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Full Length Research Paper

What is the best approximation of reference normal for NT-proBNP? Clinical levels for enhanced assessment of NT-proBNP (CLEAN)

Larry H. Bernstein[1]*, Michael Y. Zions[1,4], Mohammed E. Alam[1,5], Salman A. Haq[1], John F. Heitner[1], Stuart Zarich[2], Bette Seamonds[3] and Stanley Berger[3]

[1]New York Methodist Hospital, Brooklyn, New York, USA.
[2]Bridgeport Hospital, Bridgeport, Connecticut, USA.
[3]Mercy Catholic Medical Center, Darby, Philadelphia, Pennsylvania, USA.
[4]Touro College, Brooklyn, New York, USA.
[5]Medgar Evers College, Brooklyn, New York, USA.

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The natriuretic peptides, B-type natriuretic peptide (BNP) and NT-proBNP that have emerged as tools for diagnosing congestive heart failure (CHF) are affected by age and renal insufficiency (RI). NT-proBNP is used in rejecting CHF and as a marker of risk for patients with acute coronary syndromes. This observational study was undertaken to evaluate the reference value for interpreting NT-proBNP concentrations. The hypothesis is that increasing concentrations of NT-proBNP are associated with the effects of multiple co-morbidities, not merely CHF, resulting in altered volume status or myocardial filling pressures. NT-proBNP was measured in a population with normal trans-thoracic echocardiograms (TTE) and free of anemia or renal impairment. Study participants were seen in acute care for symptoms of shortness of breath suspicious for CHF requiring evaluation with cardiac NT-proBNP assay. The median NT-proBNP for patients under 50 years is 60.5 pg/ml with an upper limit of 462 pg/ml, and for patients over 50 years the median was 272.8 pg/ml with an upper limit of 998.2 pg/ml. We suggest that NT-proBNP levels can be more accurately interpreted only after removal of the major co-morbidities that affect an increase in this peptide in serum. The PRIDE study guidelines should be applied until presence or absence of comorbidities is diagnosed. With no comorbidities, the reference range for normal over 50 years of age remains steady at ~1000 pg/ml. The effect shown in previous papers likely is due to increasing concurrent comorbidity with age.

Key words: Congestive heart failure, natriuretic peptides, anemia, chronic renal insufficiency.

INTRODUCTION

B-type natriuretic peptide (BNP) and N-terminal pro BNP tests are widely used on immunochemistry and point-of-care platforms for early stratification of patients with shortness of breath from either lung-related function or cardiac output abnormality (Januzzi et al., 2005; Bay et al., 2003; Januzzi et al., 2006). The interpretation of NT-proBNP may be affected by age (Raymond et al., 2003) or the presence of one or more other comorbidities, such as chronic renal insufficiency (RI) (Raymond et al., 2003;

*Corresponding author. E-mail: larry.bernstein@gmail.com. Tel: 203-261-8671.

Luchner et al., 2005; Anwaruddin et al., 2006), type 2 diabetes (Magnusson et al., 2004) and acute coronary syndrome (ACS) (Weber et al., 2006) among others. The determination of a useful decision value for reporting NT-proBNP therefore poses a significant challenge. Raymond et al. (2003) found greater age, increasing dyspnea, high plasma creatinine and a LVEF < 45% to be independently associated with an elevated NT-proBNP plasma level by multiple linear regression analysis. NT-proBNP almost doubled per age decade in the undivided study sample, likely due to age related increases in left ventricular stiffness, as well as associated comorbidities. We decided to establish the relative importance of the effects they found.

It is reasonable to expect the occurrence of confounders in patients presenting to emergency care with dyspnea. Januzzi et al. (2005) demonstrated an age effect and proposed age dependent concentration cutpoints at ages 50, 65, and 75. The aim of this study is to reevaluate the reference range for interpreting an NT-proBNP elevation assuming the interpretation derived from the PRIDE study (Januzzi et al., 2005) cannot be dependent solely on age and does not account sufficiently for concomitant morbidities which can raise NT-pro BNP levels independent of CHF.

