The Role of Serum NT-proBNP for Predicting Left Ventricular Systolic Dysfunction in Hospitalized Patients in Sri Lanka

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Keywords

Abstract

Background/aims:

Only a few studies have addressed the role of NT-proBNP in identifying Left Ventricular Systolic Dysfunction (LVSD) in South Asian populations. Therefore, the current study was aimed at assessing the use of serum NT-proBNP in predicting LVSD in a hospitalized population in Sri Lanka.

Methods:

A random sample of 278 individuals referred for echocardiography at a major Teaching Hospital consented for venous blood samples to be collected for serum NT-proBNP assay by sandwich ELISA. Based on the ejection fraction (LVEF) and fractional shortening (FS), participants were differentiated as LVSD (LVEF<50%, FS≤ 29%) and non-LVSD individuals (LVEF>60%). According to inclusion/exclusion criteria, the final study sample consisted of 100 LVSD patients and 41 non-LVSD individuals.

Results:

The mean ages of the LVSD and non-LVSD groups were 69.1 (±6.2 years) and 71.4 (±2.4 years) (p=0.066) respectively. The median NT-proBNP value (with IQR) among LVSD patients (528.2 pg/mL,355.2–924.2) was comparatively higher than that of non-LVSD individuals (207.3 pg/mL,177.5–343.0). Strong correlations of NT-proBNP level with LVEF (Spearman *rho*= -0.84, p<0.001) and FS (*rho*= -0.81, *p*<0.001) suggested that serum NT-proBNP concentration increases in parallel to deteriorating left ventricular functions. The AUROC of serum NT-proBNP for differentiating LVSD was 0.859 (95% CI:0.79 – 0.92) and the optimal cut-off level for predicting LVSD was 265pg/mL, with 90% sensitivity and 70% specificity.

Conclusion:
Current Sri Lankan study revealed a considerable correlation of serum NT-proBNP level with LVSD and utilizing such an assay for screening will facilitate adequate evidence to rule-out LVSD among high-risk residents.

Introduction
The high incidence and prevalence of different cardiovascular diseases (CVDs) are due to the significant presence of risk factors in the respective communities, for instance, chronic Heart Failure (HF); has been observed mostly in communities where there are increasingly ageing populations (1). According to the 2013 American Heart Association (AHA) HF guidelines (page e153), HF is a "complex clinical syndrome caused by structural or functional impairment of ventricular filling or ejection of blood" (2). Left Ventricular Systolic Dysfunction (LVSD) precede the incidence of HF (3) (4) because of the impaired left ventricular function with reduced ejection fraction (LVEF) during systole (5). Approximately 50% of LVSD patients exhibit no apparent clinical manifestations making early diagnosis difficult (6). In Sri Lanka, CVDs are the leading cause of death among patients with non-communicable diseases (NCDs). Such incidents are augmented by the rapid expansion of the ageing community, the sedentary lifestyle of the local residents, unplanned urbanization and the effects of globalization (7). The impact on the quality of life of affected individuals is further complicated by the unequal distribution of healthcare facilities and poor affordability of high-level healthcare by the public in South Asia (8). Therefore, the most critical and vulnerable individuals face both delays in the identification of cardiovascular risks at an early stage and the benefit from advanced definitive diagnostic procedures such as 3D echocardiography. However, such consequences could be prevented to a considerable extent by utilizing alternative screening methods. Although previous studies have emphasized that considering serum N-terminal pro-Brain Natriuretic Peptide (NT-proBNP) measurement and electrocardiography (ECG) abnormalities is a cost-effective, early diagnostic methods for detecting LVSD (9), in Sri Lanka serum NT-proBNP test is still not widely used as a routine clinical diagnostic test in the state health sector.

