

# Circulating biomarkers and cardiac function over 3 years after chemotherapy with anthracyclines: the ICOS-ONE trial

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## Abstract

**Aims** A multicentre trial, ICOS-ONE, showed increases above the upper limit of normality of cardiac troponin (cTn) in 27% of patients within 12 months after the end of cancer chemotherapy (CT) with anthracyclines, whether cardiac protection with enalapril was started at study entry in all (*prevention arm*) or only upon first occurrence on supra-normal cTn (*troponin-triggered arm*). The aims of the present *post hoc* analysis were (i) to assess whether anthracycline-based treatment could induce cardiotoxicity over 36 month follow-up and (ii) to describe the time course of three cardiovascular biomarkers (i.e. troponin I cTnI-Ultra, B-type natriuretic peptide BNP, and pentraxin 3 PTX3) and of left ventricular (LV) function up to 36 months.

**Methods and results** Eligible patients were those prescribed first-in-life CT, without evidence of cardiovascular disease, normal cTn, LV ejection fraction (EF) >50%, not on renin-angiotensin aldosterone system antagonists. Patients underwent echocardiography and blood sampling at 24 and 36 months. No differences were observed in biomarker concentration between the two study arms, ‘prevention’ vs. ‘troponin-triggered’. During additional follow-up 13 more deaths occurred, leading to a total of 23 (9.5%), all due to a non-cardiovascular cause. No new occurrences of LV-dysfunction were reported. Two additional patients were admitted to the hospital for cardiovascular causes, both for acute pulmonary embolism. No first onset of raised cTnI-Ultra was reported in the extended follow-up. BNP remained within normal range: at 36 months was 23.4 ng/L, higher (N.S.) than at baseline, 17.6 ng/L. PTX3 peaked at 5.2 ng/mL 1 month after CT and returned to baseline values thereafter. cTnI-Ultra peaked at 26 ng/L 1 month after CT and returned to 3 ng/L until the last measurement at 36 months. All echocardiographic variables remained stable during follow-up with a median LVEF of 63% and left atrial volume index about 24 mL/m<sup>2</sup>.

**Conclusions** First-in-life CT with median cumulative dose of anthracyclines of 180 mg/m<sup>2</sup> does not seem to cause clinically significant cardiac injury, as assessed by circulating biomarkers and echocardiography, in patients aged 51 years (median),

without pre-existing cardiac disease. This may suggest either a 100% efficacy of enalapril (given as preventive or troponin-triggered) or a reassuringly low absolute cardiovascular risk in this cohort of patients, which may not require intensive cardiologic follow-up.

**Keywords** Cardio-oncology; Anthracyclines; Troponin; Echocardiography; Biomarkers; Cardiac dysfunction

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## Introduction

Cancer chemotherapy (CT) containing anthracyclines (AC) is known to cause myocardial injury in some patients, which can lead over time to left ventricular dysfunction (LVD) and even clinical heart failure (HF).<sup>1–3</sup> Serial echocardiographic exams are routinely used to monitor cardiac function after CT, but the measurement of left ventricular ejection fraction (LVEF) is recognized as being not sensitive enough to predict the occurrence of overt LVD and although other echocardiographic methods to evaluate left ventricular function, such as strain, have been proposed,<sup>4</sup> they are still not routinely used.

Monitoring of circulating cardiac biomarkers, cardiac troponins (cTn) in particular, has been suggested to be a sensitive tool for early diagnosis of LVD after anthracycline-based CT.<sup>5–7</sup> Specifically, the use of high-sensitive assays to detect cTn has been shown to predict anthracycline-induced LVD<sup>8,9</sup> and to guide cardioprotective therapy with enalapril.<sup>10</sup>

Based on these assumptions, a recent multicentre controlled trial, ICOS-ONE,<sup>11</sup> has compared two different prevention strategies using enalapril against AC-induced cardiotoxicity over 12 month follow-up. Patients were randomly assigned to the prevention arm (enalapril started in all patients before CT) and the troponin-triggered arm (enalapril started only in patients with an increase in troponin during or after CT). They did not have evidence of cardiovascular disease, their age averaged 51 years, and they all were prescribed first-in-life CT with a cumulative dose of anthracycline of 180 mg/m<sup>2</sup>. In good agreement with previous reports,<sup>6,7</sup> the occurrence of troponin rise was 23% and 26% in prevention and troponin-triggered arms, respectively. Even so, overall 12 month incidence of LVD was 1.1%.

While cTn was measured in local clinical chemistry laboratories, serial blood samples were collected in a biobank to be analysed in two core laboratories to measure circulating cardiovascular biomarkers. In addition to the cardiac-specific ultra-sensitive troponin I (cTnI-Ultra) and B-type natriuretic peptide (BNP), pentraxin 3 (PTX3), a known multifunctional protein also produced by endothelial cells under inflammatory stimuli,<sup>12</sup> prognostic marker in cardiovascular diseases,<sup>13–15</sup> was assayed in all samples available.

In order to limit potential bias related to the specialty of the investigators,<sup>16</sup> ICOS-ONE directly involved with equal

proportion cardiologists and oncologists in each of the 21 participating centres.

The aims of the present study were to describe the time course of three cardiovascular biomarkers (i.e. BNP, PTX3, and cTnI-Ultra), up to 36 months in the two study groups, and to assess possible relations with cardiac function and clinical events.

## Methods

### Study design and patients

Study design and patients' characteristics have been described extensively in a previous publication.<sup>11</sup> In short, the ICOS-ONE trial was a controlled open-label trial randomizing first diagnosis cancer patients with an indication for therapy with anthracyclines into either enalapril started right before chemotherapy (prevention group) or enalapril started at the time of the first occurrence of an abnormal plasma concentration of troponin (troponin-triggered group) and continued for 12 months. Eligible patients did not have evidence of cardiovascular disease, and they all were prescribed first-in-life CT. Moreover, their cTn had to be within the normal range at baseline and the LVEF >50%. They had to not be on therapy with renin-angiotensin aldosterone system (RAAS) antagonists.

At the conclusion of the 12 month follow-up, participating centres were invited to extend the study for an additional 24 months, leading to a total follow-up of 36 months. This decision was taken mainly because of the unexpectedly low incidence of cardiovascular adverse events attributable to anthracyclines.<sup>11</sup>

Data were analysed according to the pre-specified criteria, by study group, but, given the lack of any difference between the two study groups, even after inclusion of 24 additional months of follow-up, data have also been analysed and presented as a single cohort.

All primary and secondary endpoints were adjudicated by an independent committee blind to patient identification and study arm.

