Table of contents

Original articles

87 Comparative study between dye assisted microsurgical Subinguinal Varicocelectomy versus Subinguinal Conventional Technique for treatment of primary varicocele
Wafi I. Aborahma, Mohamed M. Elwageh, Ahmed H. Elbarbary, Mohamed A. Elhenedy

94 Role of interventional radiology in maintaining the vascular access for hemodialysis patients
Amany Elkharboutly, Mohamed Raslan

102 Relation of metabolic syndrome to the presence and severity of coronary artery ectasia
Mohamed Naseem, Sameh Samir

107 Role of heparin-binding protein and brachial artery reactivity as prognostic tests in critically ill patients with sepsis
H. Ibrahim, M. Ragab, F. Rizk, I. Abbas, Talal I.M. Hagag

119 The role of multislice computed tomography in the diagnosis of gastric malignant tumors
Atf H. Teama, Amr M. El-Badry, Eslam S. Youssef

127 SYNTAX score II as a predictor of incomplete ST-segment resolution in patients with acute myocardial infarction treated with primary percutaneous intervention
Mohamed Naseem
Role of heparin-binding protein and brachial artery reactivity as prognostic tests in critically ill patients with sepsis

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Context
Early detection of severe sepsis is crucial for successful outcome.

We hypothesized that the progression of sepsis to severe sepsis is preceded by vascular leakage, which may be caused by neutrophil-derived mediators such as heparin-binding protein (HBP). Also, vascular leakage can be assessed by flow-mediated dilatation (FMD) and reactive hyperemia. Thus, both HBP and brachial artery reactivity may predict the progression of sepsis to severe sepsis and septic shock.

Aim
The aim of the study was to identify the role of both HBP and brachial artery reactivity as predictors of morbidity and mortality in critically ill septic patients.

Settings and design
This is an observational prospective controlled study.

Patients and methods
Patients were classified into two groups. Group I included 40 patients with evident sepsis. Group II included 10 critically ill nonseptic patients who constituted the control group. HBP blood samples were collected at three time points over 6 days after admission. Brachial artery reactivity measurements were also taken.

Statistical analysis
Statistical analysis was carried out on a personal computer using IBM SPSS Statistics (version 22).

Results
Significant difference was detected between survivors and nonsurvivors in maximum sequential organ failure assessment (SOFA) score, white blood cells, HBP at baseline and that at 48 and 96 h. The receiver operating characteristic curve using baseline HBP for prediction of severe sepsis shows a sensitivity of 94.7% and a specificity of 100% at cutoff level more than 1.9 ng/ml, and that for prediction of mortality shows a sensitivity of 91.6% and specificity of 100% at levels more than 1.9 ng/ml. Highly significant difference exists between survivors and nonsurvivors in FMD, baseline velocity, hyperemic velocity, velocity difference, and postdeflation resistance index (RI). Receiver operating characteristic curve analysis for prediction of severe sepsis/septic shock using FMD showed a sensitivity of 94.7%, specificity of 100%, and associated criterion 3.4% or less. Hyperemic velocity had a sensitivity of 100%, specificity of 100%, and associated criterion 39 cm/cardiac cycle or less.

Conclusion
Plasma HBP levels and brachial artery reactivity can predict severe sepsis and mortality in patients with sepsis.

Keywords:
brachial artery reactivity, flow-mediated dilatation, heparin-binding protein, hyperemic velocity, morbidity and mortality, septic shock, severe sepsis, survival analysis

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Introduction
Septic shock is the leading cause of mortality in patients admitted to the ICU [1,2]. Most severe infections are due to bacteria [3].

Heparin-binding protein (HBP) (also known as azurocidin and CAP37) is a multifunctional inflammatory mediator [4] released from neutrophils and induces vascular leakage [5]. HBP was recently proposed as a biomarker for the early detection of severe sepsis [6].

