentially impacting on the effects of chemotherapy on PCr/ATP.

While we observed a significant decrease in LVEF between pre and end chemotherapy by approximately 5% we also observed a negative association between changes in PCr/ATP ratio and LVEF between pre- and end-chemotherapy. This finding is not consistent with studies in dilated cardiomyopathy (unrelated to anthracyclines) where low PCr/ATP ratios were associated with reduced LVEF [31, 32] and while this was a pilot study, we had predicted a fall in PCr/ATP ratios in line with or perhaps preceding a fall in LVEF. The reasons for this are not clear. There is possibly a more complex relationship between energetics and LVEF in the setting of anthracycline chemotherapy compared to other forms of cardiomyopathy or the relatively modest reduction in mean LVEF of 5% was not sufficient to reveal a measurable change in PCr/ATP ratio during the course of the chemotherapy regimen. A further reason for the lack of clear change in PCr/ATP ratio is the possibility of a type 2 statistical error. Since the number of patients clinically well enough to have a ³¹P-MRS scan at mid-chemo time point fell from 20 to 11, this small number was at the predicted limit to allow us to detect a significant change in PCr/ATP ratio. Furthermore, this mid-chemo time point was important in that all 20 patients had been receiving anthracyclines over the previous 6 weeks in the run up to this mid-point scan. Thereafter, most patients (14) switched to docetaxel for the last 3 chemo-cycles thus diminishing our chances of detecting an effect on the final scan. It is therefore possible that cardiac energetics recovered in the 14 patients treated with Docetaxel for the final 3 chemo cycles. In addition, the relatively low cumulative dose of anthracyclines used may have limited cardiotoxicity.

Overall, the fall in LVEF, increase in QT interval on ECG and the rise in cardiac troponin together suggest that there was some degree of cardiac toxicity in our cohort. The very slight fall in PCr/ATP ratio at mid-chemo, while non-significant, is therefore highly intriguing and merits further study addressing the issues highlighted in our pilot. Furthermore, the sensitivity of ³¹P-MRS scans could be significantly improved by using higher magnetic field strengths (7T) [33,34].

Conclusions

This study investigated ³¹P MRS in assessing cardiac energetics of breast cancer patients undergoing chemotherapy in a pilot cohort. Our findings have highlighted the challenges of serial cardiac imaging studies during complex chemotherapy regimens and outlined a pathway by which this technique could be further explored to establish its potential for detection of cardiac energetics during chemotherapy. Future studies should take account of a potentially high level of patient drop-out, background factors that could influence cardiac PCr/ATP ratio, such as age, physical fitness and regular medications, and should seek to improve sensitivity using 7T MRI.

Declarations

Funding: This study was funded by the Edinburgh and Lothians Health Foundation and The Margaret Lee Breast Cancer Fund. Funding for the ³¹P array used in this study was provided by the British Heart Foundation Centre of Research Excellence award. CTR is funded by a Sir Henry Dale Fellowship from the Wellcome Trust and Royal Society [098436/Z/12/Z].

Conflicts and competing interest: All authors declare no conflict of interest and no competing interests in relation to the work presented in this manuscript

Consent for publication: all authors consent to publication of this manuscript.

Acknowledgements: the authors wish to thank and acknowledge the huge contribution made by the 20 patients with breast cancer who chose to help us during their chemotherapy with this research study.

Author contributions: GM, SS, OO and MAD conceived and designed the study and wrote the manuscript. GM, SS, AC, CR, SM, WC performed and analysed the heart scans. OO, LH, OO recruited and consented patients, took blood samples and booked the scans. GM, SS and MAD analysed and graphed the data. All authors reviewed and edited the final manuscript.

Ethics statement

This study was approved by the South East Scotland Research Ethics Committee (14/SS/1041) and all participants gave full written informed consent.

References

1. Bristow MR, Mason JW, Billingham ME, Daniels JR. Doxorubicin cardiomyopathy: evaluation by phonocardiography, endomyocardial biopsy, and cardiac catheterization. *Ann Intern Med.* 1978 Feb;88(2):168-75
2. Friedman MA, Bozdech MJ, Billingham ME, Rider AK. Doxorubicin cardiotoxicity. Serial endomyocardial biopsies and systolic time intervals. *JAMA.* 1978 Oct 6;240(15):1603-6
3. Praga C, Beretta G, Vigo PL, Lenaz GR, Pollini C, Bonadonna G, Canetta R, Castellani R, Villa E, Gallagher CG, von Melchner H, Hayat M, Ribaud P, De Wasch G, Mattsson W, Heinz R, Waldner R, Kolaric K, Buehner R, Ten Bokkel-Huyninck W, Pervodchikova NI, Manziuk LA, Senn HJ, Mayr AC. Adriamycin cardiotoxicity: a survey of 1273 patients. *Cancer Treat Rep.* 1979 May;63(5):827-34
4. Steinherz LJ, Steinherz PG, Tan CT, Heller G, Murphy ML. Cardiac toxicity 4 to 20 years after completing anthracycline therapy. *JAMA* 1991 Sep 25;266(12):1672-7.
5. Pein F, Sakiroglu O, Dahan M, Lebidou J, Merlet P, Shamsaldin A, Villain E, de Vathaire F, Sidi D, Hartmann O. Cardiac abnormalities 15 years and more after adriamycin therapy in 229 childhood survivors of a solid tumour at the Institut Gustave Roussy. *Br J Cancer.* 2004;91:37–44
6. Cardinale D, Colombo A, Bacchiani G, Tedeschi I, Meroni CA, Veglia F, Civelli M, Lamantia G, Colombo N, Curigliano G, Fiorentini C. Early detection of anthracycline cardiotoxicity and improvement with heart failure therapy. *Circulation.* 2015 Jun 2;131(22):1981-8.
7. Florescu M, Magda LS, Enescu OA, Jinga D, Vinereanu D. Early detection of epirubicin-induced cardiotoxicity in patients with breast cancer. *J Am Soc Echocardiogr.* 2014 Jan;27(1):83-92
8. Charbonnel C, Convers-Domart R, Rigaudeau S, Taksin AL, Baron N, Lambert J, Ghez S, Georges JL, Farhat H, Lambert J, Rousselot P. Assessment of global longitudinal strain at low-dose anthracycline-based chemotherapy, for the prediction of subsequent cardiotoxicity. *Eur Heart J Cardiovasc Imaging.* 2017 Apr 1;18(4):392-401
9. Mokuyasu S, Suzuki Y, Kawahara E, Seto T, Tokuda Y. High-sensitivity cardiac troponin I detection for 2 types of drug-induced cardiotoxicity in patients with breast cancer. *Breast Cancer.* 2015;22:563–9.
10. Seidman A, Hudis C, Pierri MK, Shak S, Paton V, Ashby M, Murphy M, Stewart SJ, Keefe D. Cardiac dysfunction in the trastuzumab clinical trials experience. *J Clin Oncol.* 2002 Mar 1; 20(5):1215-21