PATIENTS AND METHODS

Our study population consisted initially of 725 patients who were seen in acute care for symptoms suspicious for shortness of breath or decompensated CHF requiring evaluation with cardiac NT-proBNP assay and for whom TTE was available. Exclusion conditions were the following co-morbidities: anemia as defined by WHO, atrial fibrillation (AF), elevated troponin T exceeding 0.070 mg/dl, systolic or diastolic blood pressure exceeding 140 and 90 respectively, ejection fraction less than 45%, left ventricular hypertrophy (LVH), left ventricular wall relaxation impairment, and renal insufficiency (RI) defined by creatinine clearance < 60ml/min using the MDRD formula 1.

Another population consisted of 330 voluntary blood donors age 25 to 65 who were prescreened for cardiac, lung, liver, renal disease and systemic diseases prior to phlebotomy. The majority of the first, acute symptomatic group of patients were age over 50 years, but most of the blood donors were skewed to under 50 years age. This introduces an unintended selection bias of the voluntary blood donors with respect to age, but not with respect to the medical comorbidities that are of concern for the study.

Cardiac troponin T and NT-proBNP were measured by enhanced chemiluminesence immunoassay at Methodist Hospital on the Roche Modular analyzer and at MCMC on the Roche COBAS Integra, and on the Roche Elecsys 2010 at Bridgeport Hospital (Roche Diagnostics, Inc., Indianapolis, IN). The serum creatinine was analyzed on the Roche Modular system at Methodist Hospital, on the Roche COBAS Integra at MCMC, and on the Ortho 950 at Bridgeport Hospital. All assays were harmonized across instruments so the results are comparable between institutions. The whole blood leukocyte count and the hemoglobin concentration were measured either on the Beckman Coulter Gen S or STKS at MCMC, and on the Gen S at Methodist, and the LH750 at Bridgeport Hospital (Beckman Coulter, Miami, FL). The hematology analyzers are all on a common harmonized Coulter platform. All TTE were performed on a Philips instrument.

Statistical treatment

The combined acute and blood donor study sets were kept separate and each analyzed for central tendency, distribution and variability. The two were combined after the comorbidities described above were extracted from the acute care study group. This resulted in a population that should be representative of an unaffected study population that could be used to establish a most representative reference range. The database was replicated several times and then patient rows were randomly deleted until there was an expanded combined and mixed data set with 6,700 entries. All of the database sets were used for analyses. One might conjecture that there remained any bias after the creation of this study data set.

The results are reported in means with $p < 0.05$ as the measure of significance for difference between means. Independent Student's t-tests were applied comparing NT-proBNP and anemia. Univariate ANOVAs were used to compare NT-proBNP levels with varying ranges of hemoglobin and age using SPSS 15.0 (SPSS, Chicago, IL). A linear regression analysis with linear fitting and confidence interval was performed using SYSTAT 12 (SYSTAT, San Jose, CA). The results are reported in means with $p < 0.05$ as the measure of significance for difference between means. Linear regression analysis, Independent Student's t and Mann-Whitney tests were applied comparing NT-proBNP for age intervals. Reference range was determined using MedCalc 9.2.0.0 (Mariakerke, Belgium).

RESULTS

NT-proBNP and age

The NT-proBNP statistics by age of the clean data set of donors plus three acute-site patient sets are shown in Table 1. The medians and confidence intervals of the medians in the age ranges actually suggest differences between all 3 age ranges. Linear fit and confidence interval ($r < 0.1$, beta = 2.1) shows the effect of age is weak: NT- proBNP (pg/ml) = 195.48 + 2.15*age. The likelihood of a value under 1000 pg/ml is 50 to 100:1. We do have a problem indicated by the lack of linearity, that one would have to conclude is related to an error traced to assuming the normality of the data. So the data represented by Table 1 is not as clean as the study had assumed. It appears to be over-weighted in the NT-proBNP values of the patients over 50 years of age. The final changes in the NYMH data after removal of outliers with WHO standard for anemia taken into account results in an upper limit of normal for NT-proBNP that appears to approach 1000 pg/ml in the final analysis at age 50 years. The global median, 25th and 75th percentile, and 97.5 percentile limits in pg/ml, irrespective of age are: 188.4, 64, 523 and 1065. The ANOVA result for an age at or above, and under 50 years is significant at $p < 0.002$. The NT-proBNP is less than 500 pg/ml at age under 50 years.

We have to be cautious because the ANOVA test is used in violation of the parametric distributional assumption in data that has a wide range of values that has the greatest standard deviation above the age of 50 years, but would be better fit to parametric by taking the

Table 1. NT-proBNP profile of combined population taken from 3 sites and donors.