Severe sepsis is characterized by impaired microvascular blood flow [7]. Microvascular function can be noninvasively assessed by measuring reactive hyperemia (RH), the augmentation in limb blood flow occurring after a period of stagnant ischemia [8]. Ultrasound measurement of hyperemic velocity (HV), the maximal
velocity of blood flow after cuff deflation, can be used to assess RH [9–14]. Many of these studies reported associations between indices of RH and adverse outcomes of sepsis, but did not fully explore whether these associations were confounded by important variables such as vasopressor use, blood pressure, and comorbid conditions. Flow-mediated dilatation (FMD) is largely mediated by endothelial nitric oxide production [15,16], which is dysregulated with insufficient nitric oxide activity in sepsis [17–19]. Ultrasound measurements of brachial artery reactivity have recently been used to simultaneously assess conduit artery endothelial function (with FMD) and microvascular function (with HV) [9–13]. HV generates the brachial artery shear stress that is responsible for FMD, and hence the two measurements are clearly linked [20]. Nevertheless, in some studies of simultaneous FMD and HV measurements, HV alone (not FMD) has been associated with systemic inflammation [12], cardiovascular risk factors [11], and cardiovascular events [9]. Moreover, such studies reported that FMD and HV are at best weakly correlated [11–13], suggesting that they reflect different physiologic processes: FMD estimating conduit artery endothelial function and HV estimating RH and microvascular function [12–14].

Aim
The aim of the study was to identify the role of both HBP and brachial artery reactivity as predictors of morbidity and mortality in critically ill septic patients.

Patients and methods
The study included 50 patients admitted to the Critical Care Department, Cairo University, between January 2013 and January 2014. The patients were divided into two groups. Group I (GI) included 40 patients with evident sepsis. Group II (GII) included 10 patients as a control group who did not develop sepsis during their hospital stay. Two patients were excluded because of lack of definitive data on their real survival. Patients were subjected to the following:

(1) Detailed history taking including risk factors and comorbidities and a thorough clinical examination.
(2) Assessment of infection (onset, site of infection, cultures, and causative organisms).
(3) Laboratory workup:

Serial HBP and serial high-sensitivity C-reactive protein (CRP) (at baseline, 48, and 96 h) were routinely evaluated. Blood samples were collected in 5 ml tubes at the time of inclusion or on the morning of the following day (0 h) and after 48 and 96 h. Tubes were immediately centrifuged at 3000 rpm for 10 min and separate aliquots of the serum supernatants were stored at −20°C until analysis. The concentration of HBP was determined by ELISA. The researcher was blinded to patient samples.

(4) Brachial artery reactivity measurements were taken by registered sonographers as soon as possible after diagnosis of sepsis at any stage. Measurements were taken with a Fukuda US apparatus (Japan) probe 9–11 MHz, a portable Mindray apparatus (China) probe 9–11 MHz. Nonfasting patients were placed in the supine position with ~30° head elevation for at least 10 min before taking measurements. The brachial artery was imaged using a medial approach 2 cm above the antecubital fossa with the arm extended and the thumb pointed to the ceiling. The pulse-wave Doppler gate was positioned at a 60° angle within the center of the arterial lumen. A sphygmomanometric cuff was placed at the widest part of the forearm 1–2 cm distal to the antecubital fossa. Preocclusion two-dimensional gray-scale images and pulse-wave spectral Doppler recordings were obtained. The cuff was rapidly inflated to 200 mmHg (or to 50 mmHg above systolic blood pressure if the systolic blood pressure was >150 mmHg) for 5 min, and then rapidly and completely deflated. Pulse-wave spectral Doppler recordings were acquired for 15 s after cuff deflation. Two-dimensional images were obtained 30–90 s after deflation. The brachial artery diameter was measured at end-diastole, determined by the R wave from external monitoring. The diameter of the brachial artery was measured from the media–adventitia interface in the near field to the media–adventitia interface in the far field. A series of three diameter measurements were averaged at baseline and after deflation. The three maximal postdeflation diameter measurements were used. The percentage brachial artery FMD was calculated as the difference between brachial artery diameter after and that before occlusion divided by preocclusion brachial artery diameter. The velocity–time integral over a single cardiac cycle was calculated from the pulse-wave spectral Doppler tracing (cm/cardiac cycle). Baseline velocity is considered the average of three representative Doppler tracings before brachial artery occlusion. HV was considered the average of the three maximal Doppler tracings 0–15 s after cuff release [20].
Inclusion criteria
All septic patients at any stage of sepsis within 48 h of sepsis diagnosis were eligible for inclusion into the study [8].

Exclusion criteria
Patients with severe cardiomyopathy with left ventricular ejection fraction less than 30%, chronic hemodialysis patients, those with a history of solid organ or bone marrow/stem cell transplantation, patients with advanced liver failure (Child–Pugh grade C), patients taking drugs such as nitrate, β-blocker, Ca-channel blocker, which affects the heart rate and in response affect the velocity–time integral, and organic nitrate (vasodilators), those with active bleeding, patients with hematocrit less than 22% or less than 25% while taking vasopressors agents, pregnant women, and those undergoing hormone replacement therapy were excluded from the study.

