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TITLE PAGE

Pharmacology of cardio-oncology:

Chronotropic and lusitropic effects of B-type natriuretic peptide in cancer patients with early diastolic dysfunction induced by anthracycline or nonanthracycline chemotherapy

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NONSTANDARD ABBREVIATIONS: BNP, B-type natriuretic peptide; Nt-proBNP, aminoterminal fragment of B-type natriuretic peptide pro-hormone; HF, heart failure; HR, heart rate; LVEF, left ventricular ejection fraction; PKG, protein kinase G; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; Hb, hemoglobin.

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ABSTRACT

B-type natriuretic peptide (BNP) is widely used as a diagnostic marker of systolic dysfunction. We previously conducted a clinical study in which anthracycline or nonanthracycline chemotherapy did not cause systolic dysfunction in cancer patients; however, some patients showed asymptomatic alterations of diastolic relaxation while others showed persistent elevations of BNP, measured as prohormone BNP aminoterminal fragment. Here we describe post hoc pharmacological analyses showing that i) impaired relaxation and persistent elevations of BNP were mutually exclusive manifestations of diastolic dysfunction ii), in some patients BNP elevations were induced by an early compromisation of myocardial relaxation iii), BNP elevations then halted further deterioration of relaxation in a concentration-dependent manner iv), high BNP increased heart rate (HR). BNP elevations therefore caused positive lusitropy and chronotropism, which might be explained by activation of natriuretic receptor-associated guanylyl cyclase and production of cGMP in ventricular myocytes and sinoatrial node, respectively. BNP levels also influenced responses to a lusitropic drug, ranolazine, that was given to treat diastolic dysfunction. For patients with impaired relaxation and normal or only transiently high BNP, ranolazine improved myocardial relaxation without inducing chronotropic effects. For patients in whom relaxation abnormalities were corrected by persistently high BNP, ranolazine susbstituted for BNP and decreased HR by diminishing BNP levels. These findings describe a pharmacologic scenario in which cancer drugs cause an early diastolic dysfunction that in some patients is both heralded and modulated by BNP elevations. Patients showing BNP elevations should therefore receive the adequate pharmacologic treatment for correcting diastolic dysfunction and tachycardia.

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INTRODUCTION

B-type natriuretic peptide (BNP) is widely used as a diagnostic marker of cardiac dysfunction, primarily but not exclusively in the settings of acute and chronic heart failure (HF). Under conditions of hemodynamic stress, such as when the ventricles are dilated, hypertrophic, or exposed to abnormal wall tension, cardiomyocytes secrete a prohormone BNP that is cleaved by circulating endoproteases to release active BNP (Braunwald, 2008; Daniels and Maisel, 2007). BNP then causes arterial vasodilation, stimulates diuresis and natriuresis, mitigates the activities of renin-angiotensin system and sympathetic nervous system. Hemodynamic actions of BNP therefore play an intuitively beneficial effect for patients affected by heart failure (HF) (Braunwald, 2008).

Circulating endoproteases also release the aminoterminal fragment of prohormone BNP (Nt-proBNP). This peptide is hemodynamically inactive but shows a significantly longer half-life compared to active BNP (120 vs. 20 min) (Weber and Hamm, 2006). Inasmuch Nt-proBNP is formed in (1:1) ratio to BNP, one may use Nt-proBNP for improving BNP detection and predictiveness in cardiovascular disease (Masson et al., 2006; Omland et al., 2007).

Many cancer drugs can cause cardiotoxicity. Anthracyclines induce dilative cardiomyopathy and HF (Minotti et al., 2004; Vejpongsa and Yeh, 2014). Nonanthracycline drugs, like antimetabolites or alkylators or tubuline-active agents, may cause cardiac events by inducing microvasculature dysfunction (Menna et al., 2008). BNP and Nt-proBNP have therefore been considered as potential biomarkers of cardiac dysfunction also in oncologic settings but their role in the management of cancer patients is much less defined compared to the general population (Zamorano et al., 2016).

When adopted to monitor cancer patients BNP or Nt-proBNP was used primarily for detecting or predicting the occurrence of systolic dysfunction. Conflicting results were nonetheless obtained across different studies, which was attributed to differences in chemotherapy regimens (Romano et al., 2012). Moreover, hemodynamic effects of BNP elevations were not explored for patients without systolic dysfunction, which leaves numerous questions about BNP unanswered. The pathophysiologic foundations of cardiotoxicity are in fact laid during chemotherapy but systolic dysfunction may take longer to occur, sometimes months or years after cancer diagnosis and

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treatment (Minotti et al., 2010; Menna and Salvatorelli, 2017). The current thinking is that systolic function declines when overt cardiac damage has already occurred (Ewer and Lenihan, 2008). Should BNP elevations be detected during or shortly after chemotherapy in patients with a preserved systolic dysfunction, one would have an opportunity to intercept cardiotoxicity in its early stages and to characterize the role of BNP in these settings.

Early asymptomatic diastolic dysfunction is suspected to precede systolic dysfunction and other clinical manifestations of cardiotoxicity (Carver et al. 2007; Minotti et al., 2010). BNP elevations have been considered to denote also diastolic rather than systolic dysfunction (Lukowicz et al. 2005). We recently completed a pilot multicenter study of early asymptomatic diastolic dysfunction in patients treated by anthracycline-based or nonanthracycline chemotherapy. In that study both echocardiographic imaging and Nt-proBNP elevations were used to detect diastolic dysfunction at one week after the last chemotherapy dose. We found that echocardiographic indices of impaired diastolic relaxation and Nt-proBNP elevations occurred in twenty-eight out of eighty patients but the two events were mutually exclusive, only one patient developing both impaired relaxation and out-of-range Nt-proBNP. Remarkably, all patients showed a preserved systolic function. We therefore speculated that BNP elevations, measured as Nt-proBNP, caused a positive lusitropic effect that prevented the occurrence of impaired diastolic relaxation (Calabrese et al. 2018).

In the same study Nt-proBNP was measured for investigational purposes also before each chemotherapy cycle. Here the serial measurements of Nt-proBNP were analyzed to define the pattern or patterns of BNP elevations and to characterize cause-and-effect relations between such patterns and the presence or absence of diastolic dysfunction at the end of chemotherapy. We provide evidence to support that BNP elevations, measured as Nt-proBNP, can in fact induce a lusitropic effect that mitigates diastolic dysfunction. We also show that BNP induces a positive chronotropic effect. These findings are incorporated in a pharmacologic framework where anthracyclines or nonanthracycline drugs cause cardiotoxicity, and BNP serves not only as a diagnostic marker but also as an endogenous modifier of cardiotoxicity.

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EXPERIMENTAL PROCEDURES

Patients

All data derived from a pilot, prospective, multicentre, real life study of early cardiotoxicity induced by standard dose anthracycline-based or nonanthracycline chemotherapy (Calabrese et al., 2018). Patient demographic, oncologic and cardiovascular characteristics at baseline (T0) were: 18-70 years of age; Eastern Cooperative Oncology Group performance status of 0 to 1; eligibility for anthracycline-based adjuvant therapy of breast cancer or frontline therapy of newly diagnosed nonHodgkin lymphoma, nonanthracycline (fluoropyrimidine-platinum) adjuvant therapy of colorectal cancer; normal ECG; left ventricular ejection fraction (LVEF) ≥50%; normal diastolic function at 2D echocardiography; normal systolic and diastolic blood pressure (SBP and DBP, repectively); normal levels of Nt-proBNP; absence of any cardiovascular or metabolic disease requiring pharmacologic treatment.

Eighty patients were evaluated by echocardiography and Nt.proBNP at T0 and at one week after chemotherapy (T1). Intertreatment blood samples for Nt-proBNP assays were obtained before each chemotherapy cycle from a subgroup of sixty-seven patients. Demographic and oncologic characteristics of the study subgroup were identical with the complete cohort of eighty patients (source population), both groups showing a prevalence of women treated by anthracyclines for breast cancer. In the majority of cases, women with breast cancer received four cycles of anthracycline-cyclophosphamide (60 mg of doxorubicin/m^2 or 90 mg of epirubicin/m^2 every three weeks), followed by four cycles of taxane (100 mg of docetaxel/m^2 every three weeks). Patients with non Hodgkin lymphoma received six cycles of rituximab/cyclophosphamide/doxorubicin/vincristine/prednisone (50 mg of doxorubicin/m^2 every three weeks). The study subgroup and the source population were also balanced for the incidence of impaired diastolic relaxation (grade I diastolic dysfunction at echocardiography) and/or out-of range Nt-proBNP at T1 (**TABLE 1**). The study subgroup was therefore fully representative of the source population.

The study was compliant to the Declaration of Helsinki and was approved by the Institutional

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Review Board of each participating center. Written informed consent was obtained from all patients.

