Vasopressin in Septic Shock; Assessment of Sepsis Biomarkers: A Randomized, Controlled Trial

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Abstract

Background and Aims: Vasopressin (VP) in sepsis apart from vasoconstrictive effect may have some immunomodulatory effects. The aim of this study was to evaluate the effect of VP on different aspect of sepsis by measuring of sepsis biomarkers. Materials and Methods: In this trial, a total number of 42 septic shock patients were included. The first group received norepinephrine (NE) infusion to reach the target mean arterial pressure (MAP) of ≥ 65 mm Hg and the second group received arginine vasopressin (AVP) infusion in addition to NE. Serum lactate, C-reactive protein (CRP), interleukin-6 (IL-6), IL-10, pentraxin 3 (PTX3), angiopoietin 1 and 2 (Ang 1 and 2) levels were assessed. Results: Level of IL-6 and IL-10 decreased, but there was no significant difference between the two groups after 48 h. CRP and PTX3 levels were not also significantly different between groups. Although Angs were not statistically different, there was a trend toward higher Ang-1 in and lower Ang 2 in AVP group after 24 and 48 h. In addition, lactate level did not differ between NE and AVP groups. There was no interaction between VP and hydrocortisone use on IL-6, IL-10, and PTX3, but a significant statistical interaction on Ang 1 and Ang 2 were observed. Conclusions: Although analysis of sepsis biomarkers showed no significant difference between two groups, no immunomodulatory effect for VP alone, subgroup analysis of hydrocortisone used in this study showed that the combination of glucocorticoids and AVP had a significant effect on Angs level which eventually causes less endothelial permeability and higher MAP in this group of patients.

Keywords: Sepsis biomarkers, septic shock, vasopressin

Introduction

Septic shock is one of the main reasons for intensive care unit mortality and based on the latest guidelines, norepinephrine (NE) is the first vasopressor of choice in these patients. Recognition of relative deficiency of vasopressin (VP), an endogenous peptide hormone, during septic shock led to its usage as a vasopressor. However, VP generally is added to NE or other vasopressors and is not recommended as a single initial agent. Arginine VP, through V1 receptors, increases vascular tone and mean arterial pressure (MAP). In addition, VP increases catecholamine effect on vascular tone and may result in less catecholamine requirement and also the restoration of urine output during the shock. The immunomodulatory effect has also been illustrated by early use of VP in sepsis.

Utilization of biomarkers in medical practice has a long history, but recently sepsis biomarkers became the center of attention. A biomarker could be used as a diagnostic or prognostic indicator or a tool for monitoring of therapy. Considering the complexity of sepsis pathophysiology which could trigger proinflammatory or anti-inflammatory responses, coagulopathy, microcirculatory, and endothelial dysfunction, single one biomarker is not able to represent all aspects of sepsis, even if monitored daily during ICU stay. This is why procalcitonin has failed in some studies as a diagnostic marker for sepsis. Therefore, recent studies reported usage of a panel of biomarkers to reflect evolving nature of sepsis.

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Various markers have been studied in sepsis. IL-6 is the main cytokine in the hyperinflammatory phase of sepsis which produces the features of systemic inflammatory response syndrome (SIRS). IL-10 has potent anti-inflammatory properties, which is protective against tissue damage through suppression of proinflammatory biomarkers synthesis but in excess amount is prognostic for mortality. Pentraxins (PTXs) are part of acute phase reactant proteins which include “short” PTXs such as C-reactive protein (CRP) and “long” PTXs such as PTX3. Higher levels of PTX3 during early phase of sepsis were associated with mortality. Among endothelial activation markers, angiopoietin (Ang) 1/2 system hold the most promise. The Angs are growth factors which prompt endothelial cell activation. Ang-1 is an anti-inflammatory marker whereas Ang-2 is an inflammatory marker. Multiple studies have shown the value of the Ang in sepsis. Lactate is the most commonly used biomarker of perfusion. The anaerobic condition during septic shock ends in hyperlactatemia in these patients.

