Trial Design

Rationale and Design of the ICON-RELOADED Study: International Collaborative of N-terminal pro-B-type Natriuretic Peptide Re-evaluation of Acute Diagnostic Cut-Offs in the Emergency Department

Brief Title (45 characters): **ICON-RELOADED Study Design and Rationale**

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Conflicts of Interest

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Clinical Diagnostics, and Abbott. Dr. Doros has received consulting income from Pfizer, Genzyme, and Sarepta. Dr. Hollander has received support from PCORI, NIH, Alere, Siemens, Roche, Portola, and Trinity. Dr. Levy has received support from PCORI, NIH, Siemens, Roche, Thermofisher, Amgen, Novartis, Trevena, Cardiorentis, and ZS Pharma. Dr. Nagurney has received grant support from Roche Diagnostics, Alere/Biosite, Boehringer Ingelheim, Thermo Fisher and Nanosphere. Dr. Nowak receives grant support from Roche Diagnostics, Siemens, Abbott, Novartis, and Radiometer, and is a consultant for Roche, Siemens, Abbott, Trinity, and Thermofisher. Dr. Pang is or has been in the past one year a consultant for BMS, Novartis, Trevena, scPharmaceuticals, Roche Diagnostics, and Relypsa, and has received research support from Roche, Novartis, PCORI, IUSM, Indianapolis EMS. Ms. Patel was an employee of Roche Diagnostics. Dr. Peacock has received grants support from Abbott, Alere, Banyan, Cardiorentis, Janssen, Pfizer, Roche, The Medicine's Company, ZS Pharma; has served as a consultant for Alere, Cardiorentis, Ischemia Care, Janssen, Phillips, Prevencio, The Medicine's Company, ZS Pharma; and has ownership interests in Comprehensive Research Associates LLC, and Emergencies in Medicine LLC. Dr. Walters received investigator funding for this study from Roche Diagnostics. Dr. Januzzi has received grant support from Siemens, Singulex and Prevencio; consulting income from Roche Diagnostics, Critical Diagnostics, Sphingotec, Phillips, and Novartis; and participates in clinical endpoint committees/data safety monitoring boards for Novartis, Amgen, Janssen, and Boehringer Ingelheim.

ABSTRACT:

Objectives: To re-assess use of amino-terminal pro B-type natriuretic peptide (NT-proBNP) concentrations for diagnosis and prognosis of acute heart failure (HF) in patients with acute dyspnea.

Background: NT-proBNP facilitates diagnosis, prognosis, and treatment in patients with suspected or proven acute HF. As demographics of such patients are changing, previous diagnostic NT-proBNP thresholds may need updating. Additionally, value of in-hospital NT-proBNP prognostic monitoring for HF is less understood.

Methods: In a prospective, multicenter study in the United States and Canada, patients presenting to emergency departments with acute dyspnea were enrolled, with demographic, medication, imaging and clinical course information collected. NT-proBNP analysis will be performed using the Roche Diagnostics Elecsys® proBNPII immunoassay in blood samples obtained at baseline and at discharge (if hospitalized). Primary endpoints include positive predictive value (PPV) of previously established age-stratified NT-proBNP thresholds for the adjudicated diagnosis of acute HF, and its negative predictive value (NPV) to exclude acute HF. Secondary endpoints include sensitivity, specificity, and positive and negative likelihood ratios for acute HF and among those with HF the prognostic value of baseline and pre-discharge NT-proBNP for adjudicated clinical endpoints (including all-cause death and hospitalization) at 30 and 180 days.

Results: 1461 dyspneic subjects have been enrolled and are eligible for analysis. Follow-up for clinical outcome is ongoing.

Conclusions: The International Collaborative of N-terminal pro-B-type Natriuretic Peptide Re-evaluation of Acute Diagnostic Cut-Offs in the Emergency Department study offers a contemporary opportunity to understand best diagnostic cutoff points for NT-proBNP in acute HF, and validate in-hospital monitoring of HF using NT-proBNP.

KEY WORDS:

NT-proBNP

Acute heart failure

Biomarker

Diagnosis

Prognosis

ABBREVIATIONS:

CI: confidence interval

ED: emergency department

HF: heart failure

HFpEF: heart failure with preserved ejection fraction

HFrEF: heart failure with reduced ejection fraction

ICON: International Collaborative of NT pro-BNP

LR-: negative likelihood ratio

LR+: positive likelihood ratio

NT-proBNP: N-terminal pro B-type natriuretic peptide

NPV: negative predictive value

PPV: positive predictive value

PRIDE: N-Terminal Pro-BNP Investigation of Dyspnea in the Emergency Department

Acute heart failure (HF) can be challenging to diagnose, as symptoms and signs often overlap with other conditions, leading to both under and over recognition, and incorrect treatment. Testing of blood for the presence of natriuretic peptides facilitates the diagnosis, as well as provides an objective indication of the severity and prognosis, of HF. B-type natriuretic peptide (BNP), and its amino-terminal pro-peptide cleavage equivalent, NT-proBNP, are both sensitive and specific biomarkers for the diagnosis of acute HF (1-4). In prospective, randomized clinical trials of patients presenting with symptoms of acute dyspnea, natriuretic peptides improved clinician diagnostic accuracy for acute HF,(5) reduced health care expenditures, and improved outcomes compared with clinical judgment alone.(6)