Nt-proBNP assay

Nt-proBNP was measured in heparin-litium plasma by Siemens immune-chemiluminescence assay for Dimension Vista® System (Luminescent Oxygen Channeling Immunoassay technology, LOCI®), precisely as described (Calabrese et al., 2018). The assay was linear over 5-35.000 pg/ml (Calabrese et al. 2018), and the limit of normality was set at 125 pg/ml as per manufacturer recommendation for patients 18-70 years of age (Siemens Technical Note, 2012). All assays were performed at the Clinical Pharmacology Unit of University Campus Bio-Medico of Rome. Where indicated some patients presented at T0 with Nt-proBNP >125 pg/ml that was caused by emotional distress due to cancer diagnosis (Politi et al., 2007). These patients recovered from distress and showed normalization of Nt-proBNP over the first two or three cycles of chemotherapy; they were therefore included in the study and were evaluated for BNP at T1. Few other patients presented at T1 with Nt-proBNP >125 pg/ml that was caused by non-cardiac events like e.g., fever. These patients were deemed as normal by the investigators.

Other conditions

Systolic function was determined by 2D echocardiography measurements of LVEF, as described (Calabrese et al., 2018). Heart rate was determined by the R-R interval at 12-lead ECG. Diastolic function was evaluated by the mean ratio of peak early filling (E wave) to late diastolic filling (A wave) and by the mean deceleration time of early filling velocity (DT), as also described (Calabrese et al., 2018). Only grade I diastolic dysfunction (impaired relaxation) was detected (E/A decrements and concomitant DT prolongation). Abnormalities of Doppler imaging of mitral annulus were not observed, which was anticipated for patients without HF (Calabrese et al., 2018; Previtali et al., 2012). To avoid inter-observer variability each center identified a study-dedicated operator. At the end of the study echocardiograms were then reviewed by an independent expert for completeness, quality and consistency of imaging sources. Where indicated, E/A and DT were normalized to age-related ranges by the formula

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

This was done on considering physiologic age-related changes of myocardial relaxation and to permit comparison among patients of different age (Calabrese et al. 2018). Throughout this study myocardial relaxation was expressed by E/A changes; comparable results were obtained by expressing impaired relaxation as DT prolongation.

Cumulative anthracycline doses were expressed as doxorubicin myelotoxic equivalents (Ewer, 2013). Where indicated some patients were treated for five weeks with ranolazine as recommended by the European Medicines Agency for its approved indication in chronic angina (375 mg bid for two weeks, followed by 500 mg bid for ten days and 750 mg bid for ten more days) (European Medicines Agency, 2016).

Statistical analysis

Data are expressed as median with range, or numbers and percent, and were analyzed by Kruskall Wallis one way analysis of variance with Dunn's post-hoc test, two-tailed Mann-Whitney test or Wilcoxon matched pair test, as appropriate. Categorical variables were analyzed by Chi Square test or Fisher's Exact test. Differences were considered as significant when P value was <0.05. Linear regression analyses and best fitting curves of nonlinear regressions were obtained by Prism 5®, version 5.01 (GraphPad Software Inc., La Jolla, CA). Other details are reported in legends for Figures and Tables.

RESULTS

Patterns of Nt-proBNP from T0 to T1

Nt-proBNP was measured in sixty-seven patients at baseline (T0), before each chemotherapy cycle, and one week after the last chemotherapy cycle (T1). At T1, fifty-five patients presented with Nt-proBNP below the limit of normality (125 pg/ml) (**FIGURE 1A**); interestingly, however, only twenty-eight out of such fifty-five patients showed normal levels of Nt-proBNP also during the course of chemotherapy, the remaining twenty-seven patients showing transient elevations of Nt-proBNP during chemotherapy. These two subgroups were referred to as “normal” or “transiently high” Nt-proBNP, respectively (**FIGURE 1/B-C**). Twelve other patients showed Nt-proBNP >125 pg/ml at T1 (**FIGURE 1D**). In these latter patients Nt-proBNP began to increase over the second half of chemotherapy, exceeded 125 pg/ml at T1, and was significantly higher compared to T0 ($P<0.001$) or compared to normal or transiently high Nt-proBNP at T1 ($P<0.001$). This group was referred to as “persistently high” Nt-proBNP (**FIGURE 1E**)

Patients with normal, transiently high, or persistently high Nt-proBNP were balanced for demographic and oncologic characteristics such as tumor type and anthracycline versus nonanthracycline chemotherapy, the only exceptions being that i) there were more women in the group with transiently high Nt-proBNP compared to the group with normal Nt-proBNP and ii), the group with persistently high Nt-proBNP was composed of only women (**TABLE 2**). Remarkably, however, women in the three groups were balanced for age ($P=0.712$) and menopausal status ($P=0.193$). Patients were balanced also for LVEF, SBP and DBP, which were normal for all patients (**TABLE 3**). Thus, patients with different patterns of Nt-proBNP were similar for factors that are known to influence BNP levels (Braunwald, 2008).

An inverse correlation has been suggested to occur between BMI and BNP or Nt-proBNP (Bhatt et al., 2018; Braunwald, 2008; Das et al., 2005). We characterized whether transient or persistent elevations of Nt-proBNP occurred in patients showing a lower BMI at T0 or a significant decrease of BMI at T1. Patients with normal, transiently high or persistently high Nt-proBNP presented with a similar BMI at T0 and T1 [25 (20-30), 23 (18-29), 22 (19-29) at T0; 25 (20-34), 23

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(18-32), 24 (17-29) at T1, P>0.05 at multiple comparisons]. For each patient group, BMI at T0 correlated linearly with BMI at T1, and correlation slopes were not significantly different from the lines of identity (**FIGURE 2**, upper panels). We next correlated net changes of BMI from T0 to T1 with concomitant net changes of Nt-proBNP levels. We found that i) for patients with normal Nt-proBNP there was a canonical although borderline significant inverse correlation between BMI increases and Nt-proBNP decreases (all within the range of normality) ii), for patients with transiently high Nt-proBNP, there was no correlation between Nt-proBNP and BMI iii), for patients with persistently high Nt-proBNP, there was an insignificant trend toward a positive correlation between Nt-proBNP elevations and BMI increases (**FIGURE 2**, lower panels). Thus, patients with normal, transiently high or persistently high Nt-proBNP were balanced for BMI, and changes in the levels of Nt-proBNP showed inconsistent correlations with marginal BMI modifications that occurred in the three groups of patients.

Positive lusitropic effect of Nt-proBNP

An impaired diastolic relaxation, defined as grade I diastolic dysfunction at echocardiography, was detected in one out twelve patients with persistently high Nt-proBNP (8% incidence). Impaired diastolic relaxation was more frequent for patients with normal Nt-proBNP (5/28,18% incidence) or transiently high Nt-proBNP (8/27, 30% incidence). Of note, impaired relaxation occurred also in two patients treated by nonanthracycline chemotherapy (one normal Nt-proBNP and one transiently high Nt-proBNP). When examined versus patients with normal or transiently high Nt-proBNP in aggregate, patients with persistently high Nt-proBNP showed a significantly lower risk of developing an impaired diastolic relaxation (RR= 0.333, 95% CI 0.16-0.71, P<0.01). These findings suggested that high BNP protected against the occurrence of impaired relaxation (positive lusitropic effect).

Some patients received cardiac imaging not only at T0 and T1 but also during chemotherapy. For patients treated by anthracyclines this usually occurred after a median cumulative dose of 120 mg/m^2, which was half of the planned median cumulative dose of 240 mg/m^2. We therefore explored interactions between a decline of myocardial relaxation and an increase of Nt-proBNP during the course of chemotherapy. Three patterns were observed: i) for patients with normal Nt-proBNP, the

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decline in myocardial relaxation occurred unopposed ii), for patients with transiently high Nt-proBNP, the decline in myocardial relaxation was accompanied by a peak of Nt-proBNP that subsided as relaxation continued worsening and eventually decreased below the range of normality iii), for patients with persistently high N-proBNP, the decline in relaxation stopped as Nt-proBNP began to increase. This latter pattern occurred regardless of whether patients were given anthracycline-based or nonanthracycline chemotherapy (see **FIGURE 3** for representative cases). For patients with persistently high Nt-proBNP, LVEF remained unchanged throughout chemotherapy (**FIGURE 4**). Thus, persistent elevations of Nt-proBNP seemed to correlate with a mitigation of impaired relaxation but showed no correlation with LVEF.

We next scrutinized the only patient that presented at T1 with an impaired myocardial relaxation and a concomitant persistent elevation of Nt-proBNP. Compared to eleven other patients with persistently high Nt-proBNP, this patient showed the lowest deviation of Nt-proBNP from the range of normality (**FIGURE 5A**). Myocardial relaxation improved as Nt-proBNP gradually exceeded its range of normality (**FIGURE 5B**).

