Cardiac stress biomarkers after red blood cell transfusion in patients at risk for transfusion-associated circulatory overload: a prospective observational study

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BACKGROUND: Transfusion-associated circulatory overload (TACO) is a leading cause of serious reactions. In regard to TACO, little is known regarding biomarkers as a predictor, their most informative timing, or thresholds of significance or differentiation from other reactions.

STUDY DESIGN AND METHODS: In this study of inpatients at risk for TACO (age ≥ 50 years) receiving 1 red blood cell unit, cardiac biomarkers, brain natriuretic peptide (BNP), N-terminal pro-BNP (NT-proBNP), and high-sensitivity troponin were measured at baseline, 6 to 12 hours (except troponin) posttransfusion, and 18 to 24 hours posttransfusion. Primary outcome was a critical increase in biomarkers (>1.5-fold increase and supranormal) at 18 to 24 hours.

RESULTS: Fifty-one patients were analyzed; 29% had cardiovascular disease, 73% had one or more cardiac risk factors, and 50% took cardiac or antihypertensive therapies. Although eight (16%) developed an increase in systolic pressure of at least 30 mmHg and four (8%) reported dyspnea and/or cough, none had TACO. At baseline, BNP level was more than 100 ng/L in 59% and NT-proBNP was more than 300 pg/mL in 83%. A total of 25% had a BNP critical increase, 33% had a NT-proBNP critical increase, and 2% had a troponin critical increase at 18 to 24 hours. Overall, 38% had at least one biomarker critical increase and NT-proBNP/BNP concordance was 84%. An increase in the NT-proBNP (>1.5-fold increase and >300 pg/mL) at 18 to 24 hours was the commonest biomarker change.

CONCLUSIONS: An increase of the NT-proBNP at 18 to 24 hours may be the preferred surrogate marker for identifying a patient experiencing physiologic difficulty in handling the volume challenge. Larger studies are needed to clarify the risk of TACO for a given pretransfusion biomarker profile and the correlation between TACO and increase in biomarkers after transfusion.

Transfusion-associated circulatory overload (TACO) is common (1%-8%),[1] underrecognized, and underreported[2–4] and is associated with an increased risk of in-hospital mortality in case-control studies.[2] Despite the poor reporting of this common complication, it is a leading cause of transfusion-related fatalities;[5] 46% of fatalities reported to the United Kingdom's hemovigilance system (2010-2016) were from TACO. If TACO occurs in 3% of transfused patients,[2] with only 86 cases of TACO reported to this hemovigilance system in 2016[5] (for a reporting rate at 1 in 21,140 red blood cell [RBC] units), the gap between true incidence and reporting is vast (at only one in 634 cases reported). Hence, the true magnitude of the impact of TACO on patient outcomes is not captured by hemovigilance systems. The sequelae are substantial with a review of 98 consecutive TACO cases

ABBREVIATIONS: BMI = body mass index; BNP = brain natriuretic peptide; GFR = glomerular filtration rate; IQR = interquartile range; NHSN = National Health Safety Network; NT-proBNP = N-terminal pro-brain natriuretic peptide; sBP = systolic blood pressure; TACO = transfusion-associated circulatory overload.

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finding 18% requiring transfer to the intensive care unit and 8% experiencing major complications.[6]

Although potential mitigation strategies have been advanced for RBC-induced TACO (slower rate of infusion, single-unit transfusions, peritransfusion furosemide, optimization of fluid balance before transfusion),[1] none have been proven in clinical studies. There is also little knowledge to guide the design and execution of TACO prevention trials. For example, the timing, route, and dose of furosemide as a preventative measure has not been evaluated and it is unknown if slowing the rate of transfusion would have any mitigating effect. Similarly, the optimal cardiac biomarker for volume overload and the timing of this measurement has not been clarified.

Brain natriuretic peptide (BNP), the most commonly used biomarker for TACO, is a hormone released by cardiac myocytes in the ventricles triggered by increased ventricular blood volume. Several studies have evaluated BNP and N-terminal pro-BNP (NT-proBNP) in patients receiving transfusion;[7–10] these studies primarily examined levels before and after transfusion in small cohorts with TACO or other reactions. Samples for cardiac biomarkers were drawn at variable intervals from the end of transfusion to as far as 48 hours. In these reports, the diagnosis of TACO was not standardized. In addition, there is controversy over optimal criteria for TACO, including "elevation in BNP" but without specific cutoffs or timing.[11] Whether the published cutoffs for congestive heart failure[12–14] can be utilized as predictors of risk or a diagnostic measure in TACO is unknown.