The purpose of this study was to evaluate whether VP use in septic shock patients has beneficial immunomodulatory effects. Biomarker selection in this study was based on the strength of evidence, the availability of assays, sample volume provision, stability of biomarkers due to long-term storage of samples. In addition, based on a hypothesis that combination of glucocorticoid with VP increases vasoconstrictive and anti-inflammatory effects in septic shock, we did a subgroup analysis of patients receiving both VP and hydrocortisone.

**Materials and Methods**

This study was conducted between November 2012 and April 2014 in the three general adult ICUs within three teaching hospitals. The study was registered in Iranian Registry of Clinical Trials (IRCT) with a code number of IRCT2012100311002N1. The study was approved by the Medical Ethics Committee (91-02-33-18310-63707), and informed consent was signed by patients’ next of kin.

The study was a randomized, controlled trial. Septic shock patients were enrolled if they were older than 18 years old and not >12 h had passed after ICU admission. Septic shock criteria include two or more of SIRS criteria, hypotension, infection, and organ failure.

Exclusion criteria were VP use for other indications, heart failure (class III or IV of NYHA) and acute coronary syndrome, serum sodium lower than 130 mEq/L, poor-prognosis patients (death anticipated within hours), end-stage renal disease, mesenteric ischemia, vasospastic diseases (e.g., Raynaud’s phenomenon), and pregnancy, refusal to sign the consent form.

Patients were randomly enrolled in one of the two study groups. The randomization was based on a random number list. One group received NE (Laboratorios Normon, Spain) infusion to achieve a MAP of ≥65 mm Hg. The other group received the same protocol plus VP (Exir Pharmaceutical Co. Tehran, Iran) at a rate of 0.03 u/min.

Titration of NE infusion rate to reach map of ≥65 mm/Hg and addition of other vasopressors and inotropes such as dopamine, dobutamine, and epinephrine were left to the discretion of patient’s primary physician. VP was discontinued if any life-threatening adverse effects were occurred (arrhythmias, hyponatremia, digital, and mesenteric ischemia). If target MAP was achieved for >8 h, vasopressors were slowly tapered over the next 24–48 h. There were no crossovers between groups during this study.

Within the first 6 h of septic shock, early goal-directed therapy and all the supportive measures were performed according to the SCCM guideline. Hydrocortisone (100 mg IV every 8 h) was added in patients with a highly elevated level of procalcitonin (>10 ng/mL) and if a noticeable increase in MAP was not observed in a patient who was receiving more than two vasoactive agents.

Initially, patients’ demographic data and primary diseases were recorded. Simplified Acute Physiologic Score (SAPS) II, marker of severity of illness, and the Sequential Organ Failure Assessment (SOFA), and marker of organ dysfunction were calculated once a day. Other routine measures include systolic blood pressure (SBP), diastolic blood pressure, MAP, central venous pressure (CVP), body temperature, and oxygen saturation. In addition, serum creatinine and sodium, platelet count, liver enzymes (aspartate aminotransferase, alanine aminotransferase, and bilirubin) and arterial blood gas were collected on a daily basis. The baseline procalcitonin level was also measured for each patient. A 12-lead electrocardiograph was performed daily. Other diagnostic procedures were carried out whenever indicated. Survival of patients was recorded for 28 days after randomization. Suspected adverse reactions which occurred during the trial were documented.

Blood samples were collected from study participant at baseline, 24 h, and 48 h after randomization and were centrifuged for 20 min at 2000 g and then, the plasma was separated and kept at −80°C. The levels of IL-6, IL-10, PTX3, Ang-1, and Ang-2 were determined by enzyme-linked immunosorbent assay (Crystal Day Biotech Co., LTD, Shanghai, China). Serum lactate was measured on a Roche Cobas Integra 400 analyzer (Roche Diagnostics, Indianapolis, IN).