Positive chronotropic effect of high Nt-proBNP

Chemotherapy lowered Hb count, which was similar for patients with normal, transiently high, or persistently high Nt-proBNP. Hemoglobin loss was accompanied by increased HR, which was also similar for the three groups (**TABLE 4**). For patients with normal or transiently high Nt-proBNP, increased HR (expressed as Δ bpm from T0 to T1) correlated with Hb loss (expressed as Δ Hb from T0 to T1). Patients with persistently high Nt-proBNP did not show such a correlation (**FIGURE 6**, upper panels).

The slope of Δ bpm versus Δ Hb was identical for patients with normal or transiently high Nt-proBNP (3.3 ± 1.3 versus 4.2 ± 1.6, respectively, P=0.666). A pooled slope of 3.7 bpm/g of Hb was therefore obtained and was used to calculate theoretical Δbpm, i.e., that Δ bpm one would expect to occur at T1 if all patients gained 3.7 bpm for each gram of Hb they had lost during chemotherapy. Theoretical Δbpm was therefore calculated by the formula [Δ bpm = 3.7 x ΔHb]. Next, we compared

theoretical ∆bpm to clinical ∆bpm that was measured by ECG. Theoretical and clinical ∆bpm were eventually plotted against Nt-proBNP levels at T1. For patients with normal or transiently high Nt-proBNP, theoretical ∆bpm was congruent with clinical ∆bpm. For patients with persistently high Nt-proBNP, clinical ∆bpm deviated from, and exceeded theoretical ∆bpm as Nt-proBNP increased above its range of normality (**FIGURE 6**, lower panels). This suggested that persistently high BNP caused an independent effect on increasing HR (positive chronotropic effect).

Effects of a lusitropic agent on myocardial relaxation, Nt-proBNP, and HR

The antianginal drug, ranolazine, causes positive lusitropic effects without inducing HR changes (Minotti, 2013; Stone 2008). Having shown that impaired relaxation and high Nt-proBNP might be mutually exclusive manifestations of diastolic dysfunction, we preliminarily assessed the impact of ranolazine in patients with impaired relaxation/Nt-proBNP<125 pg/ml (n=6) or normal relaxation/persistently high Nt-proBNP (n=5).

After five-weeks on ranolazine (T5) patients with impaired relaxation and NT-proBNP <125 pg/ml (which was either normal or transiently high during chemotherapy) showed a significant improvement of myocardial relaxation, which returned in normal range. In the same patients ranolazine caused negligible fluctuations of Nt-proBNP, which remained in normal range; moreover, these patients showed an HR decrease that was congruent with the Hb recovery occurring from T1 to T5 (i.e., clinical ∆ bpm was not significantly different to theoretical ∆ bpm calculated by assuming [∆ bpm = 3.7 x ∆Hb]) (**FIGURE 7**, left panels). Thus, for patients with impaired relaxation/Nt-proBNP <125 pg/ml, ranolazine exhibited a canonical lusitropic effect.

For patients with normal relaxation and persistently high Nt-proBNP, ranolazine did not cause significant changes of myocardial relaxation, which remained in normal range; interestingly, however, ranolazine caused remarkable decrements of Nt-proBNP. This latter finding suggested that ranolazine, being a lusitropic agent, could substitute for BNP in preserving diastolic function, and hence it relieved the diastolic distress that stimulated BNP elevations. These patients also exhibited HR decrements that exceeded the effect due Hb recovery (i.e., clinical ∆ bpm was significantly higher

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than theoretical Δ bpm) (**FIGURE 7**, right panels). Having acknowkedged that ranolazine per se lacks effects on HR, we considered that Hb-independent HR decrements were caused by ranolazine through diminishing BNP levels and removing its chronotropic effect.

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DISCUSSION

Dual effects of BNP elevations in cancer patients with diastolic dysfunction

We have shown that chemotherapy-induced BNP elevations may help to mitigate diastolic dysfunction (lusitropic effect); however, this beneficial effect of BNP comes at the cost of inappropriate tachycardia that is also caused by high BNP (chronotropic effect) (**FIGURE 8**). These findings shed a new light on the role of BNP in cancer patients.

Evidence and pharmacologic foundations for the lusitropic effect of BNP in cancer patients

The positive lusitropic effect of BNP was inferred from the lower incidence and relative risk of impaired myocardial relaxation in patients with persistently high Nt-proBNP compared to patients with normal or transiently high Nt-proBNP. The time courses of myocardial relaxation versus Nt-proBNP levels were highly suggestive of a sequence of events in which an early decline of relaxation was intercepted and mitigated by persistent BNP elevations before diastolic dysfunction eventually occurred (see Figure 3). Two lines of evidence also suggest that BNP caused its lusitropic effect in a concentration-dependent manner: i) positive lusitropy did not occur in a patient showing the lowest deviation of Nt-proBNP from the range of normality ii), myocardial relaxation gradually improved as Nt-proBNP exceeded its range of normality (see Figure 5).

The pharmacologic foundations of BNP lusitropy may rest with a variety of mechanisms. Similar to other natriuretic peptides, BNP binds to receptors associated with particulate guanylyl cyclase and cGMP production (Azer et al. 2014; Molkentin, 2003; Munagala et al., 2004). cGMP can then induce relaxant effects through protein kinase G (PKG)-mediated phosphorylation of titin, which results in a reduced passive tension of cardiomyocytes (Bishu et al., 2011). Evidence for a BNP-dependent phosphorylation of other relaxation-coupled proteins is less solid. In comparative studies C-type natriuretic peptide was more effective than BNP in promoting phosphorylation of troponin and phospholamban (Moltzau et al., 2014). Interestingly, however, BNP mobilizes endothelial C-type natriuretic peptide from endothelial cells in humans (Hillock et al., 2008) and measurably increases its lusitropic effect in preclinical models (Moltzau et al., 2014). Beneficial cross talks between BNP and other natriuretic peptides may therefore occur, and accordingly, defects in the BNP-PKG

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cascade were shown to occur in patients with severe diastolic dysfunction (van Heerebeek et al. 2012).

The BNP-PKG signaling cascade can also promote catecholamine exocytosis from cardiac sympathetic nerve endings, an effect mediated by inhibition of phosphodiesterase type 3-catalyzed hydrolysis of cAMP (Chan et al., 2012). Catecholamine activation of cardiac β_1 or β_2 receptors might then promote concomitant lusitropic and inotropic effects (Molenaar et al., 2007). Here an adrenergic positive inotropism was not observed; in fact, LVEF did not decrease when chemotherapy was started and did not increase when Nt-proBNP began to rise (see Figure 4). An involvement of catecholamines cannot therefore be established at this point in time.

Evidence and pharmacologic foundations for the chronotropic effect of BNP in cancer patients

Myelotoxicity and low Hb count are common consequences of chemotherapy. Low Hb count may increase HR through complex reflexes that at least in part originate from carotid body (Lahiri et al., 2006). Here the positive chronotropic effect of BNP was inferred from the following findings: i) a correlation between Hb loss and increased HR occurred for patients with normal or transiently high Nt-proBNP but not for patients with persistently high Nt-proBNP ii), high Nt-proBNP levels caused HR to increase independent of Hb loss (see Figure 6). Regardless of a possible involvement of exocytosed catecholamines, BNP can increase HR via receptor-mediated mechanisms. In fact, the binding of BNP to natriuretic peptide receptor A increases conduction velocity within the sinoatrial node and toward the surrounding atrial myocardium (Azer et al., 2014). These effects probably involve cGMP activation of L-type Ca^{2+} current (Springer et al., 2012).

Probing the role of BNP with ranolazine

Ranolazine has been approved to treat patients with stable angina, in whom it works by relieving diastolic myocardial tension and by improving coronary conductance. Of note, ranolazine induces its beneficial effects without inducing concomitant changes of SBP, DBP or HR (Stone, 2008). We described previously the rationale for a phase 2 study in which patients presenting at T1 with diastolic dysfunction were treated with ranolazine. In brief, we suggested that ranolazine, inhibitor of the late inward sodium current, could interrupt vicious cycles that caused Ca^{2+} overload

and impaired relaxation in cardiomyocytes exposed to anthracyclines or nonanthracycline chemotherapeutics (Minotti et al., 2013). Here we presented preliminary data that recapitulate connections between diastolic relaxation, BNP, and HR. For patients in whom impaired relaxation was not corrected by persistent elevations of BNP, relaxation was improved by the lusitropic effect of ranolazine while HR decreased in response to an Hb recovery that occurred over the time period of ranolazine administration. For patients in whom diastolic dysfunction was corrected by persistent elevations of BNP, ranolazine relieved the diastolic distress that stimulated BNP elevations; by so doing, ranolazine diminished BNP levels and caused HR decrements that reflected an abrogation of the chronotropic effect of BNP. Thus, the effects of a lusitropic drug (ranolazine) were influenced by the levels of an endogenous lusitropic and chronotropic agent (BNP). Of note, the two lusitropic agents might share some mechanism of action. BNP-driven phosphorylation of relaxation coupled proteins diminishes their sensitivity to Ca^{2+} (Zhang et al., 1995); in a similar manner, sodium current-independent effects of ranolazine may diminish myofilament sensitivity to Ca^{2+} (Lovelock et al., 2012).