Biomarkers are sought as objective indicators to differentiate dyspneic transfusion reactions, such as TACO versus transfusion-related acute lung injury (TRALI).[15] Bedside assessment of fluid status is hindered by poorly documented fluid balance or patient weights,[6] lack of sensitivity and specificity of the clinical examination,[16,17] infrequent use of central venous catheters to measure the central venous pressure,[18] and infrequent use of cardiac biomarkers for investigation of dyspneic reactions. The US National Health Safety Network (NHSN) definition for TACO requires three of the following within 6 hours of the end of transfusion:[11] acute respiratory distress (dyspnea, orthopnea, cough), elevated BNP (no guidance on timing or levels), elevated central venous pressure, evidence of left heart failure (no criteria), positive fluid balance (undefined), and chest x-ray evidence of pulmonary edema. The transfusion medicine physician or nurse usually has no more than the following three lines of evidence to make the diagnosis in retrospect: evidence of respiratory distress in clinical notes, a documented physical examination, and a chest x-ray (if ordered). The practical limits of clinical data are therefore themselves an obstacle to a diagnosis of TACO. In addition, whether the chest x-ray and BNP testing must be performed within 6 hours to meet the NHSN definition is unclear. Hence, many potential cases of TACO are relegated to the category of "transfusion-associated dyspnea" by shortfalls in timely and complete testing, imaging, and documentation. In addition, proposed criteria are not harmonized by different organizations, including from NHSN,[11] the International Society of Blood Transfusion,[19] and UK Serious Hazards of Transfusion.[20]

This report characterizes the kinetics of BNP, NT-proBNP, and high-sensitivity troponin levels in patients at higher risk for TACO to inform studies evaluating TACO prevention strategies. The primary outcome was to identify rates of change in candidate biomarkers (defined as >1.5-fold and supranormal)[10] from baseline to 18 to 24 hours posttransfusion in patients at risk for TACO.

MATERIALS AND METHODS

This was a prospective, observational cohort study conducted at three academic hospitals in Toronto (Sunnybrook Health Sciences Centre, Toronto General Hospital, and Princess Margaret Cancer Centre); orders for single-unit RBC transfusions for inpatients, aged 50 years or older, Monday to Friday, from 8 AM to 5 PM were identified by technologists (when research personnel were available due to cost constraints). The technologists notified research personnel immediately, who then approached the patient (or substitute decision maker) for consent, usually in parallel with the processing of the pretransfusion blood bank sample to minimize transfusion delays. The protocol was approved by all institutional review ethics boards. The goal was to determine the kinetics of BNP, NT-proBNP, and high-sensitivity troponin levels in patients receiving a single RBC transfusion.

Patients had to meet the following eligibility criteria: age at least 50 years,[2] inpatient, and receiving a single RBC unit. Exclusion criteria applied were anticipated major surgical procedure within 24 hours, active bleeding (active visible bleeding, >2 units of RBCs in the preceding 24 hr for bleeding, >20 g/L decrease in hemoglobin [Hb] in the preceding 24 hr), hemodynamic instability with systolic blood pressure (sBP) of less than 90 mmHg or requiring inotropic support, diagnosis of acute myocardial infarction during admission as per the clinical notes, glomerular filtration rate (GFR) of less than 30 mL/min, order for additional furosemide in the 6 hours before transfusion in addition to standing orders, concomitant order for other blood products, plan for discharge at less than 36 hours, patient or substitute decision maker unable to provide consent, and patient previously enrolled in the study. The criteria excluded surgical patients (bleeding) and hypovolemic and hypotensive patients as we were simultaneously evaluating the inclusion criteria for a subsequent randomized trial involving furosemide (Transfusion-Associated Circulatory Overload Best Eliminated with Lasix [TACO-BEL]; Clinical Trial NCT02802696).

The following baseline data were collected: demographics, reason for admission, comorbid conditions,

cardiac history, left ventricular ejection fraction (and diastolic dysfunction where reported on the echocardiogram report), cardiac risk factors, cardiac medications, chest x-ray results within 48 hours of transfusion, ordered and actual infusion rate, product modifications, posttransfusion signs and symptoms (dyspnea, cough, orthopnea, chest pain), and any transfusion reactions. All patients were under active surveillance for transfusion reactions by research personnel and if there were any changes in vital signs or patient symptoms, the case underwent medical review. TACO was defined by the NHSN Criteria as detailed above.[11] Fluid balance 24 hours before and 24 hours after transfusion were collected where recorded. Additional data included: chest x-ray results in the 24 hours postenrollment, and hypertension within 24 hours (≥30 mmHg change from baseline[10]). Patient vital signs were recorded at four time points: baseline, end of transfusion, 6 to 12 hours posttransfusion, and at 18 to 24 hours posttransfusion.

Cardiac biomarkers (BNP and NT-proBNP) were collected at three time points: immediately before, 6 to 12 hours after, and 18 to 24 hours after transfusion. The high-sensitivity troponin (I or T)[21] was measured immediately before and 18 to 24 hours after, with site-based real-time testing in accordance with local methods/reagents and each patient was tested at both time points with the same troponin assay. The 6- to 12-hour troponin was not measured due to cost constraints. Sample handling details and institution-specific methods are outlined in the supplementary material (Table S1, available as supporting information in the online version of this paper).

A total of 40 patients with complete biomarker profile was considered a convenient sample that could be enrolled during the 6-month study period. There were no data available to inform expected mean BNP levels or anticipated delta BNP before and after transfusion, so a formal sample size calculation was not possible.