11. Cardinale D, Sandri MT, Martinoni A, Tricca A, Civelli M, Lamantia G, Cinieri S, Martinelli G, Cipolla CM, Fiorentini C. Left ventricular dysfunction predicted by early troponin I release after high-dose chemotherapy. *J Am Coll Cardiol.* 2000;36:517–522.
12. Thavendiranathan P¹, Wintersperger BJ, Flamm SD, Marwick TH. Cardiac MRI in the Assessment of cardiac injury and toxicity from cancer chemotherapy. A systematic review. *Circ cardiovasc Imaging.* 2013 Nov; 6(6):1080-91.
13. Ventura-Clapier R, Garnier A, eksler V, Joubert F. Bioenergetics of the failing heart. *Biochim biophys Acta* 2011 Jul; 1813(7):1360-72.
14. Neubauer S. Metabolic imaging with cardiac magnetic resonance spectroscopy. *Heart Metab.* 2009; 44:17-2015.
15. Shah AS, Anand A, Sandoval Y, Lee KK, Smith SW, Adamson PD et al. High-sensitivity cardiac troponin I at presentation in patients with suspected acute coronary syndrome: a cohort study. *Lancet* 2015;386(10012):2481-8
16. Rodgers CT, Robson MD. Coil combination for receive array spectroscopy: Are data-driven methods superior to methods using computed field maps? *Magnetic resonance in medicine.* 2016 Feb 1;75(2):473-87.
17. Purvis L, Clarke W, Biasioli L, Robson M, Rodgers C. Linewidth constraints in Matlab AMARES using per-metabolite T₂ and per-voxel ΔBo. P2885 International Society of Magnetic Resonance in Medicine 2014
18. Tyler DJ, Emmanuel Y, Cochlin LE, Hudsmith LE, Holloway CJ, Neubauer S, Clarke K, Robson MD. Reproducibility of 31P cardiac magnetic resonance spectroscopy at 3 T. *NMR in biomedicine.* 2009 May 1;22(4):405-13.
19. Ichikawa Y, Ghanefar M, Bayeva M, Wu R, Khechaduri A, Naga Prasad SV, Mutharasan RK, Naik TJ, Ardehali H. Cardiotoxicity of doxorubicin is mediated through mitochondrial iron accumulation. *J Clin Invest.* 2014 Feb;124(2):617-30
20. Zhou S, Starkov A, Froberg MK, Leino RL, Wallace KB. Cumulative and irreversible cardiac mitochondrial dysfunction induced by doxorubicin. *Cancer Res.* 2001 Jan 15;61(2):771-7.
21. Weiss RG, Chatham JC, Georgakopolous D, Charron MJ, Wallimann T, Kay L, Walzel B, Wang Y, Kass DA, Gerstenblith G, Chacko VP. An increase in the myocardial PCr/ATP ratio in GLUT4 null mice. *The FASEB Journal.* 2002 Apr 1;16(6):613-5.
22. de Lima Junior EA, Yamashita AS, Pimental GD, De Sousa LG, Santos RV, Goncalves RV et al. Doxorubicin caused severe hyperglycaemia and insulin resistance, mediated by inhibition in AMPk signalling in skeletal muscle. *J. Cachexia Sarcopenia Muscle.* 2016; 7(5): 615-625.
23. Cardinale D, Colombo A, Sandri MT, Lamantia G, Colombo N, Civelli M, Martinelli G, Veglia F, Fiorentini C, Cipolla CM. Prevention of high-dose chemotherapy–induced cardiotoxicity in high-risk patients by angiotensin-converting enzyme inhibition. *Circulation.* 2006 Dec 5;114(23):2474-81.
24. Acar Z, Kale A, Turgut M, Demircan S, Durna K, Demir S, Meriç M, Ağaç MT. Efficiency of atorvastatin in the protection of anthracycline-induced cardiomyopathy. *Journal of the American College of Cardiology.* 2011 Aug 23;58(9):988-9.
25. Gulati G, Heck SL, Ree AH, Hoffmann P, Schulz-Menger J, Fagerland MW, Gravdehaug B, von Knobelsdorff-Brenkenhoff F, Bratland Å, Storås TH, Hagve TA. Prevention of cardiac dysfunction during adjuvant breast cancer therapy (PRADA): a 2x2 factorial, randomized, placebo-controlled, double-blind clinical trial of candesartan and metoprolol. *European heart journal.* 2016 Feb 21;37(21):1671-80.
26. Lipshultz SE, Rifai N, Dalton VM, Levy DE, Silverman LB, Lipsitz SR, Colan SD, Asselin BL, Barr RD, Clavell LA, Hurwitz CA, Moghrabi A, Samson Y, Schorin MA, Gelber RD, Sallan SE. The effect of dexrazoxane on myocardial injury in doxorubicin-treated children with acute lymphoblastic leukemia. *N Engl J Med.* 2004 Jul 8;35(2):145-53
27. A. Hirsch, P. A. Bottomley, G. Gerstenblith and R. Weiss. Allopurinol acutely increases ATP energy delivery in failing human hearts. *J Am Coll Cardiol.* 2012; 59(9): 802-808
28. Kobashiwaga LC, Xu YC, Padbury JF, Y-T Tseng Y-T, Yano N. Metformin protects cardiac myocytes from doxorubicin induced cytotoxicity through an AMP-activated protein kinase dependent signalling pathway: An in vitro study. *PlosOne* 9(8): e104888.
29. Jakovljevic DG, Papakonstantinou L, Blamire AM, MacGowan GA, Taylor R, Hollingsworth KG, Trenell MI. Effect of Physical Activity on Age-Related Changes in Cardiac Function and Performance in Women. *Circulation: Cardiovascular*

Imaging. 2015 Jan 30;8(1):e002086.