The primary outcome was to compare sepsis biomarkers (Ang 1 and 2, PTX3, IL-6 and IL-10 and lactate) in these two groups and evaluate the effect of VP on these markers. Systemic hemodynamics, ICU mortality and 28-day mortality, organ failure, NE requirements for each group and the effect of corticosteroid on the biomarkers were also assessed as secondary end-points.

**Statistical analysis**

The results were reported as mean ± standard deviation or number (%). One sample Kolmogorov-Smirnov test was used for assessment of the normality distribution of variables. Student t-test was performed for the comparison of groups with normal distribution and Mann-Whitney U-test for nonnormal distribution. For categorical variables, the Chi-square test.
RESULTS

The number of patients who met the inclusion criteria and those who excluded from the study is depicted in Figure 1. Ultimately, 21 patients were randomly assigned to NE or VP group.

Table 1 shows the demographic and clinical variables at baseline. There are no significant differences between the two groups. Patients in NE group had a mean SAPS II score of 55.5, and this was 52.4 for patients in VP group. Based on this data, the severity of illness in both groups was high and equals to predicted mortality of >50%. Furthermore, level of procalcitonin in both groups was not different (11.4 µg/ml vs. 9.3 µg/ml, P = 0.43).

Patients in VP group had significantly lower heart rate (HR) and higher SBP and MAP compared to NE group during the first 24 h [Table 2]. However, CVP was comparable during the survey.

While the rate of VP infusion did not changed during the study, infusion rate of NE was titrated to reach the target MAP. After 24 h, it was 13.5 µg/min in the NE group versus 5.2 µg/min in VP group (P < 0.001), and after 48 h, it was 8.3 µg/min in the NE group versus 4.5 µg/min in VP group (P = 0.013). Renal function was comparable between groups. About 33.3% in NE group and 23.8% in the VP group needed hemodialysis during the first 24 h [Table 2]. Similarly, the rate of organ dysfunction between both groups was not different based on SOFA score at 48 h (12.3 vs. 11.3, P = 0.35).

Mortality during the first 48 h was 8 patients in the NE group and 4 patients in the VP group (P = 0.17). In addition, ICU mortality, 28-day mortality and length of ICU stay were similar between groups. Adverse effects in both groups were assessed on a daily basis, which was also similar.

Seven Biomarkers were checked at baseline, 24 h, and 48 h after randomization and the results are listed in Table 3.

As it is demonstrated in Table 3, levels of inflammatory and anti-inflammatory markers (IL-6 and IL-10) were decreased during the study but there were no significant difference between the two groups after 48 h (IL-6; NE vs. VP 116 vs. 74 pg/ml, P = 0.24 and IL-10; NE vs. VP 125 vs. 107 pg/ml, P = 0.53).

Acute-Phase Protein Biomarkers were also not significantly different between groups (CRP; NE vs. VP 74 vs.