The primary outcome was to identify rates of change in candidate biomarkers (defined as >1.5-fold[10] and exceeding a reference threshold) from baseline to 18 to 24 hours posttransfusion in patients at risk for TACO. The 1.5-fold increase was utilized due to its predictive value in a previous report.[10] The secondary objective of the trial was to inform the design of a multicenter randomized control trial of furosemide versus placebo for the prevention of TACO, in terms of test selection and timing. Other measures included baseline demographics to confirm selection of patients at higher risk for TACO, vital signs at three posttransfusion time points, proportion of patients with posttransfusion hypertension (≥30 mmHg increase in sBP), proportion of patients with a 1.5-fold increase[10] in any of the three biomarkers (and higher than the normal range for troponin, >100 ng/L for BNP, and >300 pg/mL for NT-proBNP) at 24 hours, a level higher than 100 ng/L[12] and 400 ng/L[14] for BNP at any time point, a level above 300 pg/mL[13] and 900 pg/mL[14] for NT-proBNP at any time point, and a 20% increase in troponin from baseline.[12] The aforementioned low and high thresholds utilized in the analysis were based on large studies in patients presenting to hospital with dyspnea, with the low threshold useful in ruling out heart failure and the high threshold having a high diagnostic accuracy for ruling in heart failure.

Means with standard deviation (SD) (or median and interquartile range [IQR] as appropriate) and proportions for demographics and clinical data were tabulated for the cohort. The prevalence of elevated biomarkers in patients with and without elevations in sBP were tabulated and comparisons made by two-tailed Fisher's exact test. Biomarker levels were explored in relation to subgroups of clinical relevance (by GFR, body mass index [BMI], cardiac risk factors, cardiac disease, other blood products received in preceding 24 hours, cardiac medications, and age; unpaired t-test). Multivariate analysis was performed to determine factors predictive of baseline BNP and NT-proBNP level and a 1.5-fold increase (and over the thresholds detailed above) in the three biomarkers at 24 hours. Variables included in the model to predict baseline biomarker level were age, BMI, GFR, presence of underlying malignancy, presence of cardiac disease, and any cardiac risk factors. Variables included in the model to predict increase were age, BMI, GFR, presence of cardiac disease, any cardiac risk factors, treatment with cardiac medications, baseline BNP, and NT-proBNP. All analyses were performed with computer software (SAS Version 9.4, SAS Institute). A p value of less than 0.05 was used to denote significance.

RESULTS

Between June and November 2017, a total of 78 patients were approached to participate and 52 consented. The primary reason for refusal of consent was due to the concern regarding multiple blood draws. One patient withdrew consent before any study activities commenced, leaving 51 patients for analysis. All 51 patients met the inclusion and exclusion criteria. One patient refused the final blood draw measurements but other results were included in the analysis with their consent. Some blood draws were not achieved within the required time window or were not processed by the lab appropriately; missing data are noted in the result tables and recruitment continued until 40 patients with complete biomarkers was achieved. Biomarker data were analyzed on all patients where data were available at each time point. The demographics and baseline characteristics of the patients are presented in Table 1. Baseline and 24-hour fluid calculations were not completed by the nursing staff in 65% of patients at both time points. Half of the patients were admitted for the management of malignancy and had severe comorbid conditions; 29% had preexisting cardiovascular disease, 73% had cardiac risk factors, 50% were receiving cardiac and/or antihypertensive therapies, and 18% had a reduction in GFR (<60 mL/min).

The mean Hb level at enrollment was 70.3 ± 5.4 g/L with a reduction from 76.2 ± 5.9 g/L 24 hours previously.

TABLE 1. Baseline characteristics of the 51 participating patients*

Characteristic	Central tendency and variation or proportion
Age (years)	65.4 (57.5-73.2)
Sex (female)	24 (47)
Weight (kg)	70.0 (55.6-84.4)
BMI	23.7 (21.0-28.2)
BMI ≥ 25	20 (39)
Days from admission to enrollment	6 (3-13.5)
Reason for admission†	
Noncardiac surgery	6 (12)
Cardiovascular surgery	3 (5)
Trauma or thermal injury	2 (4)
Infection or sepsis	4 (7)
Respiratory failure	3 (5)
Malignancy for chemotherapy	16 (31)
Febrile neutropenia	3 (5)
Cancer-related complications	11 (22)
Anemia management	4 (7)
Other	1 (2)
Comorbidities	29 (57)
Severe lung disease (symptoms at rest)	3 (6)
Renal dysfunction (GRF < 60 mL/min)	9 (18)
Metastatic cancer	13 (25)
Immunosuppressive therapy	10 (20)
Diabetes with end-organ failure	1 (2)
ECOG performance status	
0-1	30 (59)
2-4	21 (41)
GFR (mL/min)	97.5 ± 35.6
Cardiac disease	15 (29)
Coronary artery disease or angina	4 (8)
Previous myocardial infarction	2 (4)
Prior coronary bypass grafting	1 (2)
Aortocoronary stent	2 (4)
Valvular heart disease	3 (6)
Arrhythmia	12 (24)
Diastolic heart failure	2 (4)
Left ventricular ejection fraction (n = 28)	61 ± 9%
Left ventricular ejection fraction < 40%	1 (2)
Chest x-ray within 48 hours (n = 13)	13 (25)
Consistent with CHF (1 of 13)	1 (8)
Cardiac risk factors	37 (73)
Current or past smoker	20 (40)
Diabetes	13 (26)
Hypertension	18 (36)
Dyslipidemia	11 (22)
Family history of ischemic heart disease	2 (4)
Obesity (BMI > 30)	9 (18)
Cardiac and anti-hypertensive therapies	25 (50)
Diuretics	10 (20)
Beta-blocker	16 (32)
ACE inhibitor	8 (16)
Calcium channel blocker	9 (18)
Antiarrhythmia therapy	7 (14)
Other cardiac medication	2 (4)