NT- pro-BNP is an active type of neurohormone belonging to the Natriuretic Peptides (NP) produced by the cardiomyocytes of the left ventricle and then secreted into the blood to induce diuresis, natriuresis and vasodilation. Further, these neurohormones suppress fibrosis, hypertrophy and remodeling of heart muscle after myocardial infarction (10). NT-pro-BNP is more stable in blood in fact, three days in whole blood and up to 24 hours in EDTA samples (11) and exhibits a wider detection range than other NPs (12). NT-pro-BNP also exhibits less inter-patient variability (13), and accurately differentiates normal and impaired LVEF. Therefore, it can also be used to detect lesser degrees of LVSD (14).
In contrast, it was noted that the blood NT-pro-BNP levels vary according to comorbidities, age, gender, genetics, assay method. Therefore, its usage alone as a screening tool for detecting LVSD is debated and deciding a clear universal cut-off value is a challenge (15). As South Asians differ from western ethnicities in terms of anthropology, genetics, and socioeconomic background that affect health status, it is doubtful whether the NT-proBNP reference ranges and cut-off level, as determined in the previous western studies, can directly be applied to the South Asian communities. Although the use of NT-proBNP is beneficial for screening LVSD or HF, in Sri Lanka, serum NT-proBNP is still not widely used as a routine clinical diagnostic test. Therefore, the current study aimed specifically to assess the use of serum NT-pro-BNP in predicting LVSD in a hospitalized Sri Lankan population.

Materials and Methods
Study design, study population and the study sample
We conducted a hospital based prospective study to assess the associations of NT-proBNP with echocardiography findings and the utility of serum NT-pro-BNP in predicting LVSD in a high risk hospitalized population. This population were those referred for echocardiography to assess left ventricular function with a history of acute coronary syndrome (ACS), or other cardiovascular disorders/risk factors such as Hypertension, Hypercholesterolemia, Diabetes Mellitus (DM), Heart Block, Carditis. It also included patients with undefined chest pain, dyspnea, fever, or post-covid syndrome, admitted to the Teaching Hospital in Peradeniya, Sri Lanka. There were about 1000 echocardiography tests performed in each three-month period; hence the estimated sample size for the study was 278 individuals in total (16) who were selected by random sampling.

Blood sample collection, serum separation, and storage
Initially, with the informed written consent obtained from each selected individual referred for echocardiography, a venous blood sample of 3 ml was withdrawn into a clot activator tube; serum was then separated and stored at -80ºC freezer until analysis of NT-pro-BNP.

The Enzyme-Linked Immunosorbent Assay (ELISA) procedure for serum NT-pro-BNP level analysis
The serum samples were analyzed for NT-pro-BNP using the sandwich enzyme-linked immunosorbent assay (ELISA) kits (11) (17) imported from Wuhan Elabscience Biotechnology Company Ltd, Wuhan, Peoples Republic of China (Catalog No: E-EL-H6126). This ELISA kit uses Biotinylated Detection Antibody specific for human NT-proBNP and Avidin-Horseradish Peroxidase (HRP) conjugate to detect NT-proBNP. The skilled professional who carried out the ELISA procedure was blinded to the study participants' demographic and clinical data, and echocardiography findings. The analytical sensitivity of the ELISA method was 0.09 ng/mL (90 pg/mL) with a detection range of 0.16 -10 ng/mL (160 -10,000 pg/mL). The manufacturer claims that there is no significant cross-reactivity or interference

between Human NT-pro-BNP and analogues. The coefficient of variation (CV) is <10%. The ELISA kits were stored below 4°C until unpacking and all reagents were brought to room temperature (~25 °C) and the microplates were preheated (~25 °C) for 15 min, before the use. All the laboratory standards were maintained throughout the ELISA procedure, as instructed in the kit manual. The estimation of raw NT-proBNP values was based on the average optical density (OD) of raw absorbance in each microplate well which was then matched with standard curve formulated for each ELISA plate. All the NT-proBNP measurements less than 160pg/ml were considered as "results below the detection range".

The Echocardiography

On the same day of, or the day after the blood sample collection, each individual underwent echocardiography to identify left ventricular function. The medical officer who performed the echocardiography was blinded to the selected individuals' serum NT-proBNP values. The echocardiography studies were performed by Toshiba XarioXG SSA-680A echocardiography machine in 2D mode when the patient was in the left lateral position. The echocardiography report consisted of several measurements (e.g, LVEF, End Diastolic Volume, End Systolic Volume, diagnoses made through the presence of left ventricular hypertrophy (LVH), left ventricular dilatation, good/impaired left ventricular systolic function, good/impaired left ventricular diastolic function, good/impaired right ventricular function, the function of atrial valve/mitral valve, or the presence of congenital heart diseases).