Surgical patients with short ICU stay – for example, those with extradural hemorrhage with encephalopathy – were studied as controls.

Study design
This was a prospective case–control observational study.

Statistical analysis
Statistical analysis was carried out on a personal computer using IBM SPSS Statistics (version 22; IBM Corp., Armonk, New York, USA). Numerical variables were presented as median and interquartile range, and between-group differences were compared using the Mann–Whitney U-test. Categorical variables were presented as number and percentage and intergroup differences were compared using the Pearson χ²-test or Fisher’s exact test as appropriate. Receiver operating characteristic (ROC) curve analysis was used to examine the value of continuous variables for prediction of binary outcomes.

Survival analysis was carried out using the Kaplan–Meier method, and the log-rank test was used to compare individual survival curves. Multivariable binary logistic regression analysis was used to build up a predictive model for prediction of 28-day mortality using a combination of predictors. The predicted probability of 28-day mortality as estimated from the regression model was then used for plotting a ROC curve to assess the predictive value of the model. ROC curves derived from various combinations of predictors were compared using the DeLong method.

Results
On the basis of the hypothesis of use of both HBP and brachial artery reactivity as prognostic tests, we suggested that GI include patients with evident sepsis only so that measurements of HBP and brachial artery reactivity at admission, 48, and 96 h as well as patient outcomes could evaluate both tests for the development of severe sepsis and septic shock. GII included 10 critically ill patients who did not develop sepsis during their hospital stay (Table 1).

A significant difference was found between patients and controls in terms of age [GI (n=38): age=48.5 years (range: 48–54 years) and GII (n=10): age=60 years (range: 50–62 years); P=0.001] and number of organ dysfunctions [GI (n=38): 2 (range: 2–2) and GII (n=10): 1 (range: 1–1); P=0.001], whereas no significant difference was detected as regards sex, history of smoking, diabetes, hypertension, cardiac diseases, and time to duplex or logistic organ score (LOS) in the ICU.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group I (n=38)</th>
<th>Group II (n=10)</th>
<th>P-value</th>
<th>Survivors</th>
<th>Nonsurvivors</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48.5 (48–54)</td>
<td>60 (50–62)</td>
<td>0.001</td>
<td>48.5 (48–54.5)</td>
<td>60 (50–62)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>30</td>
<td>9</td>
<td>0.661</td>
<td>11 (91.7)</td>
<td>28 (77.8)</td>
<td>0.416</td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td>1</td>
<td></td>
<td>11 (8.3)</td>
<td>8 (22.2)</td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>14</td>
<td>1</td>
<td>0.14</td>
<td>2 (16.7)</td>
<td>13 (36.1)</td>
<td>0.292</td>
</tr>
<tr>
<td>HTN</td>
<td>15</td>
<td>2</td>
<td>0.298</td>
<td>2 (16.7)</td>
<td>15 (41.7)</td>
<td>0.169</td>
</tr>
<tr>
<td>Smoking</td>
<td>15</td>
<td>3</td>
<td>0.722</td>
<td>3 (25)</td>
<td>15 (41.7)</td>
<td>0.493</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>2</td>
<td>0</td>
<td>1.00</td>
<td>0 (0.0)</td>
<td>2 (5.6)</td>
<td>1.00</td>
</tr>
<tr>
<td>Time to duplex</td>
<td>26 (24–30)</td>
<td>24 (20–26)</td>
<td>0.215</td>
<td>24 (22–28)</td>
<td>26 (24–30.5)</td>
<td>0.382</td>
</tr>
<tr>
<td>LOS in ICU</td>
<td>10.5 (5–20)</td>
<td>6 (6–8)</td>
<td>0.055</td>
<td>6 (4–7)</td>
<td>11 (6–21)</td>
<td>0.008</td>
</tr>
<tr>
<td>Number of dysfunctional organ</td>
<td>2 (2–2)</td>
<td>1 (1–1)</td>
<td>0.001</td>
<td>1 (0–1)</td>
<td>2 (2–2)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are given as median (range) or n (%). DM, diabetes mellitus; HTN, hypertension.
A highly significant difference was detected between survivors and nonsurvivors in age [GI (n=38): age=48.5 years (range: 48–54 years) and GII (n=10): age=60 years (range: 50–62 years); P<0.001] and number of dysfunctional organ survivors [1 (range: 0–1)] and nonsurvivors [2 (range: 2–2)] (P<0.001).