Age	Under 50 years	50-69 years	70 and over
NT-proBNP			
N	209	126	82
Mean	35.9	182.4	611.7
95% CI of MEAN	29.8-43.3	132.1-251.9	425.2-880.1
Median	27.6	142.3	564.2
95% CI of median	24.8-33.6	92.3-219.0	419.7-1007.7
2.5-97.5 percentile	5.0-1364	10.8-11604	28.8-14242
25-75 percentile	14.9-55.8	42.1-565	210.2-2062

log of the NT-proBNP. Keeping in mind that the primary aim is to establish a normal reference, the decision value obtained has to also delimit an optimal separation between the workable reference normal, and an adjacent population that has as yet unestimated medical risk. When we compare the mean and median NT-proBNP values between ages 65 and under and over 65 years, the results are not significant ($p = 0.275$) (age 65 and under [408, 378]; age over 65 years [331, 191]). The result for an age of 50 years cut off being significant at $p < 0.002$ is the result of posthoc analysis, which is the equivalent of doing separate t-tests on each pair within the three groups.

If this presentation is confusing so far, it is partly due to viewing the NT-proBNP without using the log transform of the test results to minimize hidden effects, and to clarify the effect of factors needed to deal with the reference range for NT-proBNP.

We observe the following changes with respect to NT-proBNP and age:

(i) Sharp increase in NT-proBNP at over age 50
(ii) Increase in NT-proBNP at 7% per decade over 50
(iii) Decrease in eGFR at 4% per decade over 50
(iv) Slope of NT-proBNP increase with age is related to proportion of patients with eGFR less than 90
(v) NT-proBNP increase can be delayed or accelerated based on disease comorbidities

NT-proBNP elevation with eGFR decline

A receiver operator characteristic curve was plotted keeping the CHF patients but removing the patients with creatinine clearance below 60 ml/min (RI). The area under the curve (AUC) is 0.706 whether the RI patients are excluded or not The specificity is 71% at the large cutoff of 1500 pg/ml. Patients with impaired renal function at eGFR between 61 and 90 ml/min are included in the calculation so that further exclusion by the eGFR would bring the cut off to 1100 mcg/ml. This is illustrated by the ROC curve for eGFR which exceed 80 ml/min. Criterion >1100 (Exclusion: GFR < 80, LEU, Hgb < 10); AUC: 75.6%, Sensitivity was 73.68 (95% CI 56.9 to 86.6), Specificity was 70.39 (95% CI 63.6 to 76.5), and the negative predictive value was 93.5%. Figure 1 shows the specificity of NT-proBNP is slightly sensitive to eGFR with decreasing prevalence of renal function loss from eGFR of 40 to 80 ml/min and drops significantly at eGFR above 80 ml/min. Figure 2 plots the mean and 95% CI of NT-proBNP (CHF removed) by the National Kidney Foundation staging for eGFR interval (eGFR scale: 0, > 120; 1, 90 to119;2, 60 to 89; 3, 40 to 59; 4, 15 to 39; 5, under 15 ml/min). We created a new variable to minimize the effects of age and eGFR variability by correcting these large effects in the whole sample population.

Adjustment of the NT-proBNP for eGFR and for age over 50 differences. We have carried out a normalization to adjust for both eGFR and for age over 50:

(i) Take Log of NT-proBNP and multiply by 1000
(ii) Divide the result by eGFR (using MDRD[9] or Cockroft Gault[10])
(iii) Compare results for age under 50, 50-70, and over 70 years
(iv) Adjust to age under 50 years by multiplying by 0.66 and 0.56.

The equation does not require weight because the results are reported normalized to 1.73 m^2 body surface area, which is an accepted average adult surface area.

NT-proBNP comparison of donors and non-donors

When we compare the NT-proBNP for the combined 198 donors and 219 acute care patients without associated co-morbidities there is a significant difference ($p < 0.0001$) in the donor and non-donor means (44.3 and 1645.1 pg/ml). If we look at age under 50 years, the means comparison is also significant ($p = 0.010$, unequal variance assumed) (63 non-donors, 613.8 pg/ml; 146 donors, 28.7 pg/ml). The donor comparison with non-donor indicates that the reference range can be lowered for screening purposes.