Though embedded as a Class I Level of Evidence A in clinical practice guidelines for diagnostic evaluation of HF (7), questions remain regarding the current use of natriuretic peptides. In the past few decades, considerable changes in HF demographics have occurred. Patients with HF are generally older, with more co-morbidities such as atrial fibrillation, anemia, and chronic kidney disease;(8) all of these differences may cause higher than expected natriuretic peptide concentrations.(9) Drugs for HF (i.e., sacubitril/valsartan), which directly raise BNP concentrations and simultaneously lower NT-proBNP, are now available. Conversely, compared to earlier decades, there is increasing incidence and prevalence of HF with preserved ejection fraction (HFpEF) and patients are generally more obese,(10, 11) both of which may cause lower-than-expected natriuretic peptide concentrations.(12, 13) Accordingly, presently utilized diagnostic thresholds for natriuretic peptides may require a re-assessment.

For NT-proBNP, historical regulatory-approved thresholds were based on data generated from testing in non-acute patients, a population different from typical patients encountered in the emergency department (ED); Moreover, these cutoffs were developed for their high negative predictive value (NPV), with the intent of excluding HF, rather than for their positive predictive value (PPV) in identifying HF. While NPV is of value, identification of PPV-optimized cut-offs is important as well. In this regard, the N-Terminal Pro-BNP Investigation of Dyspnea in the Emergency Department (PRIDE) and International Collaborative of NT-proBNP (ICON) studies identified PPV-optimized cut-offs for the identification of acute HF. In these studies, an approach of using diagnostic thresholds of 450 pg/mL, 900 pg/mL and 1800 pg/mL for age categories of <50, 50–75 and >75 years, respectively, was considerably more accurate, improving specificity without sacrificing sensitivity(14). While both the age-stratified NT-proBNP approach and the NPV-optimized cut off of 300 pg/mL to exclude acute HF has been endorsed by clinical practice guidelines (7), consensus statements, (15) and top-tier research journal summaries (16), regulatory-approved thresholds for NT-proBNP remain unchanged for use in acute dyspnea, leading to considerable confusion. Thus, opportunities exists not only to validate age-stratified cut-points for NT-proBNP, but to do so in a more contemporary cohort.

Beyond diagnosis, NT-proBNP also facilitates assessment of HF severity as well as the prediction of prognosis in patients with acute HF(14, 17, 18). While concentrations of NT-proBNP at presentation with acute HF are strongly prognostic, post-treatment concentrations are

even more powerfully predictive of hospitalization or death; accordingly, recent enthusiasm has grown in use of NT-proBNP for prognostic monitoring during hospital treatment for acute HF. However, although retrospective data suggest in-hospital change in NT-proBNP may be useful for prognosis,(19-23), prospectively collected data are limited.

Accordingly, the primary aim of this study is to validate the ICON cut-offs in a contemporary cohort. Additionally, as a secondary aim, this study will prospectively evaluate the value of in-hospital NT-proBNP monitoring for prediction of clinical outcomes. The design, population, endpoints, and statistical considerations for the International Collaborative of N-terminal pro-B-type Natriuretic Peptide Re-evaluation of Acute Diagnostic Cut-Offs in the Emergency Department (ICON-RELOADED) Study are described.

METHODS

The institutional review board at each participating institution approved the study, and all patients provided written informed consent prior to enrollment. Funding was provided by Roche Diagnostics. Joanna Suomi, MSc, contributed to the writing and editing of this manuscript as an employee of the Baim Institute for Clinical Research.

Study Design, Setting, and Evaluations

The ICON-RELOADED Study is a prospective, multicenter clinical trial conducted at 19 sites in the United States and Canada (**Figure 1**).

Figure 1: Study site distribution. A wide range of sites enrolled 1461 patients and provided a balanced demographic of patients.

Abbreviations: BIDMC, Beth Israel Deaconess Medical Center; MGH, Massachusetts General Hospital.

A total of 1461 patients presenting to ED with acute dyspnea were enrolled between October 2015 and October 2016. As seen in **Table 1**, blood samples were collected at enrollment and at hospital discharge (unless the patient was discharged from the emergency department). Post discharge clinical events were obtained at 180 days (±14 days) via phone call with patient or primary care physician when needed.

Table 1. ICON-RELOADED timeline.

	Evaluations at Enrollment	**Evaluations at Discharge**	**Evaluations at 180 ±14 Days**
	Oct 2015 – Oct 2016		**Apr 2016 – Apr 2017**

	◆——→		
NT-proBNP and biorepository blood samples	X	X*	
DNA sample**	X		
Clinical course	X	X	X
Concomitant medications	X	X	X
Adverse events	X	X	X

*If patients were discharged from ED directly home, no discharge sample had to be collected. There may have been an additional pre-discharge blood draw if the subject remained in hospital for more than 24 hours since the previous pre-discharge blood draw.