## Biomarkers of cardiotoxicity

Blood samples were collected at each CT cycle—up to the 5th cycle—and during follow-up visits at 1, 6, 12, 24, and 36 months after CT and centrifuged. Plasma shipped on dry ice to a central repository was stored at  $-70^{\circ}\text{C}$  until assayed. The following cardiovascular biomarkers were assayed in a core laboratory by personnel blind to patients' characteristics and study group:

- BNP: as a marker of stretch of the heart chambers;
- PTX3: as an index of the extent of systemic inflammatory activation, in particular at the vascular level; and
- cTnI-Ultra: as a marker of myocardial injury.

## Echocardiography

Patients underwent echocardiographic exams at entry and 1, 3, 6, 12, 24 and 36 months after the end of CT as described.<sup>11</sup> Echocardiographic exams were read locally first and then sent as digital recordings to the Echocardiographic Core Laboratory at the European Institute of Oncology, where a quality control on randomly selected recordings was performed.

## Cardiac magnetic resonance

In a subset of nine patients who accepted to undergo serial cardiac MRI in one center (Philips 3T, IRCCS Ospedale San Raffaele, Università Vita-Salute, Milano, Italy), serial exams were performed at randomization and during follow-up, median time 12 months, [3–27] months, according to standard protocol for functional and structural evaluation.

## Statistical methods

Intra-observer concordance between local and centrally determined raised troponin was assessed by means of Cohen's kappa. To allow for asymmetrical imbalance, the maximal kappa was calculated by the method of Feinstein and Cicchetti.<sup>17</sup>

Biomarkers are reported for each time point as median and [Q1–Q3]. The within-subjects effect of time was assessed by means of one-way ANOVA for repeated measurements (after log-transformation of data) with *post hoc* Sidak-correction. If the assumption of sphericity was violated, a Greenhouse–Geisser correction was included. As only cases are included who have all measurements, data from the following time points are included: baseline, 1, 12, 24, and 36 months follow-up. Upon stratification by different variables (raised troponin, age, type of chemotherapy, chemotherapy dose, type of cancer, and echocardiographic

characteristics), biomarker concentrations were compared per time point by means of Kruskal–Wallis.

Differences over time between groups and the interaction with time of biomarkers (after log-transformation of data) were assessed by mixed model analysis. Included were patients with complete collection of blood samples at the following time points: baseline and 1, 12, 24, and 36 months follow-up; 3 and 6 months data were not included due to the excessive number of missing cases. A violation of sphericity (as tested by Mauchly's test) was corrected by means of Greenhouse–Geisser and concentrations were log-transformed to approach normality. If a significant interaction with time was found, *post hoc* analyses were performed with Sidak-correction.

## Results

### Patients

Of the 273 patients included in ICOS-ONE, 242 provided at least one biological sample for central assays. Baseline clinical characteristics were similar between the total ICOS-ONE population and those who provided biological samples (*Table 1* and *Figure 1*).

### Study treatments

At 1-year follow-up, 173 patients were on enalapril: 136 randomized to 'prevention' arm, and 37 randomized to 'troponin-triggered' arm with raised cTn. Fifty-one patients were still taking the drug at 36 months follow-up for decision of the attending physician: 13 in the troponin-triggered arm, 38 in the prevention arm.

No differences were observed in biomarker concentration (*Table 2* and Supportig Informartion, *Figure S1*) or in any of the echocardiographic or clinical endpoints (data not shown) between the two study treatments of ICOS-ONE. Therefore, patients were also analysed as a single cohort.

### First occurrence of a raised local troponin

Cardiac troponins (cTn) were measured at the local clinical chemistry laboratories of each hospital as primary endpoint of the trial in 261 patients.<sup>11</sup> In addition to the 67 incident cases observed within the first 12 months of follow-up, only one patient presented first time raised cTn at 24 months follow-up.

**Table 1** Patient characteristics

|  | All (N = 273)    | With biomarkers assayed (N = 242) |
|--|------------------|-----------------------------------|
| Demographics                             |                  |                                   |
| Sex (female)                             | 241 (88.3)       | 214 (88.4)                        |
| Age (years)                              | 51.3 ± 11.9      | 51.2 ± 11.8                       |
| BMI (kg/m <sup>2</sup> )                 | 23.9 [21.7–26.9] | 24.6 [21.7–27.1]                  |
| Heart rate (b.p.m.)                      | 75 [68–80]       | 75 [68–81]                        |
| Systolic BP (mmHg)                       | 120 [115–130]    | 120 [115–130]                     |
| Diastolic BP (mmHg)                      | 80 [70–80]       | 75 [70–80]                        |
| EF (%)                                   | 63 [60–67]       | 63 [60–67]                        |
| Clinical history                         |                  |                                   |
| Hospitalization for any reason last year | 140 (51.3)       | 130 (53.7)                        |
| Hypertension                             | 7 (2.6)          | 6 (2.5)                           |
| Dyslipidemia                             | 17 (6.2)         | 17 (7.0)                          |
| Diabetes                                 | 11 (4.0)         | 9 (3.7)                           |
| Smoker                                   | 44 (16.1)        | 42 (17.4)                         |
| Ex-smoker                                | 35 (12.8)        | 26 (10.7)                         |
| Stroke                                   | 2 (0.7)          | 2 (0.8)                           |
| Atrial Fibrillation                      | 2 (0.7)          | 2 (0.8)                           |
| Cancer type and CT                       |                  |                                   |
| Breast cancer                            | 207 (75.8)       | 183 (75.6)                        |
| Leukaemia                                | 29 (10.6)        | 26 (10.7)                         |
| Lymphoma                                 | 35 (12.8)        | 31 (12.8)                         |
| Sarcoma                                  | 2 (0.7)          | 2 (0.8)                           |
| Cycles of CT (# of rounds)               | 4 [3–4]          | 4 [3–4]                           |
| Dose* (mg/m <sup>2</sup> )               | 180 [135–240]    | 180 [135–240]                     |

Data are shown as n (%), mean ± standard deviation, or median [interquartile range]. BMI, body mass index; BP, blood pressure; EF, ejection fraction; CT, chemotherapy.

\*Cumulative anthracycline equivalent dose.

## Clinical events

Clinical events in ICOS-ONE study are listed in *Table 3*. During the extended follow-up from 12 to 36 months additional 13 patients died, accumulating to a total of 23 (9.5%), all due to a non-cardiovascular cause. Fourteen out of 183 breast cancer patients (7.7%), 8/26 leukaemia (30.8%), and 1/31 lymphoma (3.2%) died. No new occurrences of left ventricular dysfunctions were reported. Two additional patients were admitted to the hospital for acute pulmonary embolism; no other new cardiovascular event was reported.