30. Linschoten M, Teske AJ, Cramer MJ, van der Wall E, Asselbergs FW. Chemotherapy-Related Cardiac Dysfunction: A Systematic Review of Genetic Variants Modulating Individual Risk. *Circulation: Genomic and Precision Medicine*. 2018 Jan;11(1):e001753.
31. Bottomley PA, Panjath GS, Lai S, Hirsch GA, Wu K, Najjar SS, Steinberg A, Gerstenblith G, Weiss RG. Metabolic rates of ATP transfer through creatine kinase (CK Flux) predict clinical heart failure events and death. *Sci Transl Med*. 2013 Dec 11;5(215):215re3. doi: 10.1126/scitranslmed.3007328
32. Neubauer S, Horn M, Cramer M, Harre K, Newell JB, Peters W, Pabst T, Ertl G, Hahn D, Ingwall JS, Kochsiek K. Myocardial phosphocreatine-to-ATP ratio is a predictor of mortality in patients with dilated cardiomyopathy. *Circulation*. 1997 Oct 7;96(7):2190-6.
33. Rodgers CT, Clarke WT, Snyder C, Vaughan JT, Neubauer S, Robson MD. Human cardiac 31P magnetic resonance spectroscopy at 7 Tesla. *Magnetic resonance in medicine*. 2014 Aug 1;72(2):304-15.
34. Stoll VM, Clarke WT, Levelt E, Liu A, Myerson SG, Robson MD, Neubauer S, Rodgers CT. Dilated Cardiomyopathy: Phosphorus 31 MR Spectroscopy at 7 T. *Radiology*. 2016 Nov;281(2):409-417

Tables

Table 1: Patient population clinical characteristics and treatment regimens

Patient	Age range (years)	Smoking history	BMI	Menopausal status	Cardiac history	Chemotherapy	Herceptin	Cancer side	Radiotherapy
1	60-70	No	28	Post	No	FEC-T	No	R	Yes
2	60-70	No	31	Post	No	FEC	No	R	Yes
3	40-50	No	24	Pre	No	FEC-T	No	R	No
4	50-60	No	31	Post	No	FEC80	No	L	Yes
5	40-50	Yes	28	Pre	No	FEC-T	No	R	Yes
6	50-60	No	38	Post	No	FEC-T	No	L	Yes
7	30-40	No	24	Pre	No	FEC-T	No	L	No
8	50-60	Yes	32	Post	No	FEC-T	No	L	Yes
9	60-70	No	26	Post	Yes*	FEC80	No	R	Yes
10	50-60	No	25	Pre	No	FEC-T	No	R	Yes
11	30-40	No	28	Pre	No	FEC80	No	L	Yes
12	50-60	No	30	Pre	No	FEC-T	No	R	Yes
13	50-60	Yes	24	Post	No	FEC-T	Yes	L	Yes
14	40-50	No	36	Pre	No	FEC-T	No	R	Yes
15	50-60	No	35	Post	Yes*	FEC-T	No	L	Yes
16	40-50	No	23	Pre	Yes**	FEC80	No	R	Yes
17	50-60	No	27	Post	Yes**	FEC80	No	L	Yes
18	40-50	No	26	Pre	No	FEC-T	Yes	L+R	Yes
19	30-40	Yes	30	Pre	No	FEC-T	No	L	Yes
20	50-60	No	32	Pre	No	FEC-T	No	L	Yes

Key: Cardiac history of any of angina, hypertension, previous myocardial infarction, heart failure. * hypertension treated with ramipril, **hypertension treated with Bendroflumethiazide. Chemotherapy regimen: FEC80 – 6 cycles of Fluorouracil, Epirubicin (80mg/m³), cyclophosphamide. FEC-T – 3 cycles of Fluorouracil, Epirubicin (100mg/m³), cyclophosphamide followed by 3 cycles of docetaxel.

Figures

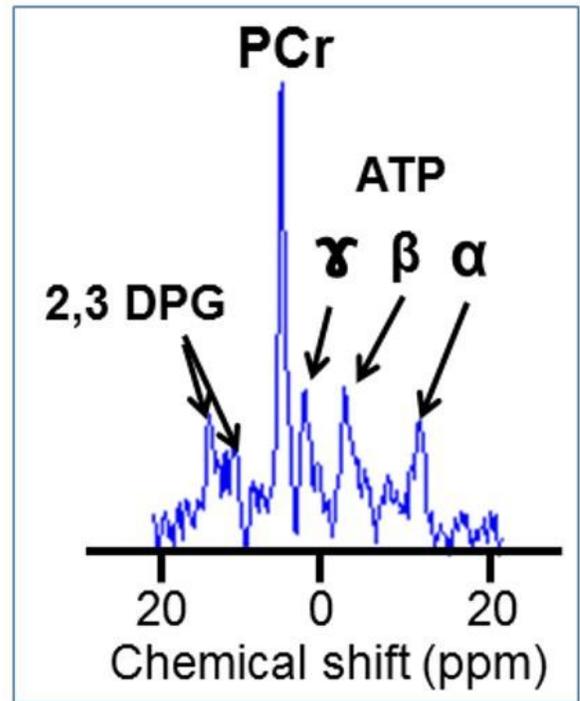
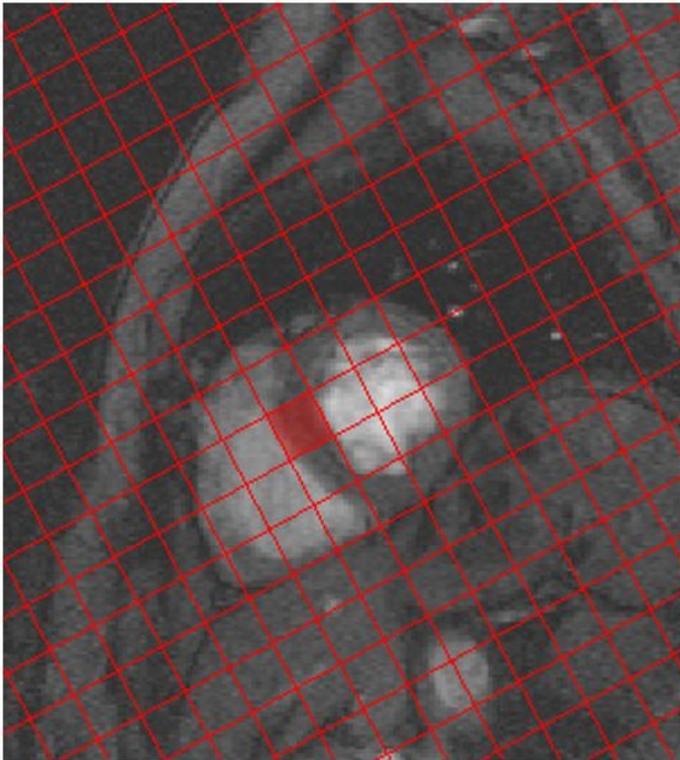


Figure 1

31P-MRS spectrum acquisition An example of the voxel positioning for 31P-MRS acquisition spectrum, the spectrum acquired at the mid-septum, denoted by the red square, is analysed. An example of a 31P MR spectrum is also shown (B) with resonances corresponding to phosphocreatine (PCr), γ , β and α adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3 DPG) labelled.