* Data are reported as median (IQR), number (%), or mean ± SD.
† Fifty-five reasons for admission in 51 patients.
ECOG = Eastern Cooperative Oncology Group; CHF = congestive heart failure; ACE = angiotensin-converting enzyme.

The RBC transfusions were prescribed over 2.7 ± 0.9 hours (rate ordered in 90%) and were infused over 2.8 ± 1.1 hours. No products underwent volume reduction or washing and 39% were irradiated. No partial units were issued for the study patients (i.e., use of a sterile docking device to split a unit in half for smaller-volume transfusion).

There were no clinically significant differences in vital signs from before transfusion to 18 to 24 hours posttransfusion (Table 2). An increase of more than 30 mmHg in sBP at any time point in the 24-hour period was seen in eight (16%) patients; three (6%) patients at end of transfusion, three (6%) patients at 6 to 12 hours, and three (6%) patients at 18 to 24 hours (one patient had a >30 mmHg increase at two time points). No patient developed TACO by the NHSN definition or any other acute transfusion reaction, although four (8%) patients developed dyspnea or cough in the 24 hours posttransfusion.

Absolute values for biomarkers are shown in Table 3 and percent increase in Table 4 and Fig. 1. At baseline, the BNP level was more than 100 ng/L in 59% and more than 400 ng/L in 14%. At 6 to 12 hours, the BNP level was more than 100 ng/L in 55% and more than 400 ng/L in 8%. At 18 to 24 hours, the BNP level was more than 100 ng/L in 63% and more than 400 ng/L in 17%. There was poor correlation between the 6- to 12-hour BNP and the 18- to 24-hour BNP (R^2 = 0.04; Fig. 2). Mean time from collection to assay run time was 45.3 (±39.5) hours.

At baseline, the NT-proBNP was more than 300 pg/mL in 83% and more than 900 pg/mL in 48%. At 6 to 12 hours, the NT-proBNP level was more than 300 pg/L in 83% and more than 900 pg/L in 46%. At 18 to 24 hours, the NT-proBNP level was more than 300 pg/L in 89% and more than 900 pg/L in 46%. There was good correlation between the 6- to 12-hour NT-proBNP and the 18- to 24-hour NT-proBNP (R^2 = 0.84; Fig. 2). There was poor correlation between the 24-hour NT-proBNP with either of the 6- to 12-hour BNP (R^2 = 0.02) and the 18- to 24-hour BNP (R^2 = 0.10). In multivariate analysis, only age was predictive of the baseline level of both BNP (p = 0.02) and NT-proBNP (p = 0.009). The troponin level was above the reference range in 34% at baseline and 42% at 18 to 24 hours. There was an increase of 1.5-fold and above the normal range in one patient (2%) at 18 to 24 hours, while a more than 20% increase was seen in 25%.

Overall, there was considerable overlap between patients with elevated biomarkers (Fig. 3). For those with complete data on BNP and NT-proBNP at 18 to 24 hours, concordance was 84% (37 of 44 patients either had no increase in either or an increase in both; seven patients had an isolated increase in either BNP or NT-proBNP alone). Of the eight patients with a sBP increase of at least 30 mmHg at any time point in the 24 hours after transfusion, six had complete biomarkers; five of six (83%) had an elevation in one or more biomarkers (Fig. 3). Only elevation of NT-proBNP at 18 to 24 hours was associated with an elevation in sBP after transfusion (five of 15 patients with elevated NT-pro BNP vs. two of 32 patients without elevation in NT-proBNP had an increase in blood pressure; Fisher's exact

TABLE 2. Vital signs before transfusion, at end of transfusion, 6 to 12 hours after transfusion, and 18 to 24 hours after transfusion

Vital sign	Before transfusion	After transfusion	6-12 hr	18-24 hr
sBP (mmHg)	117.5 ± 19.5 (n = 51)	124.3 ± 22.5 (n = 51)	122.0 ± 18.5 (n = 50)	124.5 + 20.9 (n = 49)
dBP (mmHg)	67.0 ± 10.3 (n = 51)	69.1 ± 11.4 (n = 51)	67.8 ± 9.3 (n = 50)	68.4 ± 12.6 (n = 49)
Heart rate (bpm)	85.3 ± 14.0 (n = 50)	83.7 ± 13.5 (n = 50)	83.8 ± 14.3 (n = 50)	82.6 ± 14.7 (n = 48)
RR (per min)	18.3 ± 1.9 (n = 50)	18.2 ± 2.0 (n = 48)	18.0 ± 2.1 (n = 49)	18.4 ± 2.1 (n = 47)
Temperature (°C)	36.8 ± 0.6 (n = 51)	36.8 ± 0.5 (n = 51)	36.9 + 0.7 (n = 49)	36.7 ± 0.5 (n = 28)
O_2 saturation	97.1 ± 2.4 (n = 51)	97.4 ± 2.3 (n = 51)	97.0 ± 1.8 (n = 50)	97.4 ± 2.3 (n = 49)
% of patients on O_2	10/51 (19.6%) (n = 51)	9/51 (17.6%) (n = 51)	9/51 (17.6%) (n = 51)	7/51 (13.7%) (n = 51)

dBP = diastolic blood pressure; RR = respiratory rate.