## Central troponin

cTn-Ultra was measured in 242 patients at the centralized laboratory. Fifty-eight patients experienced raised troponin, 15 experienced their first elevated level during CT, and the remaining 43 patients presented raised troponin during follow-up of whom 39 at 1 month FU. No first onset of raised cTn-Ultra was reported in the extended follow-up period. One patient, who already experienced raised cTn-Ultra in the first 12 months, was found to have raised cTn-Ultra at 24 months. However, cTn returned also in this patient to normal values at 36 months. At 36 months, cTn-Ultra levels did not exceed 13 ng/L, which is well below the ULN of 40 ng/L.

The observed agreement between local and central measurements of cTn, defined as ‘raised’ in 1382 samples was 96% with a Cohen’s kappa of 0.58. This high agreement but low kappa can be explained by the symmetrical imbalance, which is present in our population.<sup>17</sup> Due to this imbalance, the maximum possible kappa is 0.66, which means that our observed kappa of 0.58 is 88% of the maximum possible value.

## Time course of BNP, PTX3, and cTn-Ultra

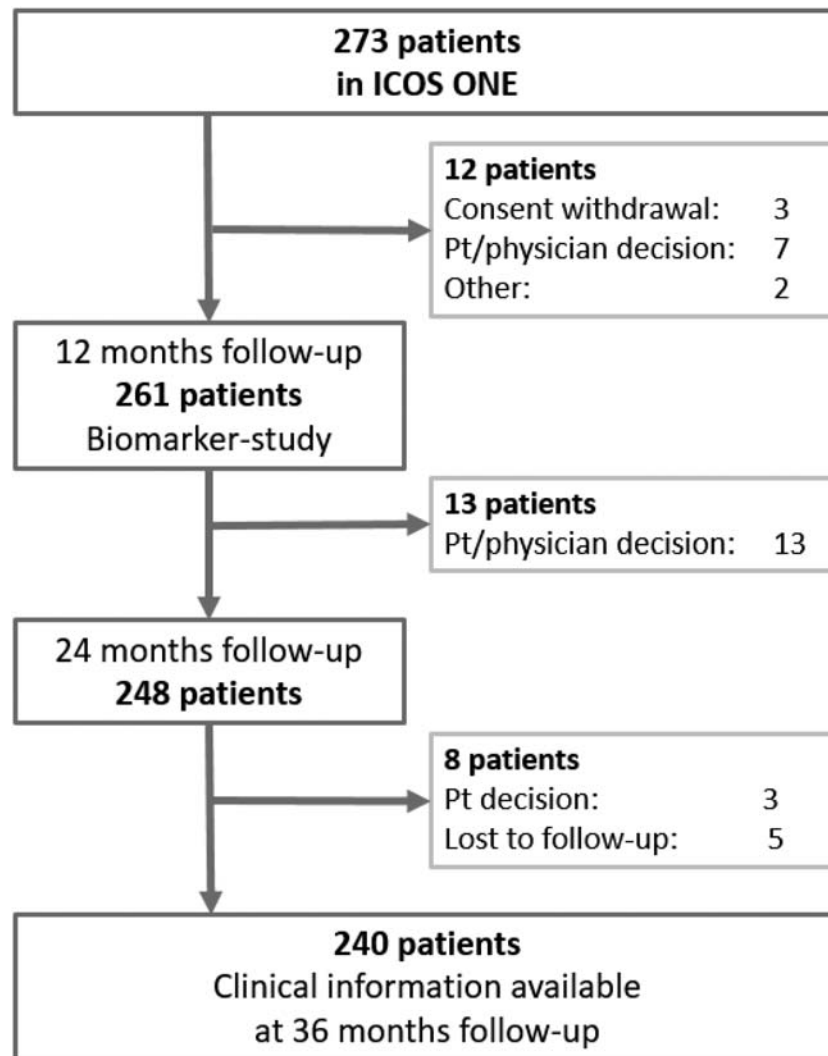
Baseline concentrations of BNP and cTn-Ultra were respectively 17.6 and 4.0 ng/L, well within the range of normality (*Table 2*). On the other hand, the median baseline concentration of PTX3 was 3.7 ng/mL, definitely higher than the upper limit of normality of 2 ng/mL.<sup>18</sup> Time courses of the three biomarkers showed peculiar features (*Figure 2*):

BNP remained within the normal range with minor fluctuations. The 36 month value was 23.4 ng/L, similar to the baseline value of 17.6 ng/L.

- PTX3 reached a peak of 5.2 ng/mL 1 month after CT and returned to baseline values from 12 months onwards (median 3.7 ng/mL at baseline, 3.5 ng/mL at 12 months, 3.4 ng/mL at 24 months, and 3.8 ng/mL at 36 months). A significant effect of time ( $F_{2,4, 103.5} = 14.30, P < 0.001$ ) was present in the PTX3 measurements and *post hoc* analyses showed significant differences for 1 month follow-up with all other measurements ( $P < 0.001$ ).
- cTn-Ultra significantly increased during CT, reaching a peak of 26 ng/L 1 month after the final CT; while at 12 months after the final CT the concentration of cTn-Ultra returned to 3 ng/L, similar to baseline value, and remained at this very low level until the last measurement at 36 months. A significant effect of time was found ( $F_{3,4, 140.3} = 104.2, P < 0.001$ ). *Post hoc* analyses showed that cTn-Ultra was significantly different from all other time points at 1 month follow-up. cTn-Ultra was significantly lower than baseline at 24 and 36 months follow-up ( $P$ -value respectively 0.023 and 0.011).

## Age

Patients were split by median age (51.3 years) into two groups. As expected, BNP was consistently and significantly higher at each time point in the older patients. Younger persons had higher levels of PTX3 at 12 ( $P = 0.002$ ), 24 ( $P = 0.003$ ), and 36 ( $P = 0.012$ ) months follow-up. cTn-Ultra was significantly higher in the older patients at baseline ( $P < 0.001$ ), 1 month follow-up ( $P = 0.008$ ), and 36 months follow-up ( $P = 0.016$ ). None of the biomarkers showed any

**Figure 1** Patient disposition.

interaction of age and time course (Supporting Information, *Figure S2*).