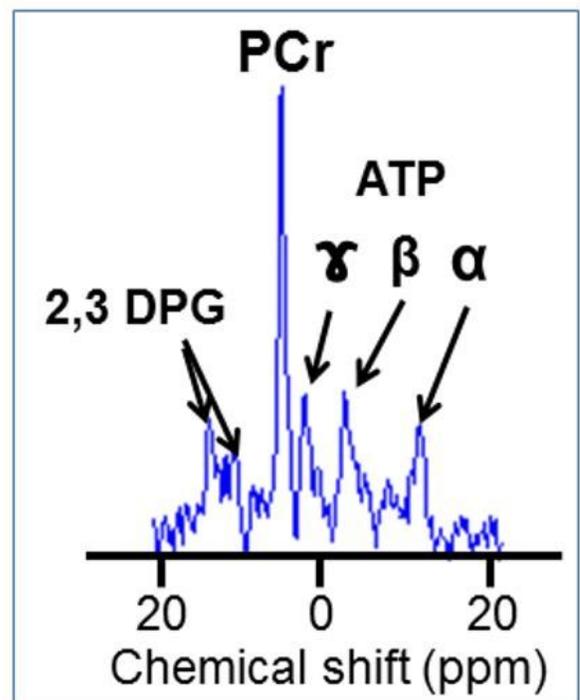
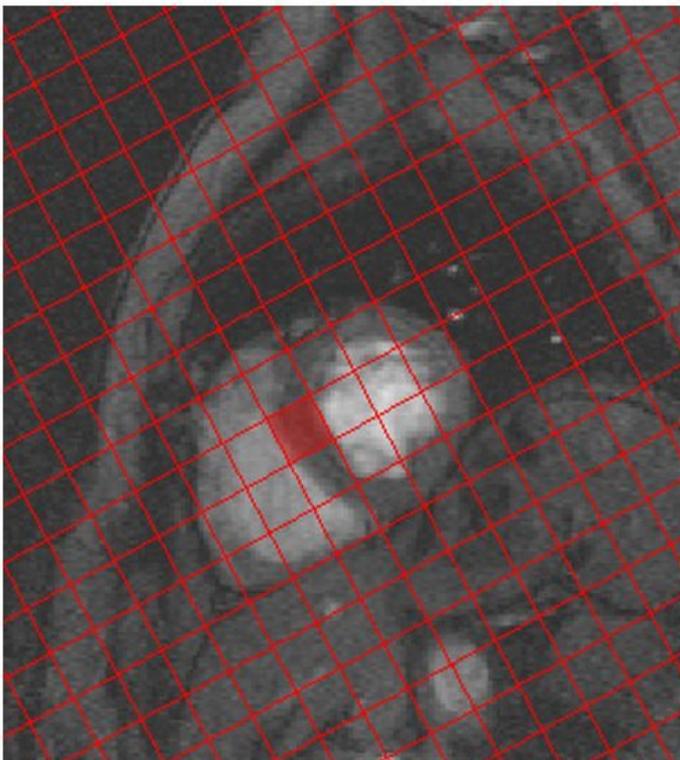


Figure 1

31P-MRS spectrum acquisition An example of the voxel positioning for 31P-MRS acquisition spectrum, the spectrum acquired at the mid-septum, denoted by the red square, is analysed. An example of a 31P MR spectrum is also shown (B) with resonances corresponding to phosphocreatine (PCr), γ , β and α adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3 DPG) labelled.

