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Predictors of early or delayed diastolic dysfunction after anthracycline-based or nonanthracycline chemotherapy: A pharmacological appraisal

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NONSTANDARD ABBREVIATIONS: DD, diastolic dysfunction; HF, heart failure; BMI, body mass index; CV, cardiovascular; LVEF, left ventricular ejection fraction; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; Hb, hemoglobin; E/A, mean ratio of peak early filling (E wave) to late diastolic filling (A wave); DT, mean deceleration time of early filling velocity; BNP, B-type natriuretic peptide (BNP); cTn, cardiac troponin.

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ABSTRACT

Diastolic dysfunction (DD) is an early manifestation of cancer drugs cardiotoxicity. Anthracyclines are considered as more cardiotoxic than other chemotherapeutics but previous studies have shown that both anthracycline-based and nonanthracycline chemotherapy can cause an early DD, detected one week after the end of chemotherapy. Here we characterized if DD also occurred in a delayed form, detected six months after chemotherapy. Sixty-seven comorbidity-free patients were examined. DD was diagnosed by echocardiography and cardiac biomarkers. Early or delayed DD occurred in 26 or 13 patients, respectively, sharing a pattern of grade I DD (impaired relaxation at echocardiography) or elevated B-type natriuretic peptide. Binary logistic analysis showed that age, gender and type of chemotherapy (anthracycline-based versus nonanthracycline) did not independently increase the probability of early or delayed DD. Early DD was predicted by the patient's cardiovascular profile, and in particular by diastolic indices that were in ranges of normality but showed measurable discrepancies from mean control values. Delayed DD was not predicted by the patient's cardiovascular profile but was predicted by post-chemotherapy adjuvant treatments (e.g., chest radiation or hormone therapy). Early and delayed DD were accompanied by moderate LVEF decrements. These findings show that anthracycline-based and nonanthracycline chemotherapy can induce early or delayed DD, which are governed by different patient- or treatment- related factors. Pharmacologic interventions that prevent DD or mitigate its progression toward a more serious cardiac dysfunction should be considered.

SIGNIFICANCE STATEMENT

Predictors of early or delayed diastolic dysfunction (DD) were investigated in cancer patients treated with anthracyline-based or nonanthracycline chemotherapy. The type of chemotherapy did not predict the risk of DD. Early DD was predicted by the patient's cardiovascular profile. Delayed DD was predicted by the adjuvant treatments the patient received after chemotherapy. These findings show that any chemotherapeutic can cause DD; however, the trajectories of DD are differently influenced by patients' characteristics or post-chemotherapy exposure to additional cardiotoxic hits.

INTRODUCTION

Asymptomatic diastolic dysfunction (DD) has long been suspected to represent an early manifestation of cardiotoxicity induced by cancer drugs. DD preceded systolic dysfunction in breast cancer patients treated with anthracyclines, with or without subsequent treatment with the anti-ErbB2 monoclonal antibody, trastuzumab (Klein et al., 2019). DD was also shown to precede, or to accompany with heart failure (HF) and other cardiac events that occurred years or decades after anthracycline treatment of childhood or adult cancer (Carver et al., 2007; Armstrong et al., 2015).

Studies of cancer treatment-related DD usually included subgroups of patients showing the cardiovascular (CV) risk factors that predispose to DD in the general population (e.g., hypertension, diabetes, dislipidemia, overweight) (Borlaug et al., 2011). Predictors of cancer treatment-related DD are nonetheless uncertain at this point in time (Armenian et al., 2017). In a study of 85 breast cancer patients treated with anthracycline-based chemotherapy, with or without subsequent trastuzumab, age and baseline body mass index (BMI) were independently associated with treatment-related DD (Serrano et al., 2015); however, neither age nor BMI predicted DD in a larger study of breast cancer patients who also received anthracycline, anthracycline followed by trastuzumab, or trastuzumab alone (Upshaw et al., 2020).

Other areas of debate pertain to the role of anthracyclines versus nonanthracycline chemotherapeutics in the settings of DD. Microvascular dysfunction and inappropriate interactions of Ca^{2+} with myofilaments in diastole are important determinants of DD (Redfield, 2016). Anthracyclines cause endothelial dysfunction in cancer patients (Finkelman et al., 2017), downregulate the expression and Ca^{2+} sequestering activity of sarcoplasmic Ca^{2+} ATPase (Minotti et al., 2004), promote calcium-dependent protease degradation of the giant protein, titin, which plays a crucial role in myocardial relaxation (Lim et al., 2004). Also nonanthracycline chemotherapeutics, mainly alkylators and antimetabolites and tubuline-active agents, can cause endothelial or microvascular dysfunction (Menna et al., 2008; Zamorano et al., 2020); however, clinical evidence of cardiotoxicity of these agents has been questioned, either because data were confounded by prior or concurrent administration of anthracyclines or because cardiotoxicity

occurred after high dose regimens (Kamphuis et al., 2019). Long standing DD was reported to occur in long term survivors of platinum-treated testicular cancer but this finding may have been confounded by comorbidities that accumulate in cancer survivors (Altena et al., 2009; Carver et al., 2007; Minotti et al., 2010).

We recently reported the results of a prospective pilot study of DD induced by cancer therapy. In that study 29 out 80 patients showed DD with a preserved left ventricular ejection fraction (LVEF) as early as 1 week after cancer chemotherapy, i.e. before they developed comorbidities or received other treatments (Calabrese et al., 2018). The patients presented at study entry without pre-existing CV risk factors, and showed oncologic diseases that required anthracycline-based or nonanthracycline chemotherapy; however, DD occurred in patients treated with either type of chemotherapy. These findings suggested that any chemotherapeutic can cause an early DD, regardless of patient's risk factors, subsequent exposure to other cardiotoxic agents, or comorbidities that develop after cancer treatment.

The unique characteristics of our study population prompted us to investigate predictors of early DD. We used binary logistic regressions and probability scores that minimized biases from the limited sample size and helped to characterize whether the probability of early DD was determined by unrecognized characteristics of patient's CV profile, defined as the combined effects of all CV parameters that were screened before chemotherapy. The effects of patient's age, gender, and chemotherapy type on increasing the probability of DD associated with the CV profile were then evaluated. Importantly, the analyses of predictors was extended to patients who showed normal diastolic function at 1 week and were re-evaluated after 6 months.

SUBJECTS AND METHODS

Subjects and source data

All data derived from the patients recruited in a pilot prospective study of the incidence of DD after anthracyline-based or nonanthracycline chemotherapy (Calabrese et al., 2018). Patients' demographic and oncologic characteristics were: 18-70 years of age; absence of CV risk factors; anthracycline indication for the adjuvant treatment of early operable breast cancer [usually doxorubicin (or epirubicin)-cyclophosphamide followed by a taxane)]; anthracycline indication for the frontline treatment of non Hodgkin lymphoma [R-CHOP regimen (rituximab-cyclophosphamide-doxorubicin-vincristine-prednisone)]; fluoropyrimidine/platinum indication for the adjuvant treatment of operable colorectal cancer [FOLFOX regimen (folinate-fluorouracil-oxaliplatin) or XELOX regimen (capecitabine-oxaliplatin)]. Based on our interest in the incidence and trajectories of DD induced by conventional frontline chemotherapy, patients with Erbb2 overespressing breast cancer, requiring post-chemotherapy exposure to potentially cardiotoxic trastuzumab, were excluded. Patients requiring debulking radiation for the treatment of mediastinal lymphoma were also excluded. Breast cancer patients requiring post-chemotherapy left-sided chest radiation or hormone therapy (gonadotrophin releasing hormone agonists, antiestrogens, aromatase inhibitors) were included. Antiemetics and granulocyte colony-stimulating factors were allowed as per standard procedures. Allopurinol was used in non Hodgkin lymphoma patients to prevent hyperuricemia from the glucocorticoid component of R-CHOP. Given the absence of risk factors, no patient was taking CV drugs at study entry or required CV drugs during chemotherapy. No specific diet was recommended but a healthy lifestyle with an adequate physical activity was recommended. All patients quit smoking at the time of cancer diagnosis.