Study limitations and strengths

We acknowledge that our study shows limitations, primarily due to the relatively limited sample size of these analyses. Moreover, our study cannot elucidate the reason(s) some patients showed a persistently high Nt-proBNP while others showed normal or only transiently high Nt-proBNP. For the latter patients, the reason(s) Nt-proBNP failed to remain elevated and to compensate for impaired relaxation call for further investigations. The three patient groups were similar for factors that might have caused differences in prohormone BNP production (age, blood pressure, hormonal status, oncologic regimens). The so-called “BNP diluting effect” of high BMI, provisionally but not unequivocally attributed to a receptor-mediated partitioning of Nt-proBNP or BNP in adipose tissue (Das et al., 2005), has been excluded. Genetic factors governing individual changes in prohormone BNP production and/or BNP or Nt-proBNP pharmacokinetics might be considered (Lanfear et al., 2014). On a different note we emphasize that all blood samples for Nt-proBNP assays were taken before chemotherapy infusions were started. We therefore exclude

confounding factors due to fluid overload and cardiomyocyte stretch occurring in some patients but not in others.

Our work shows strengths as well. First, this work modifies and extends our current perception of BNP elevations in cancer patients treated by chemotherapy, showing that BNP increases in consequence diastolic rather than systolic dysfunction. Second, this work characterizes a potentially beneficial loop in which BNP elevations mitigate diastolic dysfunction. Finally, this work shows that beneficial effects from BNP lusitropy might be counteracted by inappropriate tachycardia due to BNP chronotropism. BNP should therefore be considered not only as a biomarker but also as a cardiovascular agent with both positive and potentially negative effects. This concept echoes the outcome of a clinical trial in which the administration of recombinant human BNP did not improve net clinical outcomes of patients with acute HF (O'Connor et al., 2011).

From BNP to pharmacology of cardio-oncology

We recently developed translational models of human heart in which HF risk from anthracyclines was predicted to occur after lower cumulative doses than previously reported and regardless of patient risk factors. This was attributed to the ease with which anthracyclines accumulate and generate toxic metabolites in human myocardium (Salvatorelli et al., 2017; Salvatorelli et al., 2018). Here we have shown that impaired myocardial relaxation or Nt-proBNP elevations occurred in comorbid free patients treated by a median cumulative anthracycline dose of 240 mg/m^2, which was significantly lower than the cumulative dose reported to cause 5% risk of HF (400 mg of doxorubicin/m^2) (Swain et al., 2003). Myocardial relaxation actually began to decline after a cumulative anthracycline dose of 120 mg/m^2 (see Figure 3). Clinical pharmacology studies, described in this present study, validate conclusions of basic-translational pharmacology studies. Impaired myocardial relaxation or persistently high Nt-proBNP occurred also in a small group of patients treated by standard doses of nonanthracycline drugs. This latter finding supports pharmacological reasonings that warned against cardiotoxicity from any cancer drug (Minotti et al., 2010).

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Monitoring and protecting the cardiac function of cancer patients is therefore important. An early decline of myocardial relaxation should be considered for treatment before diastolic dysfunction progresses toward a more serious stage. BNP or Nt-proBNP elevations should also be considered for treatment as they denote a compensatory mechanism that attempts to mitigate diastolic dysfunction. Inappropriate tachycardia due to BNP chronotropism represents another reason patients with high BNP should receive the adequate treatment to reduce HR. This is not routine in current days and requires evidence from ad hoc studies. This will be described in the final report of our phase 2 study of ranolazine versus β blockers and other common cardiovascular drugs in cancer patients (Minotti, 2013).

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AUTHORSHIP CONTRIBUTIONS

Participated in research design: Menna and Minotti

Conducted experiments: Menna, Calabrese, Armento, Annibali, Greco

Contributed new reagents or analytic tools: Menna

Performed data analysis: Salvatorelli, Reggiardo, Minotti

Wrote the manuscript: Minotti

Reviewed and approved the manuscript: All authors

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FOOTNOTES

This study was promoted by Menarini International Operations Luxembourg S.A. and was registered at the European Clinical Trials Database (EUDRACT 2009-016930-29).

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LEGENDS FOR FIGURES

Figure 1 Patients with normal, transiently high, or persistently high Nt-proBNP

Panel (A) shows patients with normal (<125 pg/ml) Nt-proBNP at T1. This group of patients included a subgroup in which Nt-proBNP was normal throughout chemotherapy (panel B) or showed transient elevations during chemotherapy (panel C). Panel D shows patient wih high (>125 pg/ml) Nt-proBNP at T1 (the asterisk denotes P<0.001 for Nt-proBNP at T1 versus T0). In these latter patients Nt-proBNP gradually increased over the second half of chemotherapy (panel E). Values are shown in whisker plots with medians (A,D) or medians with ranges (B,C,E). Shaded areas indicate the range of normality. Chemotherapy was expressed as percent of length to permit comparisons among oncologic regimens of different durations. Open symbols denote out-of-range levels that the investigators considered as normal according to patients characteristics at T0 or T1. Out-of-range values at T0 normalized over the first two or three cycles of chemotherapy and were therefore included in the analyses.

Figure 2 Nt-proBNP versus BMI in patients with normal, transiently high, or persistently high Nt-proBNP

The upper panels show that BMI at T1 correlated linearly with BMI at T0 and no significant difference occurred versus the line of identity (dashed line). The lower panels show net changes of BMI from T0 to T1 versus net changes of Nt-proBNP. A borderline significant inverse correlation between BMI and Nt-proBNP was observed only for patients with normal Nt-proBNP. Shaded areas indicate 95% CI of linear regressions.

Figure 3 Time courses of myocardial relaxation versus Nt-proBNP

The upper panels show changes in myocardial relaxation from T0 to T1 (best fitting curves). The arrows indicate the time when cardiac imaging was done during chemotherapy and the median cumulative anthracycline dose reached at that time. The bottom panels show the time courses of Nt-proBNP from T0 to T1 (best fitting curves). For patients with persistently high Nt-proBNP the solid

line indicates an anthracycline-based oncologic regimen while the dashed line indicates a nonanthracycline regimen. Shaded areas indicate the range of normality of myocardial relaxation or Nt-proBNP. For best fitting curves, the standard deviation of residuals (Sy.x) was ≤2.0, making in-range values significantly different to out-of-range values.

Figure 4 Time courses of LVEF and Nt-proBNP in patients with persistently high Nt-proBNP

Panel A shows LVEF from T0 to T1. The arrows indicate the time when cardiac imaging was done during chemotherapy and the median cumulative anthracycline dose reached at that time. Panel B shows the time course of Nt-proBNP from T0 to T1 (best fitting curves). The shaded area indicates the range of normality of Nt-proBNP.

Figure 5 Nt-proBNP levels and myocardial relaxation in twelve patients with persistently high Nt-proBNP at T1

The upper panels show individual Nt-proBNP levels in twelve patients with persistently high Nt-proBNP at T1. The bottom panel shows individual values of myocardial relaxation for the same twelve patients (columns and best fitting curve). The asterisks indicate the only patient who developed a persistently high Nt-proBNP and a concomitant impairment of myocardial relaxation. Shaded areas indicate ranges of normality for Nt-proBNP or myocardial relaxation.

Figure 6 Hemoglobin loss and Δbpm for patients with normal, transiently high, or persistently high Nt-proBNP

The upper panels show that at T1, Δbpm correlated with ΔHb for patients with normal or transiently high Nt-proBNP but not for patients with persistently high Nt-proBNP. Shaded areas indicate 95% CI intervals of linear regression. The lower panels (best fitting curves) show that theoretical Δbpm was congruent with clinical Δbpm for patients with normal or transiently high Nt-proBNP. For patients with persistently high Nt-proBNP, clinical Δbpm deviated from, and exceeded theoretical Δbpm when Nt-proBNP increased above its range of normality. See also text for explanations.

