Abstract: To determine plasma concentrations of angiopoietin (Ang) -1, Ang-2, Tie-2, and vascular endothelial growth factor (VEGF) in patients with sepsis-induced multiple organ dysfunction syndrome (MODS) and determine their association with mortality.

The study prospectively recruited 96 consecutive patients with severe sepsis in a tertiary care unit of a tertiary hospital. Plasma Ang-1, Ang-2, Tie-2, and VEGF levels and MODS were determined in patients on days 1, 3, and 7 of sepsis. Univariate and Cox proportional hazards analyses were performed to develop a prognostic model.

Days 1, 3, and 7 plasma Ang-1 concentrations were persistently decreased in MODS patients than in non-MODS patients (day 1: 4.0 ± 0.5 vs 8.0 ± 0.5 ng/mL, P = 0.0001; day 3: 3.2 ± 0.6 vs 7.3 ± 0.5 ng/mL, P < 0.0001; day 7: 2.8 ± 0.6 vs 10.4 ± 0.7 ng/mL, P < 0.0001). In patients with resolved MODS on day 7 of sepsis, Ang-1 levels were increased from day 1 (4.7 ± 0.6 ng/mL vs 9.1 ± 1.4 ng/mL, n = 43, P = 0.004). Plasma Ang-1 levels were lower in nonsurvivors than in survivors on days 1 (4.0 ± 0.5 vs 7.1 ± 0.5 ng/mL, P < 0.0001), 3 (3.8 ± 0.6 vs 7.1 ± 0.5 ng/mL, P < 0.0001), and 7 (4.7 ± 0.7 vs 11.0 ± 0.8 ng/mL, P < 0.0001) of severe sepsis. In contrast, plasma Ang-2 levels were higher in non-survivors than in survivors only on day 1 (15.8 ± 2.0 vs 9.5 ± 1.2 ng/mL, P = 0.035). VEGF and Tie-2 levels were not associated with MODS and mortality. Ang-1 level less than the median value was the only independent predictor of mortality (hazard ratio, 2.57; 95% CI 1.12–5.90, P = 0.025).

Persistently decreased Ang-1 levels are associated with MODS and consequently, mortality in patients with sepsis.

Abbreviations: Ang = angiopoietin, APACHE II = acute physiology and chronic health evaluation II, ARDS = acute respiratory distress syndrome, CI = confidence interval, CRP = C-reactive protein, DIC = disseminated intravascular coagulation, HR

INTRODUCTION

Emerging evidences disclose that endothelial activation and injury are involved in the development of multiple organ dysfunction syndrome (MODS) during sepsis. In addition to its function as vascular barrier, endothelial cells are implicated in inflammatory responses and coagulation imbalance in sepsis.1 A study showed that endothelial injury is an independent predictor for MODS and mortality in patients with severe sepsis. The degree of endothelial injury correlates with the numbers of organ failure in those patients.2 Taken together, sepsis-induced endothelial injury plays a critical role in mediating illness progression and outcome.

The angiopoietin (Ang) family contains 3 members in humans, including Ang-1, Ang-2, and Ang-4.3,4 Both Ang-1 and Ang-2 bind to the same site of Tie2 receptor with similar affinity.5 After binding to Tie-2 receptor, Ang-1 causes activation of Tie-2.6 Ang-1 inhibits the endothelial response to inflammatory cytokines and exerts protective effects. In addition, Ang-1 inhibits vascular endothelial growth factor (VEGF)-induced blood-vessel formation and adhesion molecule expression, and attenuates increased VEGF and thrombin-induced permeability.7,8 However, Ang-2 triggers inflammatory responses by activating the endothelial cell and increasing permeability.9,10 Because of the important role of Angs in endothelial activation and vascular barrier breakdown, many studies have explored their role as biomarkers of sepsis.11–13 Evidence has consistently shown increased Ang-2 levels in patients with sepsis compared with those without. But discrepancy exists in the levels of Ang-1 in patients with sepsis. Previous reports demonstrate that circulating Ang-1 levels remain unchanged, or even decrease, in septic patients.11–13 Although decreased Ang-1 levels have recently been reported as an independent predictor for mortality in pediatric patients with severe bacterial infection,13 a few comprehensive clinical investigations have examined the role of circulating Ang-1 during the clinical course from MODS to subsequent mortality in sepsis. Whether longitudinally measured Ang-1 levels correlate with the resolution or aggravation of MODS during sepsis remains unclear.

The study aimed to measure plasma concentrations of Ang-1, Ang-2, Tie-2, and VEGF in patients with sepsis-induced organ failure and examine the independent association between these markers and mortality. These markers were also serially
measured to determine their correlation with evolutionary change of MODS status during the course of severe sepsis.