Design of the pilot study

Details have been reported elsewhere (Calabrese et al., 2018). In brief, the patients were evaluated at study entry for the following CV parameters: LVEF (which had to be ≥50% to permit patient's recruitment); systolic and diastolic blood pressure (SBP, DBP); heart rate (HR) on 12-lead ECG; hemoglobin count (Hb); BMI (which had to be <30 kg/m^2 to permit patient recruitment); cardiac biomarkers like B-type natriuretic peptide (BNP) and troponin (cTn) (which had to be within

ranges of normality to permit patient's recruitment); echocardiographic indices of diastolic function that were considered as doable in the real life of each participating center [mean ratio of peak early filling (E wave) to late diastolic filling (A wave) (E/A), and mean deceleration time of early filling velocity (DT)]. Inasmuch as E/A decreases with age, and DT increases with age, baseline E/A and DT had to be within ranges of normality for age (Nagueh et al., 2009). To permit comparisons between patients of different age, E/A and DT values were eventually normalized to age-related ranges according to the formula (Calabrese et al., 2018)

$$\{\text{E/A (or DT)} = 100 \times ([\text{absolute value} - \text{lower limit of range}]/\text{range})\}.$$

Patients were re-evaluated 1 week after the last chemotherapy cycle, and DD with a preserved LVEF was diagnosed if patients presented with an LVEF ≥50% vis-à-vis alterations of E/A and DT, and/or elevations of BNP and cTn above the upper limit of normal. Patients without DD at 1 week after chemotherapy were asked to be re-evaluated 6 months later. Given the exploratory nature of the pilot study, a formal power analysis was not performed; however, on the basis of limited findings obtained before the pilot study was started (Carboni et al., 2009), we anticipated that 100 patients had to be recruited for the study to identify 40 patients with one or more protocol-defined indices of DD.

The study conformed with the principles outlined in the Declaration of Helsinki and was approved by the Institutional Review Board of each participating center. Written informed consent was obtained from all patients.

Predictive probability analysis of DD

For the purpose of this present study, data from 67 of the 80 patients recruited in the pilot study were used. These patients had in fact received serial measurements of BNP and cTn also during the course of chemotherapy, and therefore represented the study subgroup in which interactions between gradually increasing biomarkers and changes in diastolic function were fully characterized (Menna, Calabrese et al., 2018; Menna, Salvatorelli et al., 2018). Furthermore, in this study subgroup, all patients without DD at 1 week accepted to be re-evaluated at 6 months. The study subgroup was therefore most suitable for comparisons between patients with or without DD at 6 months after chemotherapy. The study subgroup was balanced with the source population

with respect to baseline characteristics, both groups showing a prevalence of women with breast cancer candidate to anthracycline-based chemotherapy (**Table 1**).

To limit potential biases from the small sample size, we used a logistic regression analysis that required fewer assumptions as compared to multiple regression or Analysis of Covariance. Sample size was expressed in terms of events per variable, defined by the ratio of the number of events to the number of parameters (degrees of freedom) required to represent the predicted outcome with data models. Prediction models developed with regression shrinkage techniques showed good predictive performance with events per variable << 10 (Pavlou et al., 2016; Puhr et al., 2017). Probability scores, estimated using a logistic regression model in which the risk of chemotherapy-related DD was regressed on patient's baseline CV parameters, were used as covariates to produce more reliable results than matching procedures (Elze et al., 2017). The primary objective was to fit a binary logistic regression model for defining whether the probability of DD at 1 week or 6 months ("early" or "delayed" DD) was predicted by the patient's CV profile, defined as the complete set of CV parameters at baseline. The secondary objective was to define whether the probabilty of early or delayed DD was determined also by patient demographic and oncologic characteristics. The model results confirmed that the Type I error rate was not significantly affected by the sample size and relative biases were <10%. Probability plots were used to characterize how the probability of DD changed with given values of independent variables. Performance evaluation was modelled with Area Under the Curves and Receiver Operator Characteristics (ROC) curves that represented how much the model was powered to distinguish between the probability of early or delayed DD. For each ROC curve, the Youden's index was calculated. Regression adjustments were used to reduce bias due to residual differences in observed baseline covariates between the probability of early or delayed diastolic dysfunction.

Individual CV discrepancy

CV discrepancy was determined to characterize how much the CV profile of patients with early or delayed DD, although deemed as normal at study entry, deviated from that of matched controls. For each patient with DD the discrepancy of a given CV parameter was calculated by the

percentage difference of that parameter with the mean value of the same parameter in matched controls. Next, the discrepancies calculated for all CV parameters were averaged to obtain a mean individual CV discrepancy.

Other conditions

To minimize inter-observer variabilty and to improve reproducibility of echocardiographic end points, each center designated a study-dedicated cardiologist and echocardiographic tracings from all centers were centralized for review by a blinded operator. This was especially important for measurements of E/A and DT, potentially subject to many confounders (Nagueh et al., 2009). For patients who did not develop DD at echocardiography, this approach gave reproducible age-related values of E/A and DT at baseline and after chemotherapy (**Supplementary Figure 1**). Moreover, in these patients, the age-related slopes of baseline E/A and DT were similar to the corresponding baselines of patients who eventually presented with DD after chemotherapy (**Supplementary Figure 2**). LVEF was calculated by the modified Simpson's rule. Greater than 10% decrements from baseline were defined as systolic events. To minimize interoperator variabilty each center designated a study-dedicated cardiologists and tracings from all centers were centralized for review by a blinded operator. Cardiac troponin was measured by the circulating levels of cTnI isoform (Vista Siemens®, upper limit of normal at 0.050 ng/ml). BNP was measured by the circulating levels of the aminoterminal fragment of BNP prohormone, which is formed in 1:1 ratio to mature BNP but shows a longer circulating half life (Vista Siemens®, upper limit of normal at 125 pg/ml) (Menna, Calabrese et al., 2018). Anthracycline dose was expressed as doxorubicin equivalents (Calabrese et al., 2018). Unless otherwise indicated, all values were means with 95% confidence intervals (CI). Continuous variables were analyzed by two-tailed Mann Whitney test or Kruskal-Wallis analysis of variance with Dunn's post test, as appropriate. Categorical variables were analyzed by two-tailed χ^2 square or Fisher's exact tests, as appropriate. Individual CV discrepancies were analyzed by Wilcoxon signed rank test. Differences were considered as statistically significant when P values were <0.05. Probability analyses were done by IBM SPSS Statistics for Windows, Version 21.0 (Armonk, NY). All other analyses were done by Prism 5 Program, version 5.01, ®GraphPad Software Inc. (La Jolla, CA).

RESULTS

Early and delayed DD with a preserved LVEF

Early DD was detected 1 week after chemotherapy in 26 of the 67 patients included in the analysis ("early cases"), according to a study power of >80% (one proportion test) (Chow et al., 2008). Delayed DD was detected 6 months after chemotherapy in 13 of the remaining 41 patients ("delayed cases"). Both early and delayed cases showed a prevalence of BNP elevation or grade I DD at echocardiography. The latter, also referred to as impaired relaxation (Nagueh et al., 2009), was characterized by E/A decrements toward or below the lower limit of normal, and by concomitant DT prolongations toward or above the upper limit of normal. Higher grade DD was not observed at either 1 week or 6 months after chemotherapy. Very few cases showed a combination of impaired relaxation and cTn elevation, and only one case showed both BNP and cTn elevations (**Figure 1**). The overall incidence of DD was 58%. Having considered differences in follow-up duration across studies, this figure compared reasonably well with results reported by others (Serrano et al., 2015; Upshaw et al., 2020).