TABLE 3. Cardiac biomarker results and fluid balance at three time points (before transfusion, 6 to 12 hr after transfusion, and 18 to 24 hr after transfusion)*

		After transfusion	
Test	Before transfusion	6-12 hr	18-24 hr
Hb (g/L)	70.5 ± 5.5 (n = 51)	80.3 ± 7.5 (n = 47)	83.4 ± 9.1 (n = 18)
BNP (ng/L)	116 (60 to 220) (n = 51)	116 (65 to 231) (n = 49)	155 (63 to 1985) (n = 48)
NT-proBNP (pg/mL)	776 (402 to 1629) (n = 48)	837 (427 to 1721) (n = 48)	841 (469 to 1985) (n = 46)
Troponin T (ng/L)	17 (8 to 33) (n = 27)	NA	17 (9 to 44) (n = 25)
Troponin I (ng/L)	8 (3 to 21) (n = 20)	NA	6 (3 to 23) (n = 20)
Fluid balance (mL)	–210 (–377 to 1109) (n = 18)	NA	51 (–318 to 2003) (n = 18)

* Data are reported as mean ± SD or median (IQR).

test, p = 0.02). Of the four patients who developed either dyspnea or cough, two had a critical increase in BNP markers.

In subgroup analysis (Table S2, available as supporting information in the online version of this paper), patients with a history of cardiac disease had higher BNP and NT-proBNP at all three time points. Patients with cardiac disease history had higher troponin T levels at both time points. Patients on cardiovascular medications had higher troponin I levels at both time points and higher NT-proBNP at 18 to 24 hours. Patients older than 70 years, compared to patients aged 50 to 69, had higher biomarkers at all time points. In multivariate analysis, none of the baseline demographics or baseline biomarker levels were statistically

TABLE 4. Cardiac biomarker percent increase and proportion with 1.5-fold elevation (and above critical threshold) or greater at 6 to 12 hours and 18 to 24 hours

	Median percentage increase	
Test	At 6-12 hr	At 18-24 hr
BNP (ng/L)	n = 49 3.5% (IQR = –23% to 28%) >1.5-fold and >100 ng/L: 2%	n = 48 19.2% (IQR = –22.2% to 69.0%) >1.5-fold and >100 ng/L: 25%
NT-proBNP (pg/mL)	n = 47 5.0% (IQR = –13.4% to 30.9%) >1.5-fold and >300 pg/mL: 8%	n = 45 22.1% (IQR = –8.8% to 55.7%) >1.5-fold and > 300 pg/mL: 33%
Troponin I or T (ng/L)	NA	n = 47 0% (IQR = –12% to 14%) >1.5-fold and above normal range: 2%

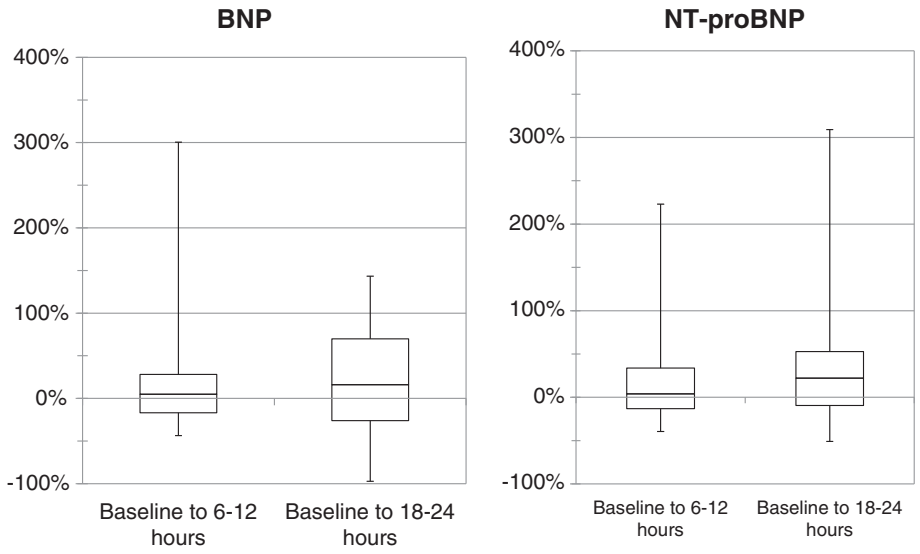

Fig. 1. Percent change from baseline for BNP and NT-proBNP at both time points (6-12 and 18-24 hr).

associated with a critical increase in BNP, NT-proBNP, or any of the three markers combined at 18 to 24 hours.