** To be drawn from those that consented to this procedure.

Study Objectives

The primary objective of this study is to externally validate the use of Elecsys® proBNP (Roche Diagnostics, Indianapolis, IN, USA) concentrations to aid in the diagnosis of HF in patients presenting emergently through use of the ICON cut-point strategy(14). This will identify ("rule in") or exclude ("rule out") the diagnosis of acute HF (**Table 2**).

Table 2: Proposed NT-proBNP cut-points for diagnosis or exclusion of acute heart failure. Based on earlier studies, these thresholds will be re-evaluated in the ICON-RELOADED Study.

Threshold for Diagnosis of Acute HF	**Patient Age Group**
450 pg/mL	<50 years
900 pg/mL	50–75 years
1800 pg/mL	≥75 years
Threshold for Exclusion of Acute HF	**Patient Age Group**
300 pg/mL	N/A

As a secondary aim, we will determine the value of in-hospital change of NT-proBNP relative to other clinical and biochemical variables predicting post-discharge outcomes.

Study Population

Patients presenting to emergency departments with acute dyspnea were enrolled if they met all of the inclusion criteria and had no exclusion criteria, as listed in **Table 3**.

Table 3. Patient eligibility criteria**.** A patient had to meet all of the listed inclusion criteria to participate in the study. A patient was excluded from participation if s/he met any of the clinical exclusion criteria, and a sample was excluded from the study if it met any of the sample exclusion criteria.

Enrollment Inclusion Criteria	Age ≥ 22 years Admission to the emergency department with acute dyspnea Written informed consent
Clinical Exclusion Criteria	Renal insufficiency requiring dialysis or known estimated glomerular filtration rate <15 mL/min/1.73 m^2 prior to enrollment Dyspnea after chest trauma Subject unable to donate up to 50 mL of blood at one time Known pregnancy Unreliable or non-compliant, including patients with known history of active alcoholism, drug abuse, or serious psychiatric disorder that may lead to noncompliance; as well as patients unwilling to abide by the requirements of the protocol Any known conditions (e.g. psychiatric history, language barrier, absence of legal representative, etc.) that would interfere with the patient's ability to comply with study instructions, might confound the interpretation of the study, or put the patient at risk Personnel, or any relative of personnel of the Sponsor, the Contract Research Organization, or the investigative site(s)
Sample Exclusion Criteria	Visible hemolysis, lipemia or icterus Known interferents with NT-proBNP assay such as high dose biotin use. Visible particulates Insufficient quantity Specimens not collected, handled, stored or shipped properly

Biorepository

Fifty (50) mL of blood was collected from each patient enrolled using serum, Lithium-Heparin (Li-Hep) Plasma, and ethylene-diamineteraacetic acid (EDTA) tubes, with DNA extraction performed from the EDTA tube. Serum tubes sat at room temperature to allow the clot to form. Plasma tubes were immediately stored at 2-8°C prior to centrifugation. A subject that did not have at least 8, 0.30 mL aliquots from the Li-Hep Plasma tube was designated as a "dropped" subject for enrollment status. If less than 8, 0.30 mL aliquots were obtained from the EDTA tubes, the subject was not "dropped" as the primary matrix of interest for regulatory approval will be Li-Hep; the EDTA samples will be utilized for creation of a biorepository for assessing novel HF markers of interest while cross-validating quality performance NT-proBNP assays in

different matrices. Samples were stored at -70°C or colder, and shipped to the ICON-RELOADED Biorepository.

For discharge blood samples, 50 mL of blood was collected, also the same protocol, within 24 hours of planned discharge.

Samples will be maintained in the ICON-RELOADED Biorepository until the time of NT-proBNP testing (<1 year), which will be performed using the commercially available Elecsys proBNP II (Roche Diagnostics, Indianapolis, IN) immunoassay, according to established methods.

Study Endpoints

Primary Efficacy Endpoints

The primary efficacy endpoints for this study are the positive predictive value (PPV) of age-specific rule-in thresholds of 450/900/1800 pg/mL for ages <50/50-75/≥75 years for the diagnosis of acute HF, and the negative predictive value (NPV) of the rule-out threshold of 300 pg/mL to exclude the adjudicated diagnosis of acute HF.

Secondary Efficacy Endpoints.

Secondary endpoints include the NPV, positive likelihood ratio (LR+), negative likelihood ratio (LR–), sensitivity and specificity for the age-specific rule-in thresholds as well as the PPV, LR+, LR-, sensitivity and the specificity for the rule-out threshold.

In addition, all efficacy endpoints will be evaluated by region (United States and Canada) for regulatory purposes.