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Figure 7 Effects of five weeks ranolazine on myocardial relaxation, Nt-proBNP, and clinical versus theoretical Δ bpm in patients with impaired relaxation/Nt-proBNP <125 pg/ml or normal relaxation /persistently high Nt-proBNP

After five weeks on ranolazine (T5), patients with impaired relaxation/Nt-proBNP <125 pg/ml at T1 (normal or transiently high Nt-proBNP during chemotherapy, n=6) showed improved myocardial relaxation, insignificant increases or decreases of Nt-proBNP, and a negative clinical Δ bpm that was congruent with theoretical Δ bpm due to Hb recovery from T1 to T5 (Δ Hb = 1.3 (-0.1 to 2.2) g/dl). For patients with normal relaxation/persistently high Nt-proBNP at T1 (n=5), ranolazine did not change myocardial relaxation but diminished Nt-proBNP levels and caused clinical Δ bpm that exceeded theoretical Δ bpm due to Hb recovery (median Δ Hb = 1.1 (1.0 -1.3) g/dl).

Figure 8 Schematic representation of the mechanisms and consequences of BNP elevations in cancer patients

Cancer chemotherapy causes diastolic dysfunction (1), which represents a stimulus to BNP elevations (2). High BNP improves diastolic function (3) but causes also an undesired tachycardia (4).

Table 1.

Demographic and oncologic characteristics of source population and study subgroup

Characteristics	Source population (n=80)	Study subgroup (n=67)	P
Age (years, median, range)	49 (28-68)	49 (28-68)	0.652
Gender (n,%) male Female	 12 (15%) 68 (85%)	 11 (16%) 56 (84%)	0.981
Oncologic disease (n,%) breast cancer non Hodgkin lymphoma colorectal cancer	 55 (69%) 17 (21%) 8 (10%)	 43 (64%) 17 (25%) 7 (11%)	0.747
Chemotherapy (n,%) anthracycline-based[a)] nonanthracycline[b)]	 72 (90%) 8 (10%)	 60 (90%) 7 (10%)	1.000
Anthracycline dose[c)] (mg/m^2, median, range)	240 (0-317)	240 (0-317)	0.683
Diastolic events at T1 Impaired relaxation[d)] High Nt-proBNP[e)] Impaired relaxation + high Nt-proBNP	 13 (16%) 14 (18%) 1 (1.3%)	 13 (19%) 12 (18%) 1 (1.5%)	0.829

Table 1 (continues)

Data were analyzed by two tailed Mann Whitney test, two tailed square Chi test or Fisher's exact test, as appropriate.

[a)]doxorubicin (or epirubicin)/cyclophosphamide followed by docetaxel; epirubicin/cyclophosphamide/docetaxel; 5-fluorouracil/epirubicin/cyclophosphamide, with or without a subsequent docetaxel; rituximab/cyclophosphamide/doxorubicin/vincristine/prednisone (for non Hodgkin lymphoma).

[b)]Folinate/5-fluorouracil/oxaliplatin; capecitabine/oxaliplatin.

[c)]Expressed as doxorubicin myelotoxic equivalents.

[d)]Grade I diastolic dysfunction at echocardiography.

[e)]Nt-proBNP >125 pg/ml.

Table 2.

Demographic and oncologic characteristics of patients with normal, transiently high, or persistently high Nt-proBNP

Characteristics	Normal (n=28)	Transiently high (n=27)	Persistently high (n=12)	P
Age (years, median, range)	47 (28-62)	51 (31-68)	50 (36-60)	0.255
Gender (n,%) male Female	 9 (32%) 19 (68%)	 2 (7%) 25 (93%)*	 - 12 (100%)**	<0.0001
Oncologic disease (n,%) breast cancer non Hodgkin lymphoma colorectal cancer	 13 (46%) 11 (39%) 4(15%)	 20 (74%) 5 (19%) 2 (7%)	 10 (84%) 1 (8%) 1 (8%)	0.127
Chemotherapy (n,%) anthracycline-based nonanthracycline	 24 (86%) 4 (14%)	 25 (93%) 2 (7%)	 11 (92%) 1 (8%)	0.682
Anthracycline dose[a)] (mg/m^2, median, range)	240 (0-317)	240 (0-317)	240 (0-300)	0.227

Data were analyzed by Kruskall Wallis one way analysis of variance with Dunn's post-hoc test or two tailed Chi Square test or Fisher's exact test, as appropriate. *P<0.0001 versus normal, **P<0.0001 versus transiently high.

[a)]Expressed as doxorubicin myelotoxic equivalents.

Table 3

Cardiovascular characteristics of patients with normal, transiently high, or persistently high levels of Nt-proBNP

Parameter	Normal (n=28)	Transiently high (n=27)	Persistently high (n=12)	P
LVEF (%) T0 T1 P (T0 - T1)	 62 (55-76) 62 (55-73) 0.917	 60 (55-75) 60 (52-69) 0.983	 60 (50-78) 60 (55-65) 1.000	 0.120 0.104
SBP (mm Hg) T0 T1 P (T0 - T1)	 120 (100-140) 120 (100-150) 0.961	 120 (90-145) 120 (100-140) 0.614	 120 (100-140) 120 (105-135) 0.519	 0.899 0.320
DBP (mm Hg) T0 T1 P (T0 - T1)	 80 (60-90) 80 (65-90) 0.455	 80 (60-85) 80 (60-90) 0.184	 80 (60-85) 73 (60-85) 0.335	 0.629 0.260

Data were analyzed by Kruskall Wallis one way analysis of variance with Dunn's post-hoc test, or by Wilcoxon matched paired t test (for T0 - T1 differences).

Table 4

Hemoglobin and heart rate in patients with normal, transiently highly, or persistently high Nt-proBNP

Parameter	Normal (n=28)	Transiently high (n=27)	Persistently high (n=12)	P
Hb (g/dl) T0 T1 P (T0 - T1)	 12.9 (10-16) 11.6 (9.3-15.8) <0.001	 13.3 (9.2-15.5) 11.5 (8.8-13.5) <0.001	 13.1 (9.9-15.2) 10.5 (9.5-13.1) 0.001	 0.937 0.522
Δ Hb (g/dl)	-1.3 (-4.9 to 3.4)	-1.4 (-5.9 to 2.8)	-1.8 (-4.2 to -0.4)	0.488
HR (bpm) T0 T1 P (T0 - T1)	 80 (59-110) 85 (57-113) 0.012	 75 (61-100) 85 (57-106) <0.01	 75 (60-96) 80 (71-122) 0.045	 0.557 0.876
Δ bpm	4 (-22 to 37)	8 (-15 to 35)	10 (-16 to 42)	0.728

Data were analyzed by Kruskall Wallis one way analysis of variance with Dunn's post-hoc test or by two-tailed square Chi test or Wilcoxon matched paired t test (for T0 - T1 differences).