The means comparison of the normalized NT-proBNP

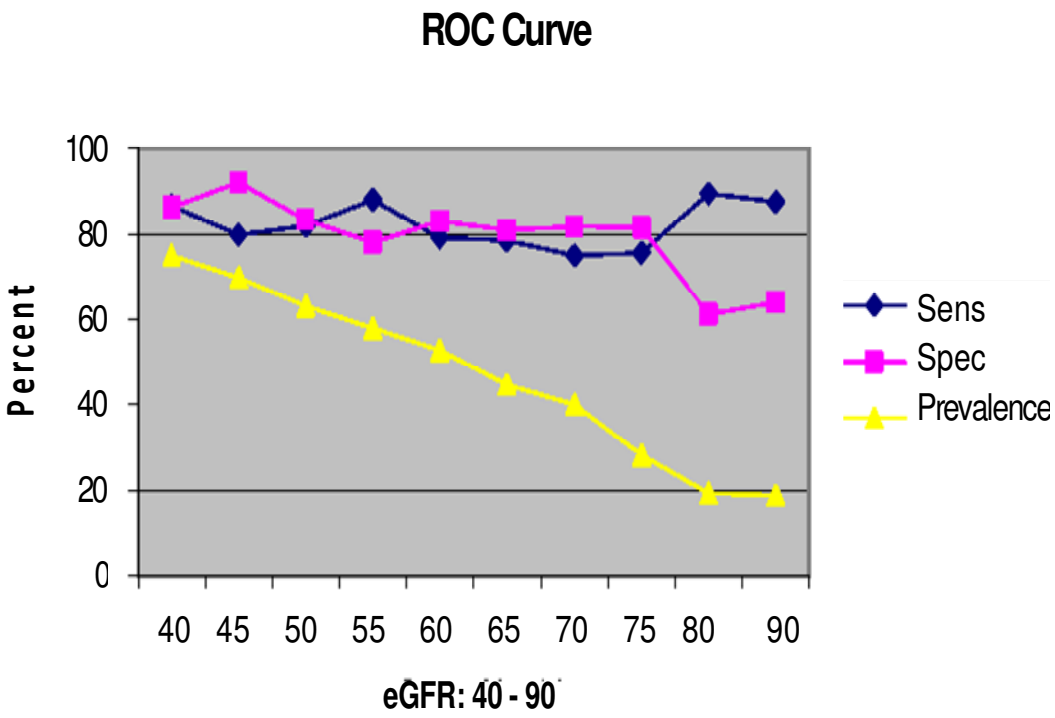

Figure 1. Plot of NT-proBNP sensitivity and specificity with RI prevalence. GFRe scale: 0, > 120; 1, 90-119; 2, 60-89; 3, 40-59; 4, 15-39; 5, under 15 ml/min.