Secondary Clinical Endpoints

We will assess the following clinical outcomes at 30- and 180-days post discharge: major adverse cardiac events following index presentation or hospitalization (MACE, a composite of all-cause death or HF hospitalization), all-cause death, cardiac death, all-cause hospitalization and HF hospitalization. Using baseline and discharge characteristics as well as NT-proBNP concentrations at baseline and discharge, we will develop a multivariable model for the prediction of clinical outcomes.

Statistical Considerations

Sample Size Calculation

The study was designed to include 1765 patients to ensure a sample of at least 1500 analyzable patients, accounting for an assumed attrition rate of approximately 15%. A maximum of 15% of patients were to be enrolled from sites in Canada, thereby intending 1500 enrolled and 1275

analyzable patients from the United States, and 265 enrolled and 225 analyzable patients from Canada.

Statistical Analyses

Efficacy Endpoints

Operating characteristics of the proposed age-based thresholds combined (for rule-in) and the proposed threshold for rule-out relative to the gold-standard diagnosis will be evaluated, including sensitivity, specificity, PPV, NPV, LR+ and LR–. Estimates and CIs for the NPV and PPV parameters will be determined.(24) Estimates, CIs and tests for the likelihood ratios parameters, LR+ and LR–, will be carried out using a non-linear mixed effects model. Based on data generated in the ICON study,(14) overall sensitivity and specificity of the proposed age-dependent thresholds are expected to be 90.0% and 84.0%, respectively. The expected PPV is 85.0% and the LR+ is estimated at 5.62.

With a planned sample size of 1,500 analyzable patients and an assumed prevalence of HF of 50% in this population,(14) the lower bound of a 95% two-sided CI for the hypothesized PPV is 82.6% while for lower bound for the hypothesized LR+ is expected to be 4.77, which would establish the proposed test as a useful tool to aid diagnosis of acute HF in this population.(25) A planned sample of 1,275 analyzable patients from the United States would result in lower bounds of a two-sided 95% CI for the PPV of 82.5% and for LR+ of 4.6, therefore providing substantial precision.

With a proposed rule-out threshold of 300 pg/mL, the expected sensitivity and specificity values are 99.0% and 60.0%, respectively.(14) Thus, the expected NPV is 98.5% and the LR– is 0.017, with a lower bound for a two-sided 95% CI for the NPV of 96.7% and a upper bound for a two-sided 95% CI of 0.034, thereby establishing the proposed test as a useful tool in the aid of excluding acute HF in this population.(25)

Clinical Endpoints

Univariable comparison of baseline and discharge characteristics (NT-proBNP concentrations, as below) between patients with and without a composite endpoint of all-cause death or repeat HF hospitalization will be assessed at 30 and 180 days post discharge using χ^2 test for categorical variables and Mann-Whitney for continuous variables. Normally distributed variables are presented as mean ± standard deviation and non-normally distributed variables are shown as median with interquartile range. Age, sex and other variables that are statistically significant will be considered as a candidate variable in a multivariable prediction of the time-to composite endpoint with a retention value of $p>0.1$ in a Cox proportional hazard model. Co-linearity will be assessed and non-parametric continuous variables will be transformed to fit a normal curve. Concentrations of NT-proBNP will be assessed as absolute values at baseline and discharge as well as change from baseline to discharge (absolute difference as well as relative percent change

from baseline value). A responder will be defined as relative percent difference in NT-proBNP from baseline to discharge ≥30% and a non-responder will be defined as NT-proBNP change <30%. In addition, multivariable prediction models for re-hospitalization will be performed using multivariable logistic regression.

Diagnostic Subgroups of Interest

As it is well-known that NT-proBNP may be influenced by certain important medical co-morbidities, diagnostic subgroups of interest have been pre-specified. These include (1) HF with reduced EF (HFrEF) versus HFpEF (defined as left ventricular EF <50% vs. ≥50%); (2) sex; (3) race (black vs. non-black); (4) obesity (body mass index <30.0 vs. ≥30.0 kg/m^2); (5) with or without a history of atrial fibrillation; and (6) with or without renal insufficiency (eGFR ≤60.0 mL/min/1.73m^2 vs. eGFR >60.0 mL/min/1.73m^2). Because decompensation of chronic HF is a very important predictor of the diagnosis of acute HF, we will evaluate discrimination and operating characteristics of NT-proBNP in patients with acute on chronic as well as *de novo* HF.

Clinical Event Adjudication

The Clinical Events Committee (CEC) includes a panel of physicians (cardiologists and emergency physicians) who are independent from the study. CEC meetings will include a minimum of 2 emergency physicians and 1 cardiologist or interventional cardiologist for the adjudication of pre-specified clinical endpoints based on charter-based criteria used for the categorization of clinical events in the study (see **Appendix** for definitions).

Assessment of HF

For the assessment of HF diagnosis, the members of the CEC will be blinded to local site NT-proBNP or BNP concentrations during index hospitalization, if performed for standard of care. Adjudication will be performed prior to the performance of central laboratory NT-proBNP testing for regulatory submission. The CEC will adjudicate the diagnosis for each patient at presentation, categorized as acute HF, acute HF and additional active diagnosis (e.g., pulmonary, other cardiac, anemia), other diagnosis and no HF (incident or prevalent), or other diagnosis and prior background of HF without acute decompensation.