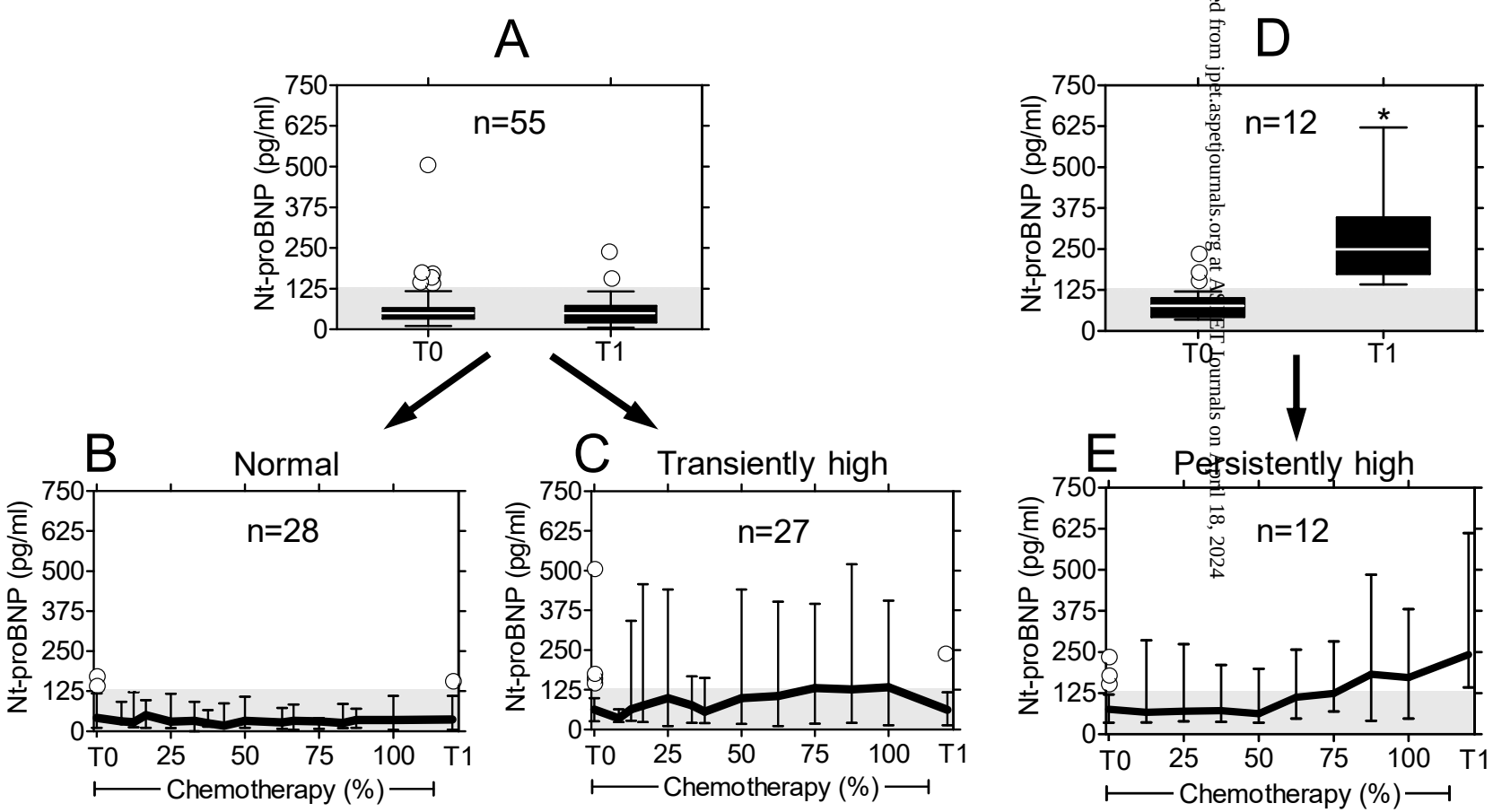

FIGURE 1

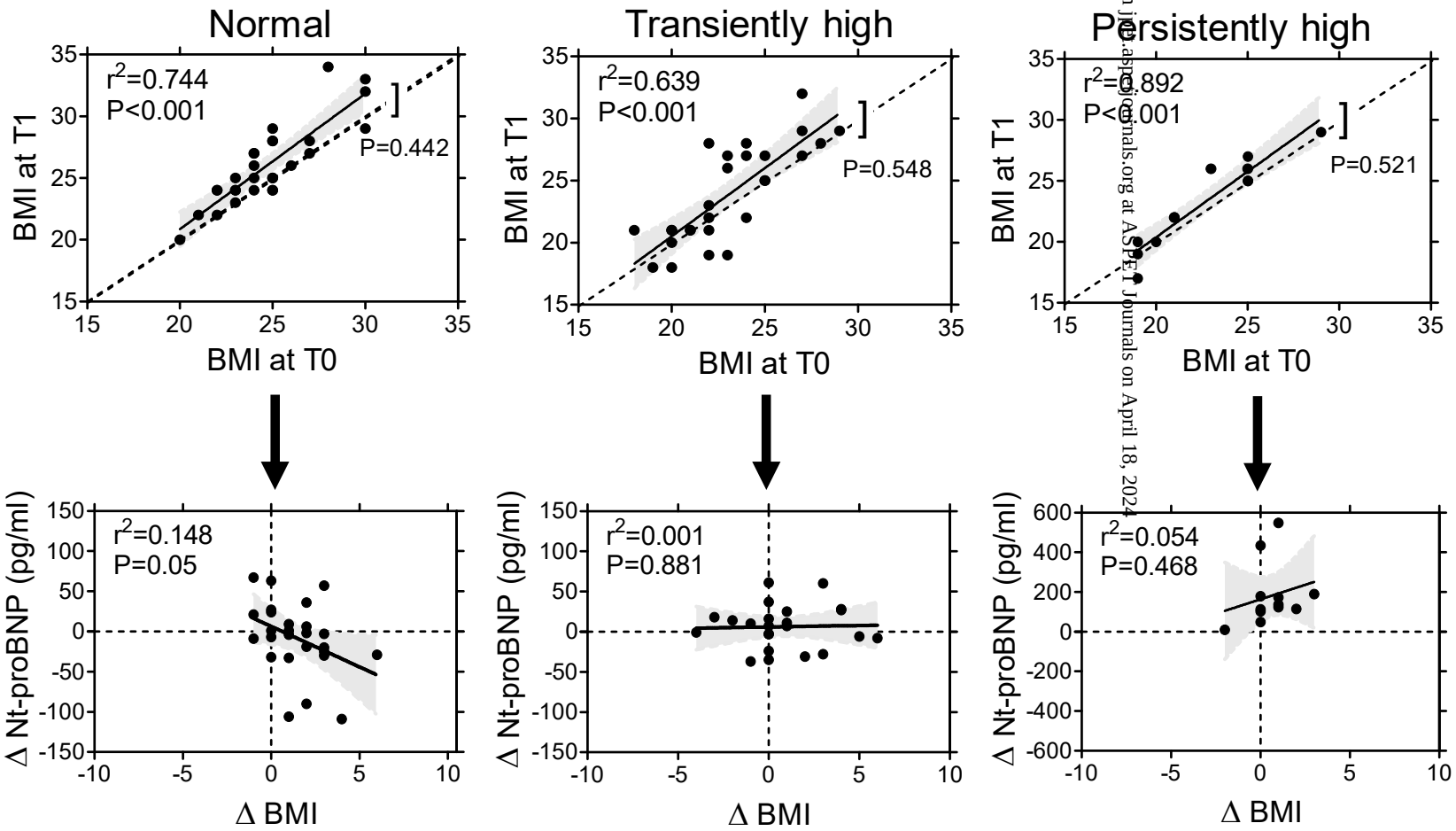

FIGURE 2

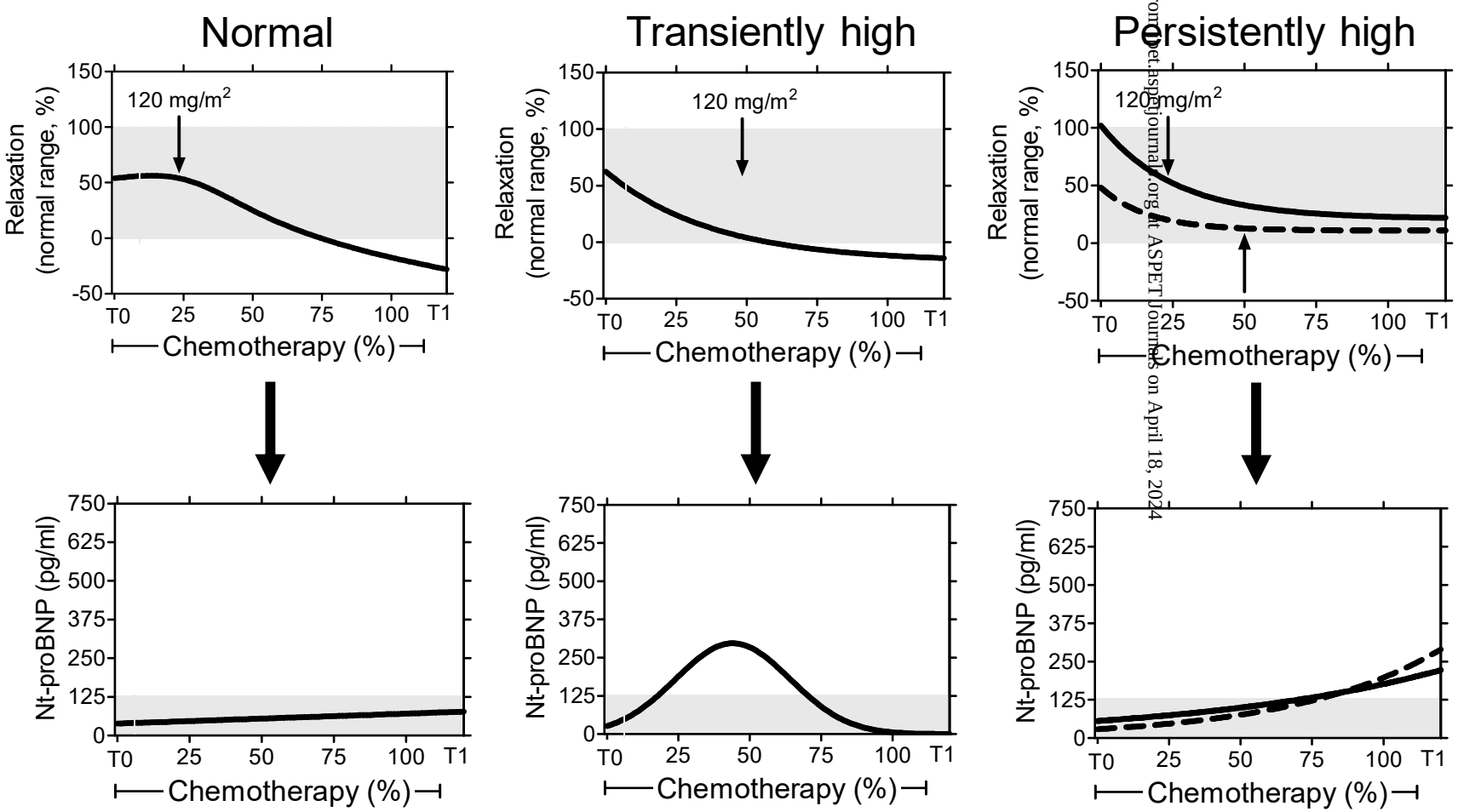

FIGURE 3