DISCUSSION

This study characterized the cardiac biomarker kinetics in 51 patients at higher risk for TACO. Restricting enrollment to recipients over the age of 50 years identified a population with a high incidence of cardiac disease, cardiac risk factors, and odds of receiving concomitant cardiac medications. Baseline elevation of cardiac biomarkers was common and an increase of more than 1.5-fold from at 18 to 24 hours was common (38%). There was an 84% concordance for the increase of these two cardiac biomarkers (NT-proBNP increasing without the BNP explaining the majority of the nonconcordance) and increasing almost always in parallel with other abnormalities (troponin, BNP, or sBP ≥ 30 mmHg). Increased sBP is commonly observed in patients with TACO.[6] In this study, an increase in sBP was associated with an increase in cardiac biomarkers at 18 to 24 hours, raising the possibility that biomarker increase may be a surrogate marker for TACO risk. Significant increases in BNP, NT-proBNP, or both were found in 26, 33, and 38% of patients, respectively, raising the question of best marker or best value to use in a clinical trial, where dual testing brings additional logistic complexity and expense. There was no added value in our cohort of patients of the 6- to 12-hour NT-proBNP measurement due to excellent correlation with

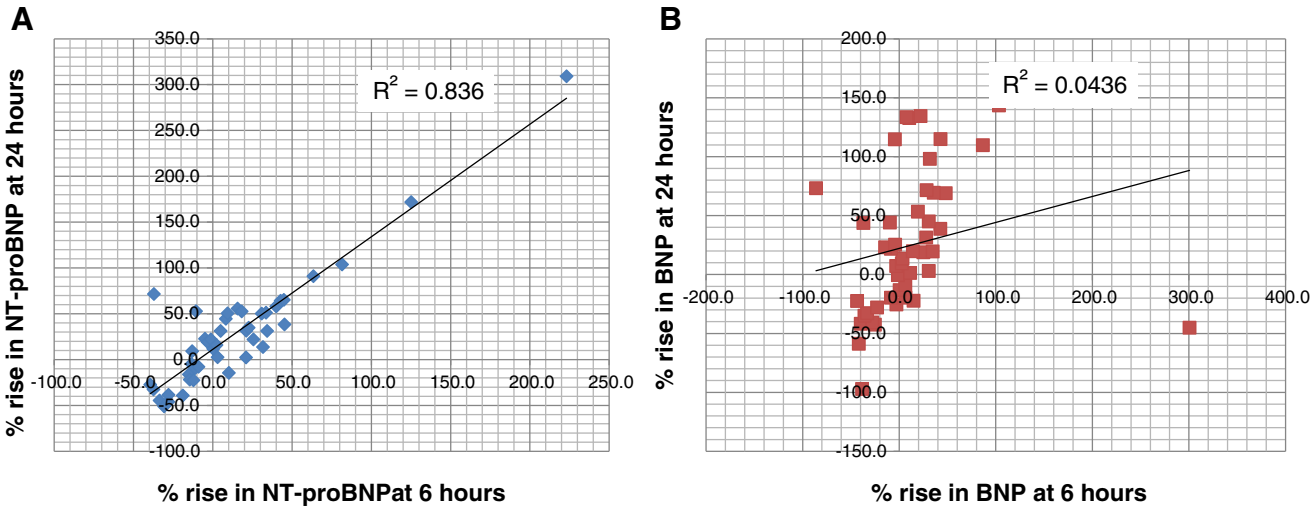

Fig. 2. Relationship between 6- to 12- and 18- to 24-hour levels of NT-proBNP (A) and BNP (B). [Color figure can be viewed at wileyonlinelibrary.com]

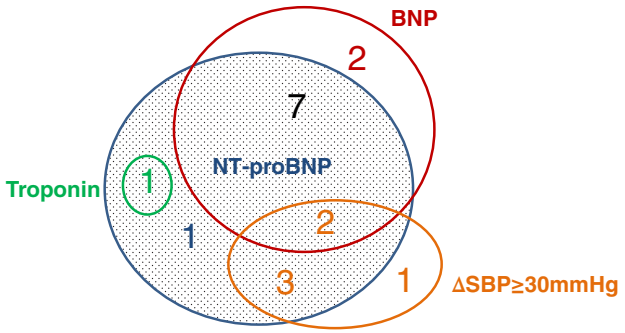

Fig. 3. Intersection of patients' biomarker increases of significance, as defined by exceeding threshold and increasing by more than 1.5-fold, at the 18- to 24-hour assessment point compared to baseline for patients with complete biomarkers (n = 42). The overlap of patients with elevated sBP (≥Δ30 mmHg at any point after transfusion) is also shown. An elevation in any one marker occurred in 16 (38%), with overlap (two markers rising significantly in parallel) in 10 (63% of those with an increase). One additional patient had an elevation in blood pressure without an increase in a cardiac biomarker. [Color figure can be viewed at wileyonlinelibrary.com]

the 18- to 24-hour NT-proBNP, although the time course of biomarker alterations in patients with TACO may be different. Furthermore, the largest increase in the proportion of patients with a 1.5-fold increase was at 18 to 24 hours, suggesting that the greatest yield is at this later time point.