The definition of LVSD

Based on echocardiography findings, the selected individuals were grouped as LVSD patients and non-LVSD individuals. LVSD was defined as LVEF ≤ 50% and Fractional Shortening (FS) ≤ 29% [18] [19] [20] while those with LVEF ≥ 60% who did not suffer from any concurrent major cardiovascular disorders (ACS, MI, Heart Block, Carditis, etc.) or have multiple risk factors for cardiovascular disorders (e.g. Hypertension, Hypercholesterolemia, Diabetes Mellitus (DM)) within the same patient, were considered as non-LVSD individuals. The following types of patients were excluded from the study sample.

- Patients diagnosed as having HF with preserved EF (HFpEF) or left ventricular diastolic dysfunction (LVDD), and valvular heart diseases.
- Patients whose LVEF was between 51% - 59% as we wanted to select LVSD patients and non-LVSD individuals according to the stated definition of LVSD.
- Patients diagnosed with bi-ventricular dysfunction and concurrent valvular heart disease.
- Patients diagnosed with Acute Kidney Injury, Chronic Kidney Disease or End Stage Renal Disease.
- Patients already on beta-blocker therapy
- Patients whose NT-proBNP level could not be detected by ELISA method or whose raw NT-proBNP level is less than the detection range of used ELISA kit.

Selected individuals were then interviewed for their demographic information, medical/surgical history, and clinical records.

Ethical Approval

The study was performed in accordance with the declaration of Helsinki, thus the ethical clearance (AHS/ERC/2018/097) for the study was obtained from the Ethics Review Committee of the Faculty of Allied Health Sciences, University of Peradeniya, Sri Lanka. The purpose of the study, potential benefits to the patient and to the society, the steps of data collection were explained in lay terms and the written informed consent from each participant was obtained prior to the venipuncture and echocardiography.

The statistical analysis

The 95% Confidence Interval (CI) and p=0.05 were used as the significance level to describe the results of the statistical analysis. Since the serum NT-pro-BNP level of the study population did not meet the parametric assumptions, nonparametric statistical tests were considered for data analysis. Comparison of NT-pro-BNP levels was done using independent samples Mann Whitney u test, median values (with interquartile range (IQR) and Kruskal -Wallis H test. Since the ELISA method was used for serum analysis, and there were no clear NT-pro-BNP cut-off values related to the ELISA method in Sri Lankan adult population, it was necessary to decide on an appropriate serum NT-pro-BNP level to differentiate LVSD in the current study population. Therefore, a receiver operating characteristic (ROC) curve was drawn in respect of serum NT-pro-BNP level and LVSD and the area under ROC curve (AUROC), varying degrees of sensitivities and specificities of NT-pro-BNP levels were considered to determine the optimum cut-off level to discriminate LVSD (LVEF < 50%) [21]. The correlations of NT-pro-BNP level with continuous variables (eg: echocardiography measures, age, routine blood investigations, blood pressure, etc.) were determined by Spearman's correlation coefficient (*rho*) and binary logistic regression was considered to determine the independent predictors of NT-pro-BNP above that of the proposed cut-off level. All the statistical analysis was performed by IBM SPSS version 26.

Results

The associations of clinico-epidemiological characteristics, comorbidities, and predisposing risk factors with LVSD

Of the 278 individuals who provided blood samples and underwent echocardiography, 78 were excluded as per the inclusion/exclusion criteria. Of the rest, serum NT-pro-BNP values could not be estimated at all in 27 samples and 32 samples indicated raw NT-proBNP level less than the lower detection limit (i.e. 160 pg/ml). Individuals with both types of samples were excluded from further data analysis. Therefore, from the remaining, 100 individuals were confirmed as LVSD patients, and 41 individuals were non-LVSD individuals. The baseline clinico-epidemiological characteristics of the study sample are

as depicted in table 1. No statistically significant difference was observed between the LVSD group and the non-LVSD group in terms of age distribution and gender. Several cardiovascular risk factors were observed in LVSD patients including prior episodes of coronary heart disease (CHD) in a major proportion (75%).

Based on the NYHA functional classification of HF, 10% of the LVSD patients and 97% (n=40) of the non-LVSD individuals belonged to class I followed by 51% of the LVSD patients and 3% (n=1) of the non-LVSD individuals in class II (Table 1).