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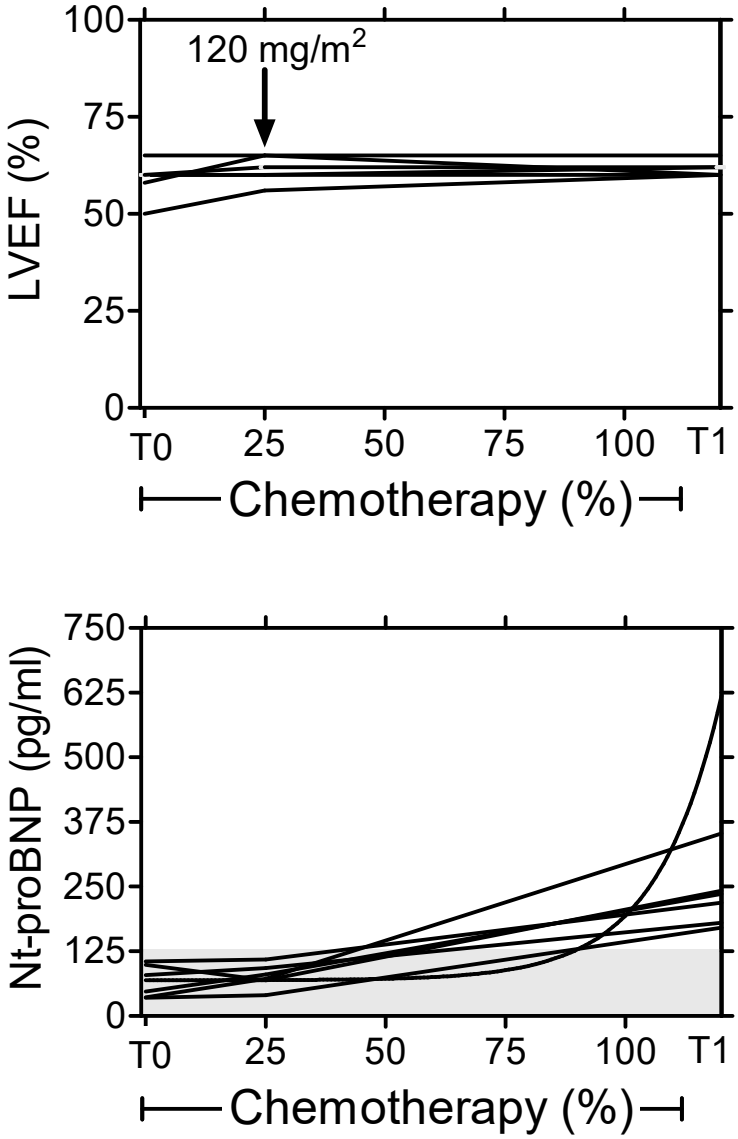

FIGURE 4

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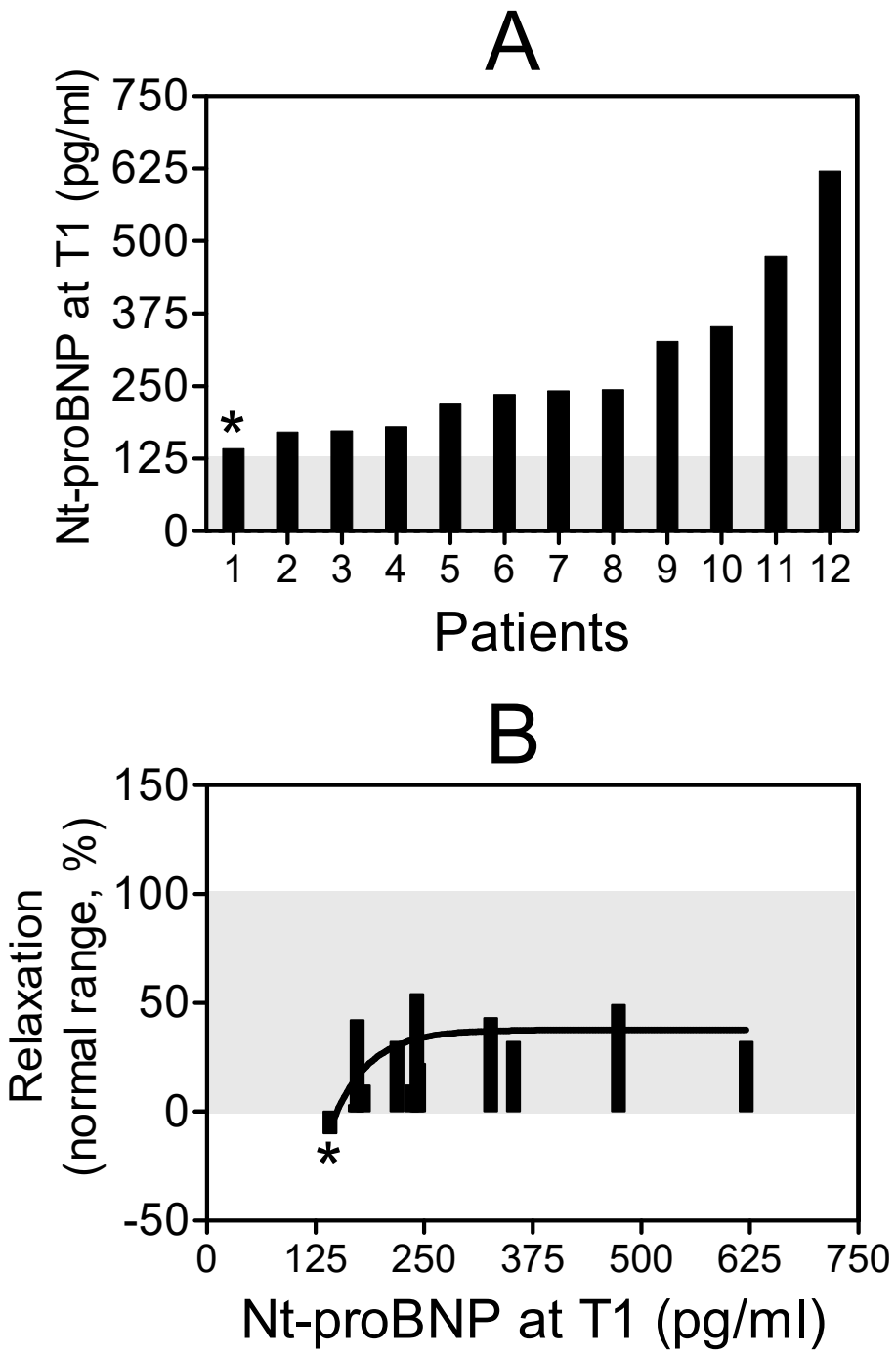

