Clinical Research

Mid-regional pro-atrial natriuretic peptide as a biomarker of left ventricular systolic dysfunction in patients with sepsis

Trisulo Wasyanto,[1] Guntur Hermawan[2]

pISSN: 0853-1773 • eISSN: 2252-8083
https://doi.org/10.13181/mji.v28i2.1952
Med J Indones. 2019;28:129–33

Received: May 03, 2017
Accepted: June 18, 2019

Authors' affiliations:
[1]*Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Sebelas Maret, Dr. Moewardi Hospital, Surakarta, Indonesia*
[2]*Department of Internal Medicine, Faculty of Medicine, Universitas Sebelas Maret, Dr. Moewardi Hospital, Surakarta, Indonesia*

Corresponding author:
Trisulo Wasyanto
Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Sebelas Maret, Dr. Moewardi Hospital, Jalan Kolonel Sutarto 132, Surakarta 57126, Indonesia
Tel/Fax: +62-271-663188
E-mail: trisulo.wasyanto@gmail.com

ABSTRACT

BACKGROUND Releasing cytokine pro inflammation in patients with sepsis (tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β) and IL-6) with other factors (mid regional pro atrial natriuretic peptide [MR-proANP] and TNF-α) will cause left ventricular systolic dysfunction (LVSD). This research aimed to prove MR-proANP as a biomarker of LVSD in sepsis, area under the curve (AUC), sensitivity, specificity, cut-off point and probability of MR-proANP and TNF-α as a biomarker of LVSD.

METHODS Non-experimental diagnostic test with cross sectional design and simple random sampling. Variable examined consisted of MR pro ANP, TNF-α and left ventricular ejection fraction (LVEF). LVSD if LVEF was ≤45%. Statistical analysis using 2 × 2 table and receiver operating characteristic curve using SPSS 22 for window.

RESULTS There were examined 71 patients from November 2013 to March 2014 in tertiary ICU of Moewardi Hospital. There were 22 patients with mild sepsis (30.9%), 40 patients with severe sepsis (56.4%) and 9 patients with septic shock (12.7%). The AUC value of MR-proANP level was 0.84 (95% CI 0.73–0.95), $p < 0.001$. Optimal cut off point was ≥225.95 pmol/l and diagnostic odd ratio (DOR) was 12.11. The AUC value of TNF-α level was 0.73 (95% CI 0.60–0.86), $p < 0.002$. Optimal cut-off point was ≥7.36 pg/ml and DOR was 5.03. Multivariate analysis was resulted that MR-proANP was the best predictor of LVSD (AUC 0.78), and TNF-α (0.69).

CONCLUSIONS MR-proANP could be used as a biomarker and the best diagnostic predictor of LVSD.

KEYWORDS biomarkers, left ventricular dysfunction, atrial natriuretic peptide, tumor necrosis factor-alpha

Sepsis remains a major health problem because of its high mortality and morbidity rates.[1] Sepsis may be associated with concurrent left ventricular systolic dysfunction (LVSD), and patients with LVSD that occurs during sepsis may have a poor prognosis. The release of proinflammatory cytokines with other factors such as local cytokine products, excessive production of nitric oxide, hypocalcemia, mitochondrial dysfunction, and apoptosis will cause myocardial depression characterized as LVSD.[2,3] Tumor necrosis factor-alpha (TNF-α) is the main proinflammatory cytokine during sepsis.[4] Atrial natriuretic peptide (ANP) represents a promising candidate biomarker, because the peptide inhibits both nuclear factor-kappa B (NF-kB) activation and TNF-α production in macrophages *in vitro*. ANP and ANP receptors are regulated by inflammatory stimuli, suggesting that ANP functions as a regulatory protein in inflammatory processes.[5] ANP is secreted into circulation by activated stimuli macrophages when sepsis occurs.[5,6] This process is also carried out by the atrium as the effect of either compliance or contraction–relaxation disorders.[6] ANP and pro-ANP are markers for congestive heart failure, and their pathophysiology and prognostics in severe sepsis and septic shock are not fully understood.[5]