Changes in LVEF are reported in **Figure 2**. Apparent fluctuations of LVEF occurred from baseline to post-chemotherapy assessments. Inasmuch as appropriate measures were taken to avoid inter-observer variability (see Subjects and Methods), these fluctuations likely reflected transient changes in patient's hydration, hemoglobin count or neurohormonal status, which are normal in oncologic settings (Yeh et al., 2014). Overall, LVEF remained ≥50% in all patients but one delayed case (LVEF 45%). A total of 38 out of 39 cases with early or delayed DD therefore met the protocol definition of DD with a preserved LVEF (see also Figure 2). Greater than 10% decrements of LVEF (systolic events) occurred in 12 patients, equally distributed across controls and early or delayed cases (P=0.118). At a cumulative analysis, controls did not show net LVEF decrements; a trend toward an LVEF decline was observed in DD cases, and particularly in delayed cases, but these changes were not statistically significant (**Figure 3**).

Baseline CV profile predicts the probability of early DD but not of delayed DD

Probability scores were used to characterize whether baseline CV profile predicted the probability of early or delayed DD. Probability was regressed on the panel of CV parameters

evaluated at study entry (LVEF, SBP, DBP, HR, Hb, BMI, E/A, DT, cTn, BNP). Early cases, but not delayed cases, showed a significantly higher probability score than their matched controls (**Figure 4**).

We explored reasons our analysis could detect a probability of early DD but not of delayed DD. Controls and cases presented at study entry with comparable mean values of all CV parameters, the only exception being borderline higher BNP values in cases as compared to controls (**Table 2**). Differences were nonetheless observed in terms of individual CV discrepancy of cases from controls. Early cases were congruent with their matched controls when the CV profile included all CV parameters evaluated at baseline; however, a significant discrepancy occurred when the 4 pre-specified indices of diastolic function (E/A, DT, Tn, BNP) were considered in isolation, the remaining CV parameters being congruent with those of controls (**Figure 5**). Also delayed cases showed a trend toward dispersed baseline indices of diastolic function, but the net discrepancy from controls was not significant (see also Figure 5). For early cases, the discrepancy of baseline diastolic indices was caused by a significant discrepancy of E/A (toward lower values) and by a concomitant albeit not significant discrepancy of Tn and BNP (toward higher values) (**Table 3**).

Effects of demographic and oncologic characteristics on the probability of early DD

Early cases showed a borderline significant older age as compared to matched controls; however, controls and cases showed the same distribution in age groups of ≤40, 41-60, or 61-70 years and were balanced also with respect to gender, tumor type, anthracycline exposure (**Table 4**). Having shown that the CV profile of early cases associated with a higher probability score as compared with controls (Δ score = 0.322, 95% CI 0.099-0.544, P=0.024), we used probability plots to evaluate whether demographic and oncologic parameters increased the Δ score. Neither age nor gender or anthracycline exposure caused significantly increased Δ scores but the combination of all parameters with the CV profile resulted in a significantly increased Δ score (**Figure 6**).

Effects of demographic and oncologic characteristics on the probability of delayed DD

Delayed cases showed a trend toward older age, similar to that observed for early cases, but again this was not significant. Distribution in age groups, gender, tumor type, and anthracycline

exposure were well balanced across controls and cases (**Table 5**). Delayed cases and controls were also balanced with respect to post-chemotherapy left-sided chest radiation and/or hormone therapy for women with breast cancer (see also Table 5). Having shown that the CV profile of delayed cases did not cause an increased probability score as compared to matched controls (Δ score = 0.133, 95% CI from -0.279 to 0.412, P=0.867), we used probability plots to evaluate whether age, gender, anthracycline exposure and post-chemotherapy treatments increased the Δ score. Age, gender, and anthracycline exposure did not increase the Δ score nor was the score increased when such factors were combined with CV profile (0.186, 95% CI from -0.151 to 0.523, P=0.418). A significant increase occurred when left-sided chest radiation and/or hormone therapy were considered (**Figure 7**).

DISCUSSION

Main findings

We have shown that anthracycline-based or nonanthracycline chemotherapy can cause early DD in some patients or delayed DD in other patients. Early and delayed DD showed the same prevailing pattern of BNP elevation or impaired relaxation at echocardiography, which appeared to be mutually exclusive. This was consistent with BNP elevations inducing lusitropic effects that compensated for impaired relaxation before echocardiographic abnormalities could be detected (Menna, Calabrese et al., 2018). The lusitropic effects of BNP could also mitigate cTn release otherwise associated with impared relaxation (Menna, Salvatorelli et al., 2018); accordingly, only one of thirthy-nine cases showed concomitant elevations of BNP and cTn. Early and delayed DD therefore shared echocardiographic or biohumoral manifestations of DD. Early and delayed DD were nonetheless characterized by remarkably different predictors. Whereas early DD was predicted by the patient's CV profile at study entry, the risk of delayed DD was predicted by post-chemotherapy treatments, like left-sided chest radiation or hormone therapy for breast cancer.

Discrepancies of diastolic indices and probability of early DD

By regressing DD probability on the patient's CV profile we characterized if in-range deviations of one or more CV parameter from control values interacted with concomitant changes of one or more other parameters, eventually building a risk of DD. Patients with an increased probability score for early DD were in fact characterized by a significant discrepancy of E/A toward lower values, and by concomitant albeit not significant discrepancies of BNP and cTn toward higher values (see Table 3). Such in-range discrepancies mirrored the out-of-range deviations that eventually occurred 1 week after chemotherapy, possibly identifying the subjects at an increased risk of early DD. Deviations of E/A toward lower values were also observed in childhood cancer survivors years before they developed cardiomyopathy (Border et al., 2020).

Age, gender and anthracycline exposure did not independently increase the probability of early DD associated with patient's CV profile; however, the combination of such characteristics

with CV profile did result in an increased probability score for early DD (see Figure 5). This again showed that probability analyses intercepted combinations of patient's characteristics predisposing to DD.

Post-chemotherapy treatments and probability of delayed DD

The probability of delayed DD only increased when left-sided chest radiation for breast cancer was considered. Both preclinical and clinical studies show that cardiac irradiation increases the risk of DD with a preserved LVEF (Cao et al., 2015; Saiki et al., 2017), with imaging studies demonstrating microvascular dysfunction in irradiated areas (Song et al., 2017). The probability of delayed DD was increased also by hormone therapy that was started after chemotherapy for breast cancer. This finding is less obvious to explain. Studies of DD during the first few months of hormone therapy are lacking. Aromatase inhibitors were shown to synergize with left-sided chest radiation in impairing myocardial deformability (Skyttä et al., 2015), and preclinical studies showed that hypothalamic blockers might relieve some beneficial effects of gonadotrophin releasing hormone on the contraction-relaxation cycle of cardiomyocytes (Dong et al., 2011). In preclinical models, loss of estrogens reduced the cardiac levels of tetrahydrobiopterin, thereby uncoupling cardiac neuronal nitric oxide synthase and exposing cardiomyocytes to an oxidative stress that contributed to impairing myocardial relaxation (Jessup et al., 2011). Our study was not powered to characterize which of these factors contributed a probability of delayed DD and whether such factor(s) interacted with effects of prior chemotherapy.

Study limitations

The sample size of this study was small. The pilot study from which all data derived had been designed to recruit 100 patients but patients' refusal to participate or to attend cardiac assessment at 1 week after chemotherapy reduced the sample size to 80 patients. These facts are not unusual in prospective cardio-oncology studies (Pituskin et al., 2017) and denote that much needs to be done for improving patients' awareness and collaboration in cardio-oncology studies (Zamorano et al., 2020). The sample size was further narrowed to 67 patients for the purposes of this present analysis (see Subjects and Methods). Probability analyses were nonetheless shown to minimize biases from a small sample size (Pirracchio et al., 2012) and could in fact identify the

association of patients' CV profile with the probability of early DD. Probability analyses were also powered to intercept left-sided chest radiation or hormone therapy as predictors of delayed DD but this was true for the subgroup of women with breast cancer. The sample size was too small for probability analyses to identify DD predictors also for those fewer patients not treated with chest radiation or hormone therapy.

The study population requires some considerations as well. The patients were not homogeneous with respect to oncologic disease, which in principle might be viewed as a source of confounders. This having been recognized, we note that controls and early or delayed cases were consistently well balanced with respect to oncologic disease and all other demographic, CV and treatment-related characteristics.