Table 1: Baseline demographic and clinical characteristics of the LVSD and non-LVSD groups

Baseline demographic and clinical characteristics		LVSD group (N=100)	Non-LVSD group (N=41)	*p* value
Age in years		69.3 (±6.1)	71.4 (±2.4)	0.066**
Gender	Male	63	22	0.303*
	Female	37	19	
Alcohol Consumption	Current Alcohol Consumer	17	9	0.158*
	Ex-Alcoholic	25	4	
	Non-Alcoholic	58	28	
Tobacco Smoking	Current Smoker	7	4	0.204*
	Ex-Smoker	25	6	
	Non-Smoker	68	31	
Clinical manifestation	Shortness of Breath (SOB)	45	1	<0.001*
	SOB on Exertion	74	4	<0.001*
	Paroxysmal Nocturnal Dyspnea	70	2	<0.001*
	Orthopnea	73	5	<0.001*
	Lower leg swelling	48	3	<0.001*
	Fatigue	62	9	<0.001*
NYHA HF Classification	Class I	10	40	<0.001*
	Class II	51	1	<0.001*
	Class III	31	-	
	Class IV	8	-	
Hypertension (BP> 140/90 mmHg)		69	3	<0.001*
Coronary Heart Disease		75	-	<0.001*
Hypercholesterolemia		60	2	<0.001*
Diabetes Mellitus		32	3	0.002*

The frequency data for each categorical variable have been presented as number (n). The data for each continuous variable have been presented as mean ±SD. *p* value as derived by Pearson's chi squared test. ***p** value as derived by independent sample *t* test.

The routine blood investigation findings, blood pressure measurements, echocardiography measurements and ECG findings were also significantly higher among LVSD patients than that of non-LVSD individuals (Table 2).

Table 2: The average routine blood investigation findings, blood pressure measurements, echocardiography measurements and selected ECG parameters in LVSD and non-LVSD individuals

Investigation parameter	LVSD group (N=100)	Non-LVSD group (N=41)	*p* value
Hb level (g/dL)	11.65 (±1.6)	13.9 (±1.17)	<0.001*
Serum Creatinine (μmol/L)	90.08 (±27.7)	74.7 (±14.7)	0.001*
Blood Urea Nitrogen (mmol/L)	8.27 (±4.2)	5.08 (±2.45)	<0.001*
Serum K+ (mmol/L)	4.37 (±0.6)	4.0 (±0.24)	0.001*
C-Reactive Protein (mg/L)	46.28 (±45.6)	93.5 (±55.7)	<0.001*
Systolic BP (mmHg)	145.5 (± 18.0)	117.2 ±9.6	<0.001*
Diastolic BP	90.4 (±11.5)	75.9 ±8.0	<0.001*
Mean Arterial Pressure (mmHg)	107.4 (±13.9)	89.7 ±7.8	<0.001*
LVEF (%)	35.47 (±8.2)	62.3 ±1.4	<0.001*
FS (%)	18.35 (±3.9)	33.09 ±2.1	<0.001*
LV Mass (g)	237.38 (±46.1)	143.1 ±18.23	<0.001*
End Diastolic Volume (mL)	169.1 (±37.1)	79.3 ±14.1	<0.001*
End Systolic Volume (mL)	111.2 (±38.6)	29.9 ±5.8	<0.001*
QRS Duration (ms)	137.5 (±16.8)	107.3±9.5	<0.001*
Corrected QT interval (ms)	465.1(± 19.8)	425.7 ±24.8	<0.001*
Goldberger's 1st criterion (mV)	3.86(±0.8)	2.7 ±0.5	<0.001*

The data for each continuous variable have been presented as mean ±SD. *p value as derived by independent sample *t* test.