Lipinska-Gediga et al[7] studied pro-ANP levels in patients with severe sepsis and septic shock, indicating that the pro-ANP level is significantly lower in survivors compared with those who died in the hospital. In a study conducted by Morgenthaler et al[8] on patients with sepsis treated in the intensive care unit (ICU), they proved that mid-regional pro-ANP (MR-proANP) has the same ability as the Acute Physiology and Chronic Health Evaluation score in predicting prognosis.[8] Thus, researchers are highly interested in examining the role of MR-proANP in sepsis that complicates the heart. This study is the first diagnostic research to investigate the main biomarker from MR-proANP and TNF-α in patients with sepsis to diagnose LVSD. Currently, clinicians use echocardiography to diagnose LVSD. Unfortunately, not all hospitals in Indonesia have an echocardiogram device. The discovery of biomarkers that can predict LVSD in patients with sepsis is expected to decrease morbidity and mortality rates, but a biomarker that detects LVSD is currently lacking. The diagnosis of LVSD may be possible in a peripheral hospital by simply measuring MR-proANP or TNF-α levels in serum. This research was aimed to prove the applicability of MR-proANP as a biomarker of LVSD in sepsis by analyzing the area under the curve (AUC), sensitivity, specificity, cut-off point, and probability of MR-proANP and TNF-α as biomarkers of LVSD in patients with sepsis.

METHODS

This research employed for diagnostic test a cross-sectional design. All patients with sepsis over 18 years old were included in this study after following simple random sampling by lottery. Sepsis was defined by the American College of Chest Physicians/ Society of Critical Care Medicine consensus criteria.[1] The exclusion criteria were all patients with sepsis and ischemic heart disease, heart failure, chronic kidney disease, and valvular heart disease, because these patients presented with LVSD. This study was approved by the Ethical Review Committee from Universitas Sebelas Maret, Dr. Moewardi Hospital (Approval number 124/X/ERC/2013) and informed consent was obtained from the patients or their legal representatives.

On the basis of the large formula of diagnostic research sample with AUC output, the sample size for this research was 68. We analyzed 71 patients so that the sample size remained sufficient if there were patients who dropped out. Blood samples were obtained immediately after the diagnosis of sepsis was established. Variables checked by the researcher were MR-proANP and TNF-α, which were measured by extracting blood serum from patients. All blood samples were centrifuged within 45 min after collection; the resulting plasma was frozen at -20°C until analyzed in a blinded fashion for this study by clinical laboratories.

MR-proANP levels were determined by using an automated immunofluorescent assay with reagent from B.R.A.H.M.S GmbH (16761 Hennigsdorf, Germany, Cat: BR819050, Lot: 19032B, with KRYPTOR tool, Thermo Scientific). The normal value was 85.2 pmol/l. TNF-α levels were examined by applying quantitative sandwich enzyme immuno-assay with human TNF-α/TNFSF1A HS (R&D systems). The reagent kit used for human TNF-α examination was a product of R&D Systems, Inc. (Minneapolis, MN 55413, USA, Cat No.: DTA00C, Lot: 312629). Immunoassay technique with human TNF-α was conducted with a Microplate Reader 680 series device. The normal value was 0.5 pg/ml. Transthoracic echocardiography examination was carried out by two cardiologists to determine LV systolic function/ejection fraction with Simpson method, and diastolic function was assessed by measuring E and A peak velocities using spectral Doppler of mitral inflow. LVSD occurred if left ventricular ejection fraction (LVEF) was ≤45%, whereas diastolic dysfunction occurred if E/A ratio <1 with Dct >240 ms or E/A ratio >2 with Dct <160 ms.[9] For statistical analysis, data were analyzed using 2 × 2 table, and receiver operating characteristic (ROC) curve was drawn using Statistical Package for the Social Sciences (SPSS) 22 for Windows.

RESULTS

This research was conducted from November 2013 to March 2014 in a tertiary ICU at Moewardi Hospital Surakarta Indonesia. The characteristics of research subjects are shown in Table 1.

The characteristics of patients with sepsis were grouped based on LVSD. No differences in gender (p = 0.520) and age (p = 0.160) were observed. First, the severity of sepsis (p < 0.001), TNF-α levels (p = 0.003), and MR-proANP levels (p < 0.001) for patients with LVSD differed from those without LVSD.