Conclusions and pharmacologic perspectives

Different predictors of early or delayed DD were identified for patients treated with anthracycline-based or nonanthracycline chemotherapy. To highlight the intrinsic cardiotoxicity of cancer drugs the study excluded patients with CV risk factors or candidate to post-chemotherapy trastuzumab or other cancer drugs which could have influenced DD trajectories over time. The probability of delayed DD introduced by left-sided chest radiation and/or hormone therapy nonetheless shows that DD is in fact a dynamic process, influenced by patient's exposure to sequential cardiotoxic hits. This scenario fits nicely in the so-called multiple hits hypothesis of cardiotoxicity of cancer drugs (Menna et al., 2008).

Thirty-eight of 39 cases with early or delayed DD showed a preserved LVEF. A trend toward LVEF decrements was nonetheless observed. Albeit not significant, this trend was similar to that observed in the largest study of cancer treatment-related DD (Upshaw et al. 2020). Concerns about impaired relaxation progressing toward higher grade DD should therefore be extended to include the possible development of systolic dysfunction. These facts, and the dynamic process of DD, raise questions about the opportunity and efficacy of primary or secondary prevention strategies with CV drugs. In the general population, DD or HF with preserved LVEF do not always respond to common CV drugs (Borlaug et al., 2011). The angiotensin receptor and neprilysin inhibitor, sacubitril/valsartan, offers important opportunities to treat HF with reduced LVEF

and shows efficacy also in oncologic patients (Camilli et al. 2020); however, the role of sacubitril/valsartan in the treatment of HF with preserved LVEF is uncertain (Solomon et al., 2019). Eplerenone, an oral mineralocorticoid antagonist, did not prevent DD in women receiving anthracycline for breast cancer treatment (Davis et al., 2019). Ranolazine, an antianginal drug that diminishes intracellular Ca^{2+} accumulation and diastolic tension by inhibiting the late inward Na^{+} current, showed promising effects in relieving chemotherapy-related DD; unfortunately, this was a preliminary observation that involved only few of the early cases we described in this study (Minotti et al., 2019).

In awaiting for drugs with an evidence of efficacy against DD, a reasonable strategy should be to monitor cancer survivors and to intercept CV and metabolic morbidities that accumulate over the years after cancer treatment. This surveillance requires patient's awareness and collaboration of cardio-oncologists with pharmacologists conversant in cancer drugs toxicity and the multiple hits hypothesis of cardiotoxicity (Minotti et al., 2010; Zamorano et al., 2020).

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AUTHORSHIP CONTRIBUTIONS

Participated in research design: Menna and Minotti

Conducted experiments: Menna, Salvatorelli, Minotti

Contributed new reagents or analytic tools: Menna

Performed data analysis: Reggiardo, Camilli, Minotti

Wrote the manuscript: Minotti

Reviewed and approved the manuscript: All authors

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FOOTNOTES

The pilot study from which this data analysis was originated was promoted by Menarini International Operations Luxembourg S.A. and was registered at the European Clinical Trials Database [EUDRACT 2009-016930-29]. No other relationship with industry is declared.

FIGURE LEGENDS

Figure 1 Early or delayed cases of DD

Early or delayed cases of DD were identified 1 week or 6 months after the last chemotherapy cycle. Early and delayed cases showed similar patterns of DD, characterized by a prevalence of impaired relaxation or BNP elevation (two-tailed χ^2 test). IR, impaired relaxation.

Modified and expanded after Calabrese et al., 2018.

Figure 2 LVEF in controls and early or delayed cases of diastolic dysfunction

The asterisk in bottom right panel denotes the only patient with DD and LVEF <50%.

Figure 3 LVEF decrements in controls and early or delayed cases

Decrements were means with 95% CI of percentages differences between LVEF at 1 week or 6 months after chemotherapy and LVEF at study entry. Differences between controls and cases were not significant (P=0.309 by Kruskall Wallis analysis of variance with Dunn's post test for multiple comparisons).

Figure 4 CV profile and probability scores for DD in controls and early or delayed cases

DD probability was regressed on the CV profile of controls and cases, defined as the complete set of CV parameters evaluated at baseline (LVEF, SBP, DBP, HR, Hb, BMI, E/A, DT, cTn, BNP). Differences were calculated by Area Under the Curves and ROC curves (see Subjects and Methods). Youden's index was 0.49 for the ROC curve of early DD, and 0.31 for the ROC curve of delayed DD.

CFD, cumulative frequency of distribution.

Figure 5 Individual CV discrepancy of early or delayed cases

Individual CV discrepancies from controls were calculated for early or delayed cases as described in Subjects and Methods. CV discrepancy was calculated for all parameters considered at baseline (left panel), for the 4 pre-specified indices of diastolic function in isolation (central panel), and for the remaining parameters in isolation (right panel). Statistical significance of mean discrepancy was calculated by Wilcoxon signed rank test.

Figure 6 Effects of demographic and oncologic characteristics on the probability Δ score of early cases versus controls

Probability plots were determined by introducing the effect of each demographic and oncologic parameter on the Δ score calculated from the probability curves reported for early cases in Figure 4, left panel. *P* values were calculated from paired sample t-test to compare the probability score mean difference between the reference model (probability score regressed on baseline CV parameters) and other models (additive effect of demographic or oncologic characteristics).

Figure 7 Effects of demographic and oncologic characteristics or post-chemotherapy treatment on the probability Δ score of delayed cases versus controls

Probability plots were determined by introducing the effects of demographic and oncologic parameters or post-chemotherapy treatments on the Δ score calculated from the probability curves reported for delayed cases in Figure 4, right panel. *P* values were calculated as described in the legend to Figure 6.

RT, left-sided chest radiation; HT, hormone therapy (gonadotropin-releasing hormone agonists, anti-estrogens, aromatase inhibitors).

Visual Abstract

Concept of chemotherapy-related early or delayed diastolic dysfunction and different predictors

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Table 1

Demographic, oncologic and baseline CV characteristics of source population and study subgroup

Characteristic	**Source population** (n=80)	**Study subgroup** (n=67)	***P***
age (mean)	49 (47-51)	49 (47-52)	0.652
age (groups)			
18-40 years	11 (14%)	11 (15%)	
41-60 years	59 (74%)	46 (69%)	0.793*
61-70 years	10 (12%)	10 (16%)	
female	68 (85%)	56 (84%)	0.824†
male	12 (15%)	11 (16%)	
current smokers	-	-	-
past smokers	21 (26%)	15 (23%)	0.701†
breast cancer	55 (69%)	43 (64%)	
non Hodgkin lymphoma	17 (21%)	17 (25%)	0.823*
colorectal cancer	8 (10%)	7 (11%)	
anthracycline indication	72 (90%)	60 (90%)	1.000†
fluoropyrimidine-platinum indication	8 (10%)	7 (10%)	
LVEF (%)	62 (61-64)	62 (61-64)	0.961
SBP (mm Hg)	121 (119-123)	121 (118-123)	0.774
DBP (mm Hg)	76 (75-78)	76 (74-78)	0.869
HR (bpm)	77 (75-79)	76 (74-79)	0.405
Hb (g/dl)	12.9 (12.5-13.2)	12.9 (12.6-13.3)	0.754
BMI (kg/m^2)	23.7 (23.0-24.3)	23.7 (23.0-24.4)	0.991
E/A (%)	41.1 (34.8-47.3)	39.5 (32.8-46.3)	0.705
DT (%)	32.9 (24.4-41.4)	28.1 (19.0-37.2)	0.456
cTn (ng/ml)	0.011 (0.010-0.012)	0.011 (0.010-0.012)	0.804
BNP (pg/ml)	73.1 (58.6-87.6)	75.5 (58.5-92.6)	0.975

Data were number of patients (with percentages of total) or means with 95% CI, and were analyzed by two-tailed Mann Whitney test, χ^2 test (*) or Fisher's exact test (†) . E/A and DT were expressed as percentages of normal-for-age ranges (see Subjects and Methods).

Table 2

Baseline CV parameters of controls and cases.