### Primary tumour of patients enrolled

Patients with breast cancer were more frequently hospitalized in the year before inclusion in the study, mostly due to scheduled surgery such as mastectomy and quadrectomy (Supporting Information, *Table S1*). Patients with lymphoma received on average 5.7 cycles of CT with a median dose of 300 mg/m<sup>2</sup> whereas leukaemia patients received on average 2 cycles accumulating to a median dose of 50 mg/m<sup>2</sup>. Upon comparing breast cancer patients ( $N = 183$ ) with lymphoma ( $N = 31$ ) and leukaemia ( $N = 26$ ), BNP levels were significantly higher in leukaemia patients at baseline, while they were significantly lower in leukaemia patients at 24 months FU (Supporting Information, *Table S2*).

PTX3 concentrations were significantly higher in patients with leukaemia than in those with breast cancer or lymphoma, at baseline (6.1, 4.0 and 3.5 ng/mL respectively,  $P = 0.003$ ). The levels of PTX3 were consistently higher in leukaemia patients during CT, although these differences were not statistically significant. Troponin was significantly raised at baseline in leukaemia as compared with breast cancer patients (8.5 vs. 3.0 ng/mL,  $P < 0.001$ ) whereas the peak of troponin at 1 month follow-up was higher in lymphoma and breast cancer (37.0 and 26.5 ng/mL) than in leukaemia patients (8.0 ng/mL,  $P < 0.001$ ) (*Figure 3* and Supporting Information, *Table S2*).

### Chemotherapy: doxorubicin/epirubicin

Because 40% of the patients received doxorubicin and 48% epirubicin, the time courses of the concentrations of the three



**Table 2** Plasma concentration of the three circulating biomarkers in 242 patients with at least one blood sample centrally assayed

|                          | No. of samples/No. of pts* | All patients     | Troponin-triggered | Prevention group | P**   |
|--------------------------|----------------------------|------------------|--------------------|------------------|-------|
| <b>BNP (ng/L)</b>        |                            |                  |                    |                  |       |
| Baseline                 | 230/242                    | 17.6 [9.4–20.1]  | 16.8 [8.6–27.9]    | 18.6 [10.1–28.5] | 0.285 |
| 2nd CT cycle             | 200                        | 16.3 [10.4–29.3] | 18.4 [11.2–29.2]   | 14.5 [9.7–30.7]  | 0.465 |
| 3rd CT cycle             | 184                        | 18.6 [9.9–30.6]  | 18.9 [10.4–29.5]   | 17.6 [9.2–32.7]  | 0.880 |
| 4th CT cycle             | 151                        | 17.5 [9.5–25.5]  | 18.4 [9.3–26.3]    | 17.2 [11.0–23.8] | 0.982 |
| 1 month FU               | 179/234                    | 18.3 [11.1–31.8] | 19.0 [11.6–32.5]   | 16.3 [9.6–31.3]  | 0.492 |
| 6 months FU              | 137/223                    | 18.8 [9.7–30.6]  | 19.9 [9.5–29.3]    | 18.0 [9.8–33.6]  | 0.919 |
| 12 months FU             | 146/217                    | 16.9 [7.5–30.4]  | 13.8 [7.4–30.3]    | 17.9 [7.6–30.5]  | 0.521 |
| 24 months FU             | 94/191                     | 19.1 [11.8–31.3] | 19.3 [13.1–29.4]   | 17.7 [11.3–38.2] | 0.921 |
| 36 months FU             | 82/173                     | 23.4 [14.4–38.3] | 23.0 [14.3–38.0]   | 23.4 [14.5–40.1] | 0.635 |
| <b>PTX3 (ng/mL)</b>      |                            |                  |                    |                  |       |
| Baseline                 | 230/242                    | 3.7 [2.5–5.5]    | 3.8 [2.6–5.9]      | 3.6 [2.4–5.8]    | 0.210 |
| 2 <sup>nd</sup> CT cycle | 200                        | 4.1 [2.7–5.4]    | 4.0 [2.8–5.1]      | 4.2 [2.6–5.9]    | 0.524 |
| 3 <sup>rd</sup> CT cycle | 184                        | 4.1 [2.6–5.8]    | 4.0 [2.7–5.8]      | 4.2 [2.5–5.8]    | 0.899 |
| 4 <sup>th</sup> CT cycle | 151                        | 4.1 [2.9–5.9]    | 4.3 [3.1–6.1]      | 4.0 [2.6–5.5]    | 0.200 |
| 1 month FU               | 179/234                    | 5.2 [3.8–8.2]    | 5.3 [4.2–9.0]      | 5.2 [3.3–8.0]    | 0.263 |
| 6 months FU              | 137/223                    | 4.1 [3.0–6.7]    | 4.4 [3.1–6.7]      | 3.9 [3.0–6.7]    | 0.506 |
| 12 months FU             | 146/217                    | 3.5 [2.6–5.4]    | 3.9 [2.7–5.5]      | 3.1 [2.3–5.4]    | 0.082 |
| 24 months FU             | 94/191                     | 3.4 [2.5–5.2]    | 3.4 [2.4–4.9]      | 3.4 [2.6–5.4]    | 0.797 |
| 36 months FU             | 82/173                     | 3.8 [2.6–5.1]    | 3.8 [2.9–5.2]      | 3.8 [2.5–5.0]    | 0.738 |
| <b>cTnI-Ultra (ng/L)</b> |                            |                  |                    |                  |       |
| Baseline                 | 230/242                    | 4.0 [2.0–7.0]    | 4.0 [2.0–7.0]      | 4.0 [2.0–7.0]    | 0.545 |
| 2nd CT cycle             | 200                        | 7.0 [4.0–10.0]   | 7.0 [4.0–10.5]     | 7.0 [4.0–10.0]   | 0.809 |
| 3rd CT cycle             | 184                        | 9.0 [6.0–12.0]   | 9.0 [6.0–12.0]     | 9.5 [6.0–14.0]   | 0.862 |
| 4th CT cycle             | 151                        | 15.0 [10.0–24.0] | 14.0 [10.0–21.0]   | 15.0 [10.0–25.0] | 0.429 |
| 1 month FU               | 179/234                    | 26.0 [16.0–42.0] | 26.0 [16.0–42.0]   | 25.0 [15.5–42.5] | 0.999 |
| 6 months FU              | 137/223                    | 7.0 [3.0–12.0]   | 7.0 [3.0–12.0]     | 7.0 [2.3–13.5]   | 0.941 |
| 12 months FU             | 146/217                    | 3.3 [0–7.0]      | 3.0 [0.0–7.0]      | 4.3 [0.0–7.1]    | 0.686 |
| 24 months FU             | 94/191                     | 0.0 [0–3.5]      | 0.0 [0.0–4.7]      | 0.0 [0.0–3.1]    | 0.528 |
| 36 months FU             | 82/173                     | 0.8 [0–3.2]      | 0.0 [0.0–3.3]      | 0.9 [0.0–3.1]    | 0.748 |

Data are shown as median [interquartile range].