Table 1. Characteristics of research subjects

Variable	LVSD n (%), (n = 48)	Non-LVSD (n = 23)	*p*
Gender			
Male	18 (37.5)	8 (34.8)	0.520
Female	30 (62.5)	15 (65.2)	
Age (years), mean (SD)	57.2 (15.3)	51.5 (16.7)	0.160
Sepsis severity			
Mild sepsis	0 (0.0)	22 (95.7)	**<0.001**
Severe sepsis	39 (81.3)	1 (4.3)	
Septic shock	9 (18.8)	0 (0.0)	
TNF-α	12.62 (8.80–18.10)	5.04 (3.23–7.86)	**0.003**
MR-proANP	357.27 (285.43–447.30)	107.99 (78.05–149.45)	**<0.001**

LVSD=left ventricular systolic dysfunction; SD=standard deviation; TNF-α=tumor necrosis factor-alpha; MR-proANP=mid-regional pro-atrial natriuretic peptide

Table 2. Comparison of AUC in MR-proANP and TNF-α

Cut-off variable	AUC	*p*	95% CI
MR-proANP	0.84	<0.001	0.73–0.95
TNF-α	0.73	0.002	0.60–0.86

AUC=area under the curve; MR-proANP=mid-regional pro-atrial natriuretic peptide; TNF-α=tumor necrosis factor-alpha; CI=confidence interval

Table 3. Multivariate analysis of the AUC of MR-proANP and the combination of MR-proANP + TNF-α as a predictor of LVSD in patients with sepsis

Cut-off variable	AUC	*p*	95% CI
TNF-α	0.69	0.009	0.56–0.83
MR-proANP	0.78	<0.001	0.66–0.90
TNF-α + MR-proANP	0.85	<0.001	0.76–0.95

AUC=area under the curve; MR-proANP=mid-regional pro-atrial natriuretic peptide; TNF-α=tumor necrosis factor-alpha; LVSD=left ventricular systolic dysfunction, CI=confidence interval

The AUC of MR-proANP level was 0.84 (95% CI 0.73–0.95; $p < 0.001$). The optimal cut-off point was ≥225.95 pmol/l, with sensitivity of 77.1% and specificity of 78.3%. The diagnostic odds ratio (DOR) was 12.11 with MR-proANP probability ≥225.95 pmol/l. The occurrence of LVSD was 92.4%.

The AUC of TNF-α level was 0.73 (95% CI 0.60–0.86; $p = 0.002$) (Table 2). The optimal cut-off point was ≥7.36 pg/ml, with sensitivity of 68.8% and specificity of 69.6%. The DOR was 5.03 with TNF-α probability ≥7.36 pg/ml, and LVSD occurrence was 83.4%.

Bivariate analysis of LVSD and factors such as age, severity of sepsis, and levels of MR-proANP and TNF-α showed that MR-proANP and TNF-α had a value of $p < 0.25$. These variables were included in multivariate analysis.

Multivariate analysis demonstrated that MR-proANP was the best predictor of LVSD (AUC 0.78) and TNF-α (0.69). The combination of MR-proANP + TNF-α could increase the diagnostic value with AUC of 0.85, sensitivity of 77.1%, and specificity of 78.3% (Table 3).

DISCUSSION

This research was aimed to demonstrate the efficacy of MR-proANP and TNF-α as biomarkers of LVSD in sepsis, as well as identify the AUC, sensitivity, specificity, optimal cut-off points, and probability for MR-proANP and TNF-α as predictors of LVSD in sepsis. Furthermore, the goal of this study was to identify the best diagnostic predictor of LVSD in sepsis. Sepsis is the host's response to the infection process that occurs systemically and involves various organs of the body, including the heart. Sepsis occurs upon contact between pathogens and hosts. Pattern recognition receptors (PRRs) consisting of Toll-like receptor (TLR) and CD14 in the monocyte surface will specifically recognize pathogen-associated molecular patterns (PAMPs) that enter the body. The interaction between PAMPs and PRRs in the monocyte surface will result in the activation of proinflammatory (TNF-α, interleukin-1β [IL-1β], and IL-6) and anti-inflammatory cytokines (IL-4, IL-5, IL-6, and IL-10). Lipopolysaccharide will bind

with the CD14 receptor and TLR-4 in the monocyte surface. This interaction activates intracellular signal transduction involving various adaptor proteins such as myeloid differentiation protein 88, TNF receptor-associated factor, and kinase enzyme-like inhibitor kappa b kinase. This condition activates the transcription protein called NF-kB. NF-kB is a transcription protein in the cytoplasm activated by binding fragmentation with inhibitor kappa b. Subsequently, NF-kB enters into the nucleus monocyte, initiates RNA transcription, and encodes proinflammatory and anti-inflammatory cytokines. NF-kB activation plays an indispensable role in inducing the rapid production of TNF-α.[4,9] Releasing proinflammatory cytokines will cause myocardial depression characterized as LVSD.