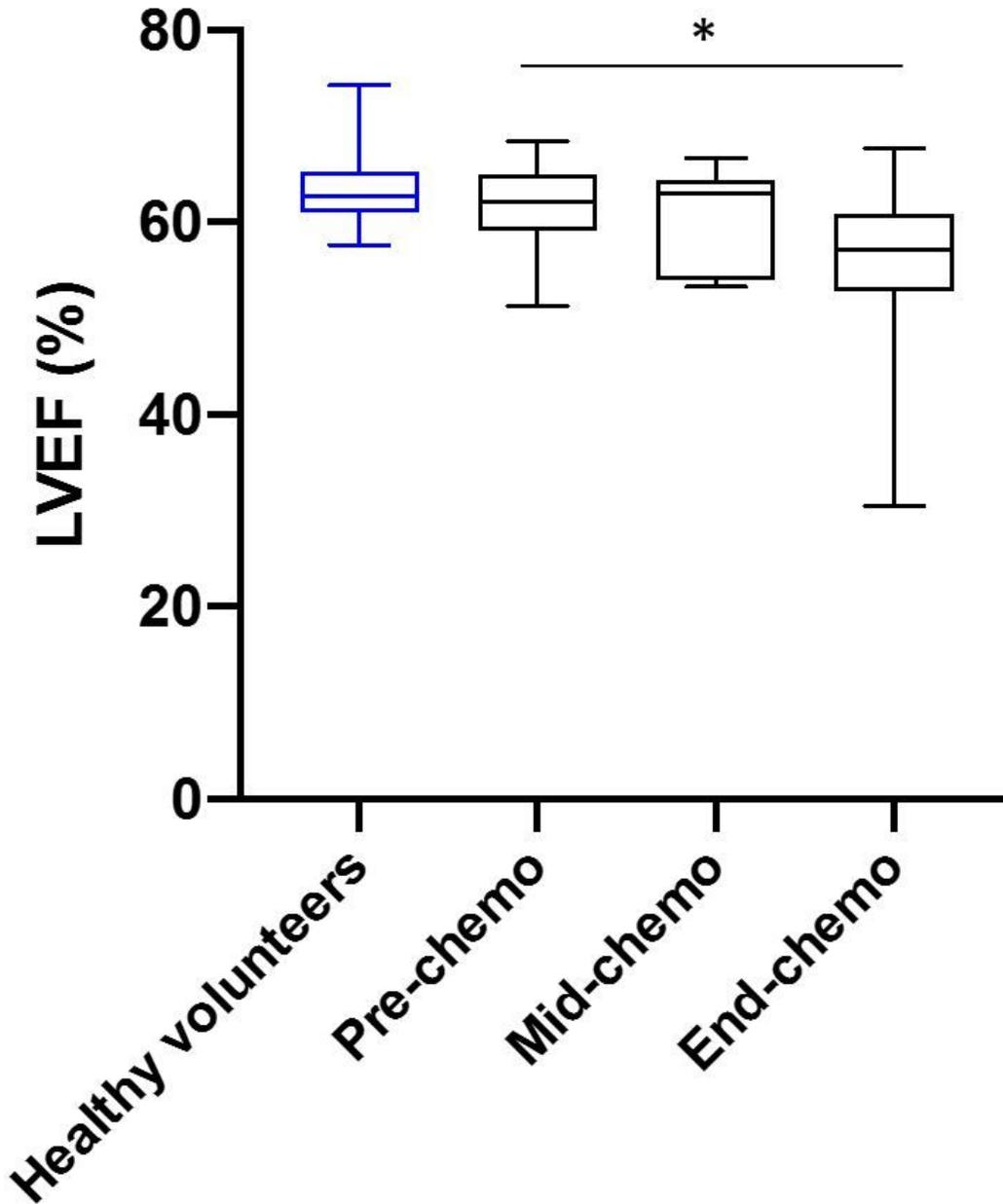


Figure 2

Left ventricular ejection fraction in healthy volunteers and in breast cancer patients during chemotherapy Healthy volunteer left ventricular ejection fraction (LVEF, blue) and sequential LVEF in patients (black) pre-, mid and end chemotherapy acquired by cardiac magnetic resonance (CMR) imaging (box and whiskers plot, values are mean \pm SD and 95% confidence intervals (* P=0.02 for change from pre-to end chemo in patients (paired t-test))).

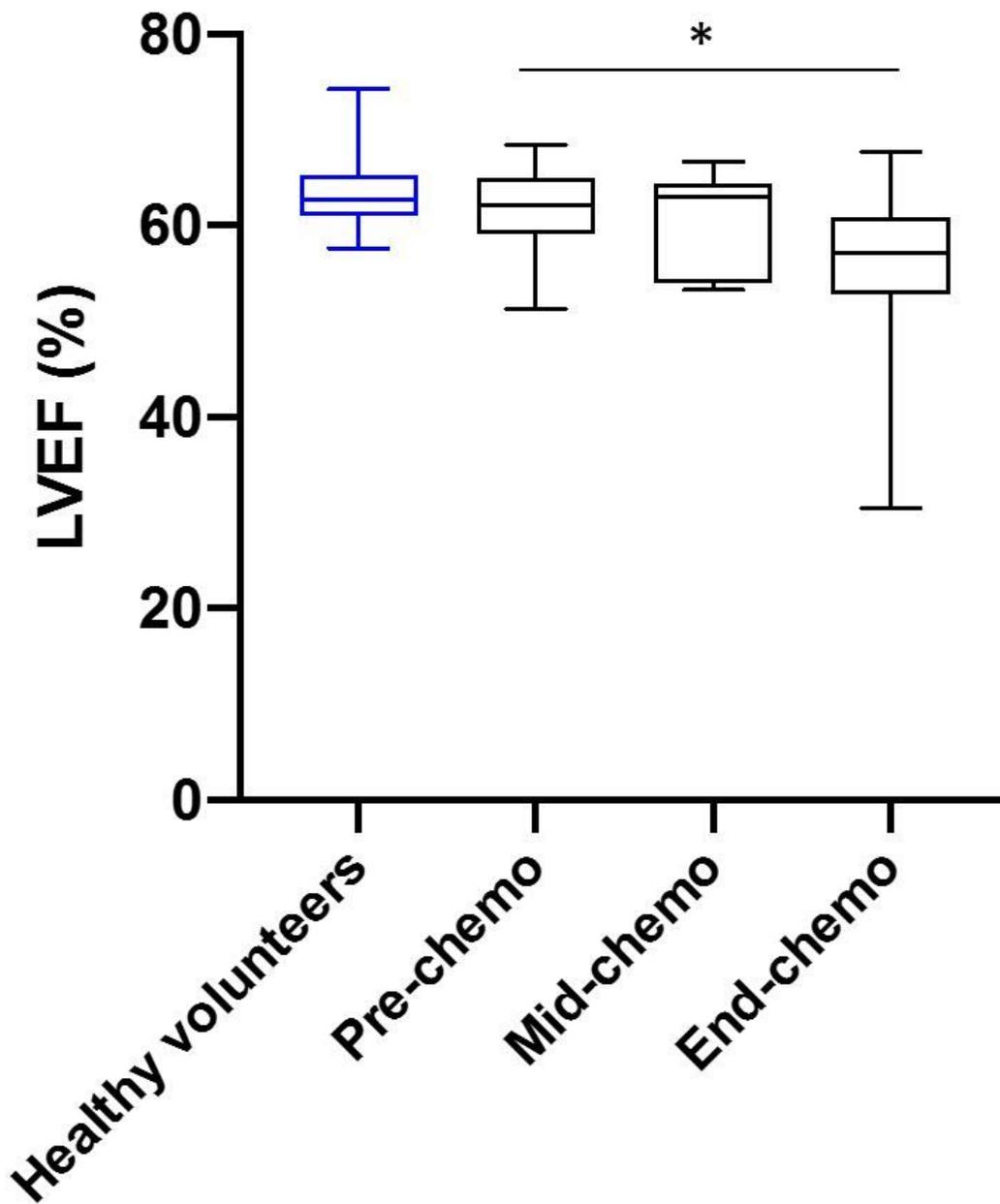


Figure 2

Left ventricular ejection fraction in healthy volunteers and in breast cancer patients during chemotherapy. Healthy volunteer left ventricular ejection fraction (LVEF, blue) and sequential LVEF in patients (black) pre-, mid and end chemotherapy acquired by cardiac magnetic resonance (CMR) imaging (box and whiskers plot, values are mean \pm SD and 95% confidence intervals (* P=0.02 for change from pre-to end chemo in patients (paired t-test)).