A highly significant difference existed between patients and controls in multigorgan dysfunction score (MODS) score (P<0.001), vasopressor use (P<0.001), ICU-free days (P<0.001), organ failure-free days (P<0.001), ventilator-free days and 28-day mortality (P<0.001) (Table 2).

Significant difference between survivors and nonsurvivors was detected in admission MODS (P<0.001), SOFA (P<0.001), acute physiology and chronic health evaluation score version 2 (APACHE II) (P<0.001), and Charlson’s (P<0.001) scores. Galasko coma score (GCS) was not significant at admission as most survivors were critically ill trauma controls (Table 3).

A highly significant difference was found between GI and GII in baseline HBP (P<0.001), HBP at 48 h (P<0.001), and that at 96 h (P<0.001) (Table 4).

We found a statistically significant difference between survivors and nonsurvivors in HBP at baseline (P=0.012), at 48 h (P=0.007), and at 96 h (P=0.004) and a highly significant difference in white blood cells at baseline (P<0.001), at 48 h (P<0.001), and at 96 h (P<0.001). CRP was not predictive of mortality at baseline, 48, and 96 h (Table 5).

Figure 1 shows ROC curve analysis for prediction of severe sepsis and septic shock using HBP levels at 96 h: area under the curve (AUC)=1, P-value less than 0.0001, sensitivity=100%, specificity=100%, and associated criterion more than 1.6 ng/ml.

Figure 2 shows ROC curve analysis for prediction of mortality using HBP level at 96 h: AUC=1, P-value less than 0.0001, sensitivity=100%, specificity=100%, positive predictive value (PPV)=100%, negative predictive value (NPV)=100%, associated criterion more than 1.6 ng/ml.

Highly significant differences existed between GI and GII in FMD (P<0.001), baseline velocity (P<0.001), HV (P<0.001), velocity difference (P<0.001), and postdeflation resistance index (RI) (P<0.001), where the results for FMD% were 2.4 (range: 1.9–2.8) in GI and 5.5 (range: 4.7–5.8) in GII, that for baseline velocity (cm/cardiac cycle) was 12 (range: 9.7–14.6) in GI and 20.05 (range: 20–22) in GII, that for HV (cm/cardiac cycle) was 21.45 (range: 18–25) in GI and 54 (range: 50–55) in GII, that for velocity difference (cm/cardiac cycle) was 8.6 (range: 8–11) in GI and 32.5

Table 2 Morbidity and mortality in comparison of cases (group I) and controls (group II)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group I [n (%)]</th>
<th>Group II [n (%)]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MODS</td>
<td>30 (78.9)</td>
<td>2 (20.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cardiac arrest</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>NA</td>
</tr>
<tr>
<td>Vasopressors used</td>
<td>36 (94.7)</td>
<td>0 (0.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Shock at duplex examination</td>
<td>3 (7.9)</td>
<td>0 (0.0)</td>
<td>0.594</td>
</tr>
<tr>
<td>ICU-free days</td>
<td>2 (5.3)</td>
<td>10 (100.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Organ failure-free days</td>
<td>2 (5.3)</td>
<td>10 (100.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ventilator-free days</td>
<td>2 (5.3)</td>
<td>10 (100.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>28-day mortality</td>
<td>36 (94.7)</td>
<td>0 (0.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Readmission to ICU</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>NA</td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survived</td>
<td>2 (5.3)</td>
<td>10 (100.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Died</td>
<td>36 (94.7)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 Scoring system in survivors and nonsurvivors on admission and discharge

<table>
<thead>
<tr>
<th>Variables</th>
<th>Admission</th>
<th>Discharge</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Survivors [n]</td>
<td>Nonsurvivors [n]</td>
<td>P-value</td>
</tr>
<tr>
<td>GCS</td>
<td>12.5 (12–13)</td>
<td>12 (9–14)</td>
<td>0.136</td>
</tr>
<tr>
<td>MODS score</td>
<td>4 (3–5)</td>
<td>6 (6–8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SOFA score</td>
<td>4 (3–5)</td>
<td>6 (6–7)</td>
<td>0.001</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>10 (7.5–11.5)</td>
<td>23 (21–27)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Charlson’s score</td>
<td>1 (0–2.5)</td>
<td>4.5 (4–6)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
### Table 4Laboratory parameters in group I and group II at baseline, 48, and 96 h