The distribution of serum NT-pro-BNP level in the study sample

The range of raw NT-proBNP among LVSD patients (n=100) was 164.52 – 6697.74 pg/mL with the mean NT-proBNP of 1073.0 pg/mL (±1345.74). NT-proBNP levels were undetectable in 27 samples while 32 samples contained raw NT-proBNP level less than the lower detection limit (160 pg/ml) of used ELISA kits. Individuals with both types of samples were excluded from further data analysis. Therefore, the range of raw NT-proBNP among non-LVSD individuals (n=41) was 160.0 – 688.84 pg/mL with the mean NT-proBNP of 277.29 pg/mL (±145.39). Considering the skewed distribution, the median NT-proBNP (with IQR) was 528.2 pg/mL (355.2 – 924.2) in the LVSD group and 207.36 pg/mL (177.52 – 343.0) among the non-LVSD individuals. The Mann Whitney U test revealed that the mean rank of NT-proBNP level in the LVSD group (85.73) was significantly different (p<0.001) than that of non-LVSD group (35.09) (Figure 1).
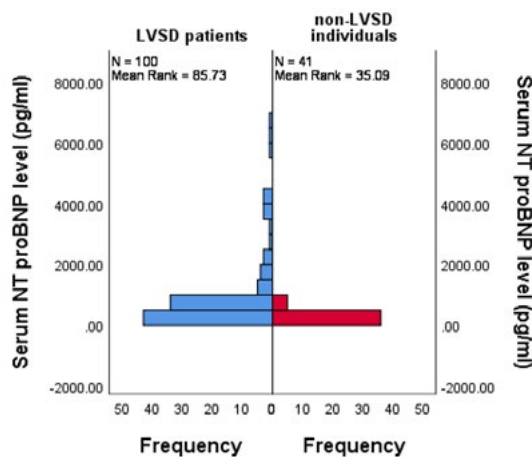

Figure 1. Independent samples Mann Whitney u test results for comparing the NT-proBNP levels in LVSD and non-LVSD groups

The Kruskal -Wallis H test (H (3) = 94.9, p<0.001) suggests that the NT-proBNP levels significantly increases through the increasing severity levels of LVSD (Figure 2). Similarly, the serum NT-proBNP levels increases significantly through the ascending levels of NYHA HF classes (H (3) = 81.4, p<0.001), and the NT-proBNP level in the NYHA HF class I is significantly different than that of NYHA HF class III and IV (Figure 2).
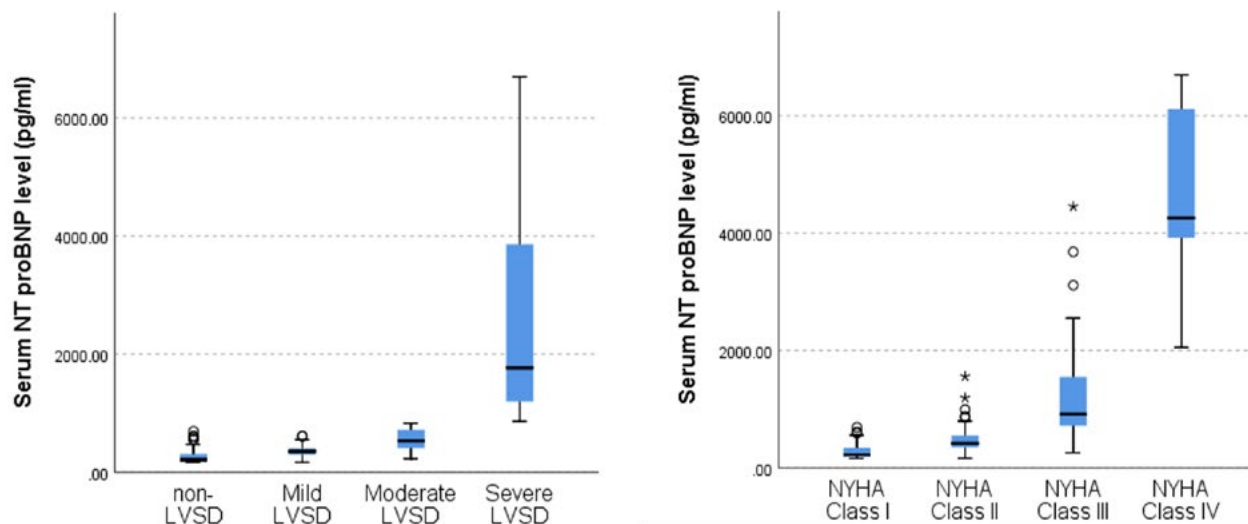

Figure 2: Median NT-proBNP levels among the LVSD severity categories: mild (LVEF= 40%-50%), moderate (LVEF= 30%-39%) and severe LVSD (LVEF= < 29%) categories (left). Median NT-proBNP levels among the NYHA HF classes (right). The probability values were derived from the independent samples Kruskal -Wallis H test. Boxes represent interquartile range (Q1 – Q3), while whiskers represent minimum and maximum values.

The NT-proBNP cut-off values and the predictive values to identify LVSD patients.