Endpoint Assessment

For assessment of clinical events and clinical endpoints, all available information is provided to the members of the CEC, excluding the CEC adjudication for index hospitalization acute HF diagnosis. The CEC will adjudicate all hospitalizations, deaths, and other clinical events that occur throughout the trial (to 180 [+/- 14] days).

RESULTS

Study enrollment ended October 15, 2016, with 1758 patients included (signed the informed consent form). Of these, 297 were excluded due to clinical or sample exclusion criteria, leaving 1461 enrolled, of which 1424 were from the United States and 37 were from Canada (**Figure 2**).

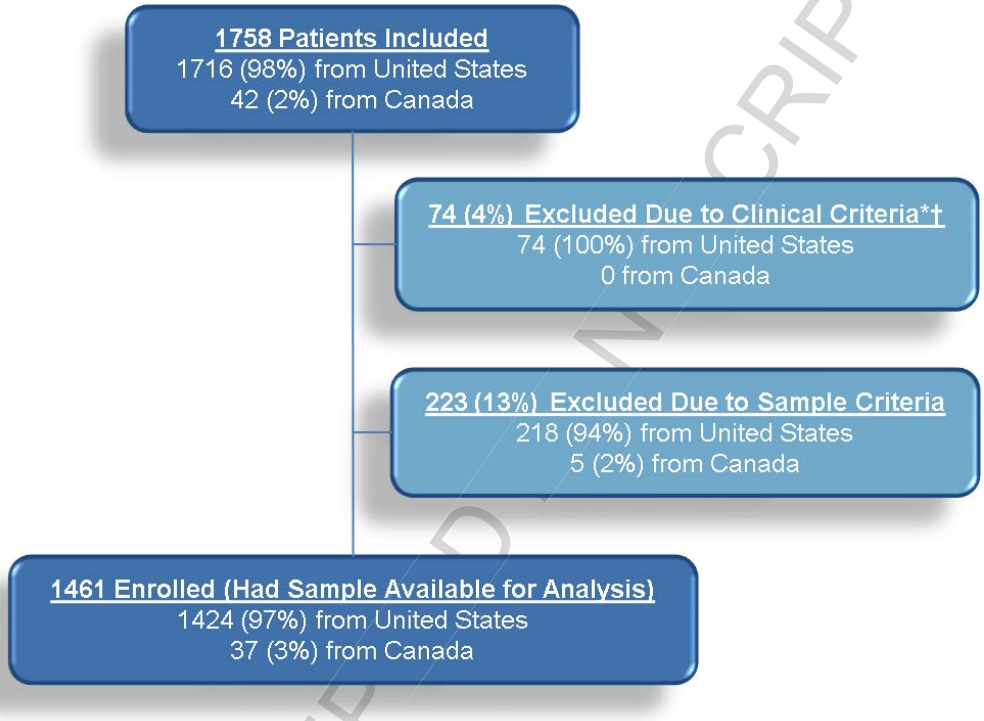

Figure 2. CONSORT Diagram. Of 1758 patients included (signed the informed consent form), 297 (17%) were excluded due to subsequent clinical or sample exclusion criteria, leaving 1461 patients enrolled, with a blood sample available for analysis.

* Data are presented non-hierarchically; 2 patients also had sample exclusion criteria but are only counted in this box.
† 57 patients included in this box met the clinical criteria but did not have a baseline blood sample.

As seen in **Table 4**, patients were a mean of 56.4 ±15.7 years old, 49.1% were female, and 40.4% were non-white race. Overall, 63.3% of patients had history of hypertension, 24.9% had prior HF, and 25.4% had left ventricular ejection fraction <40% at their most recent evaluation prior to study enrollment. Asthma was present in 30.1%, chronic obstructive pulmonary disease in 27.5%, and a history of lung cancer in 2.8%. A total of 58.5% of patients reported being current or former smokers. For the purposes of the prespecified subgroup analyses, it is notable that 36.6% of patients were black, 52.5% had BMI ≥ 30, and 24.0% had renal insufficiency (not presented). Not all cases of HF had been adjudicated at the time of publication and therefore it is not known how many subjects had HFrEF or HFpEF.

Table 4: Baseline characteristics of enrolled patients.