Table 1: Demographic information and baseline characteristics of patients

|                | NE (n=21) | AVP (n=21) | P     |
|----------------|-----------|-----------|-------|
| Age (year)    | 56.7±14.9 | 63.6±20.1 | 0.21  |
| Male sex, n (%)| 13 (61.9) | 12 (57.1) | 0.75  |
| SAPS II        | 55.5±9.8  | 52.4±12.2 | 0.53  |
| SOFA           | 12.1±2.6  | 11.1±2.8  | 0.22  |
| Procalcitonin  | 6.4±11.8  | 4.3±7.4   | 0.50  |
| Source of infection, n (%) | 10 (47.6) | 8 (48)   | 0.713 |
| Lung           | 5 (23.8)  | 7 (33.3)  | 0.690 |
| Abdomen        | 2 (9.5)   | 3 (14.2)  | 0.543 |
| Urinary        | 3 (14.2)  | 1 (4.7)   | 0.283 |
| Other          |           |           |       |
| SIRS criteria  |           |           |       |
| Temperature (°C) | 37.8±0.9 | 37.5±1.5  | 0.48  |
| Heart rate (bpm)| 87.1±18.5| 89.8±18   | 0.63  |
| Leukocyte count (×10⁹/L) | 11.4±8.7 | 14.8±10.2 | 0.20  |
| Tissue hyperperfusion/organ dysfunction |           |           |       |
| Mechanically ventilated, n (%) | 17 (81)  | 18 (86.2) | 0.70  |
| PaO₂/FIO₂ (mmHg) | 291.5±245.6 | 205.6±123.2 | 0.15  |
| Urinary output (ml/kg/h) | 1.21±0.75 | 1.1±0.77  | 0.64  |
| Lactate (mg/dl)  | 35.8±19.5 | 42.8±17.3 | 0.30  |
| pH              | 7.32±0.81 | 7.32±0.81 | 0.94  |
| Platelet counts (×10⁹/L) | 133±88  | 146±79    | 0.63  |
| GCS             | 6.7±0.7   | 7±1.7    | 0.59  |
| Time from onset of shock to randomization (h) | 6.8±2.3  | 7±3.2    | 0.51  |
| Norepinephrine dose at randomization (µg/min) | 12.7±4.2 | 13.3±4.3  | 0.62  |
| Vasoactive drugs n (%) |           |           |       |
| Dopamine        | 8 (38.1)  | 5 (23.8)  | 0.31  |
| Epinephrine     | 3 (14.3)  | 3 (14.3)  | 1  |
| Dobutamine      | 4 (19)    | 4 (19)    | 1  |
| Hydrocortisone use, n (%) | 9 (42.9) | 12 (57.1) | 0.35  |

NE: Norepinephrine; AVP: Arginine vasopressin; PaO₂: Partial pressure of oxygen; FIO₂: Fraction of inspired oxygen; GCS: Glasgow Coma Score; SAPS II: Simplified acute physiology score II; SOFA: Sepsis-related organ failure assessment; SIRS: Systemic inflammatory response syndrome
Table 2: Changes in the hemodynamic response and laboratory parameters

|                      | Baseline | P    | 24 h      | P     | 48 h      | P     |
|----------------------|----------|------|-----------|-------|-----------|-------|
| HR (beats/min)       |          |      |           |       |           |       |
| NE                   | 87.1±18.5| 0.76 | 105±10.2  | 0.001 | 106.8±8.1 | 0.001 |
| AVP                  | 89.8±18  |      | 85.4±16.2 |       | 87.2±10.1 |       |
| SBP (mmHg)           |          |      |           |       |           |       |
| NE                   | 75.7±11.1| 0.73 | 98.2±31.3 | 0.002 | 120.1±18.8| 0.40  |
| AVP                  | 77±12.8  |      | 124.4±18.6|       | 125.5±18.5|       |
| MAP (mmHg)           |          |      |           |       |           |       |
| NE                   | 62.1±6.2 | 0.32 | 77.8±12.7 | 0.008 | 74±22     | 0.10  |
| AVP                  | 64.1±6.4 |      | 87.3±9    |       | 84.4±14.6 |       |
| CVP (mmHg H2O)       |          |      |           |       |           |       |
| NE                   | 10.1±11.2| 0.67 | 16.3±5.4  | 0.90  | 12.3±8.5  | 0.10  |
| AVP                  | 11.7±8.1 |      | 16.5±4.9  |       | 16.7±5    |       |
| Creatinine (mg/dl)   |          |      |           |       |           |       |
| NE                   | 1.4±0.5  | 0.42 | 1.6±0.4   | 0.22  | 1.7±0.9   | 0.29  |
| AVP                  | 1.3±0.6  |      | 1.4±0.6   |       | 1.4±0.7   |       |
| NE infusion rate (µg/min) |      |      |           |       |           |       |
| NE                  | 12.7±4.2 | 0.62 | 13.5±5.6  | 0.001 | 8.3±4.5   | 0.013 |
| AVP                 | 13.3±4.3 |      | 5.2±4     |       | 4.5±3.8   |       |
| SOFA score          |          |      |           |       |           |       |
| NE                  | 12.1±2.6 | 0.22 | 11.7±3    | 0.18  | 12.3±4.1  | 0.35  |
| AVP                 | 11.2±2.8 |      | 10.6±1.7  |       | 11.2±2.1  |       |