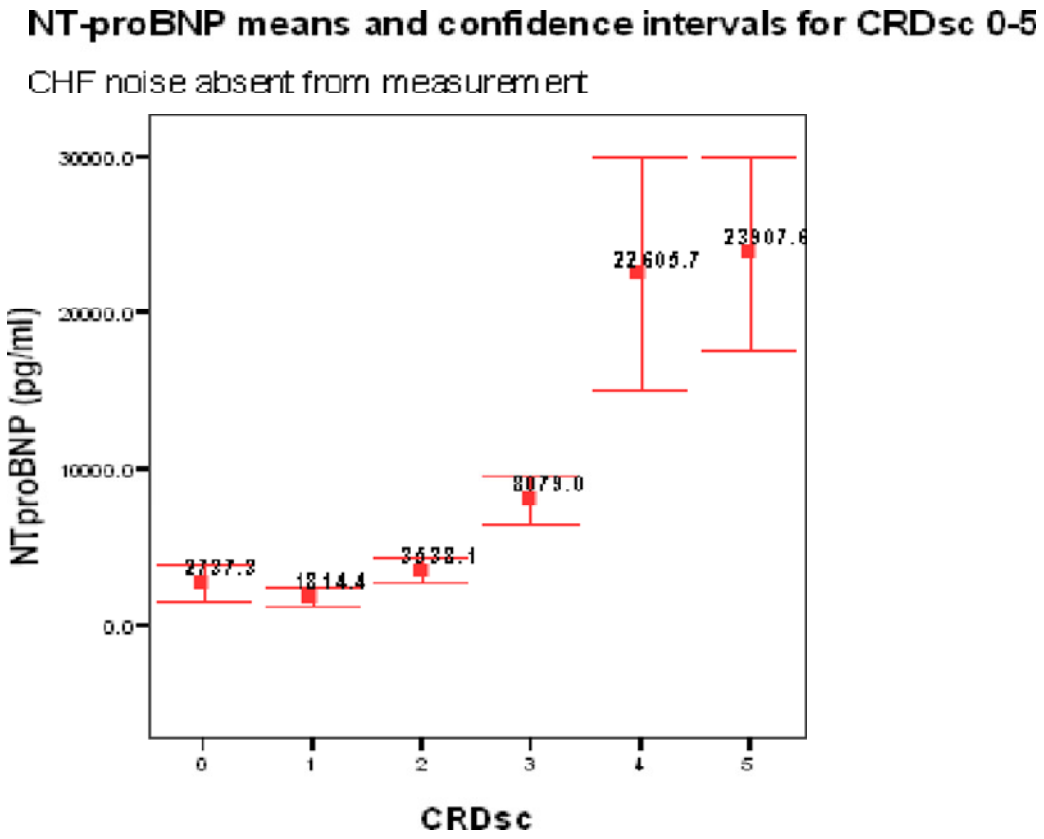

Figure 2. Plot of NKF staging by GFRe interval and NT-proBNP (CHF removed).

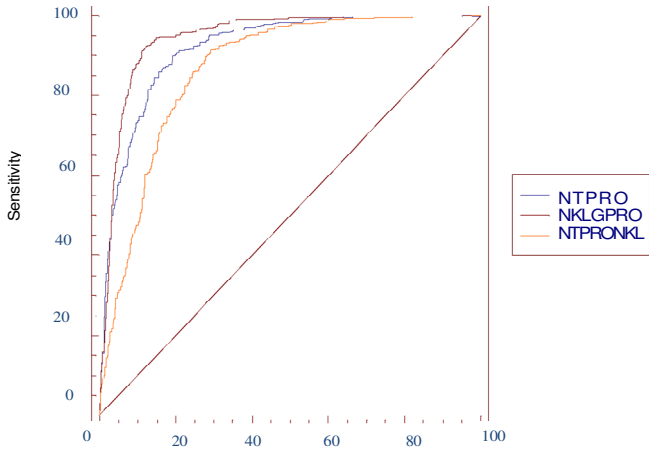

Figure 3. ROC curve comparison of NT-proBNP, NKLog(NTproBNP)/GFRe and the ratio of the two values.

Table 2. Sensitivity and specificity from ROC curve.

Criterion	Sensitivity	95% CI		Specificity	95% CI	
>15.482	99.12	98.0	- 99.7	60.87	59.6	- 62.1
>16.0075	98.95	97.7	- 99.6	63.65	62.4	- 64.9
>18.0495	96.67	94.9	- 98.0	72.31	71.2	- 73.4
>19.0053	95.80	93.8	- 97.3	77.68	76.6	- 78.7
>20.0099	94.75	92.6	- 96.4	81.24	80.2	- 82.2
>21.0043	94.05	91.8	- 95.8	85.07	84.2	- 86.0
>22.0049	92.47	90.0	- 94.5	87.13	86.3	- 88.0
>23.0001	91.59	89.0	- 93.7	88.24	87.4	- 89.0
>24.0092	89.67	86.9	- 92.0	88.90	88.1	- 89.7

(NKLog[NT-proBNP]/eGFR) results in 15.66 and 12.15 for 219 non-donors and 198 donors, significant at $p < 0.0001$, assuming unequal variance). The result for age under 50 years is 17.96 versus 11.58 for 63 non-donors and 146 donors, significant at $p = 0.0001$, but the difference is not meaningful. The means comparison for the NT-proBNP and the normalized in the age over 50 years group (N: 156 non-donors, 52 donors) is 2062.57, 14.73 [ND] vs 88.21, 13.75 [D]. The adjustment is effective in the age 50 years and over group, which has the high risk. The reference range for the normalized Klog (NT-proBNP)/eGFR is described by a mean 13.99, median 13.12, and standard deviation 6.14 with a nonparametric upper limit of 24.7. A ROC curve comparison is constructed using the expanded full database described by the methods in Figure 3. The area under the curve is 0.944 (0.938 to 0.950) for NKLog (NTproBNP)/ eGFR with a base of 571 patients with early CHF and 6115 patients without. The reference range for NKLog (proBNP)/eGFR can be referenced to the percentiles as follows: 20, 8.78; 40, 11.92; 60, 14.85; 90, 21.10; 95, 24.73; 97.5, 29.54.. The coordinates of the ROC curve are shown in Table 2. The sensitivity is 94% and specificity is 85% for NKLog (Ntpro-BNP)/eGFR from the ROC curve.