In the past, the measurement of ANP levels in blood serum was technically difficult because of its short period half-life (3–4 min). Currently, the prohormone MR-proANP has been found to have a long period half-life of about 60–120 min. Therefore, the measurement technique can be carried out easily.[7] MR-proANP is secreted into circulation by activated stimuli macrophages when sepsis occurs.[5,7] The recent issues reported about sepsis' involvement with heart complications and LVSD are due to myocardial depression and decreased cardiac index.[9] To identify complications of LVSD in sepsis, cardiologists should use echocardiography, which is marked by a decrease in the LVEF.[9–11]

Myocardial depression at the beginning of sepsis is still reversible in 7–10 days; however, if sepsis worsens, the condition becomes irreversible because of septic shock, and patients may die.[12,13] Thus, early detection of LVSD is necessary to avoid irreversible shock either through a practical examination of echocardiography or biomarker examination. Manifestations of myocardial depression fall into systolic dysfunction (including impaired contractility and reduced ejection fraction, previously called LVSD) and diastolic dysfunction. The results of this research are expected to facilitate the diagnosis of patients with sepsis and LVSD simply by checking the biomarker MR-proANP (without using echocardiogram). Such a technique that may be useful in a peripheral hospital.

This research proved that MR-proANP could be used as a biomarker of LVSD in patients with severe sepsis. MR-proANP level was ≥225.95 pmol/l, which could be used to diagnose patients with sepsis and LVSD with sensitivity of 77.1% and specificity of 78.3%. The DOR was 12.11 with 95% CI 3.66–40.12. These values indicated that patients with sepsis and elevated levels of MR-proANP ≥225.95 pmol/l have a 12-fold chance of LVSD compared with patients without elevated levels of MR-proANP. The results of this study were in line with the results of research from Lipinska-Gediga et al[7] and Morgenthaler et al.[8] Lipinska-Gediga et al[7] studied pro-ANP levels in critically ill patients with severe sepsis and septic shock; they found that the level of pro-ANP is statistically lower in survivors than in non-survivors. Their ROC curve analysis of survival on the day of admission revealed that the cut-off value was 220.58 pmol/l, AUC = 0.72, sensitivity was 91%, and specificity was 53%. Research from Lipinska-Gediga et al[7] showed that the sensitivity of pro-ANP in diagnosing sepsis is high since the first day of hospitalization, whereas specificity increases daily in hospital care.[7] Morgenthaler et al[8] obtained the median MR-proANP value of 194 pmol/l in their patients with sepsis who survived in the hospital, and this value was significantly lower than that in the non-survivors (median 853.0 pmol/l; $p < 0.001$ and AUC value of 0.88). In addition, TNF-α level was ≥7.36 pg/ml, and the patients were diagnosed with sepsis and LVSD, with sensitivity of 68.8% and specificity of 69.6%.

Multivariate analysis using the ROC curve method revealed that the use of a single biomarker MR-proANP as a predictor of LVSD yielded an AUC value of

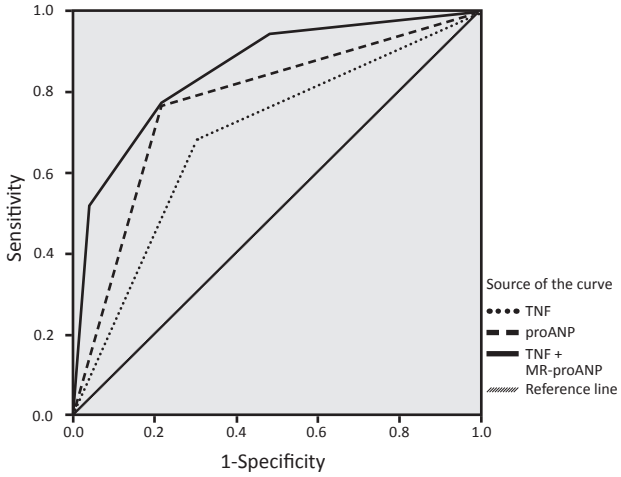

Figure 1. AUC chart of MR-proANP and the combination of MR-proANP+TNF-α as predictors of LVSD in patients with sepsis. ROC=receiver operating characteristic; TNF=tumor necrosis factor; MR-proANP=mid-regional pro-atrial natriuretic peptide; AUC=area under the curve