\*With available clinical information.

\*\*P-value for Mann–Whitney *U* test.

**Table 3** Clinical events

|                                 | In first 12 months (N = 273) | During extended FU (12–36 months) (N = 217) | Total (N = 273) |
|---------------------------------|------------------------------|---|-----------------|
| All-cause mortality             | 10 (4.1)                     | 13 (6.0)                                    | 23 (8.4)        |
| Cardiovascular mortality        | 0                            | 0   | 0               |
| Non-cardiovascular mortality    | 10 (4.1)                     | 13 (6.0)                                    | 23 (8.4)        |
| Tumour                          | 7 (2.9)                      | 10 (4.6)                                    | 17 (6.2)        |
| Infection                       | 3 (1.1)                      | 2 (0.9)                                     | 5 (1.8)         |
| Respiratory failure (COPD)      | 0                            | 1 (0.5)                                     | 1 (0.4)         |
| Left ventricular dysfunction    | 3 (1.1)                      | 0   | 3 (1.1)         |
| First CV hospitalization        |                              |   |                 |
| Acute pulmonary embolism        | 1 (0.4)                      | 2 (0.9)                                     | 3 (1.1)         |
| Other CV events                 | 1 (0.4)                      | 0   | 1 (0.4)         |
| Heart failure                   | 0                            | 0   | 0               |
| Stroke                          | 0                            | 0   | 0               |
| Acute coronary syndrome         | 0                            | 0   | 0               |
| Acute pulmonary oedema          | 1 (0.4)                      | 0   | 1 (0.4)         |
| Arrhythmias requiring treatment | 3 (1.1)                      | 0   | 3 (1.1)         |

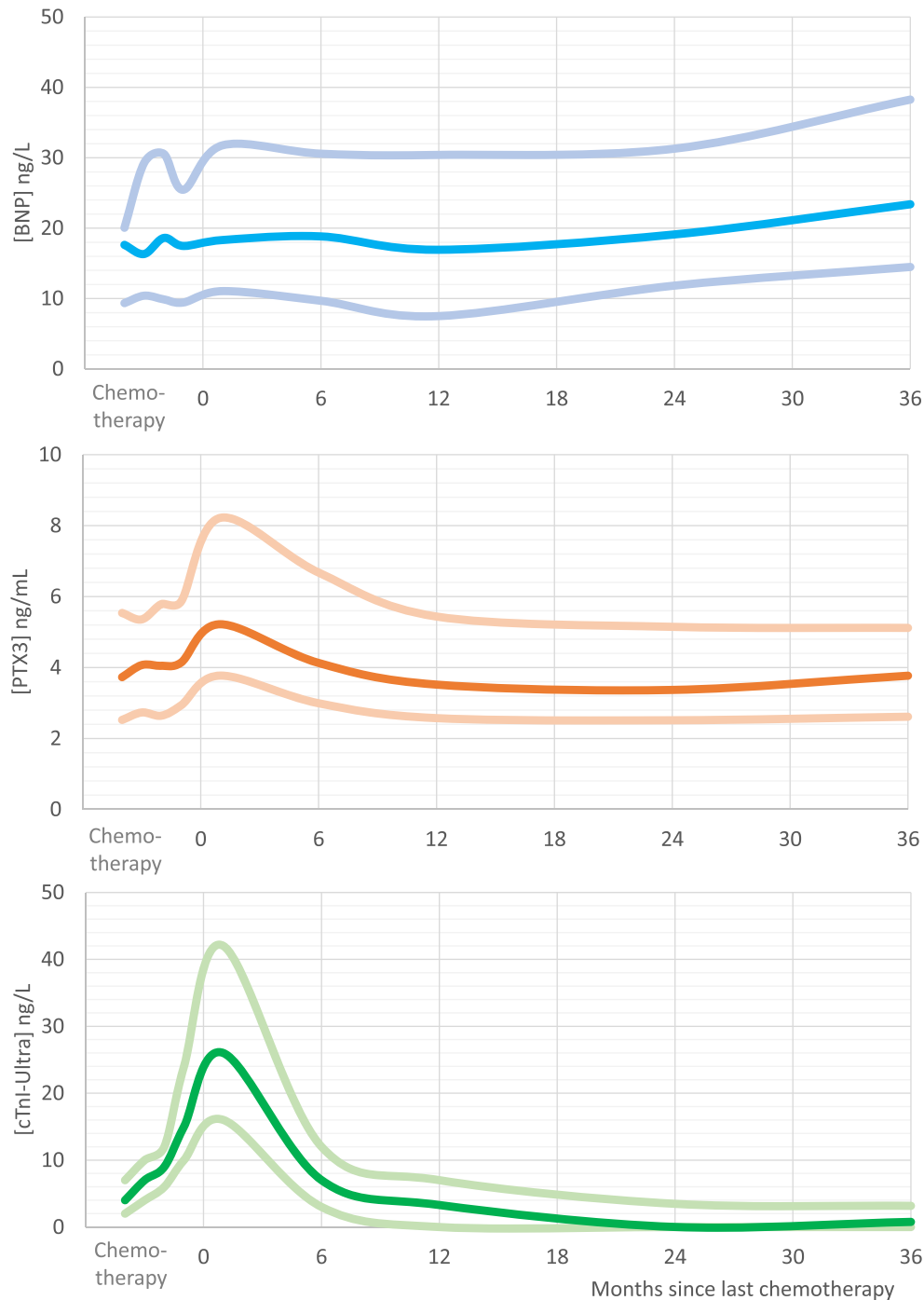
Data are shown as N (%). CV, cardiovascular; COPD, chronic obstructive pulmonary disease.

biomarkers were compared stratified by type of anthracycline (Supporting Information, *Figure S3* and *Table S3*). Troponin levels were higher at baseline and during CT in the epirubicin group; however during follow-up, at 1 and 6 months, cTnI-Ultra was significantly higher in the doxorubicin group. No differences were apparent for the other two biomarkers.