As depicted in Figure 3, The AUROC of NT-proBNP was 0.859 (95% CI: 0.79 – 0.92) which is significant in the discrimination of LVSD patients from non LVSD individuals. Based on the coordinates of ROC, 265 pg/mL presented with 90% sensitivity and 70% specificity. Therefore, with regard to the selected high-risk study sample, 265 pg/ml is considered as the optimum cut-off value of serum NT-proBNP in differentiating LVSD patients from non-LVSD individuals.
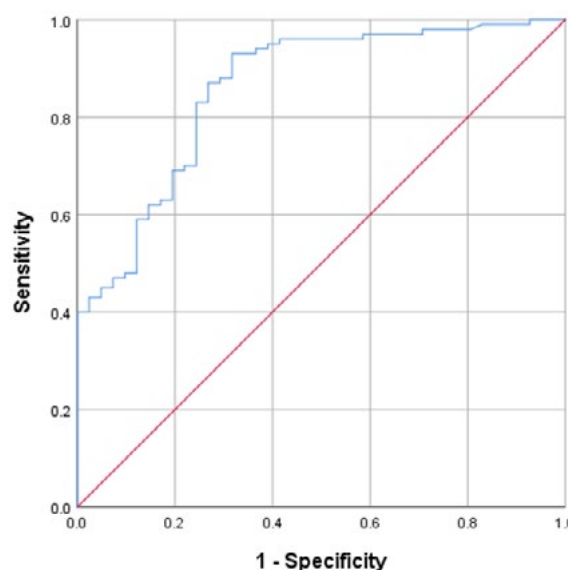

Figure 3. The ROC curve for NT-proBNP to predict LVSD in the selected elderly population. The AUROC is 0.859 (with SE = 0.034 and p < 0.001)

The correlations of serum NT-proBNP level with echocardiography, ECG, age and other covariates

Strong correlations of serum NT-proBNP level with LVEF (rho = -0.840, p <0.001), FS (rho = -0.815, p <0.001) and LV mass were (rho = 0.685, p <0.001) observed suggesting that NT-proBNP concentration in blood increases in parallel to deteriorating left ventricular functions and increasing left ventricular mass. Similarly, NT-proBNP level indicated strong positive correlations with ECG parameters such as corrected QT interval (rho = 0.69, p <0.001), Goldberger's 1st criterion (rho = 0.68, p <0.001) and QRS duration *(rho* = 0.68, p <0.001). Systolic blood pressure (rho = 0.72, p <0.001) and diastolic blood pressure (rho = 0.65, p <0.001) also indicated strong positive correlations with increased serum NT-proBNP level. It was also found that higher NT-proBNP levels are moderately correlated with lower hemoglobin level (rho = - 0.37, p <0.001). The older the study participants, the higher the NT-proBNP level was observed to be (rho = 0.40, p <0.001).

Though there were several clinical parameters that strongly correlated with NT-proBNP level, the binary logistic regression analysis revealed that elevated NT-proBNP level > 265pg/ml in the current study sample is independently associated with the age, LVEF, PND, orthopnea, history of CHD and LV mass only. Table 3 below presents the estimates of coefficients related to NT-proBNP level and associated covariates obtained by logistic regression.

Table 3: The statistically significant independent predictors of NT-proBNP level > 265 pg/ml based on binary logistic regression.

Covariate	Unstandardized Coefficients	SE	Wald	df	*p* value	*Exp(B)*	95% CI for EXP(B)	
LVEF	0.44	0.16	7.83	1	0.005	1.56	1.14	2.13
Age	-0.40	0.10	14.02	1	<0.001	0.66	0.54	0.82
PND	-2.32	1.17	3.91	1	0.048	0.09	0.01	0.97
Orthopnea	-2.45	1.23	3.93	1	0.047	0.08	0.008	0.97
CHD	-3.96	1.76	5.03	1	0.025	0.02	0.001	0.60
LV Mass	-0.05	0.02	7.04	1	0.008	0.94	0.90	0.98

SE= Standard Error, df= degree of freedom, Exp (B) = Odds ratio

Discussion

Our study is the first Sri Lankan study attempting to establish the associations of serum NT-proBNP level with demographic and echocardiography findings of LVSD patients. As hypothesized, the NT-proBNP level is significantly associated with impaired left ventricular function in the selected high risk study sample.