0.78, sensitivity of 77.1%, and specificity of 78.3%. This result was better compared with the values obtained for TNF-α (0.69). The use of double biomarkers MR-proANP + TNF-α as a predictor of LVSD showed that the AUC value of 0.85 was better compared with that of MR-proANP alone (0.78). However, the sensitivity (77.1%) and specificity (78.3%) were fixed and could increase the examination cost when compared with using MR-proANP alone (Figure 1). On the basis of the results of this study, the researchers recommend that MR-proANP examination should be performed routinely to diagnose patients with complicated sepsis to the heart and LVSD. Age does not affect the results of the MR-proANP examination.

This research had several limitations. First, some of patients with sepsis arrived late to the hospital (some patients were sick a few days at home or referred from another hospital). Thus, TNF-α was ineffective for use as a biomarker, because TNF-α levels dropped in the blood (the half-life is very short at only 18.2 min).[14] Second, this findings are descriptive in nature and need validation in future prospective studies.

In conclusion, MR-proANP could be used as a biomarker, and it was the best diagnostic predictor of LVSD in patient with sepsis (AUC 0.78). Routine MR-proANP examination is recommended to diagnose LVSD in patients with sepsis.

Conflict of Interest

The authors affirm no conflict of interest in this study.

Acknowledgment

None.

Funding Sources

None.

REFERENCES

1. Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2008. Crit Care Med. 2008;36(1):296–327.
2. Flierl MA, Rittirsch D, Huber-Lang MS, Sarma JV, Ward PA. Molecular events in the cardiomyopathy of sepsis. Mol Med. 2008;14(5–6):327–36.
3. Fernandes CJ Jr, de Assuncao MS. Myocardial dysfunction in sepsis: a large, unsolved puzzle. Crit Care Res Pract. 2012;2012:896430.
4. Kibe S, Adams K, Barlow G. Diagnostic and prognostic biomarker of sepsis in critical care. J Antimicrob Chemother. 2011;66(Suppl 2):ii33–40.
5. Ladetzki-Baehs K, Keller M, Kiemer AK, Koch E, Zahler S, Wendel A, et al. Atrial natriuretic peptide, a regulator of nuclear factor-κB activation *in vivo*. Endocrinology. 2007;148(1):332–6.
6. Kiemer AK, Vollmar AM. The atrial natriuretic peptide regulates the production of inflammatory mediators in macrophages. Ann Rheum Dis. 2001;60(Suppl 3):iii68–70.
7. Lipinska-Gediga M, Mierzchala M, Durek G. Pro-atrial natriuretic peptide (pro-ANP) level in patients with severe sepsis and septic shock: prognostic and diagnostic significance. Infection. 2012;40(3):303–9.
8. Morgenthaler NG, Struck J, Christ-Crain M, Bergmann A, Müller B. Pro-atrial natriuretic peptide is a prognostic marker in sepsis, similar to the APACHE II score: an observational study. Crit Care. 2005;9(1):R37–45.
9. Celes MR, Prado CM, Rossi MA. Sepsis: going to the heart of the matter. Pathobiology. 2013;80(2):70–86.
10. de Backer D, Cholley BP, Slama M, Vieillard-Baron A, Vignon P. Hemodynamic Monitoring Using Echocardiography in the Critically Ill. Septic Shock 2011.
11. Jozwiak M, Persichini R, Monnet X, Teboul JL. Management of myocardial dysfunction in severe sepsis. Semin Respir Crit Care Med. 2011;32(2):206–14.
12. Zanotti Cavazzoni SL, Guglielmi M, Parrillo JE, Walker T, Dellinger RP, Hollenberg SM. Ventricular dilation is associated with improved cardiovascular performance and survival in sepsis. Chest. 2010;138(4):848–55.
13. Griffee MJ, Merkel MJ, Wei KS. The role of echocardiography in hemodynamic assessment of septic shock. Crit Care Clin. 2010;26(2):365–82.
14. Oliver JC, Bland LA, Oettinger CW, Arduino MJ, McAllister SK, Aguero SM, et al. Cytokine kinetics in an in vitro whole blood model following an endotoxin challenge. Lymphokine Cytokine Res. 1993;12(2):115–20.