Materials and Methods

Patients

This study was conducted from October 2008 to April 2010 in a 37-bed medical intensive care unit (MICU) of Chang Gung Memorial Hospital, a tertiary medical center. Ninety-six patients (46 males and 50 females) were recruited within 24 hours of diagnosis of severe sepsis. Sepsis was defined as the presence of infection and at least 2 of the following criteria:14 of the American College of Chest Physicians/Society of Critical Medicine Consensus Committee: temperature >38°C or <36°C, heart rate >90 beats/minute, respiratory rate >20 breaths/minute, PaCO₂ < 32 mmHg, white blood cell >12,000/mm³ or <4000/mm³ or >10% immature (band) forms. Patients’ baseline data included age, vital signs, blood gas analysis, organ failure count, and hematologic and biochemical tests. Acute Physiology and Chronic Health Evaluation II (APACHE II) scores and Sequential Organ Failure Assessment (SOFA) score were used to assess illness severity. Underlying medical history was also collected, including diabetes mellitus, hypertension, neurologic disease, congestive heart failure, malignancy, and chronic airway obstruction disease.

The hospital’s Institutional Review Board approved the study and all patients or their legal representatives provided written informed consent. The patients were treated according to the Surviving Sepsis Guidelines.16

Definitions

Organ failure was diagnosed according to the ACCP-SCCM Consensus Committee criteria.14 Respiratory failure was defined as needing mechanical ventilation. Cardiovascular failure was defined as systolic BP ≤ 90 mmHg or mean arterial pressure ≤ 60 mmHg for 1 hour, despite fluid bolus. Renal failure was defined as low urine output (eg, <0.5 mL/kg/hour), increased creatinine (≥50% increase from baseline), or the need for acute dialysis. Hematologic failure was defined as low platelet count (<100,000/mm³) or PT/PTT > upper limits of normal. Metabolic failure was low pH with high lactate (eg, pH < 7.30 and plasma lactate > upper limits of normal), while hepatic failure was liver enzyme levels > 2 x the upper limits of normal. CNS failure was defined as altered consciousness or reduced Glasgow Coma Score. Those with more than 2 organ dysfunctions were defined as MODS.2,14 Day 1 was the day the patients were recruited.

The number of organ failure was determined on days 1, 3, and 7 of sepsis in each patient. Patients with persistently MODS on days 1 and 7 were defined as having persistent MODS. The no MODS group was defined as having organ failure counts less than 3 on days 1 and 7. Patients with MODS on day 1 but with organ failure number less than 3 on day 7 were defined as resolved MODS.

Blood Sampling and Measurement

Blood samples were taken on days 1, 3, and 7 of sepsis for plasma collection. The maximum levels of laboratory data were used for determination of organ function and comparison between survivors and nonsurvivors. Soluble Ang-1, Ang-2, VEGF, and Tie-2 plasma concentrations (Sekisui Diagnostics, MA) on days 1, 3, and 7 were determined by ELISA based on the manufacturer’s instructions. Plasma levels of these mediators were compared between patients with and without MODS, and between survivors and nonsurvivors. For sequential MODS changes, patients were classified into 3 groups (ie, no MODS, resolved MODS, and persistent MODS) and compared.

Statistical Analysis

All data were expressed as mean ± SEM or percentage. Since most continuous variables were skewed, nonparametric approaches were used. Quantitative variables between 2 groups were compared using the Mann–Whitney test for continuous and ordinal variables, and the chi-square test for nominal variables. Nonparametric tests (Wilcoxon signed rank) were used for comparison of time points within a group. Differences among comparison for more than 2 groups were determined using the Kruskal–Wallis test. The primary outcome studied was in-hospital mortality.

Univariate analyses were primarily used for the selection of variables, based on P value less than 0.1. Selected variables, including APACHE II score, SOFA score, Ang-1 levels, Ang-2 levels, C-reactive protein (CRP), and lactate were entered into a Cox proportional hazards model to identify the net effects of each individual factor. Hazard ratios (HR) with 95% confidence interval (CI) were used to assess independent contributions of significant factors. Receiver operating characteristic (ROC) curves for day 1 APACHE II scores, plasma levels of Ang-1, Ang-2, CRP, and lactate for predicting the development of MODS and mortality during ICU stay were plotted, and the respective areas under the curves were calculated. A P < 0.05 was considered statistically significant. All analysis was done using the SPSS software version 10.0 (Chicago, IL).