BNP and NT-proBNP have physiologic and test characteristics that may inform the timing of the draw and selective application in an individual patient. BNP has a very short half-life of approximately 20 minutes.[22] BNP measurement is hindered by its decay with storage (30% over 24 hr).[23] If comparing a pretransfusion sample (the blood bank sample 72 hr before), the baseline result will be substantially lower than the true value, thereby exaggerating the true shift when compared with the real-time testing of the postreaction sample. NT-proBNP has a longer half-life of 120 minutes[24] and is less affected by decay during sample storage.[23] NT-proBNP elevations are more pronounced in renal failure compared to BNP; with GFR-adjusted cut-points, the NT-proBNP result is predictive of outcomes (heart failure and mortality).[25] Troponin measures, especially high-sensitivity assays, have also been advocated as a biomarker for heart failure,[26] but there are no data in TACO. The evaluation of cardiac biomarkers is a rapidly evolving field with numerous tests under intense study,[26] potentially bringing new tools to assist with TACO risk prediction and classification of dyspneic reactions.

The poor correlation between levels of BNP at 6 to 12 and 18 to 24 hours suggests a need to better discern the most informative timing of measurement. In addition, given this biomarker's very short half-life of only 20 minutes, the relationship of values at distant time periods is expected to be more variable, especially in a hospitalized patient undergoing other interventions. Given that NT-proBNP is a fragment cleaved off a prohormone to release BNP, the only logical conclusion is that an increase of NT-proBNP without a similarly detectable increase in BNP suggests the increase was missed with the two spaced measurements. NT-proBNP is less vulnerable to degradation during storage than BNP, making it more favorable for use in clinical studies relying on batch testing for cost containment. Moreover, the NT-proBNP assay is currently available from a single manufacturer versus the numerous vendor offerings (and thus site-to-site variation) in BNP assays. Taken together, NT-proBNP is best positioned for a multicenter study and is amenable to batching and centralized testing to reduce shipping costs and optimize scaling discounts. A troponin elevation was uncommon and it warrants assessment in larger cohorts with patients at risk for development of TACO. In subgroup analysis, patients with cardiovascular disease and age more than 70 years not unexpectedly had higher biomarker levels. In multivariate analysis, none of the baseline variables were predictive of a critical increase in biomarkers, possibly due to the small number of included patients.

Previous retrospective studies have identified biomarker thresholds associated with TACO compared to patients with other transfusion reactions. Roubinian and colleagues[9] evaluated BNP levels at a median of 16 hours posttransfusion in 93 patients with reactions associated with dyspnea. In multivariate analysis, they found a BNP level of more than 1000 pg/mL (R&D Systems, Luminex Corp.), without a requirement for an increase from baseline, was associated with a 40-fold odds ratio for a TACO (vs. TRALI) diagnosis. In our series of patients at risk for TACO, 4% of patients met these criteria, although none experienced TACO. Zhou and coworkers[10] evaluated BNP levels (Triage, Biosite, Inc.) in 21 TACO patients and 19 control patients without TACO. They found a level of more than 100 pg/mL posttransfusion and a more than 1.5-fold increase to be associated with a 26-fold risk of TACO compared to control patients. We observed such increases in 2% at 6 to 12 hours and in 25% at 18 to 24 hours, although none developed TACO. Li and coworkers[8] studied BNP levels (Triage, Biosite, Inc.) in 115 patients with dyspneic transfusion reactions (no control patients) and found that the BNP level did not assist with the differentiation between TACO and TRALI. Finally, Tobian and colleagues[7] studied NT-proBNP (Roche Elecsys, Roche Diagnostics) in 40 patients with acute transfusion reactions. They found a posttransfusion level of more than 1000 pg/mL to be independently associated with TACO. In our case series of patients at high risk for TACO, 43% had a posttransfusion value of more than 1000 pg/mL at 18 to 24 hours. Clearly due to the diversity in the findings above, additional studies in patients with TACO, transfused controls, and nontransfused controls are needed to clarify the role of biomarkers in predicting risk of TACO and differentiating TACO from TRALI. Cardiac biomarkers have been

widely studied and validated in stable outpatients with cardiovascular disease,[27] rather than in the setting of an acute change in volume status. In patients presenting with flash pulmonary edema, BNP and NT-proBNP levels often only increase the day after presentation,[22] raising the possibility that an early blood draw could miss the biomarker peak. The greatest signal in cardiac biomarkers being observed at 24 hours is in line with the Serious Hazards of Blood Transfusion (SHOT) program and the International Society of Blood Transfusion recommendation to extend the interval for TACO diagnosis from 6 hours to a longer time interval of 12–24 hours,[19,20] based on their observations of TACO presenting after 6 hours.