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline</th>
<th>48 h</th>
<th>96 h</th>
<th>Group I</th>
<th>Group II</th>
<th>Group I</th>
<th>Group II</th>
<th>Group I</th>
<th>Group II</th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (μg/ml)</td>
<td>30.5 (25–35)</td>
<td>31.5 (26–35)</td>
<td>30 (26–35)</td>
<td>0.435</td>
<td>31.5 (26–35)</td>
<td>30 (27.8–35.3)</td>
<td>1.000</td>
<td>3.0 (26–35)</td>
<td>3 (25.5–31.5)</td>
<td>1.000</td>
<td>3.0 (26–35)</td>
</tr>
<tr>
<td>HBP (ng/ml)</td>
<td>5.8 (4.6–6.5)</td>
<td>6.05 (4.6–7.2)</td>
<td>6.05 (4.6–7.2)</td>
<td>&lt;0.001</td>
<td>6.05 (4.6–7.2)</td>
<td>1.2 (1–1.28)</td>
<td>&lt;0.001</td>
<td>5.95 (4.5–6.6)</td>
<td>1.12 (0.75–1.4)</td>
<td>&lt;0.001</td>
<td>6.2 (4.5–6.6)</td>
</tr>
<tr>
<td>WBC (×1000/mm³)</td>
<td>15.55 (12.5–19.2)</td>
<td>12.5 (10.7–19.5)</td>
<td>12.5 (10.7–19.5)</td>
<td>0.021</td>
<td>12.5 (10.7–19.5)</td>
<td>9 (8.9–11)</td>
<td>0.006</td>
<td>13.2 (9.9–16.8)</td>
<td>8 (7.25–8)</td>
<td>0.004</td>
<td></td>
</tr>
</tbody>
</table>

CRP, C-reactive protein; HBP, heparin-binding protein; WBC, white blood cell.

### Table 5Laboratory parameters in survivors and nonsurvivors

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline</th>
<th>48 h</th>
<th>96 h</th>
<th>Survivors</th>
<th>Nonsurvivors</th>
<th>Survivors</th>
<th>Nonsurvivors</th>
<th>Survivors</th>
<th>Nonsurvivors</th>
<th>Survivors</th>
<th>Nonsurvivors</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (μg/ml)</td>
<td>30 (25–33.5)</td>
<td>31 (26–35)</td>
<td>30 (26–35)</td>
<td>0.719</td>
<td>31 (28.25–35.75)</td>
<td>30.5 (26–34.5)</td>
<td>0.521</td>
<td>30 (25.5–31.5)</td>
<td>30 (26–35)</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>HBP (ng/ml)</td>
<td>1.2 (0.4–1.6)</td>
<td>1.2 (1.2–1.7)</td>
<td>1.2 (1.2–1.7)</td>
<td>0.012</td>
<td>1.2 (1.2–1.7)</td>
<td>6.2 (4.8–7.5)</td>
<td>0.007</td>
<td>1.12 (0.8–1.4)</td>
<td>6.0 (4.5–6.6)</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>WBC (×1000/mm³)</td>
<td>12.5 (10–14.9)</td>
<td>10.5 (9–11)</td>
<td>10.5 (9–11)</td>
<td>&lt;0.001</td>
<td>10.5 (9–11)</td>
<td>12.25 (10.7–19.6)</td>
<td>&lt;0.001</td>
<td>8 (7.25–8)</td>
<td>13.2 (9.9–16.8)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

CRP, C-reactive protein; HBP, heparin-binding protein; WBC, white blood cell.
(range: 30–35) in GII, and postdeflation RI was 0.775 (range: 0.71–0.8) in GI and 0.53 (range: 0.48–0.54) in GII (Table 6).

Figure 3 shows Box plot showing FMD in both study groups with highly significant difference between the two groups ($P<0.001$). The central box represents the values from the lower to the upper quartile (25th–75th percentile). Middle line represents the median. Error bars extend from the minimum to the maximum value.

Figure 4 shows Box plot showing highly significant difference between GI and GII in baseline velocity ($P<0.001$), HV ($P<0.001$), and velocity difference ($P<0.001$).

Figure 5 shows Box plot showing highly significant difference between survivors and nonsurvivors in FMD ($P<0.001$). The central box represents the values from the lower to the upper quartile (25th–75th percentile). The middle line represents the median. Error bars extend from the minimum to the maximum value.