Parameter	1 week		6 months		
	controls (n=41)	early cases (n=26)	controls (n=28)	delayed cases (n=13)	*P*
LVEF (%)	63 (61-65)	62 (60-64)	62 (60-64)	64 (60-67)	0.692
SBP (mm Hg)	121 (117-125)	120 (117-124)	121 (117-126)	120 (113-128)	0.867
DBP (mm Hg)	76 (73-78)	76 (73-79)	76 (73-79)	76 (72-80)	0.988
HR (bpm)	76 (72-79)	77 (73-81)	77 (72-81)	73 (69-78)	0.807
Hb (g/dl)	13.0 (12.5-13.5)	12.9 (12.3-13.5)	13.1 (12.4-13.7)	12.8 (12.1-13.5)	0.927
BMI (kg/m^2)	24 (23-25)	23 (22-25)	24 (23-25)	23 (22-25)	0.824
E/A (%)	42 (33-51)	36 (25-46)	41 (33-49)	45 (20-70)	0.453
DT (%)	31 (19-43)	23 (10-37)	37 (25-48)	41 (14-68)	0.421
cTn (ng/ml)	0.010 (0.010-0.011)	0.012 (0.010-0.013)	0.010 (0.010-0.011)	0.011 (0.010-0.012)	0.625
BNP (pg/ml)	56 (46-65)	66 (55-77)	50 (38-61)	69 (50-87)	0.078

Data were number of patients with percentages of total, or means with 95% CI, and were analyzed by Kruskal-Wallis analysis of variance with Dunn's post test for multiple comparisons. E/A and DT were expressed as percentages of normal-for-age ranges (see Subjects and Methods).

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Table 3

Baseline individual discrepancy of diastolic indices in early or delayed cases

Cases	Mean individual discrepancy (%)			
	E/A	DT	cTn	BNP
Early	-20 (-39 to -1) P=0.022	-8 (-22 to 6) P=0.241	15 (-3 to 33) P=0.098	18 (-0.5 to 37) P=0.134
Delayed	0.1 (-0.6 to 0.8) P=1.000	-3 (-57 to 51) P=0.695	0.8 (-1 to 2) P=1.000	15 (-25 to 55) P=0.470

Mean individual discrepancy was calculated as described in Subjects and Methods. Statistical significance was determined by Wilcoxon signed rank test.

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Table 4

Demographic and oncologic characteristics of controls and early cases

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Characteristic	**controls** (n=41)	**cases** (n=26)	*P*
age (mean)	47 (44-50)	52 (49-56)	0.053
age (groups)			
18-40	10 (14%)	2 (8%)	
41-60	25 (61%)	20 (77%)	0.214*
61-70	6 (15%)	4 (15%)	
female	33 (80%)	23 (88%)	0.503†
male	8 (20%)	3 (12%)	
past smokers	9 (22%)	6 (23%)	1.000†
breast cancer	26 (64%)	17 (65%)	
non Hodgkin disease	10 (24%)	7 (27%)	0.836*
colorectal cancer	5 (12%)	2 (8%)	
anthracycline exposure	36 (88%)	24 (92%)	0.697*
mean dose (mg/m^2)	260 (249-271)	234 (203-265)	0.242

Data were number of patients with percentages of total, or means with 95% CI, and were analyzed by two-tailed Mann Whitney test, χ^2 test (*) or Fischer's exact test (†).

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Table 5

Demographic and oncologic characteristics of controls and delayed cases

Characteristic	**controls** (n=28)	**delayed cases** (n=13)	*P*
age (mean)	46 (42-49)	51 (45-56)	0.169
age (groups)			
18-40	8 (29%)	2 (15%)	
41-60	18 (64%)	7 (54%)	0.124*
61-70	2 (7%)	4 (31%)	
female	22 (79%)	11 (85%)	1.000*
male	6 (21%)	2 (15%)	
past smokers	6 (21%)	3 (23%)	1.000†
breast cancer	19 (68%)	7 (54%)	
non Hodgkin disease	7 (25%)	3 (23%)	0.344*
colorectal cancer	2 (7%)	3 (23%)	
anthracycline exposure	26 (93%)	10 (77%)	0.304†
mean dose (mg/m^2)	238 (210-267)	207 (132-281)	0.928
HT	5 (22%)	2 (15%)	
left chest RT	2 (9%)	1 (8%)	0.997*
HT + left chest RT	6 (26%)	3 (23%)	

Data were number of patients with percentages of total, or means with 95% CI, and were analyzed by two-tailed Mann Whitney test, χ^2 test (*) or Fisher's exact test (†).

HT, hormone therapy (gonadotrophin releasing hormone agonists, antiestrogens, aromatase inhibitors); RT, radiation therapy.

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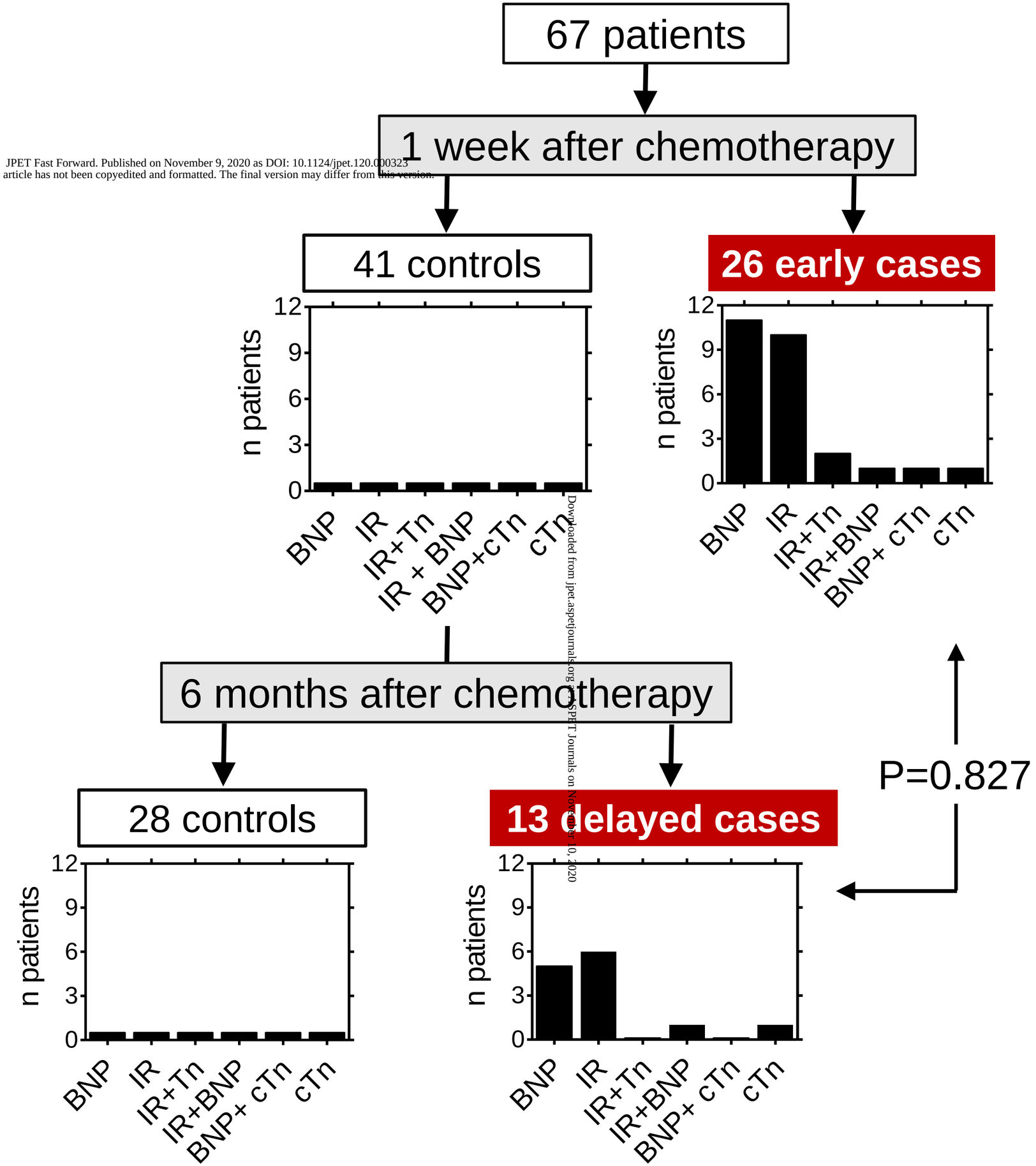