The skewed distribution of NT-proBNP level towards the lower LVEF values has been reported in several previous studies (17) (22) (23) where the mean, median and mean rank of serum NT-proBNP level were significantly higher in the LVSD group than the non-LVSD group. In a local study conducted in 2016, the mean NT-proBNP level in HF cases was relatively higher than in the current study. Though our study yielded a lower median NT-proBNP level than the previous local study, the range of NT-proBNP distribution in both studies was similar. One explanation of the different findings in two Sri Lankan studies is that NT-proBNP level is assay method dependent (24). In the previous study, this was measured by the minividas® auto analyser (25) whilst in the present study, the ELISA method was used. Use of the ELISA method for assessing NT-proBNP is not affected by the presence of different analogues such as bilirubin, hemoglobin, rheumatoid factor, triglycerides and biotin etc in the blood samples (11). A previous study that considered the ELISA method for assessing NT-proBNP emphasized that there is a 97% certainty of normal LVEF if NT-proBNP level is below the proposed cut-off level (ie: 97.2 pg/mL) in the particular study (17). In a study conducted in India in 2012, the mean NT-proBNP level was 1503.33pg/mL in left ventricular failure patients (26) which is slightly higher than the mean NT-proBNP observed in the present LVSD group. A study based in Pakistan which included congestive HF patients, reported a mean NT-proBNP level of 10 000pg/mL (27), obviously an extreme cut-off level when compared to the present study. Our study exhibited a considerable variance of serum NT-proBNP level through both the LVSD severity stages and the NYHA HF classes. Other studies have also shown increasing NT-proBNP median levels through worsening left ventricular function (17) and towards the higher NYHA HF classes (14). The results of the Dallas heart study also support that NT-proBNP level is significantly associated with the presence of LVSD (28). However, the current recommendation is that when a patient presents with a higher NYHA HF class, as evidenced by the symptoms and activity tolerance, the advanced diagnostic methods such as echocardiography should be employed directly rather than relying on NT-proBNP level (29). The main echocardiography parameters including LVEF and FS exhibited a strong negative correlation with the serum NT-proBNP, allowing for speculation that when the LVEF and FS decline, the serum NT-proBNP level gradually rises. This finding may be supported by the theory that declining LVEF and FS reflect the impaired function of the left ventricle resulting from more anatomical alterations of the myocardium with accelerating cardiomyocyte growth and increasing cardiomyocyte stretch (3)

(30). As a compensatory response to such structural alterations, higher concentrations of NT-proBNP are secreted into the blood, by the cardiomyocytes of the left ventricle (31). Similar patterns of strong negative correlations of NT-proBNP level with LVEF, as found in the present study, have also been explained by several other Western researchers (13) (32). Apart from the correlations of NT proBNP level with LVEF and FS, the findings of the present study indicated that the NT proBNP level was also significantly correlated with the abnormal ECG parameters such as corrected QT interval, Goldberger's 1st criterion and QRS duration. Therefore, it can be speculated that NT proBNP level and ECG characteristics are in accordance in predicting LVSD. Burke and Cotts in 2007 suggested that since NT-proBNP level is affected by various comorbidities and demographic factors, thus appropriate reference values for the specific individual groups must be determined (33). In the current study, the AUROC clearly distinguishes LVSD patients from non-LVSD individuals. Similarly, in the Pakistani study, the AUROC of NT-proBNP was 0.99 for diagnosing congestive HF (27). Consistent with the current study finding, the study by Verdu et al (2012) also found an AUROC of 0.94 for NT-proBNP to exclude HF (24) whilst two other overseas studies (4) (12) have also shown similar AUROCs.