Table 6  Duplex parameters in group I and group II

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group I</th>
<th>Group II</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preocclusion diameter (cm)</td>
<td>4 (3.5–4.9)</td>
<td>4.4 (3.4–5.1)</td>
<td>0.430</td>
</tr>
<tr>
<td>Postdeflation diameter (cm)</td>
<td>4.1 (3.6–5)</td>
<td>4.65 (3.6–5.4)</td>
<td>0.263</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>2.4 (1.9–2.8)</td>
<td>5.5 (4.7–5.8)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Baseline velocity (cm/cardiac cycle)</td>
<td>12 (9.7–14.6)</td>
<td>20.05 (20–22)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Hyperemic velocity (cm/cardiac cycle)</td>
<td>21.45 (18–25)</td>
<td>54 (50–55)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Velocity difference (cm/cardiac cycle)</td>
<td>8.6 (6–11)</td>
<td>32.5 (30–35)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Preocclusion peak velocity (cm/cardiac cycle)</td>
<td>61 (51–67)</td>
<td>59.5 (53–64)</td>
<td>0.453</td>
</tr>
<tr>
<td>Postdeflation peak velocity (cm/cardiac cycle)</td>
<td>65 (60–70)</td>
<td>64 (61–67)</td>
<td>0.770</td>
</tr>
<tr>
<td>Preocclusion RI</td>
<td>0.8 (0.72–0.85)</td>
<td>0.745 (0.71–0.76)</td>
<td>0.093</td>
</tr>
<tr>
<td>Postdeflation RI</td>
<td>0.775 (0.71–0.8)</td>
<td>0.53 (0.48–0.54)</td>
<td>$&lt;0.001$</td>
</tr>
</tbody>
</table>

FMD, flow-mediated dilatation; RI, resistance index.
Figure 6 shows Box plot showing baseline velocity, hyperemic velocity, and velocity difference in survivors and nonsurvivors.

Figure 7 shows Box plot showing preocclusion and postdeflation resistance index (RI) in survivors and nonsurvivors.

Figure 8 shows ROC curve analysis for prediction of severe sepsis/septic shock using flow-mediated dilatation (FMD): AUC = 0.97, P-value less than 0.0001, sensitivity = 94.7%, specificity = 100%, PPV = 100%, NPV = 83.3%, and associated criterion 3.4% or less.

Figure 5 shows Box plot showing flow-mediated dilatation (FMD) in survivors and nonsurvivors.

Figure 6 shows Box plot showing highly significant difference in baseline velocity and HV between survivors and nonsurvivors (P<0.001). The central box represents the values from the lower to the upper quartile (25th–75th percentile). The middle line represents the median. Error bars extend from the minimum to the maximum value, excluding outside values, which are displayed as separate points (rounded markers). An outside value is defined as a value that is smaller than the lower quartile minus 1.5 times the interquartile range, or larger than the upper quartile plus 1.5 times the interquartile range (inner fences).

Figure 7 shows Box plot showing preocclusion and postdeflation RI in survivors and nonsurvivors. There is a highly significant difference in postdeflation RI (P<0.0001). The central box represents the values from the lower to the upper quartile (25th–75th percentile). The middle line represents the median. Error bars extend from the minimum to the maximum value, excluding outside values, which are displayed as separate points (rounded markers). An outside value is defined as a value that is smaller than the lower quartile minus 1.5 times the interquartile range, or larger than the upper quartile plus 1.5 times the interquartile range (inner fences).

Figure 8 shows ROC curve for prediction of severe sepsis/septic shock using hyperemic velocity (HV).

Figure 9 shows ROC curve for prediction of severe sepsis/septic shock using flow-mediated dilatation (FMD).
Figure 9 shows ROC curve analysis for prediction of severe sepsis/septic shock using hyperemic velocity: AUC=1, P-value less than 0.0001, sensitivity=100%, specificity=100%, PPV=100%, NPV=100%, and associated criterion 39 cm/card cycle or less.

Figure 10 shows ROC curve analysis for prediction of mortality using flow-mediated dilatation (FMD): AUC=1, P-value less than 0.0001, sensitivity=100%, specificity=100%, PPV=100%, NPV=100%, and associated criterion 3.4% or less.

Figure 11 shows ROC curve analysis for prediction of mortality using hyperemic velocity (HV): AUC=1, P-value less than 0.0001, sensitivity=100%, specificity=100%, PPV=100%, NPV=100%, and associated criterion 39 cm/cardiac cycle or less.

Figure 12 shows ROC curve analysis for prediction of mortality using postdeflation resistance index (RI): AUC=0.999, P-value less than 0.0001, sensitivity=97.2%, specificity=100%, PPV=100%, NPV=92.3%, and associated criterion more than 0.64.