FIGURE 1

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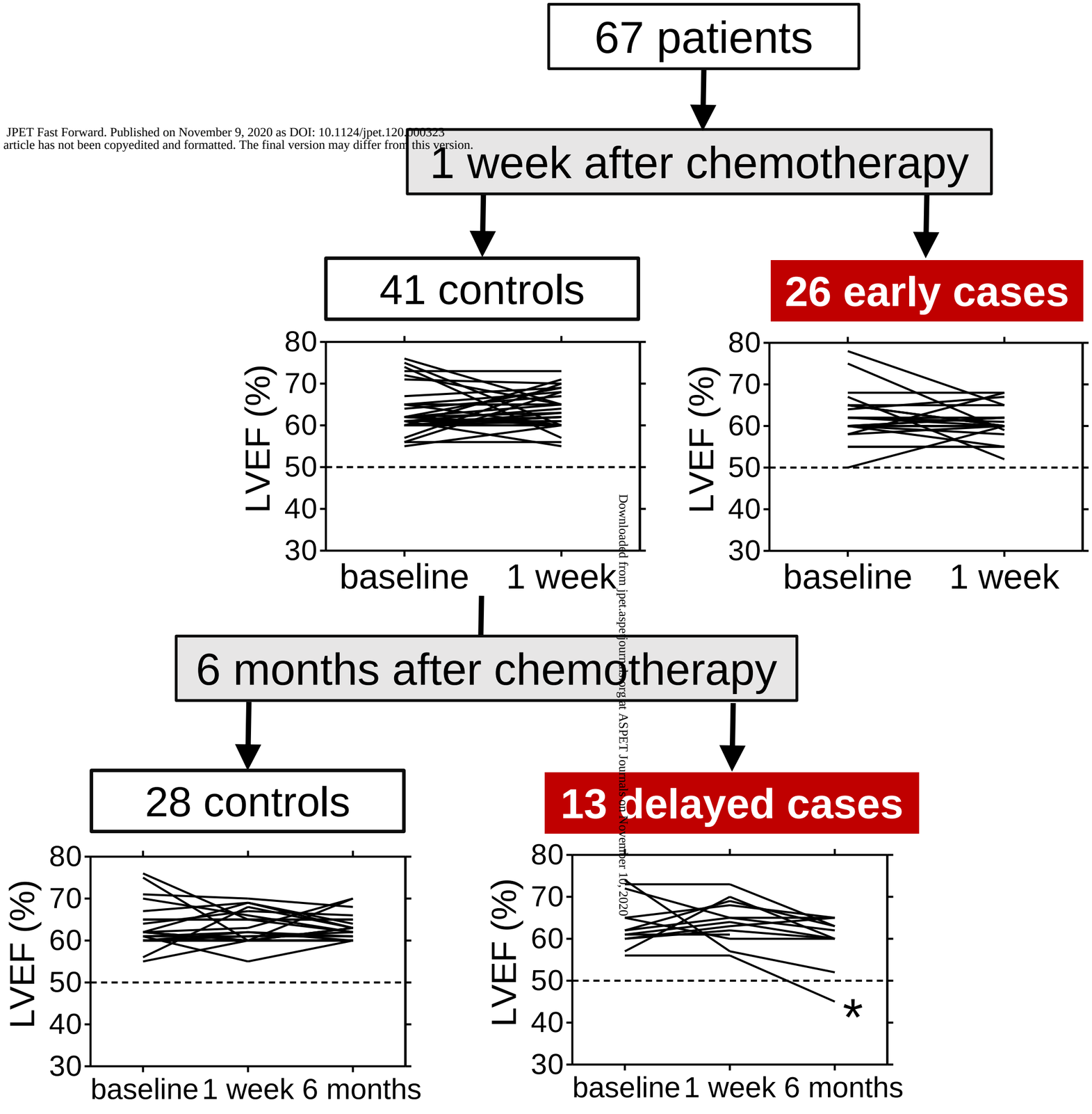

FIGURE 2

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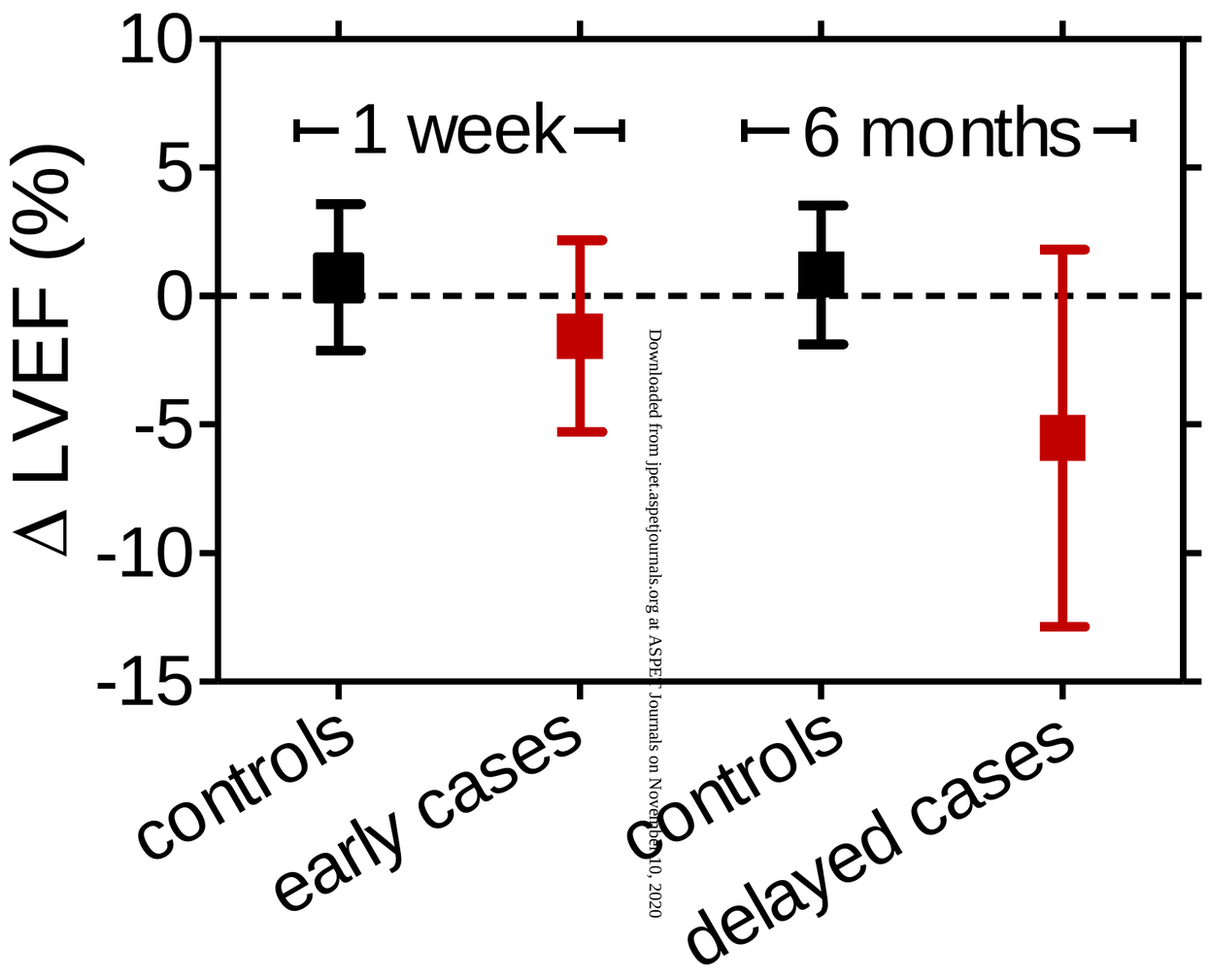