FIGURE 5

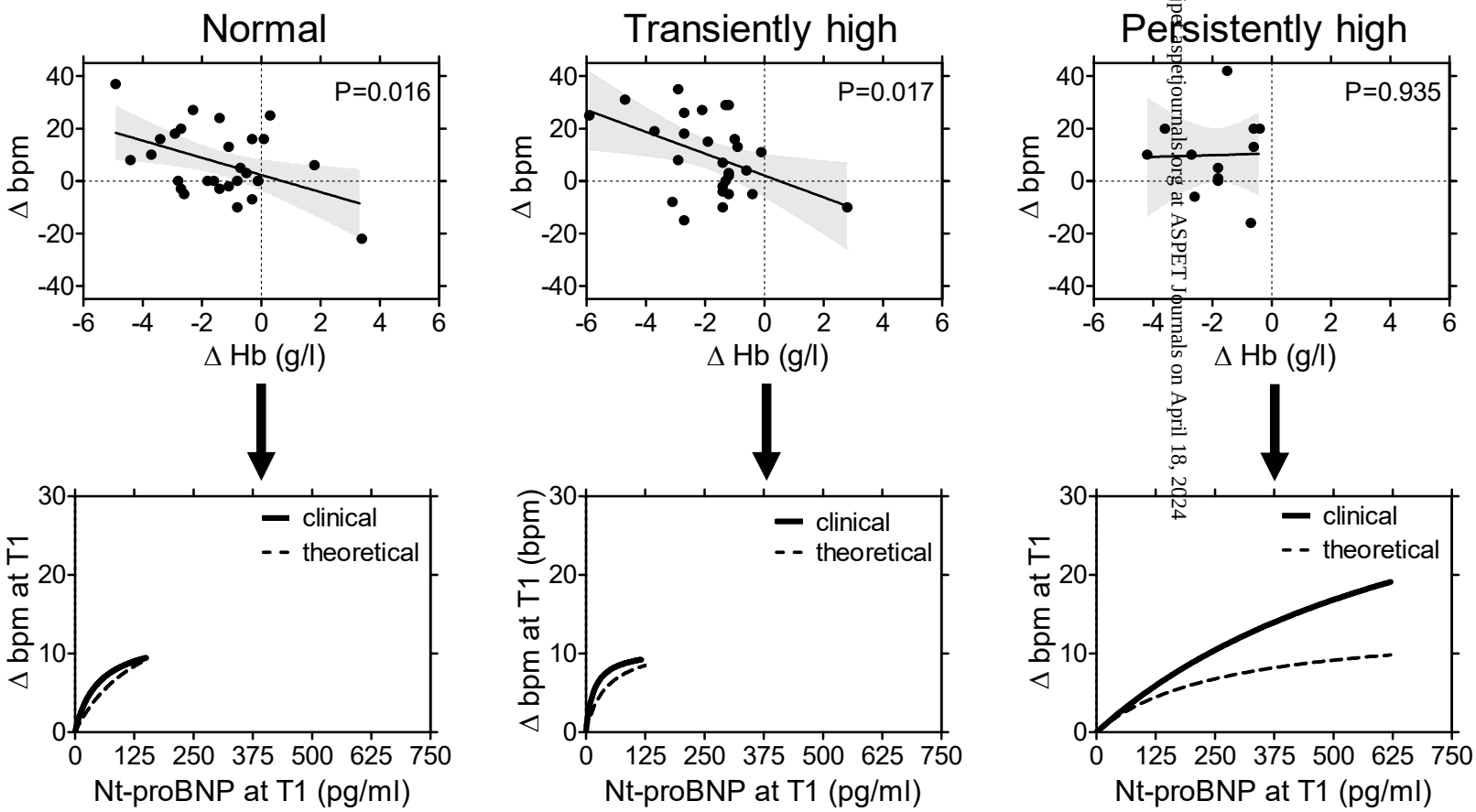

FIGURE 6

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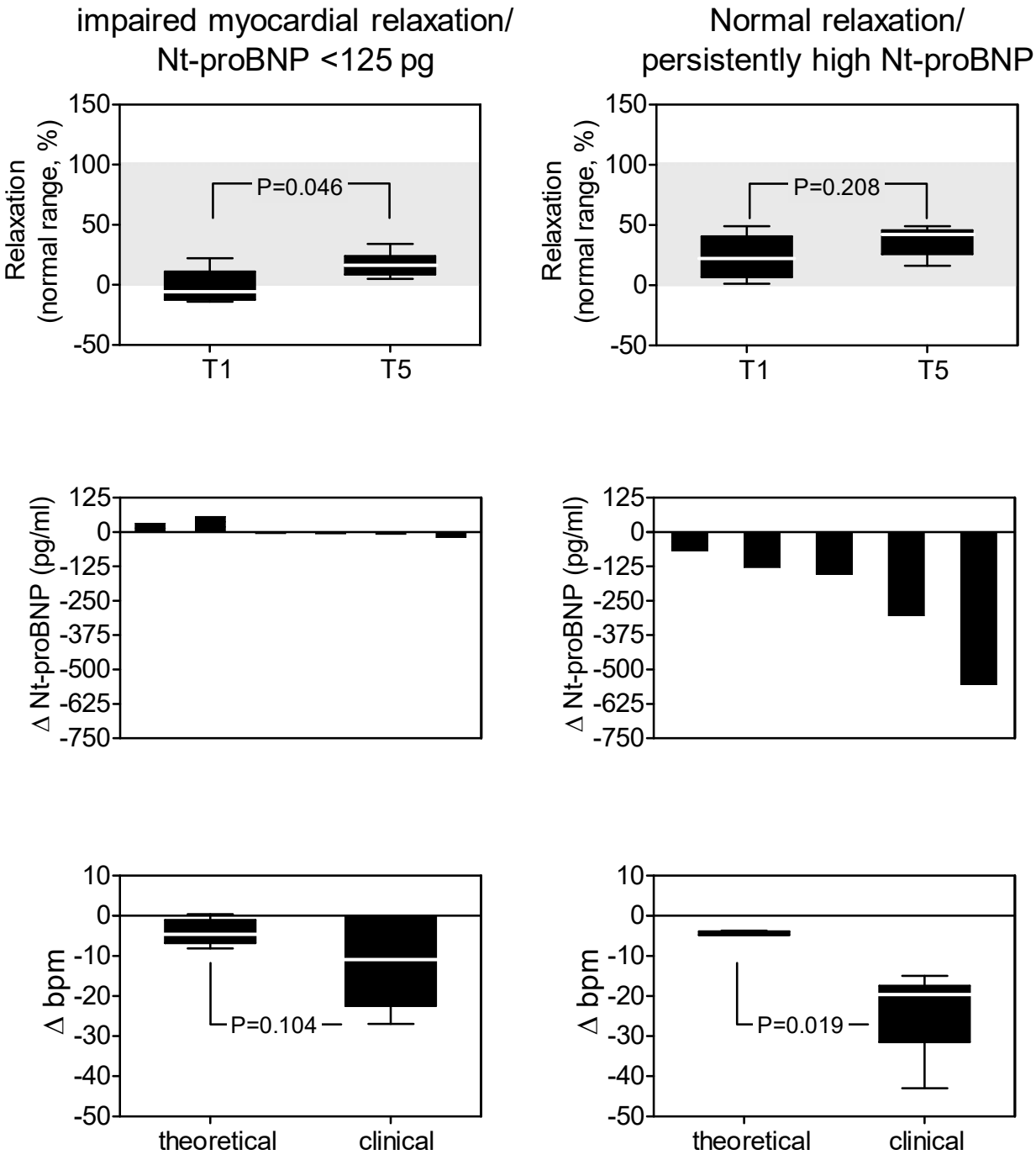

FIGURE 7

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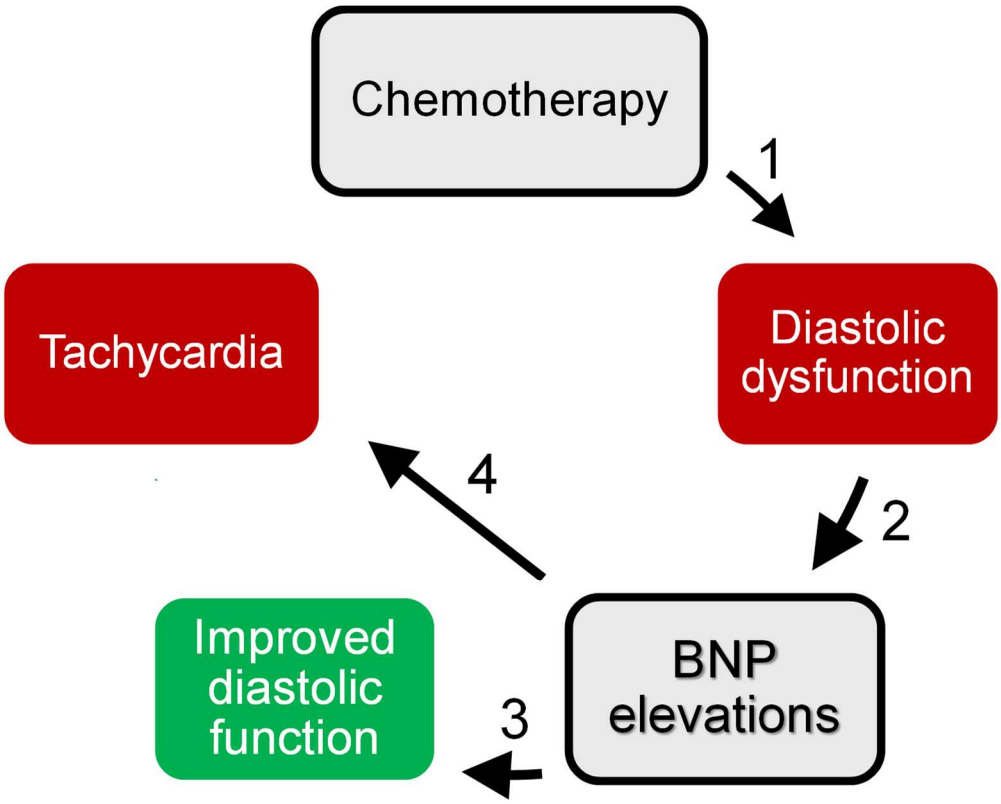

FIGURE 8