Characteristic	All Patients N=1461
Demographics	
Age (years) (Mean ± SD (N))	56.4±15.7 (1461)
Female Sex	49.1% (718/1461)
Hispanic or Latino	13.6% (191/1406)
Non-White Race	40.4% (579/1432)
Medical History	
Diabetes Mellitus	28.9% (420/1454)
Hypertension	63.3% (921/1455)
Heart Failure	24.9% (356/1431)
Peripheral Arterial Disease	4.3% (61/1434)
Implantable Cardioverter-Defibrillator	5.9% (86/1452)
Cardiac Resynchronization Therapy	1.7% (24/1442)
Coronary Artery Bypass Graft	6.6% (96/1451)
Prior Coronary Artery Disease	21.2% (307/1445)
Previous Myocardial Infarction	13.2% (188/1428)
Previous Percutaneous Coronary Intervention	8.9% (128/1431)
Renal Insufficiency/Failure	7.8% (114/1455)
Dialysis	0.0% (0/114)
eGFR <15 mL/min/1.73m^2	0.0% (0/103)
LVEF <40% at Most Recent Evaluation Prior to Enrollment	25.4% (103/405)
Atrial Fibrillation	14.9% (216/1453)
Significant Mitral Valve Disease	4.2% (57/1372)
Significant Aortic Valve Disease	2.3% (31/1338)
Asthma	30.1% (437/1450)
Chronic Obstructive Pulmonary Disease	27.5% (399/1449)
History of Lung Cancer	2.8% (40/1454)
Alcohol History	
Never	36.6% (514/1404)
Former	16.4% (230/1404)
Current	47.0% (660/1404)
Tobacco History	
Never	41.5% (591/1423)
Former	36.7% (522/1423)
Current	21.8% (310/1423)
Cocaine History	
Never	89.0% (1227/1379)
Former	10.4% (144/1379)
Current	0.6% (8/1379)

Abbreviations: eGFR, estimated glomerular filtration rate; LVEF: Left ventricular ejection fraction; SD, standard deviation.

Available demographic data for patients who were excluded from the study due to either clinical or sample criteria are presented in **Appendix Table 3**.

DISCUSSION

We describe the design and rationale of a large prospective multicenter, international trial primarily focused on NT-proBNP testing in ED patients presenting with acute dyspnea. In our study, performed in 19 North American institutions, we enrolled a population of patients representative of a contemporary cohort of patients presenting with acute dyspnea in whom natriuretic peptide testing would be contemplated. The central goal of this trial is to determine the most useful diagnostic cut-offs for this biomarker for the diagnosis of acute HF in a contemporary cohort of patients presenting with acute dyspnea. Additionally, this will be one of the first studies prospectively examining the role of in-hospital NT-proBNP monitoring for predicting prognosis after hospital-based treatment for acute HF.

To aid in early diagnosis of patients with acute dyspnea in the ED, readily available, accurate, and objective tools to support clinical judgment are needed.(26, 27) Early and correct diagnosis is essential, as delayed treatment for acute HF is associated with increased mortality.(28) Improvement in patient care after HF hospitalization is a major centerpiece of current efforts to control cost (29); accurate and rapid diagnosis of HF after admission to the emergency department would be expected to have a cascade effect on both the patient and the health care system, translating into prompt and proper treatment with subsequent reduction of the length of hospital stay and improvement in patient outcomes. Prior studies from PRIDE(30, 31) and the Canadian based Improved Management of Patients with Congestive Heart Failure (IMPROVE-CHF) study(6) suggest that prompt, accurate diagnosis would reduce indecision, potentially improve outcomes, and lead to reduction in healthcare expenditures. The validation of the ICON diagnostic cutoffs in a contemporary cohort may thus aid in enhancing diagnosis of acute HF in the emergency department.

In addition to its value in diagnosis, NT-proBNP concentrations are associated with HF severity and are guideline supported for predicting short- to intermediate-term risk for adverse outcomes, such as death or repeat hospitalization. As shown by the first ICON investigators, NT-proBNP concentrations >5180 pg/mL were associated with an adjusted odds ratio of 5.20 (95% confidence intervals [CI] = 2.20–8.10, $P<.001$) for death by 76 days.(3) Longer term follow-up of the PRIDE study demonstrated similar prognostic value of the baseline NT-proBNP measurement through four years.(18) More recent data have suggested value of serial measurement of NT-proBNP to better assess risk. Whereas a baseline measurement of NT-proBNP is useful for prognosis, subsequent re-measurement of the peptide after treatment for acute HF is of even greater predictive importance.(19-21) Although not yet supported by large-scale prospective data, retrospective research indicates that two factors appear to be independently predictive of event-free survival after hospitalization for AHF: significant reduction of NT-proBNP concentration (≥30%) and lower absolute NT-proBNP values at discharge.(20) The ICON-RELOADED study will prospectively assess the importance of in-hospital change in NT-proBNP as a prognostic indicator of short-term hazard.