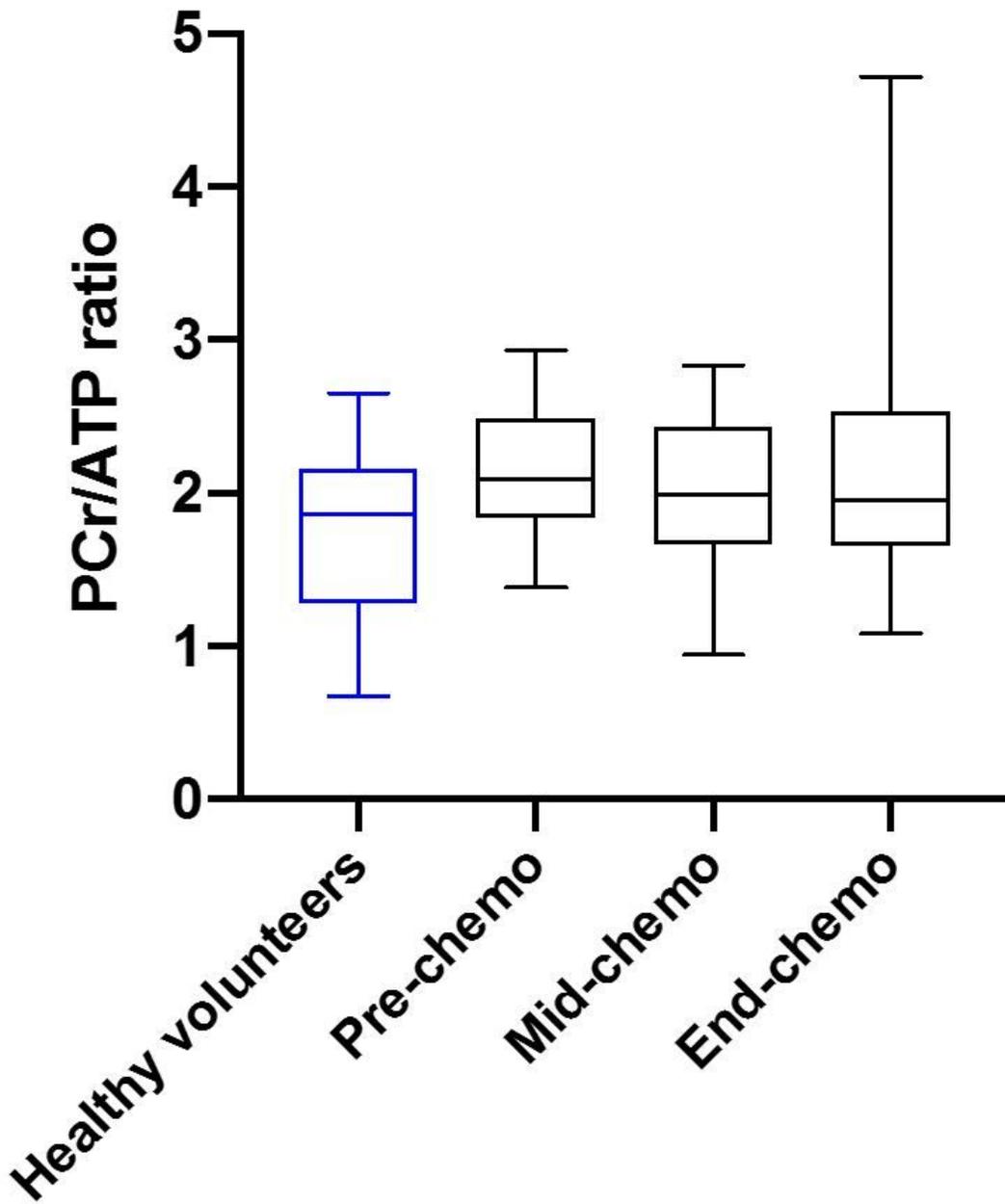


Figure 3

PCr/ATP ratio in healthy volunteers and in breast cancer patients during chemotherapy Myocardial PCr/ATP ratio (box and whiskers plot) in healthy volunteers (blue) and in breast cancer patients (black) pre, mid and at end of chemotherapy ($p=0.80$ for pre-chemo vs mid-chemo and $p=0.99$ for pre to end chemo (one way ANOVA with Tukey multiple comparison test))

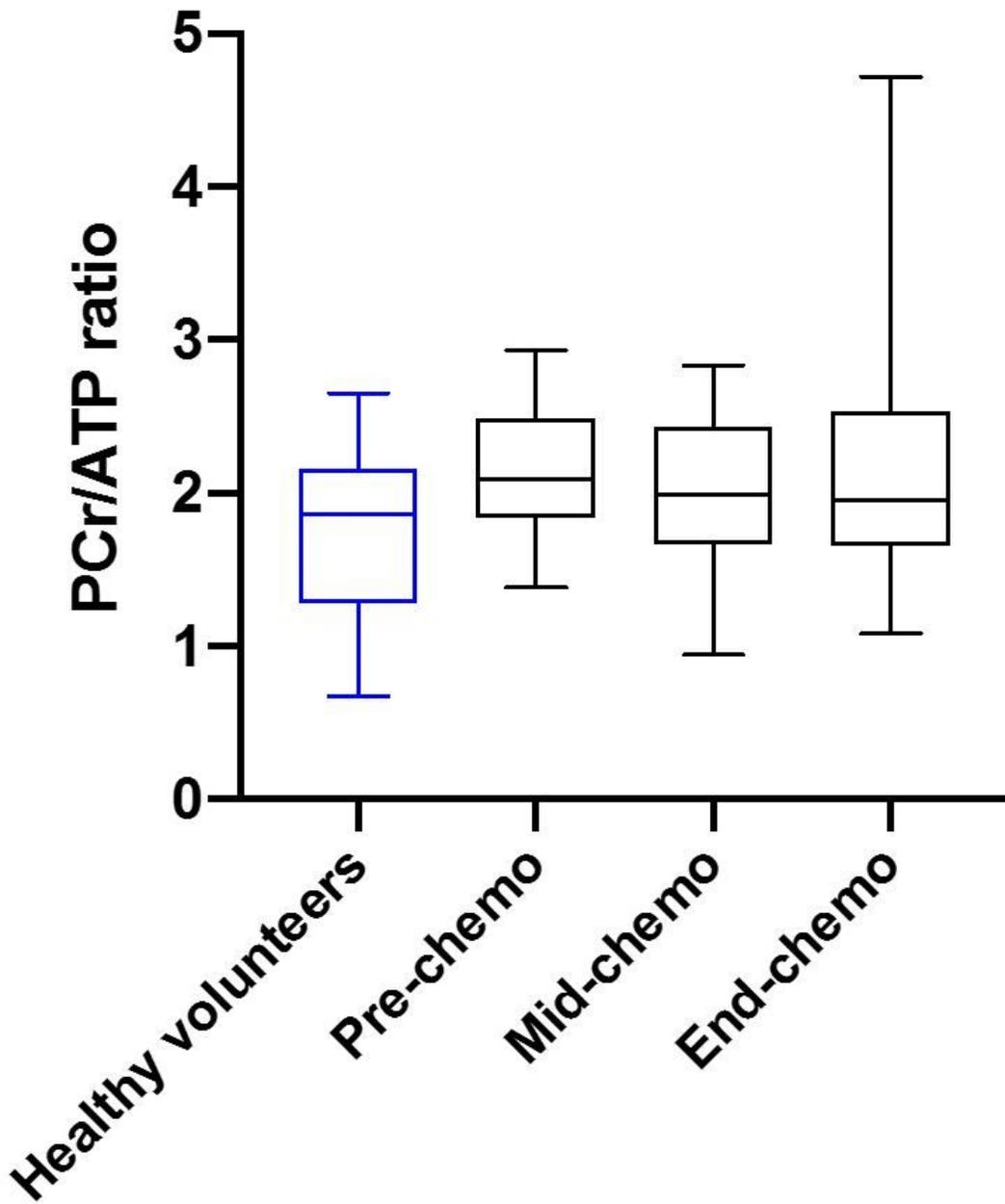


Figure 3

PCr/ATP ratio in healthy volunteers and in breast cancer patients during chemotherapy Myocardial PCr/ATP ratio (box and whiskers plot) in healthy volunteers (blue) and in breast cancer patients (black) pre, mid and at end of chemotherapy ($p=0.80$ for pre-chemo vs mid-chemo and $p=0.99$ for pre to end chemo (one way ANOVA with Tukey multiple comparison test))