### Chemotherapy: cumulative dose

The different types of anthracyclines were converted into doxorubicin equivalents.<sup>19</sup> Upon stratification by median cumulative dose (180 mg/m<sup>2</sup>), BNP plasma concentrations, which started to diverge at 6 months, were consistently

**Figure 2** Biomarker concentration during ICOS-ONE study ( $n = 242$ ) over 36 months. Median concentration of BNP (in blue), PTX3 (in orange), and troponin (in green) during chemotherapy and follow-up. Shaded lines represent the interquartile range [Q1–Q3].



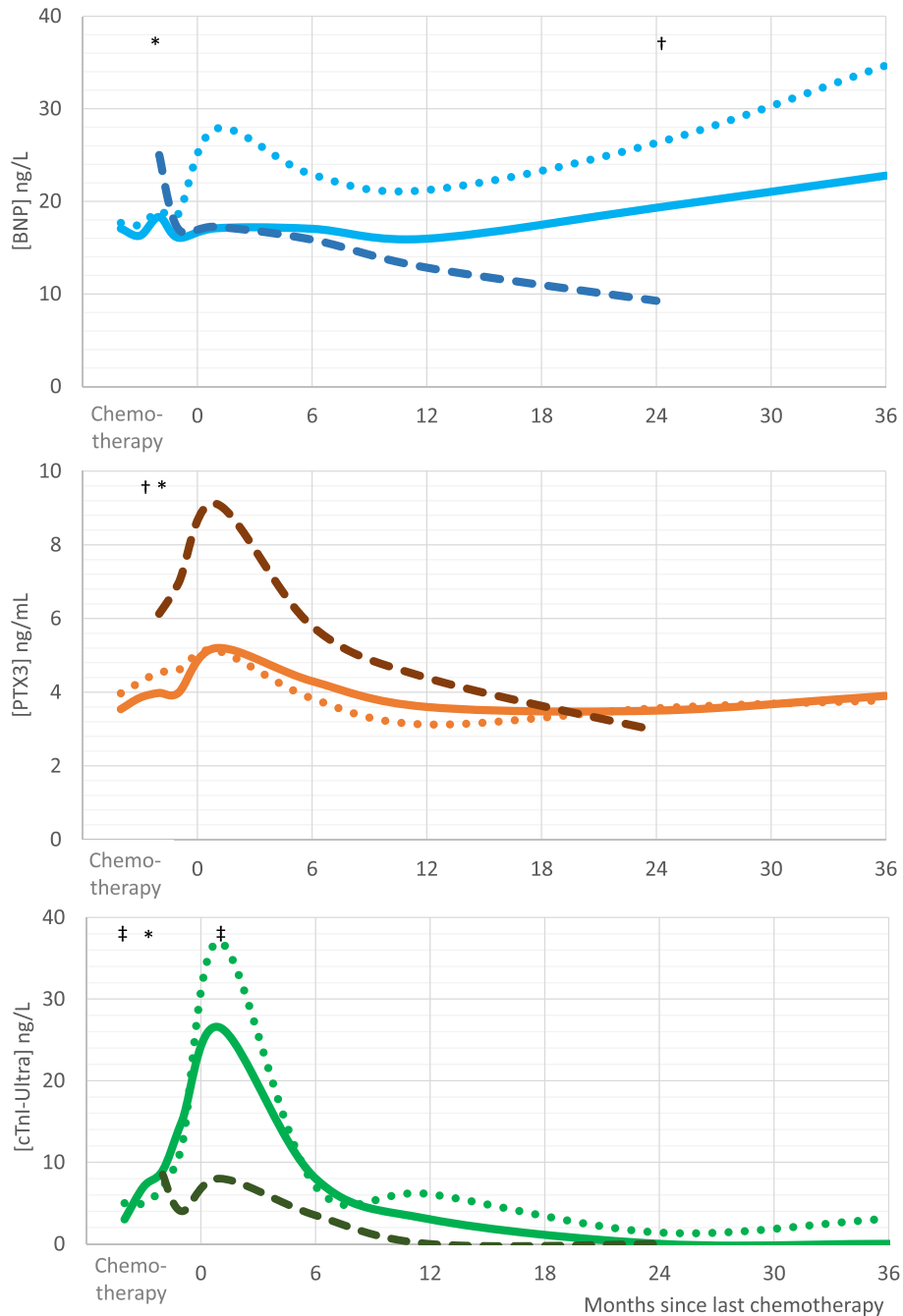
elevated over time in patients who received higher doses of anthracycline ( $F_{4, 148} = 2.385, P = 0.054$ ) (Figure 4).

The peak value of troponin at 1 month follow-up was higher in patients with higher doses of anthracyclines (21 vs. 32.5 ng/L,  $P < 0.001$ ), and a significant interaction with time was found ( $F_{3, 133} = 3.6, P = 0.012$ ).

For PTX3 no differences were observed between patients with high or low doses of anthracyclines. See also Figure 4 and Supporting Information, Table S4.

In addition to chemotherapy, 12 breast cancer patients received intra-operative radiotherapy during surgery. No significantly different levels of biomarkers were

**Figure 3** Biomarker concentration during study by type of cancer. Median concentration of BNP (in blue), PTX3 (in orange), and troponin (in green) during chemotherapy and follow-up. Stratified for cancer type, patients with breast cancer are represented by the continuous line, leukaemia by the dashed line, and lymphoma by the dotted line. \* $P < 0.05$ , † $P < 0.01$ , ‡ $P < 0.001$ .



found upon comparing these patients with other breast cancer patients who did not get intra-operative radiotherapy.