The ability to determine disease severity and subsequently tailor therapeutic response has the potential to maximize proper care, minimize resource use, and improve healthcare outcomes. For example, results from in-hospital NT-proBNP testing could be incorporated into each of the three strategies proposed by Dunbar-Yaffe *et al.* to reduce readmissions for HF: (1) identify patients at high risk of readmission prior to their index hospital discharge; (2) institute remote ambulatory monitoring strategies to identify early warning signs before acute decompensation takes place, and (3) employ strategies in the emergency department to identify low-risk patients who may not need hospital readmission.(32) Of particular importance in the current health care environment in the United States is the goal of reducing 30-day hospital readmission rates for HF(29, 33), not only to improve patient outcomes, but also to mitigate the financial penalties associated with excess readmission rates during the first 30 days after discharge.(34)

A few limitations should be considered with the eventual interpretation of this study's findings. The applicability of data generated from this North American cohort should be considered when applying the results in venues outside of the United States and Canada**.** Additionally, a relatively high percentage of samples (13%) were excluded from the analysis due to various sample exclusion criteria, possibly omitting eligible subjects. However given the central goal of this trial was focused on assay validation, such exclusions are mandated. Although not tested for significance, in comparison with the original ICON study cohort,(26) the patients in the present study were numerically younger (mean 56.4 vs. 68.3 years old), less likely to have prior heart failure (24.9% vs. 34%), prior MI (13.2% vs. 25%), or prior coronary artery disease (21.2% vs. 40%); and more likely to have hypertension (63.3% vs. 53%). Females comprised about 49% of each of the studies' patient populations. As well, though results of local natriuretic peptide testing were redacted from the CEC processes, such results could theoretically influence behaviors of clinicians during patient hospitalization and care.

CONCLUSION

The ICON-RELOADED Study will validate NT-proBNP diagnostic cutoffs in a modern multicenter cohort of patients, an important step to understanding the optimal diagnostic application of this biomarker in emergency department patients. Furthermore, the study will carefully examine the role of in-hospital NT-proBNP monitoring, which may provide insights to better manage patients following hospitalization for acute HF. More accurate and timely diagnosis combined with more precise management of the HF patient may improve outcomes, in part due to better management of higher risk patients, while simultaneously reducing the burden of healthcare expenditures.

HIGHLIGHTS

- NT-proBNP facilitates diagnosis, prognosis, and treatment of acute heart failure
- Change in patient demographics require updated diagnostic NT-proBNP cutoffs
- ICON-RELOADED aims to determine diagnostic NT-proBNP cutoffs in a sample of 1461 patients
- Prognostic value of in-hospital change in NT-proBNP will be assessed

CLINICAL PERSPECTIVES:

More accurate diagnosis of acute HF can lead to more efficient triage, more accurate therapeutic focus, reduced hospital length of stay, reduced medical expenditures, reduced disease complications, and improved clinical outcomes. In a contemporary cohort of patients presenting to the Emergency Department, the ICON-RELOADED Study will validate NT-proBNP thresholds of 450/900/1800 pg/mL for age categories of <50/50–75/≥75 years for diagnosis of acute HF in a modern cohort of patients who present to the ED with acute dyspnea. Additionally, better understanding of the use of NT-proBNP for disease prognosis in acute HF may be associated with similar positive clinical implications.

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APPENDIX

Clinical endpoint committee charter definitions for adjudication of events. Definitions are based on regulatory guidance[1]or clinical practice guidelines.

ACUTE HEART FAILURE

Clinical manifestations of heart failure:

a) New or worsening symptoms of heart failure (at least 1 of the following):
- Dyspnea
- Orthopnea
- Paroxysmal nocturnal dyspnea
- Increased fatigue or worsening exercise tolerance
- Worsening swelling of the legs
- Gastrointestinal distress related to congestion/low output

AND

b) New or worsening signs of heart failure (at least 2 of the following):
- Pulmonary edema
- Pulmonary basilar crackles
- Jugular venous distension / elevated jugular venous pressure / positive hepato-jugular reflux
- Peripheral edema
- Rapid weight gain
- New or worsening third heart sound or gallop rhythm
- Increased abdominal distension or ascites
- Hepatomegaly (not related to liver disease)
- Radiological evidence of worsening heart failure
- Changes in biomarker (e.g., B-type natriuretic peptide or amino-terminal pro-B type natriuretic peptide) above local reference limits
- Invasive or non-invasive tests showing elevated cardiac feeling pressures or low cardiac output

AND

c) Additional/increased therapy (at least 1 of the following):

- Initiation of oral diuretic, intravenous diuretic, inotrope, or vasodilator therapy
- Uptitration of oral diuretic, intravenous therapy (if already on therapy)
- Initiation of mechanical or surgical intervention (mechanical circulatory support, heart transplantation or ventricular pacing to improve cardiac function), or the use

[1] Hicks KA, Tcheng JE, Bozkurt B, et al. 2014 ACC/AHA Key Data Elements and Definitions for Cardiovascular Endpoint Events in Clinical Trials. Circulation. 2015;132(4):302-61.

of ultrafiltration, hemofiltration, or dialysis that is specifically directed at treatment of heart failure

ACUTE DYSPNEA

A subjective feeling of shortness of breath, difficult or labored breathing, arising or worsening over the course of no longer than several days.

DEATH

All deaths are considered cardiac unless an unequivocal non-cardiac cause can be established. Specifically, any unexpected death even in patients with coexisting potentially fatal non-cardiac disease (e.g., cancer, infection) should be classified as cardiac.