NE: Norepinephrine; AVP: Arginine vasopressin; HR: Heart rate; SBP: Systolic blood pressure; MAP: Mean arterial pressure; CVP: Central venous pressure; SOFA: Sepsis-related organ failure assessment

Table 3: Changes in biomarkers during the study

|                      | Baseline | P    | 24 h      | P     | 48 h      | P     |
|----------------------|----------|------|-----------|-------|-----------|-------|
| IL-6 (pg/ml)         |          |      |           |       |           |       |
| NE                   | 309.5±163.6| 0.29| 125±98.1  | 0.67  | 116.7±116.1| 0.24  |
| AVP                  | 258.1±151.5|    | 114.9±103.1|       | 74.2±93.4 |       |
| IL-10 (pg/ml)        |          |      |           |       |           |       |
| NE                   | 347.9±166.1| 0.33| 219.4±110.8| 0.62  | 125.8±80.2 | 0.53  |
| AVP                  | 297.7±170|      | 201.6±122.1|       | 107±91.7  |       |
| PTX3 (ng/ml)         |          |      |           |       |           |       |
| NE                   | 71.7±100.1| 0.55| 29.8±28.3 | 0.42  | 8.9±7.1   | 0.46  |
| AVP                  | 95.4±151|      | 23.9±18.4 |       | 11.3±10.4 |       |
| CRP (mg/dl)          |          |      |           |       |           |       |
| NE                   | 74±39.6  | 0.51 | 77.6±47.5 | 0.84  | 74±48.2   | 0.30  |
| AVP                  | 83.7±43.4|      | 71±43.2   |       | 57.3±37.8 |       |
| Ang-1 (ng/ml)        |          |      |           |       |           |       |
| NE                   | 0.93±0.61| 0.70 | 1.26±0.69 | 0.17  | 1.32±0.77 | 0.19  |
| AVP                  | 0.86±0.55|      | 1.59±0.83 |       | 1.80±0.18 |       |
| Ang-2 (ng/ml)        |          |      |           |       |           |       |
| NE                   | 22.3±8.4 | 0.72 | 15.9±8.7 | 0.07  | 13.8±6.7  | 0.09  |
| AVP                  | 21.5±6.9 |      | 11.4±6.9 |       | 9.5±7.6   |       |
| Lactate (mg/dl)      |          |      |           |       |           |       |
| NE                   | 3.98±2.17| 0.3  | 3.15±2.58 | 0.53  | 1.76±1.07 | 0.13  |
| AVP                  | 4.76±1.92|      | 2.66±1.69 |       | 1.2±0.59  |       |

NE: Norepinephrine; AVP: Arginine vasopressin; IL-6: Interleukin 6; IL-10: Interleukin 10; PTX3: Pentraxin 3; Ang-1: Angiopoietin 1; Ang-2: Angiopoietin 2; CRP: C-reactive protein

57.3 mg/dL, P = 0.3 and PTX3; Ne vs. VP 8.9 vs. 11.3 ng/ml, P = 0.46).

Although Angs as markers of endothelial damage were not statistically different, there were a trend toward higher Ang-1 in VP group after 24 and 48 h (after 24 h; NE vs. VP 1.26 vs. 1.59 ng/ml, P = 0.17 and after 48 h NE vs. VP 1.32 vs. 1.8 ng/ml, P = 0.19) and also a trend toward lower Ang-2 in VP group after 24 and 48 h (after 24 h; NE vs. VP...
In addition, Lactate level did not differ between NE and VP groups (after 24 h; 3.15 vs. 2.66 mg/dL, \(P = 0.53\) and after 48 h; 1.7 vs. 1.2 mg/dL, \(P = 0.13\); respectively).