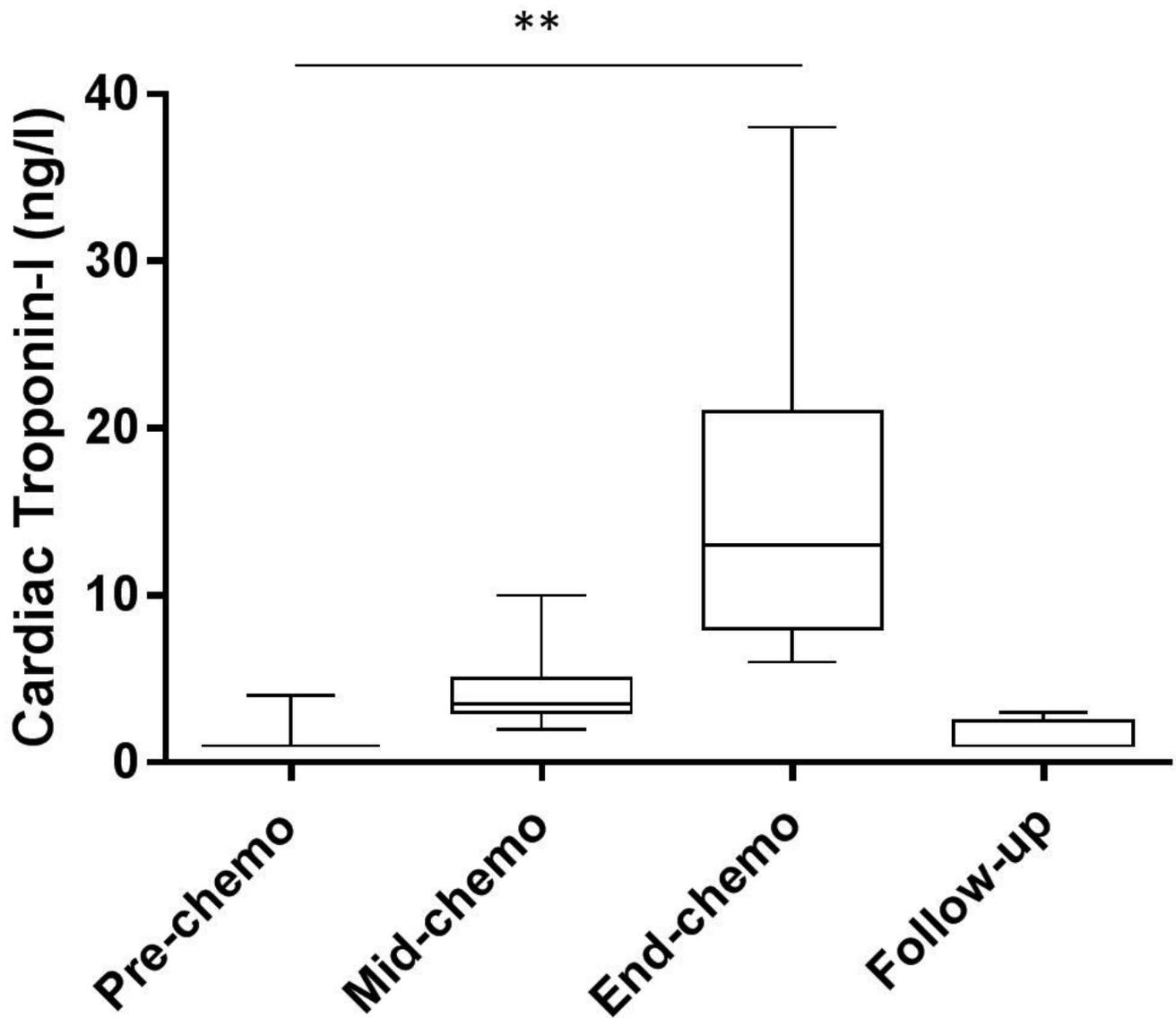


Figure 4

Plasma Cardiac troponin levels during chemotherapy in breast cancer patients High sensitivity cardiac troponin-I levels pre, mid and end-chemotherapy (box and whiskers plot, ** indicates $P < 0.001$ for change from pre-chemo (paired t-test)) and at 2-3 months follow-up in patients with values above the normal range at end-chemo (n=9).