FIGURE 3

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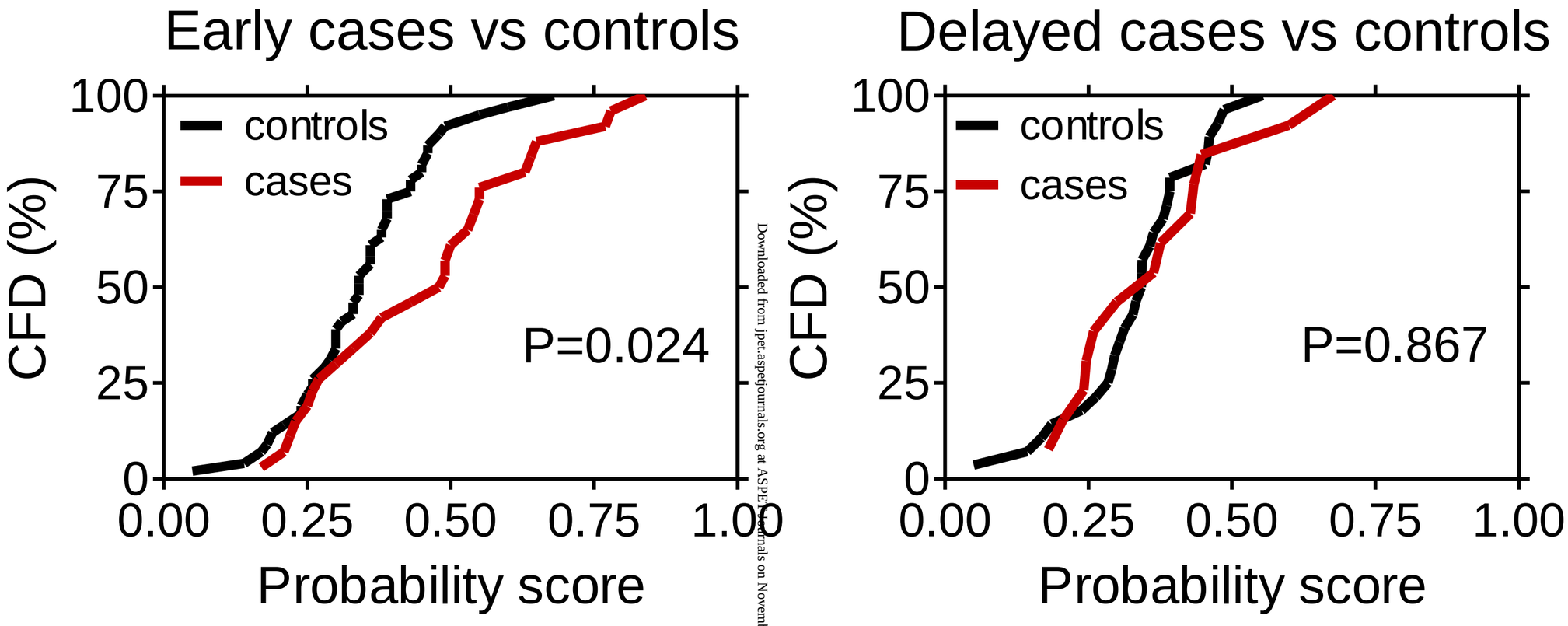

FIGURE 4

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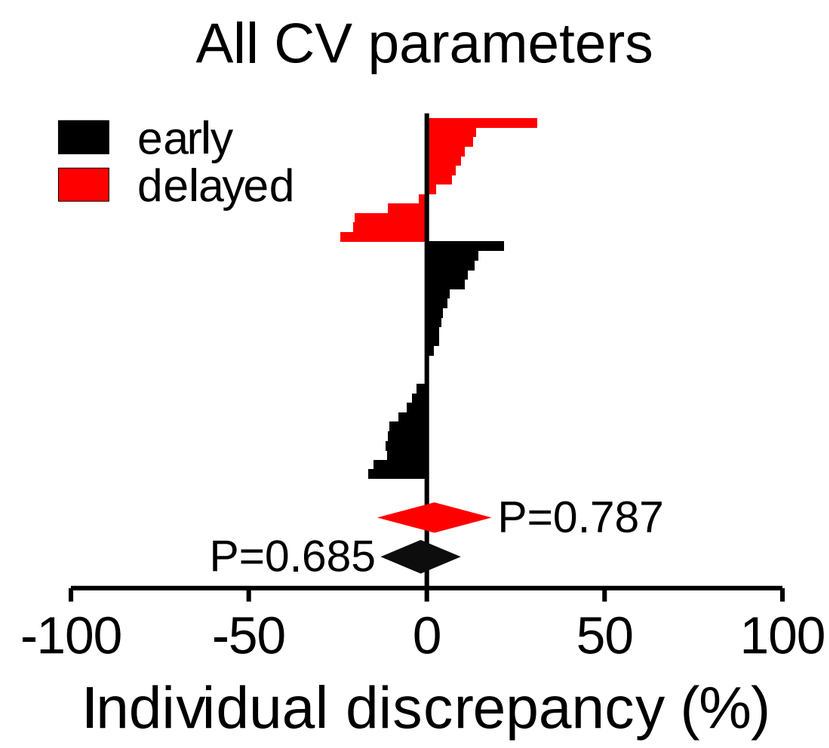