The results show that IL-6 to IL-10 ratio in each time point was also comparable in both groups.

**Corticosteroid effect**

Nine patients in NE group (42.9%) and twelve patients in VP group (57.1%) received hydrocortisone during the study. The results of repeated measures ANOVA test with a Mauchly’s test of Sphericity assumption indicate that hydrocortisone use had a positive effect on patients MAP (\(F[1,30] = 9.856, P = 0.004\)), \(\text{P}_{\text{AO}}/\text{FIO}_2\) (\(F[1,19] = 14.044, P = 0.001\)), creatinine (\(F[1,30] = 5.830, P = 0.022\)), and SOFA score (\(F[1,30] = 6.294, P = 0.018\)). However, hydrocortisone use in septic shock patients had no effect on NE dose requirements (\(F[1,30] = 1.066, P = 0.31\)).

There were no interaction between VP and hydrocortisone use on IL-6 level (\(F[1,30] = 2.261, P = 0.143\)), IL-10 (\(F[1,30] = 0.038, P = 0.847\)), CRP (\(F[1,8] = 0.456, P = 0.518\)), and PTX3 (\(F[1,30] = 0.562, P = 0.459\)). Although VP use itself had no significant effect on Ang-1 and Ang-2 (\(F[1,30] = 3.320, P = 0.078\) and \(F[1,30] = 0.002, P = 0.965\)), hydrocortisone had a significant effect on both of these biomarkers (\(F[1,30] = 22.387, P = 0.001\) and \(F[1,30] = 10.755, P = 0.003\)) and also hydrocortisone had statistical interaction with VP use which had an effect on Ang-1 and Ang-2 levels (\(F[1,30] = 13.882, P = 0.001\) and \(F[1,30] = 14.724, P = 0.001\)) [Figures 2 and 3].

**DISCUSSION**

All the measured sepsis biomarkers including IL-6, IL-10, lactate, CRP, PTX, Ang1/2 were comparable in both groups. However, patients in VP group had a higher MAP, lower HR, and lower NE infusion rate. This is in accordance to the previous studies where VP decreased the need for NE and increased MAP in septic shock patients.[22-25] Moreover, both groups had a similar rate of adverse effects such as arrhythmias, digital ischemia, and hypotremia which also in consistent with other studies.[22-25]

Although many trials targeting inhibition of inflammatory mediators have failed to show any reduction in mortality and to date, only corticosteroids and drotrecogin alfa have been able to modulate inflammatory response and demonstrated mortality benefits.[26] Researchers have shown a decrease in tumor necrosis factor alpha (TNF-\(\alpha\)), IL-1\(\beta\), IL-6, inducible nitric oxide synthase, and prostaglandin E2 with VP use indicating an anti-inflammatory property for this drug.[27]

In the VASST study, as a supporter of anti-inflammatory effects of VP, 778 adult septic shock patients randomly divided into two groups, one group received NE, and other group received VP plus open-label vasopressors to reach MAP >65 mm/Hg. That study showed significant decrease in 28 days and 90 days mortality in less severe septic shock patients but not in more severe sepsis. The reasons for speculating anti-inflammatory effect for VP were (1) Beneficial effects of VP in less severe septic shock patients that were noticeable after 10 days, which is an interval needed for anti-inflammatory effect of VP. (2) VP in combination with corticosteroid reduced mortality rate which both have anti-inflammatory effects.[28]

Based on a recent analysis of VASST trial, there were more decreases of cytokines in survivors of septic shock, and the reduction was more prominent in the VP group.[29]

VP decreased IL-1\(\beta\) and TNF-\(\alpha\) in a study on brain. Thus, authors speculated that VP may have an anti-inflammatory effect.[28] Another study on mice sepsis model showed that pro-inflammatory cytokines may have a downregulatory effect on V1A-receptor expression during sepsis.\[30\]

Some studies investigated the ratio of some cytokines and their relation to outcome. Higher IL-10/TNF-\(\alpha\) ratios were found in nonsurvivors of these studies.[31] However, we do not find such an association in the current study.
PTX3 is shown to have more significant correlation with clinical parameters and outcome compared to other markers such as IL-6, TNF-α, and CRP. Nevertheless, neither CRP nor PTX3 at any time point was different between groups in our study.