Considerable effort is under way to develop cardiac biomarkers that are sensitive and specific for diagnosing heart failure in patients presenting with dyspnea and predicting outcomes in patients with confirmed heart failure. In addition, testing must be available on a platform with rapid and high-throughput capacity for use in emergency department patients. This required capability raises the possibility of a "precision" transfusion prescription based on the pretransfusion sample, with patients at high risk based on biomarkers managed with validated TACO prevention strategies, rather than a broad application of strategies across all patients. Novel biomarkers under intense study include ST2, galectin-3, cardiac troponins, growth factor differentiation factor-15, and procalcitonin.[26] Measurement of troponin levels in TACO prevention studies should consider the challenge that positive results create for the research and clinical teams. The test must be turned around rapidly and the physician notified of a positive research test result immediately. In addition, there is a difference between troponin T and troponin I in the prediction of mortality in patients presenting to the emergency department, with troponin T more predictive of death, raising the possibility that one assay may be superior as a peritransfusion measurement.[28] Cardiac biomarkers with a high specificity are greatly needed to help differentiate TACO and TRALI, with the latter assignment having negative impacts on donor eligibility for future donations and hence the need to avoid misclassification of TACO as TRALI.

This study improved on some of the limitations of prior studies. The study enrolled patients prospectively utilizing uniform enrollment criteria. Serial measurements were done of both BNP and NT-proBNP at multiple predefined time points, rather than a range from time of the reaction to 48 hours.[8] Three biomarkers were measured in parallel to transcend single-marker reviews. Demographic details were collected to determine clinical and transfusion factors associated with biomarker levels. The study included different hospital sites to strengthen generalizability, as testing platforms, fluid management, and transfusion practices vary. This is the first report to measure cardiac biomarkers after single-unit RBC transfusions, thereby scrutinizing the physiologic challenge of the most evidence-based transfusion prescription. Previous reports on this topic describe a majority of patients receiving two or more components and, in general, a mix of products.[7,9,10]

There are several limitations to this report. First, the sample size was small and precluded any conclusive multivariate analysis. Second, although we had broad inclusion criteria, half of the patients were admitted for management of malignancy, as this reflected the involved sites' affiliations with tertiary oncology programs and the restriction of the recruitment period to just daytime hours. Patients with malignancy have increased levels of BNP possibly due to inflammation (66 pg/mL vs. 44 pg/mL, $p < 0.01$)[29] and therefore larger studies need to a priori plan for subgroup analyses to ensure results are similar for patients with and without malignancy. Future studies need to ensure that they enrich the study populations with patients at risk for TACO, particularly chronic renal failure, systolic and diastolic dysfunction, and perioperative patients. In addition, we were unable to track patients not approached for the study due to the high volume of transfusion at the three hospital sites to determine if the patient population captured was representative of nonbleeding inpatient recipients. Third, we excluded patients with acute myocardial infarction as we were evaluating troponin levels. Fourth, although we commonly found baseline elevation in cardiac biomarkers and/or a 1.5-fold or greater elevation posttransfusion, it is unknown if these markers of cardiac physiologic stress will translate into a higher risk of TACO. A large multicenter trial with baseline and posttransfusion biomarker measurements will be needed to answer this question as only 1% to 8%[1] of patients manifest TACO. No TACOs were observed to determine if all patients with TACO had elevated levels of biomarkers, although with only 51 patients, only one to two patients would be expected to experience TACO. In addition, this study and previous studies have not determined if a fold increase in the level or the absolute increase should be used as a surrogate for a patient with fluid overload. Fifth, this cost-constrained study did not have a control population of nontransfused patients to determine if acute elevations of cardiac biomarkers occur due to other physiologic stresses during the 24-hour period of measurement. Finally, we did not observe any TACO cases due to the small sample size and therefore large multicenter trials will be needed to determine if critical increases in biomarkers and blood pressure are associated with this common transfusion complication.

The hypothetical role for cardiac biomarkers in transfusion medicine is sizable: as a laboratory measure before transfusion to identify a patient at risk for TACO, as a surrogate marker in clinical trials on TACO prevention, and to help differentiate between different dyspneic reactions. This preliminary study suggests that there is likely value in the use of cardiac biomarkers in evaluating the fluid status in transfused patients, but given the elevations in many patients without any signs of fluid overload other than

increase in sBP, it is unlikely that a diagnosis of TACO can be simplified to a single laboratory measure, but that a set of TACO criteria is still a necessity.

In conclusion, this study validates the inclusion criteria to select for a higher-risk population for TACO, with a high proportion of patients with cardiac risk factors, cardiac disease, cardiac medications prescribed, and baseline elevations in cardiac biomarkers. The significance of a baseline elevation of cardiac biomarkers is unclear and needs further study. A single draw of NT-proBNP at 18 to 24 hours post-transfusion is likely sufficient to capture the greatest increase therein. The addition of BNP to the NT-proBNP, although increasing the capture of strain events (from 33% to 38%), comes at the cost of assay heterogeneity and more rapid decay. An increase in cardiac biomarkers of 1.5-fold is common at 18 to 24 hours posttransfusion and deserves attention in larger controlled studies to ascertain significance in terms of manifesting TACO.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Table S1. Cardiac biomarker testing laboratory specifications.
Table S2. Cardiac biomarkers for subgroups of patients (* = $p < 0.05$, NA = not applicable as only 1 troponin I had a GFR < 60 mL/min).