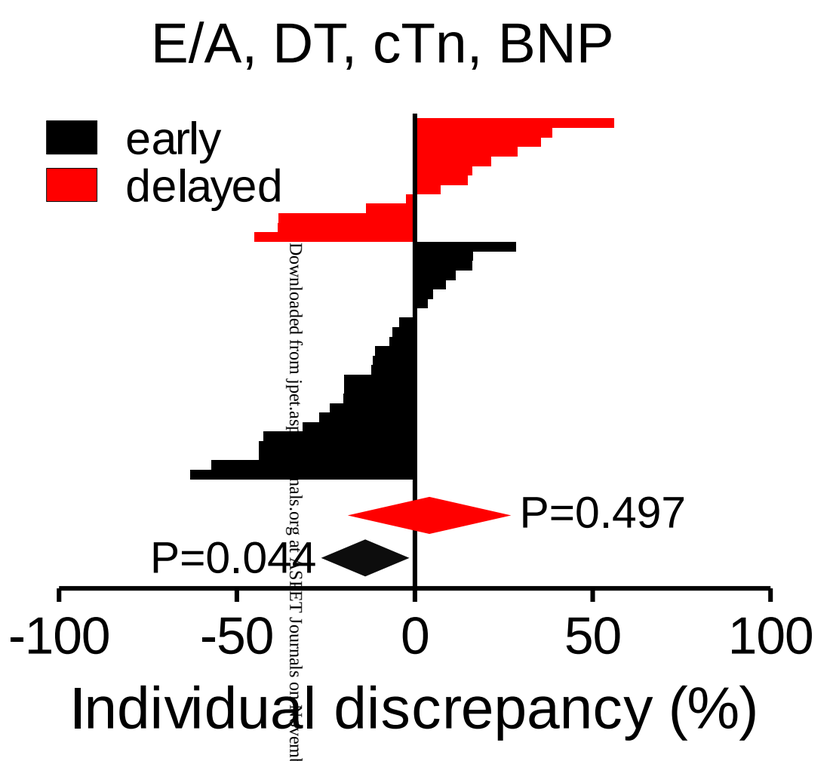

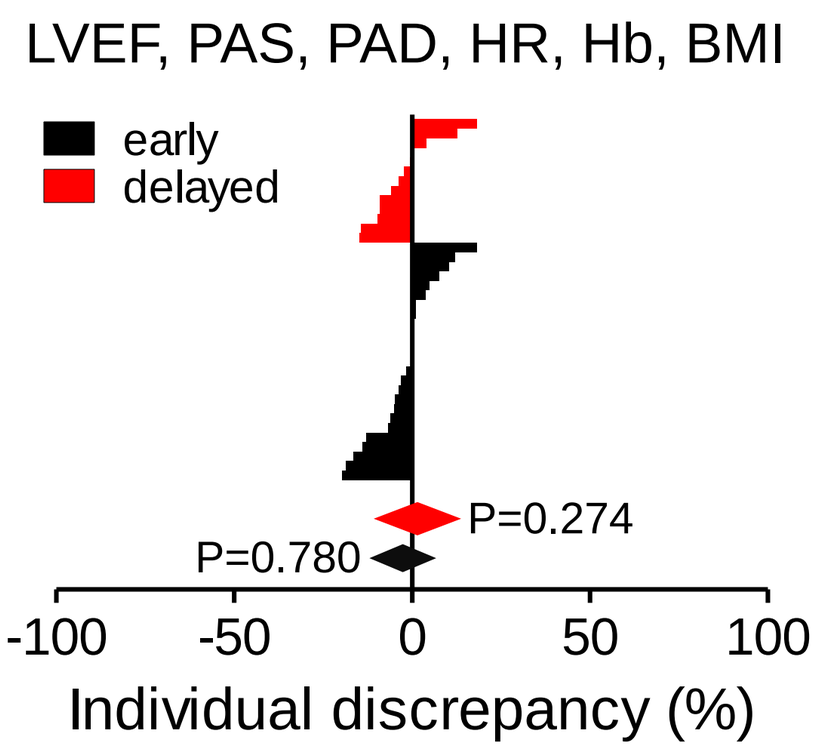

FIGURE 5

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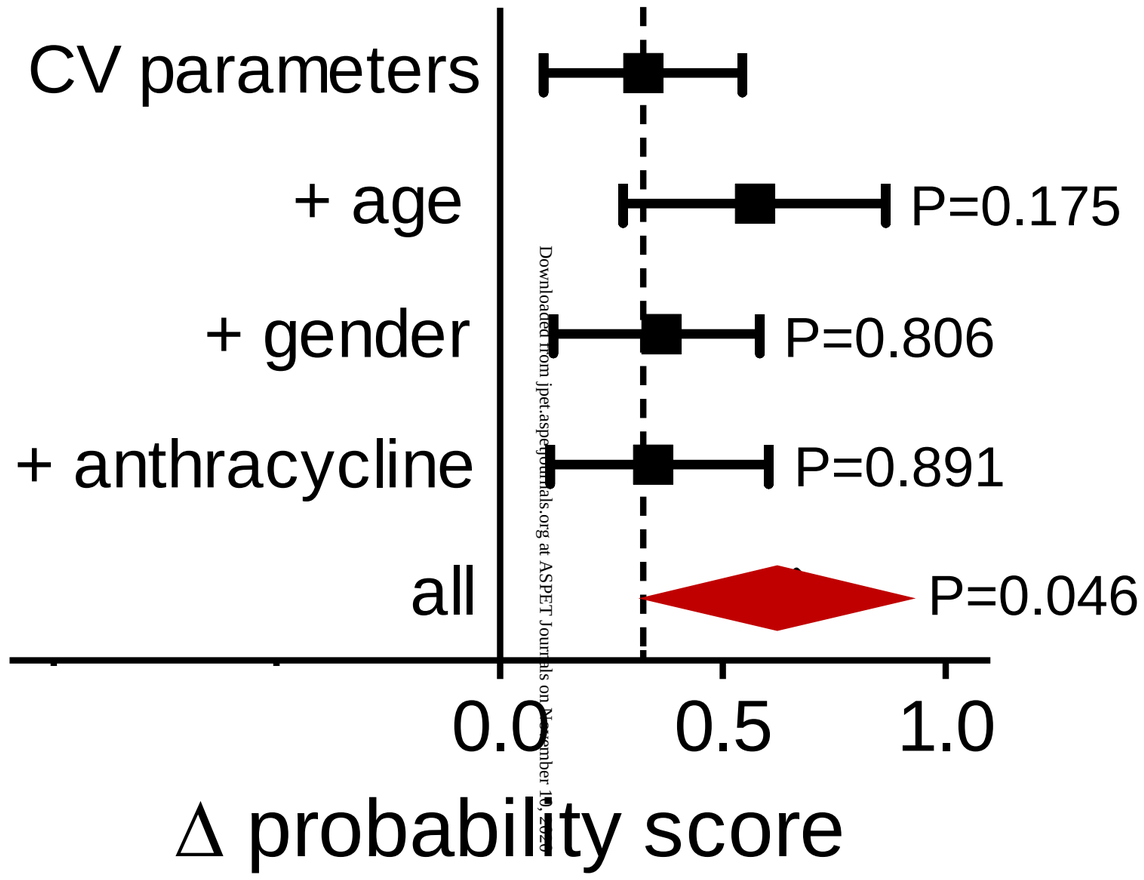

FIGURE 6

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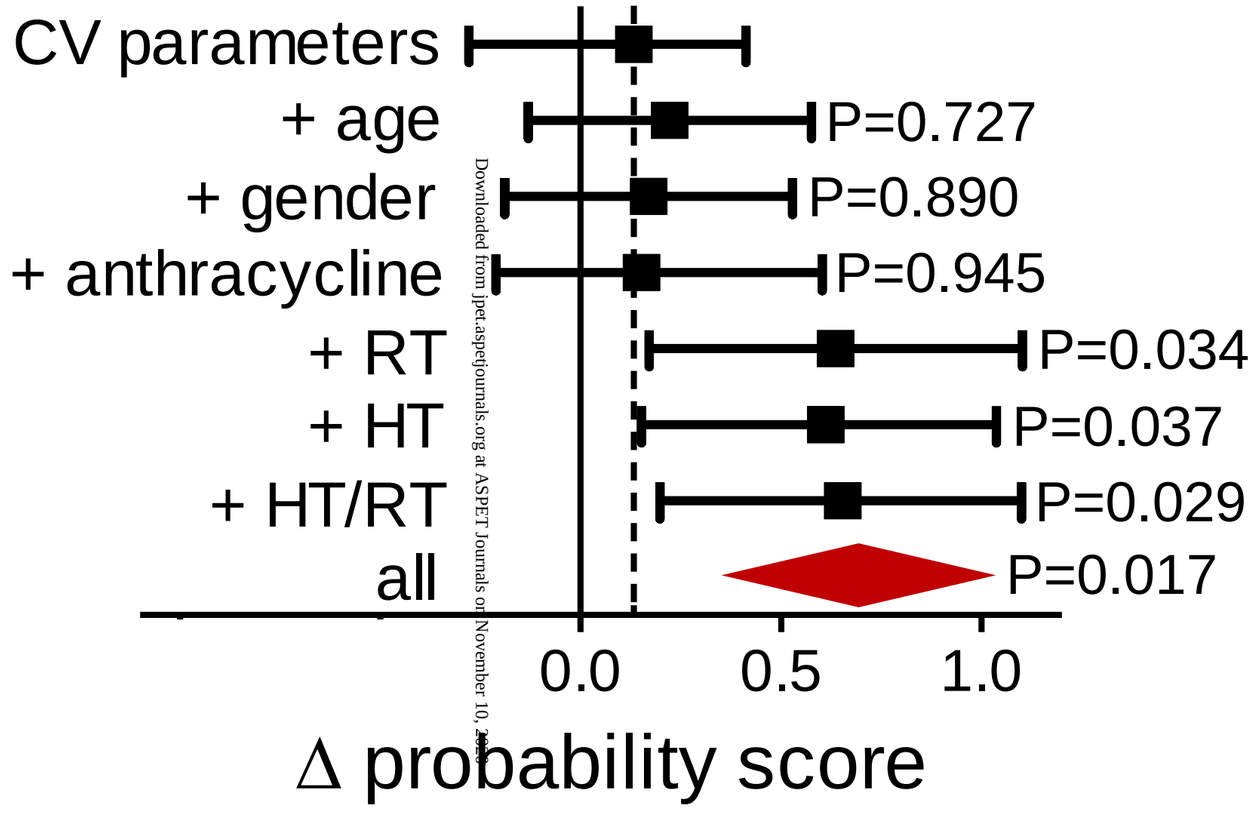

FIGURE 7