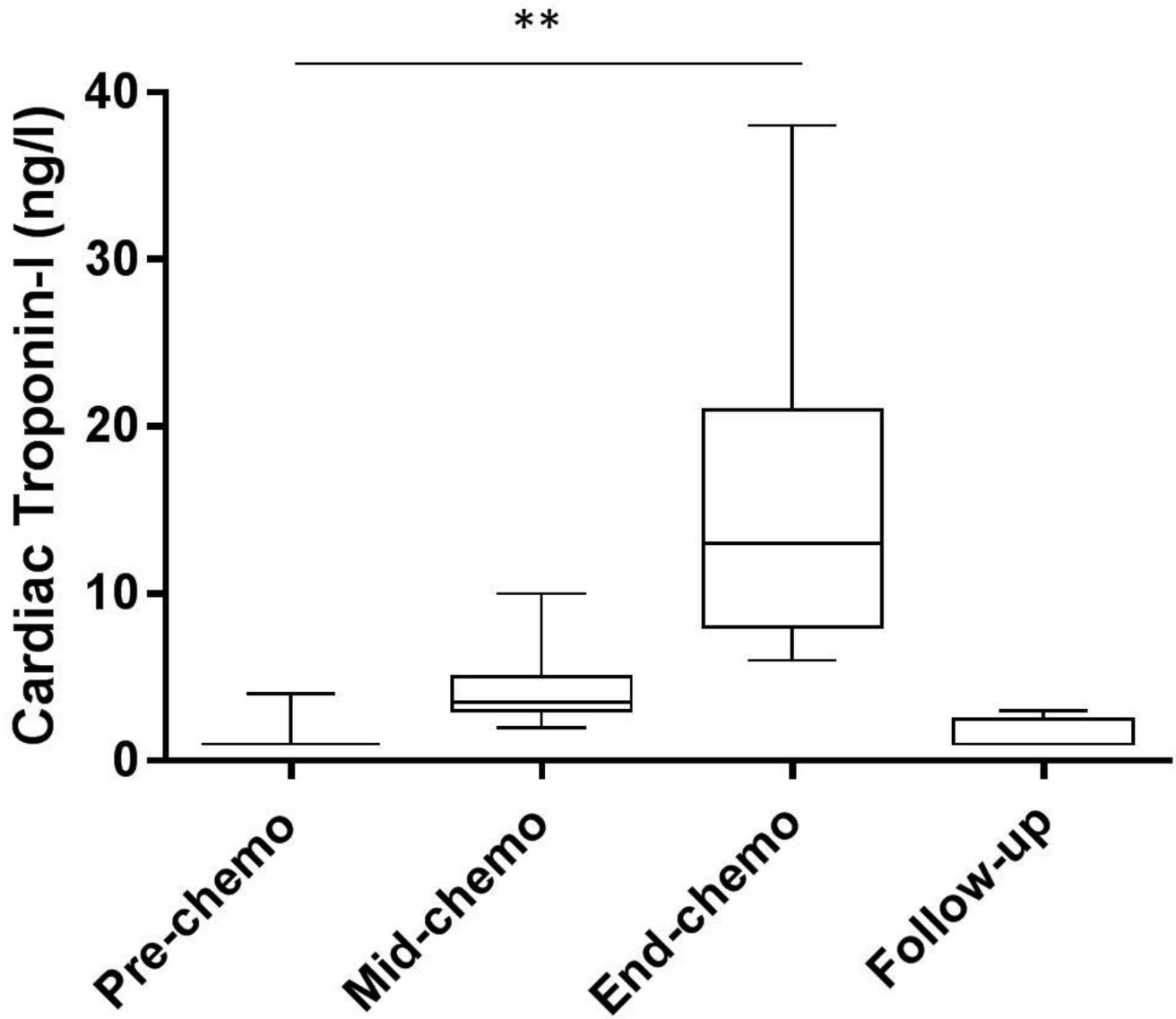
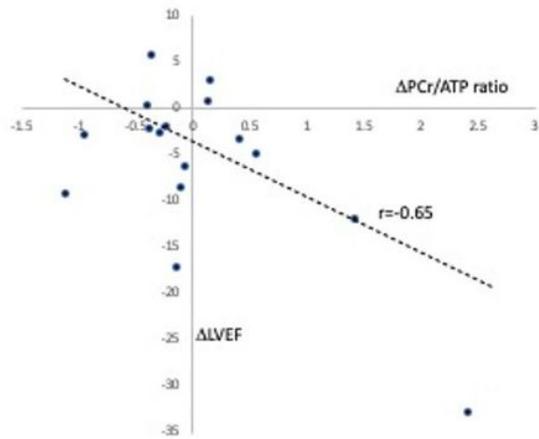


Figure 4

Plasma Cardiac troponin levels during chemotherapy in breast cancer patients High sensitivity cardiac troponin-I levels pre, mid and end-chemotherapy (box and whiskers plot, ** indicates $P < 0.001$ for change from pre-chemo (paired t-test)) and at 2-3 months follow-up in patients with values above the normal range at end-chemo (n=9).

A. Pre-end LVEF v pre-end PCr/ATP ratio



B. Mid-end LVEF v pre-end PCr/ATP ratio

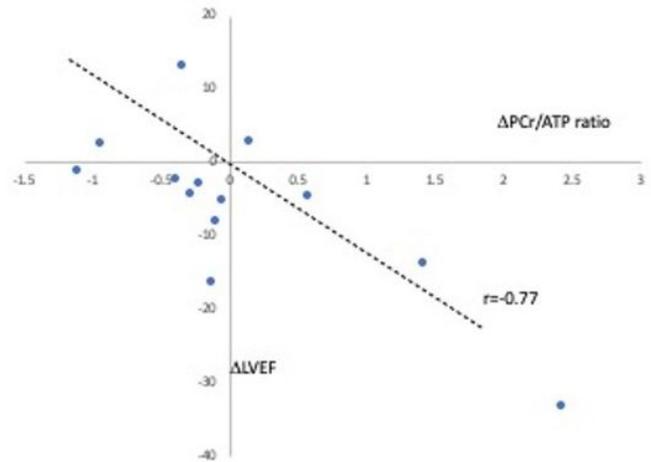
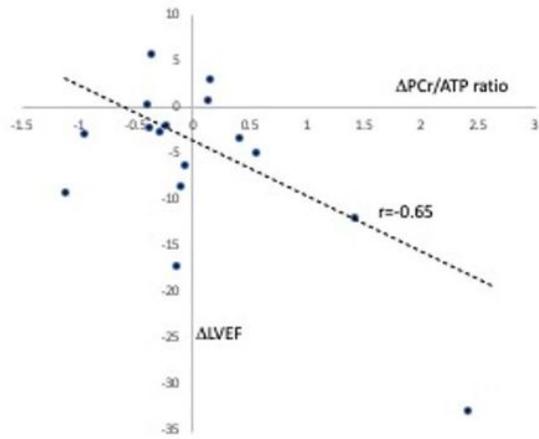


Figure 5

Associations between the change in PCr/ATP ratio and change in LVEF during chemotherapy

A. Pre-end LVEF v pre-end PCr/ATP ratio



B. Mid-end LVEF v pre-end PCr/ATP ratio

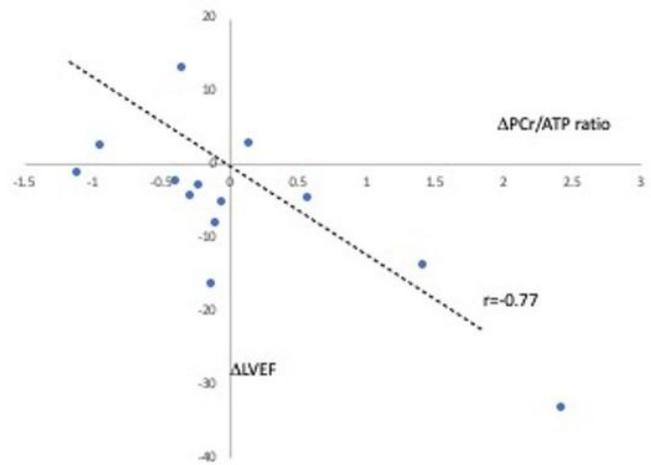


Figure 5

Associations between the change in PCr/ATP ratio and change in LVEF during chemotherapy