Kaplan–Meier survival analysis for patients with FMD less than 3.4% or more than or equal to 3.4% shows a significant P-value (0.046) (Fig. 13).

Kaplan–Meier survival analysis for patients with HV less than 30 or more than 30 cm/cardiac cycle shows a significant P-value (0.046). Velocity difference less than 13 or more than 13 cm/cardiac cycle shows a significant P-value (0.046) (Fig. 14).
In our study, brachial artery reactivity was measured in a median 24 h (interquartile range: 20–26 h) in the control group (GII) and a median 26 h (interquartile range: 24–30 h) in GI from the time of admission without any significant difference between the two groups. In a comparative study by Wexler et al. [8], brachial artery reactivity was measured 41 h (range: 30–57 h) after patients met the severe sepsis diagnostic criteria. In our study only three patients of GI were in septic shock at duplex examination and at the time of presentation without any significant difference with GII. In a comparative study [8] 28% required vasopressor infusion when brachial artery reactivity was measured.

In our study GI had significantly lower FMD versus GII (controls) \(P<0.001\). GI also had significantly lower HV versus GII \(P<0.001\).

In a comparative study GI had significantly lower FMD [2.68% (range: 0.81–4.19%)] compared with GII [4.11% (range: 3.06–6.78%)] \(P<0.001\) as well as lower HV [34 cm/cardiac cycle (range: 25–48 cm/cardiac cycle)] versus controls [63 cm/cardiac cycle (range: 52–81 cm/cardiac cycle)] \(P<0.001\).

Stratified analysis showed that association between severe sepsis and FMD depends on age category. For participants younger than 60 years (the median age of cases and controls combined) FMD is lower in severe sepsis patients [2.65 (range: 0.91–4.13)] than in controls [4.82 (range: 3.76–8.33)] \(P<0.001\).

For patients older than 60 years FMD was similar in severe sepsis patients [2.8% (range: 0.77–5.31%)] and controls [3.56% (range: 1.8–5.92%)]. The test for interaction was significant \(P<0.002\), confirming the relation between FMD and severe sepsis based on age.

Pre-existing age-related endothelial dysfunction or loss of arterial compliance, or both, may explain these findings as reported by Witte et al. [21]. In our study, the average age of survivors was 48.5 years (range: 48–54.5 years) and that in nonsurvivors was 60 years (range: 50–62 years). Thus, our study age is less than that in the compared group, which may explain the difference in FMD results. Further, the compared study examined a high percentage of shocked patients on vasopressors, which may have also been responsible for the difference in the results. Brachial artery FMD was independently associated with sepsis after controlling for all variables in patients younger than 60 years but not in older individuals [8].

Further, we used external monitor R wave to freeze, which was not synchronized with an ultrasound machine – that is, patients were monitored through externally recorded telemetry tracing. However, if the apparatus with ECG gating cable connected to the patient and apparatus not available, we can use apparatus not gating and patient connected to external monitoring not to the apparatus [22].

### Relation to outcome and severity of illness

In our study FMD% was significantly higher in survivors compared with that in nonsurvivors, which is in agreement with the study by Vaudo et al. [23].

In the study by Wexler et al. [8] FMD tended to be nonsignificantly lower in nonsurvivors \(P=0.12\), probably due to the higher percentage of shocked patients on vasopressors. In our study, HV and change in velocity (HV–baseline velocity) were statistically higher in survivors than in nonsurvivors, which is in agreement with the study by Wexler et al. [8].

### Secondary outcome and survival

In our study HV and FMD were significantly positively correlated with the number of organ failure cases, 28-day ICU, and ventilation-free days. Similar results were obtained with HV but not with FMD in the study by Wexler et al. [8].

Our finding is in agreement with that of DeBacker et al. [7], who studied 42 septic patients. FMD in septic patients was significantly lower than that in healthy controls. Most of the nonsurvivors (86%) showed a decline in sequential FMD analyses, whereas only 43% of survivors showed a reduction in FMD \(P=0.01\).

Our finding also goes hand in hand with the results of Vaudo et al. [23], who found that septic patients with lower FMD at hospital admission experienced worsening severity of illness (SOFA score) over time. Vaudo et al. [23] selected patients with Gram-negative sepsis and excluded patients with diabetes mellitus, hypertension, smoking, hyperlipidemia, and
obesity. In addition, their patients were younger and without organ dysfunction at enrollment. In contrast, Wexler et al. [8] included nonselective consecutive patients with severe sepsis who were older, had higher comorbidities, and had greater severity of illness compared with those in the study by Vaudo et al. [23]; these characteristics probably blunted FMD in the study by Wexler et al. [8].