DISCUSSION

It is reasonable to ask why the study does not support the adequacy of NT-proBNP for identifying CHP using only an age-based reference range dividing the population into two groups predicated on presence and absence of acute CHF. CHF and its severity obviously affect NT-proBNP levels. Neverthess, the simple method employed does not deal with the entanglement of the interrelated renal function loss, anemia, and CHF.

The diagnosis of CHF is generally based on symptoms and signs. But shortness of breath is the most common presentation for a patient not known to have CHF. NT-proBNP might be used before an echocardiographic examination, which then fails, even though the ECHO helps in diagnosis of etiology, classification and management of CHF.

A patient might have CHF even if he has normal LV EF. Assessment of diastolic function is difficult if only standard pulsed wave Doppler is used. But then we are almost in the position of asking which came first, the chicken or the egg. What if the Doppler fails and the eGFR is less than 45? It is perhaps better to view this condition as a set of several sub conditions within a category of anomaly that is not homogeneous in terms of the basic diagnosis, but has key features that are in common, but differ with respect to treatment strategies and outcomes. With increasing age, not only GFR declines but also the relaxation of LV impairs. So even if standard E, A waves or DT of E wave show normal patterns, still patients might have some degree of impairment. This poses the suggestion that the information has to be presented in a manner that best enables the physician looking at the tests.

We are able to find a reasonable interpretation for the NT-proBNP is dependent on age only above 50 years and that an upper limit of 1000 pg/ml is defensible. The upper limit reference interval is much lower for the population under age 50 years. Our study population is larger than the PRIDE study, and the patient characteristics appear to be similar. We do not find it critical dividing the population into two groups predicated on presence and absence of acute CHF because our study aim was not limited to a cohort recruited from the emergency department presenting with acute CHF. The effect of age on NT-proBNP plasma concentration is substantially accounted for and corrected for by adjusting for the effect of eGFR, which we do through a new function, NKLog (NT-proBNP)/eGFR.

One problem encountered with the PRIDE[1] study is the prevalence of COPD or asthma in a large part of the cohort studied, but the diagnosis is presented without any examination of coexistence of other explanatory variables with cardiac factors to explain an NT-proBNP elevation. Our approach was to include a swath of patients who had an ECHO evaluation of CHF. Patients who met sufficient criteria for CHF were removed from consideration for reference range evaluation, but were used for ROC curve analysis. Having a defensible subpopulation to work with who were free of cardiac disease, anemia (the anemia was an impressive effect after exhaustive study, but could not be considered independent of renal disease), advanced renal disease or evidence for diastolic dysfunction, we proceeded as described.

Conclusion

We suggest that NT-proBNP levels can be accurately assessed only after removal of the major confounding co-morbidities that increase this peptide in serum. Practitioners may apply PRIDE[1] criteria initially, but should consider adopting an alternative approach. We established our new range after establishing absence of co-morbidities.

The value of this approach for screening purposes is an allowance for a considerably lower reference normal with a higher specificity based on the donor studies and the mixture model. This study finds that the reference range for NT-proBNP is age-dependent past age the age of 50 years, mainly as the change relates to eGFR(large effect), and perhaps still other factors (which may be interacting).

Abbreviations: ACS, Acute coronary syndrome; **Afib,** atrial fibrillation; **AMI,** acute myocardial infarction; **ANOVA,** one-way analysis of variance; **BNP,** B-type natriuretic peptide; **CHF,** congestive heart failure; **cTnT,** cardiac troponin T; **ECG,** electrocardiogram; **eGFR,** estimated glomerular filtration rate (creatinine clearance); **Hgb,** hemoglobin; **LVEF,** left ventricular ejection fraction; **RI,** renal insufficiency; **TTE,** transthoracic echocardiogram; **BH,** Bridgeport Hospital; **MCMC,** Mercy Catholic Medical Center of Philadelphia; **NYMH,** New York Methodist Hospital.

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