Recently, endothelial activation markers such as the Ang pathway (Ang-1/2) is getting more attention as sepsis biomarkers for diagnostic and prognostic purposes. Hall et al. showed that in the first 2 h of sepsis, vascular smooth muscle is less responsive to vasoconstrictors in rats. They thought that excess endothelial nitric oxide synthase (eNOS) in endothelium may be the cause of endothelial dysfunction and Ang-1 through inhibition of eNOS production may increase vascular vasoconstriction and its responsiveness to vasopressors and decrease vasopressor requirement.

Although in our findings, the VP group had higher Ang-1 and lower Ang-2 levels, this difference did not reach the statistical significance.

In a post-hoc study of VASST trial, VP and corticosteroid interaction were analyzed. Their results showed that patients who received both VP and corticosteroid had lower mortality and organ failure. Such interaction also described in other studies.

Effect of glucocorticoid on VP is complex and may be explained by interactions between the hypothalamic pituitary-adrenal and hypothalamic-posterior pituitary VP axes. Gordon et al. in a randomized controlled trial of 61 septic shock patients showed that although glucocorticoid does not have any effect on VP level, it decrease VP requirement in these patients. In our study, NE requirement was also further decreased in glucocorticoid plus VP group. The cause may be due to the cytokine-induced downregulation of V1 receptors, which is reversed by glucocorticoid.

On the other hand, VP stimulates V3 receptor at pituitary and increase cortisol level as a result VP may have an indirect anti-inflammatory effect.

In our findings, hydrocortisone use had a positive effect on patients MAP and SOFA. However, its use had no effect on NE dose requirements. Moreover, as it is illustrated in Figures 2 and 3, addition of hydrocortisone to VP significantly increase Ang-1 and decrease Ang-2 levels in VP group while it did not convey the same result in NE group.

There are some hypotheses for significant effects we have found (a) Relative deficiency of both VP and cortisol may cause vascular smooth muscle unresponsiveness to catecholamines. As a result combination of these two may lead to a better response in septic shock patients, (b) Inflammatory markers such as TNF-α, IL-1β, IL-6, and interferon gamma may cause downregulation of V1 receptor and decreasing VP effect during inflammatory phase of sepsis. Glucocorticoid use with anti-inflammatory effect may decrease these markers and upregulate V1 receptors, (c) Combination of VP sparing effect of glucocorticoid and catecholamine and glucocorticoid sparing effect of VP may result in lower dosage requirement of these three agents, (d) There was no randomization in patients receiving corticosteroid and its administration was based on severity of shock and only patients with more severe illness received glucocorticoid. This might have caused a bias in the results of the analysis, (e) To identify the complex interaction of the infection, cytokine level and treatment, very large numbers of patients are required to find true patterns. Therefore, small sample size of this trial might not be an appropriate setting for this analysis.

Some of the limitations of our study were relatively small sample size, the study designed as an open-label trial and VP dosage was not adjusted according to body weight.

**CONCLUSION**

In summary, patients in VP group had lower NE requirements, higher MAP, and lower HR. Analysis of sepsis biomarkers (lactate, IL-6, IL-10, CRP, PTX3, Ang 1 and 2) showed no significant difference between two groups. Although sub group analysis of hydrocortisone use in this study showed that the combination of glucocorticoid and VP had a significant effect on MAP, SOFA, and Ang levels.

Future trials with a larger sample size and randomized use of glucocorticoid are needed to evaluate VP effect in septic shock patients.

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**Conflicts of interest**

There are no conflicts of interest.

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