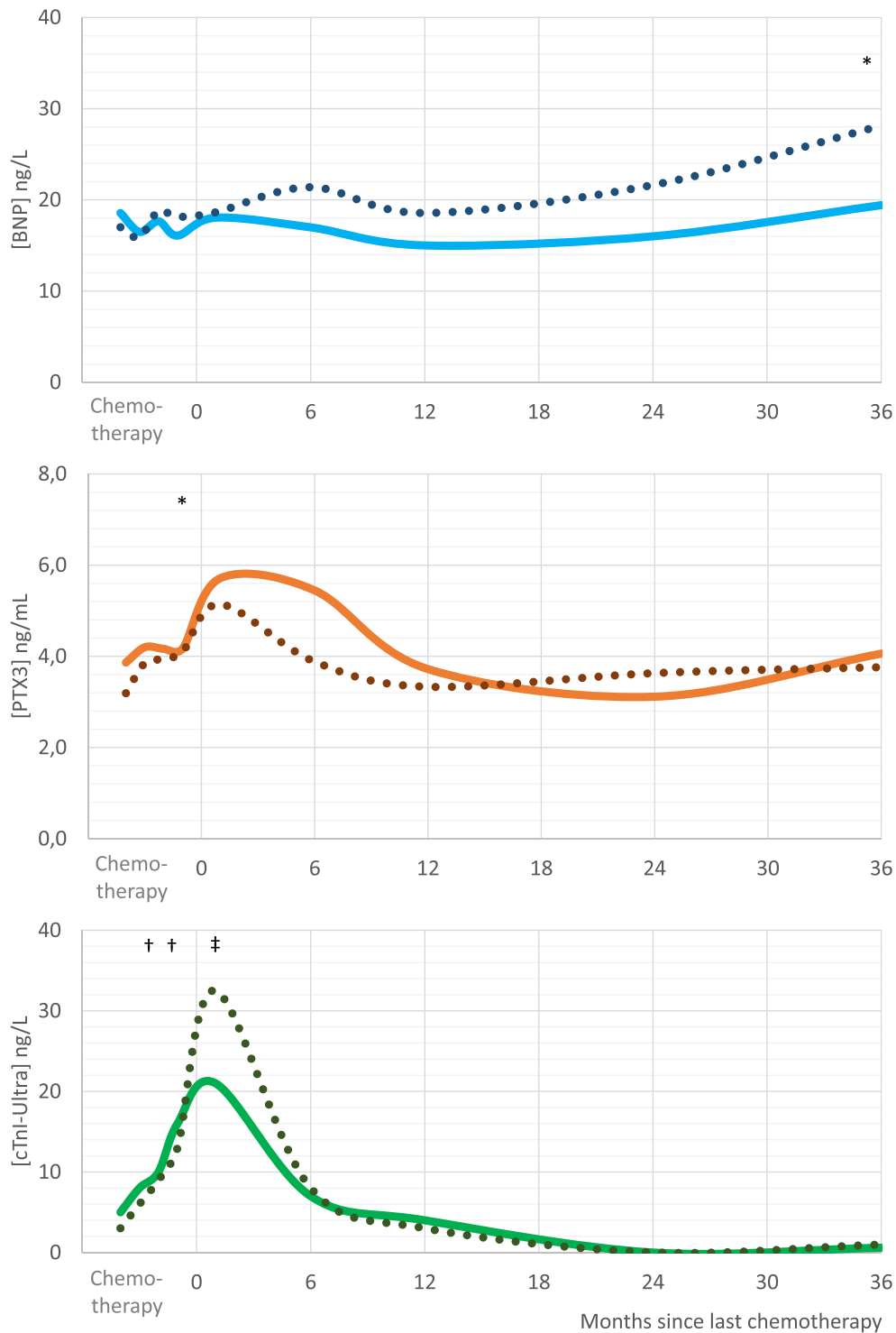
### Echocardiography

Patients underwent echocardiographic examination at baseline and during their follow-up visits. Of all locally read

echoes, 10% were randomly reassessed in the central core lab, resulting in low coefficients of variation [LVEF: 3.3%, E/e': 5.2%, E/A: 8.2%, left atrial volume index (LAVI): 12.0%] between the peripheral and core laboratories. In addition, all echocardiographic exams revealing a decrease in LVEF, according to study protocol, were validated by the core laboratory.



**Figure 4** Biomarker concentration during ICOS-ONE study by dose of anthracycline (IEO equivalent). Median concentration of BNP (in blue), PTX3 (in orange), and troponin (in green) during chemotherapy and follow-up. Stratified for anthracycline dose (by IEO equivalent). Patients who received  $\leq 180$  mg/m<sup>2</sup> are continuous line while the dashed line represents patients who received  $>180$  mg/m<sup>2</sup>. \* $P < 0.05$ , † $P < 0.01$ , ‡ $P < 0.001$ .



All echocardiographic variables stayed very stable with a median ejection fraction of 63% and a median LAVI around 24 mL/m<sup>2</sup> over the course of the study, increasing to

26 mL/m<sup>2</sup> at 36 months. Upon stratification by cumulative dose of anthracyclines, no differences were observed in LVEF or LAVI.

Fifteen patients had LAVI  $\geq 34$  mL/m<sup>2</sup> at baseline with significantly higher BNP levels than those with LAVI  $< 34$  mL/m<sup>2</sup> (40 vs. 17 ng/L,  $P < 0.001$ ). However, at other time points, no differences in concentration of BNP were observed and during follow-up LAVI returned to normal (Supporting Information, *Figure S4*). Of the 15 patients with LAVI  $\geq 34$  mL/m<sup>2</sup> at baseline, six had leukaemia and one lymphoma, a significantly greater proportion than in the LAVI  $< 34$  mL/m<sup>2</sup> group. Neither LV ejection fraction, s' wave velocity or E/e', E/A showed any relation with the three biomarkers.

## Cardiac magnetic resonance imaging

One out of nine patients who underwent serial cardiac MRI had lymphoma, all others had leukaemia, mean age was 40 years [31 to 55 years], five were female, five assigned to 'prevention' arm. Baseline LVEF averaged 64%, within the normal range for all patients. Over a median of 1 year, LVEF averaged 61%, all individual values being  $> 55\%$ . Echocardiographic LVEF showed a temporal trend superimposable to that of MRI.

## Discussion

The present analysis was focused on the time course of three cardiovascular circulating biomarkers and of echocardiographic variables in the multicentre randomized trial ICOS-ONE, over an extended follow-up of 36 months after the end of anthracycline-containing CT. Troponin, as well as the other two biomarkers (NT-proBNP and PTX3), transiently raised during CT courses, returned to baseline normal values by 12 months after CT, and remained stable thereafter. Few data are available in the literature on the time course of cardiovascular biomarkers for such an extended period after cancer chemotherapy.<sup>20,21</sup> Data on PTX3 in these patients have never been reported before.

During the whole duration of the study, troponin was higher in patients aged at least 51 years, in those who underwent CT with doxorubicin than in those with epirubicin, and in those receiving higher cumulative doses of anthracyclines indicating that cardiac injury induced by anthracycline is dose-dependent. However, in all cases, cTnI-Ultra levels returned to baseline values 12 months after CT and remained very low thereafter. In a previous study,<sup>10</sup> involving patients treated with high-dose chemotherapy, persistence of increased levels of TnI for several months was observed more frequently in patients not treated than in those treated with enalapril.