In our study, patients in GII were younger than patients in GI but were in the same age bracket and younger than 65 years, which may explain the highly significant FMD result when we compare the two groups, similar to the result of Vaudo et al. [23].

In our study Kaplan–Meier survival analysis between patients with FMD less than 3.4% and those with FMD more than 3.4% revealed significant differences (P=0.046), in contrast to the result of Wexler et al. [8].

In our study Kaplan–Meier survival analysis between patients with HV less than 30 and those with HV more than 30 cm/cardiac cycle as well as between those with velocity difference less than 13 or more than 13 cm/cardiac cycle revealed significant difference (P=0.046), similar to the result of Wexler et al. [8].

Implications of low hyperemic velocity in severe sepsis
Our findings are consistent with previous studies showing that other indices of RH (reactive hyperemia) as FMD (flow mediated dilation) and HV (hyperemic velocity) are reduced in sepsis [24–29], associated with illness severity [30–32], and associated with ICU mortality [33].

Our study shows that HV is independently associated with hospital mortality.

Heparin-binding protein levels as predictors of mortality
The 38 patients with mainly pulmonary and urinary tract infection were considered as GI and were referred to as the sepsis group. Only three patients developed septic shock at admission. GII (the control group) consisted of 10 patients who did not show any evidence of infection.

Blood culture and sensitivity was obtained from all patients but only 35 patients were positive (92.1%); there was no growth in three patients.

Linder et al. [34] studied a total of 179 patients: 151 patients formed the sepsis group (n=151) and the remaining 28 were ICU patients (the control group) who had been admitted for noninfectious conditions.

No control patient developed infections or shock at admission. However, 14 (50.0%) patients developed an infection later, and 24 (86%) were given antibiotics.

In our study HBP (ng/ml) in GII was significantly lower than that in GI at baseline, 48, and 96 h with highly significant difference (P<0.001).

The same results were obtained by Linder et al. [34], in whose study HBP levels were significantly higher in the sepsis group compared with that in controls at enrollment.

In our study no significant difference was found between Gram-positive and Gram-negative bacteremia, with similar results obtained by Linder et al. [34].

Serial heparin-binding protein measurements
The median HBP levels were higher at each sampling time point among nonsurvivors compared with survivors in contrast to high-sensitivity CRP. These results go hand with hand with those of Linder et al. [34].

ROC analysis was used for prediction of mortality at baseline, 48, and 96 h at HBP cutoff levels more than 1.9, more than 1.8, and more than 1.6, respectively (P<0.0001).

But Kaplan–Meier survival analysis of 28-day mortality at baseline, 48, and 96 h revealed no evidence for interaction. This result agrees with that of Linder et al. [34], in whose study 28-day mortality increased four-fold among sepsis patients with initial HBP more than 15 mg/ml.

Predictors of mortality
ROC curve for prediction of 28-mortality using the HBPmax gave an AUC=1, P-value less than 0.0001, sensitivity 100%, specificity 100%, PPV=100%, NPV=100%, and associated criterion more than 3.8.

ROC curve analysis for prediction of 28-mortality using HBPmax, FMD, and HV gave an AUC=1, P-value less than 0.0001, sensitivity 100%, specificity 100%, PPV=100%, NPV=100%, and associated criterion more than 0.
ROCs curves for prediction of 28-mortality using SOFA\textsubscript{max} and APACHE II score compared with those using HBP\textsubscript{max}, FMD, and HV did not show any difference between the two groups.

In our study there was no difference between variable as SOFA\textsubscript{max} and APACHE II on one side and all our tests (HBP, HV, and FMD) on the other side in prediction of mortality, but our tests (HBP, HV, and FMD) have very high predictive value for morbidity (severe sepsis and septic shock) which is lost in universal scores (SOFA\textsubscript{max} and APACHE II), our tests in the level of microvascular changes, which occurs early in the pathogenesis of sepsis before the scenario of MODS starts so the question why we delay to the start of the fatal scenario and predict that with universal scores (SOFA and APACHE II) and then start to give action (e.g. admission in ICU).

**Conclusion**

Plasma HBP levels and brachial artery reactivity can predict severe sepsis and mortality in patients with sepsis.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

**References**