In a previous Sri Lankan study, the identified optimal cut-off level of plasma NT-proBNP to exclude HF was lower (82.7pg/ml) (25); nevertheless, the relevant sensitivity, and specificity were not found to compare with the current study. In the Pakistani study, NT-proBNP level > 300pg/ml was 100% sensitive and 42% specific for identifying congestive HF (27) It has also been reported that NT-proBNP <300pg/ml is ideal for ruling out acute HF with a negative predictive value (NPV) of 99% (34). In the study by Verdu et al (2012) the optimal cut-off level to exclude HF was 280pg/ml (24). The NT-proBNP level of 150pg/ml exhibited a sensitivity of 94%, specificity of 40%, positive predictive value (PPV) 48% and NPV 92% (22). An NT-proBNP level of 100pg/ml was 88% sensitive for LVSD; however, the related AUROC was 0.77 (95% CI, 0.71 -0.82) for LVEF <50% (35). The most compelling explanation for such differences in cut-off levels in various studies is that NT-proBNP level is assay dependent (36) resulting in NT-proBNP cut off level applicable only for the selected method of assay. The report of Hammerer-Lercher et al (2004) also highlighted that there is a diversity of natriuretic peptide assays resulting in different cut-off levels, such that, importantly, the published cut-off/reference levels in literature are applicable only to the method where such studies were based (37). Apart from the age, weight and comorbidities, the diagnostic method also influences the performance characteristics leaving it a challenge when deciding universal reference values for NT-proBNP measurement (38). NT-proBNP level is also affected by the medications such as β blockers and diuretics, so it is advisable to test for NT-proBNP before the start of such medications, if facilities are available. However, the proposed cut-off level in the present study falls within the range of different cut-off levels as presented in the contemporary overseas studies. However, it cannot be claimed that the proposed cut-off level is the most suitable for screening for LVSD in the general population, because this study is limited to a high risk hospitalized population in Sri Lanka. Therefore, validation of the findings of this study should be carried out in a selected general population, perhaps outside the hospital setting to determine its applicability in the community screening. The ELISA procedure used in this study was only to obtain the serum NT-proBNP level rather than assessing the diagnostic performance of the used ELISA kits. Different studies have resulted in varying NT-proBNP levels, depending on the sample type (serum, plasma, tissue) and the assay procedure. Therefore, before utilizing a specific method of analysis, it is recommended that the diagnostic performance along with appropriate cut-off levels for each of target communities should be determined. In the present study, the advantages of assessing NT-proBNP such as availability, accessibility, and with no expertise needed for interpreting the results over echocardiography were considered for the applicability of NT-proBNP measurement in the local clinical services, regardless of their cost effectiveness. Therefore, a comprehensive validation study is recommended to evaluate the cost-effectiveness of assessing NT-proBNP in predicting LVSD. During the course of this study, we did not interfere with the pharmacological management of selected individuals; thus, the effects of medications on NT-proBNP level were not established. Equally, age standardized NT-proBNP cut-off levels must also be determined in a future study.

Conclusion

Against the Sri Lankan background of inconsistency of resources in the local health services, access to echocardiography and expertise in performing echocardiography is not always possible. Although previous studies in Western countries have reported that NT-proBNP measurement is a cost-effective strategy superseeding echocardiography, in Sri Lanka this blood investigation is still an expensive test which is performed mostly in the private sector laboratories. However, the findings of this current Sri Lankan study found that the NT-proBNP level exhibits significant diagnostic performances for LVSD patients, suggesting that NT-proBNP assessment is an appropriate test to be introduced at the local public sector hospitals. Providing the facilities to perform the NT-proBNP test at such hospitals will facilitate adequate evidence towards the early detection and the ruling out of LVSD. The NT-proBNP assay can also be used for the screening of vulnerable individuals in the community, especially at the NCD prevention clinics for periodic evaluation of 'at risk' groups such as those aged 50 years or over, living with uncontrolled hypertension, CHDs, hypercholesterolemia, and DM. By so doing those with LVSD can be ruled out or further referral for advanced diagnostic studies can be ensured at an early stage of the disease. Having observed significant correlations of NT-proBNP with other screening methods such as ECG, it is asserted that combining NT-proBNP measurement with ECG abnormalities increase the possibility for identifying

and ruling out LVSD when the patients present to health care settings that have limited diagnostic resources.

What is Already Known?

Previous studies of Western countries have reported that NT-proBNP measurement is a cost-effective strategy over echocardiography in predicting LVSD. However, there is a lack of information regarding the use of NT proBNP in South Asian region.

What this Study Adds?

The findings of this foremost Sri Lankan study found that the NT-proBNP level exhibited a significant diagnostic performance for LVSD patients in Sri Lanka, suggesting that NT-proBNP assessment is a valuable test to be introduced to the state health sector, in order to rule out LVSD at an early stage of the disease.

Declaration of Conflict of interests

The authors of the project declare that there is no conflict of interest with regard to the content of this manuscript.

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