In patients treated for early breast cancer with adjuvant anthracycline-containing regimens with or without trastuzumab and radiation, concomitant treatment with candesartan limited the decline in LVEF over 6 months from

63.2% to 60.6% in no candesartan, and from 62.1% to 61.4% in candesartan ( $P = 0.026$ ).<sup>22</sup> Candesartan but not metoprolol was effective in reducing the increase in circulating cardiac troponin I, suggesting that angiotensin receptor blockade may play a role in the myocardial remodelling process that occurs after cardiac injury.<sup>22,23</sup> Neither in ICOS-ONE nor in PRADA an association between changes in circulating biomarkers and changes in LV systolic and diastolic function was found.

In a placebo-controlled trial in 206 patients, the angiotensin receptor blocker candesartan (started with trastuzumab treatment after completion of anthracycline-containing CT and given for 18 months) did not protect against LV dysfunction and consistently neither cTnT nor NT-proBNP were affected.<sup>20</sup>

The preventive role of the combination enalapril and carvedilol was assessed in the OVERCOME study (prevention of left Ventricular dysfunction with Enalapril and carvedilol in patients submitted to intensive Chemotherapy for the treatment of Malignant Hemopathies).<sup>24</sup> The study involved 90 patients treated with anthracyclines. After 6 months, LVEF did not change in the intervention group from 63.3% to 62.9% but significantly decreased in controls from 64.6% to 57.9% ( $P = 0.04$ ). Nonetheless, the intervention group showed a lower rate of the combined event of death or heart failure, or of death, heart failure and a final LVEF  $< 45\%$  than controls.

BNP, a marker of volume overload, was higher in patients with higher LAVI at baseline and 6 months after CT, and this occurred more frequently in patients with leukaemia or lymphomas. The higher concentrations of BNP in patients receiving higher dose of anthracyclines is probably driven by the higher prevalence of lymphomas in this subgroup (23.4% vs. 3.7%) whose median dose of anthracyclines was 300 mg/m<sup>2</sup> (Supporting Information, *Tables S2 and S4, Figure 3*). Neither cTnI nor LVEF was associated with the observed slight changes in BNP over time. In fact, the incidence of abnormal cTn was comparable across quartiles of BNP at 36 months. As expected, patients aged  $\geq 51$  had higher concentrations of BNP.<sup>25</sup> Treatment with enalapril started in slightly half of the population of ICOS-ONE may have contributed to the normalization of BNP<sup>26</sup> and LAVI during follow-up. No influence of renal function on concentrations of biomarkers can be advocated because creatinine was normal and stable over study duration (data not shown).

PTX3 which was supra-normal at baseline, possibly because of the underlying cancer, seemed to weakly increase after CT but did not show any relation with type of chemotherapy, dose, or echocardiographic variables. PTX3 was significantly higher at baseline and during CT in patients with leukaemia or lymphoma than in those with breast cancer, in agreement with previous observations.<sup>27,28</sup> The higher concentration in younger patients is probably due to a higher prevalence of leukaemia in this age group. It may be speculated that the depression in circulating white blood cells induced by CT may have decreased a major storage compartment for circulating PTX3.<sup>29</sup>

The most noticeable result from ICOS-ONE is that in patients without pre-existing cardiac disease, first-in-life CT with moderate to low cumulative doses of anthracyclines receiving a treatment with enalapril (either given from the beginning of chemotherapy or after first rise of troponin) does not seem to be associated with significant cardiac dysfunction and cardiovascular (CV) events within 36 months after CT.

The increase in troponin reported by local laboratories and confirmed by central assays prompted the authors to extend the follow-up by two more years, to verify whether small, early increases in troponin may be the hallmark of delayed cardiac toxicity. The results of the present study suggest that this is not the case.

By far the most important limitation of the present study is the lack of a control group, that is, of patients not taking any cardio-protectant medication and/or not monitored for troponin. However, ICOS-ONE was designed to compare two approaches, both including enalapril given either for prevention or troponin-triggered.

Secondly, nothing can be said of patients with pre-existing cardiovascular disease or who had undergone previous CT, because they were excluded per protocol from the trial.

Thirdly, the lack of even mild depression of LVEF over the 36 month follow-up cannot exclude that more sensitive echocardiographic techniques, such as strain,<sup>4</sup> could have possibly revealed sub-clinical depression of LV function.

In ICOS-ONE, the lack of any biohumoral or echocardiographic evidence of cardiac impairment may be explained by the prescription of enalapril in half of the patients since randomization (prevention arm) and in those starting at the first troponin elevation during follow-up (troponin-triggered arm). In a single-centre clinical study, starting enalapril at the first occurrence of a supra-normal value of cTn fully prevented cardiac clinical events, and the decrease in LVEF.<sup>10</sup> These patients qualified as high-risk, because the majority had previous anthracycline-containing CT, mean cumulative dose of anthracycline was 335 mg/m<sup>2</sup>. However, not only patients without elevation of cTn, thus at presumed very low risk of cardiac injury, remained untreated, but also 22 (8%) patients who, in spite of a supra-normal cTn, either locally or centrally assayed, were not prescribed enalapril. None of these 22 patients experienced any cardiac clinical endpoint or LV dysfunction; their LVEF remained stable over 3 years, starting from a baseline value of 63% in both study arms.

However, given the lack of consistent evidence and the assumption of a marked efficacy of ACE-inhibition/beta-blocker not documented by all published studies,<sup>22,24</sup> an alternative interpretation can be suggested. The normalization of all three cardiovascular biomarkers together with the scarcity of cardiovascular clinical events and of impairments in echocardiographic variables over the whole course of the study may be explained by the lack of cardiovascular risk factors and the low dose of anthracycline, much lower than those reported in previous cohorts<sup>2,10</sup> (Supporting Information, *Table*

S5). As a consequence, it can be suggested that CT with carefully titrated anthracyclines is unlikely to lead to cardiac dysfunction responsible for an activation of the renin-angiotensin aldosterone system, a target of an ACE-inhibitor such as enalapril.

In conclusion, the usefulness of close troponin monitoring in low-risk patients deserves further investigation, considering also possible genetic determinants of susceptibility to anthracycline cardiac toxicity.

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## Conflict of interest

None declared.

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## Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** Patient Characteristics by cancer.

**Table S2.** Plasma concentration of BNP, PTX3 and cTnI-Ultra by cancer.

**Table S3.** Plasma concentration of BNP, PTX3 and cTnI-Ultra by type of anthracycline.

**Table S4.** Patient characteristics and plasma levels of biomarkers by anthracycline dose.

**Table S5.** Patient characteristics: a comparison of different cohorts.

**Figure S1.** Biomarker concentration by study treatment.

**Figure S2.** Biomarker concentration during study by age group.

**Figure S3.** Biomarker concentration during study by type of anthracycline.

**Figure S4.** BNP concentration by baseline LAVI.

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