- Cardiac death

Any death due to proximate cardiac cause (e.g., MI, low-output failure, fatal arrhythmia), unwitnessed death and death of unknown cause, and all procedure-related deaths, including those related to concomitant treatment, will be classified as cardiac death.

  - Cardiac death due to heart failure
    - New or worsening signs and/or symptoms of heart failure include any of the signs and symptoms as detailed above for ADHF
  - Cardiac death due to myocardial infarction
    - New or worsening signs and/or symptoms of myocardial infarction leading to death within 30 days
  - Sudden cardiac death or primary arrhythmia: refers to death that occurs unexpectedly in a previously stable patient and includes the following deaths:
    - Witnessed and instantaneous without new or worsening symptoms
    - Witnessed within 60 minutes of the onset of new or worsening cardiac symptoms
    - Witnessed and attributed to an identified arrhythmia (e.g., captured on an electrocardiographic (ECG) recording or witnessed on a monitor by either a medic or paramedic)
    - Subjects unsuccessfully resuscitated from cardiac arrest or successfully resuscitated from cardiac arrest but who die within 24 hours without identification of a non-cardiac etiology
  - Other cardiac death
    - Cardiac death not meeting definitions of sudden cardiac death or death due to heart failure

- Vascular death

Death caused by non-coronary vascular causes, such as cerebrovascular disease, pulmonary embolism, ruptured aortic aneurysm, dissecting aneurysm, or other vascular diseases.

- Non-cardiovascular death

Any death not covered by the above definitions, such as death caused by infection, malignancy, sepsis, pulmonary causes, accident, suicide, or trauma.

HEART FAILURE HOSPITALIZATION

Unplanned presentation to an acute care facility for an exacerbation of heart failure requiring an overnight stay (change in calendar day), due to signs and symptoms compatible with acute HF as defined above.

Appendix Table 1. Elecsys® Description

Topic	Elecsys proBNP II	Elecsys proBNP II STAT
Test Principle	The Elecsys proBNP II Assay components are a two-step sandwich immunoassay with streptavidin microparticles and electrochemiluminescent detection. The total duration of the test system is ~18 minutes.	The Elecsys proBNP II STAT Assay components are a two-step sandwich immunoassay with streptavidin microparticles and electrochemiluminescent detection. The total duration of the test system is 9 minutes.
Incubation	1st incubation: Antigen in the sample (15 µL), a biotinylated monoclonal NT-proBNP-specific antibody, and a monoclonal NT-proBNP-specific antibody labeled with a ruthenium complex form a sandwich complex. 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.	During a nine (9) minute incubation, antigen in the sample (15 µL), a biotinylated monoclonal NT-proBNP-specific antibody, a monoclonal NT-proBNP-specific antibody labeled with a ruthenium complex and streptavidin-coated microparticles react to form a sandwich complex, which is bound to the solid phase.

Appendix Table 2. Site and Investigator List

Site Name	Investigator	Location
Baylor College of Medicine	Peacock, Frank	Houston, TX, USA
Beth Israel Deaconess Medical Center	Shapiro, Nathan	Boston, MA, USA
Henry Ford Hospital	Nowak, Richard	Detroit, MI, USA
Jewish General Hospital	Chen-Tournoux, Annabel	Montreal, QC, Canada
Loma Linda University Health	Walters , Elizabeth	Loma Linda, CA, USA
Massachusetts General Hospital	Nagurney, John	Boston, MA, USA
Methodist Hospital	Pang, Peter	Indianapolis, IN, USA
Stony Brook University Medical Center	Singer, Adam	Stony Brook, NY, USA
Thomas Jefferson University	Hollander, Judd Chang, Anna Marie	Philadelphia, PA, USA
Toronto General Hospital	Ross, Heather	Toronto, ON, Canada
Torrance Memorial Medical Center	Lurie, Mark	Torrance, CA, USA
University of Kansas Hospital	Cannon, Chad	Kansas City, KS, USA
University of Maryland Medical Center	Christenson, Robert	Baltimore, MD, USA
University of Southern California	Arora, Sanjay	Los Angeles, CA, USA
University of Texas Health Science Center at San Antonio	DeLorenzo, Robert	San Antonio, TX, USA
University of Texas Southwestern Medical Center	Blomkalns, Andra	Dallas, TX, USA
University of Washington	Cheng, Richard	Seattle, WA, USA
Wake Forest Health Sciences	Hiestand, Brian	Winston-Salem, NC, USA
Wayne State University	Levy, Phillip	Detroit, MI, USA

Appendix Table 3. Characteristics of patients whose samples were excluded from analyses due to clinical or sample exclusion criteria.

Characteristic	N=297 Subjects
Demographics	
Age (years) (Mean ± SD (N))	55.1±15.5 (297)
Female Sex	52.2% (155/297)
Hispanic or Latino	17.2% (50/290)
Nonwhite Race	48.6% (140/288)

Abbreviations: eGFR, estimated glomerular filtration rate; LVEF: Left ventricular ejection fraction; SD, standard deviation