Results

Baseline Characteristics

The 96 patients enrolled were classified as survivors and nonsurvivors during their hospital stay. Their baseline characteristics were listed in (Table 1). Thirty-six (37.5%) died during their hospitalization. There were no differences between survivors and nonsurvivors in age, sex, shock, bacteremia, and origin of sepsis. However, the nonsurvivors had higher illness severity than survivors, as indicated by APACHE II score (21.7 ± 1.2 vs 17.4 ± 0.8, P = 0.002). The SOFA scores of nonsurvivors differed from those of survivors on day 1 (8.2 ± 0.6 vs 5.9 ± 0.3, P < 0.0001). They also had higher levels of lactate (24.4 ± 2.5 vs 17.4 ± 1.7 mg/dL, P = 0.025) and Ang-2 (15.8 ± 3.3 vs 9.5 ± 1.2 ng/mL, P = 0.035) but lower Ang-1 levels (4.0 ± 0.5 vs 7.1 ± 0.5 ng/mL, P < 0.0001) than survivors.

Cox Proportional Hazard Analysis for Predicting Mortality

The APACHE II and SOFA scores and CRP, lactate, and Ang-1 levels less than the median value (<5.5 ng/mL) and Ang-2 level higher than the median value (>7.0 ng/mL) were subjected to Cox proportional hazard analysis (Table 2). Except for Ang-1 level (HR, 2.57, 95% CI 1.12–5.90, P = 0.025), all other variables did not remain significant in the Cox proportional hazard analysis. Therefore, Ang-1 levels less than the median value were the only independent predictor for mortality in patients with severe sepsis.

Plasma Levels of Angiopoietins and Clinical Outcomes

The plasma levels of Ang-1 were lower in nonsurvivors than in survivors on days 1 (4.0 ± 0.5 vs 7.1 ± 0.5 ng/mL,
Table 1. Factors Associated With Mortality in Patients With Severe Sepsis

|                      | Survivors | Nonsurvivors | P Value |
|----------------------|-----------|--------------|---------|
| Baseline characteristics |           |              |         |
| Age, years           | 68.6 ± 1.8| 70.6 ± 2.4   | 0.3528  |
| Male sex             | 27 (45%)  | 19 (55.6%)   | 0.529   |
| APACHE II score      | 17.4 ± 0.8| 21.7 ± 1.2   | 0.002   |
| SOFA score           | 5.9 ± 0.3 | 8.2 ± 0.6    | <0.0001 |
| Presence of shock on day 1 | 13 (21.7%) | 10 (27.8%)  | 0.622   |
| Bacteremia           | 10 (16.7%)| 6 (16.7%)    | 1.000   |
| Primary origin of sepsis, n, % |       |              |         |
| Pneumonia            | 40 (66.7%)| 23 (63.9%)   | 0.827   |
| Skin and subcutaneous infection | 8 (13.3%)  | 5 (13.9%)   | 1.000   |
| Primary bloodstream infection | 3 (5%)   | 3 (8.3%)    | 0.669   |
| Other                | 2 (3.3%)  | 1 (2.8%)     | 1.000   |
| Laboratory data      |           |              |         |
| PaO2/FiO2 ratio      | 315.9 ± 23.5| 266.5 ± 27.7| 0.186   |
| pH value             | 7.438 ± 0.01| 7.426 ± 0.013| 0.488   |
| WBC, 10⁹/L           | 13793 ± 1203| 11513 ± 1459| 0.238   |
| CRP, g/L             | 140.7 ± 13.7| 185.6 ± 21   | 0.065   |
| Lactate, mg/mL       | 17.4 ± 1.7| 24.4 ± 2.5   | 0.025   |
| Ang-1, ng/mL         | 7.1 ± 0.5 | 4.0 ± 0.5    | <0.0001 |
| Ang-1 < median level, ng/mL | 22 (36.7%) | 26 (72.2%)  | 0.001   |
| Ang-2, ng/mL         | 9.5 ± 1.2 | 15.8 ± 3.3   | 0.035   |
| Ang-2 > median level, ng/mL | 37 (61.7%) | 11 (30.6%) | 0.006   |
| VEGF, pg/mL          | 709.2 ± 62.5| 812.8 ± 367  | 0.725   |
| Tie-2, ng/mL         | 21.2 ± 1.4| 22 ± 2.1     | 0.747   |

Ang = angiopoiotin, APACHE = acute physiology and chronic health evaluation, CRP = C-reactive protein, FiO₂ = fraction of inspired oxygen, MODS = multiple organ dysfunction syndrome, PaO₂ = partial pressure of oxygen in arterial blood, SOFA = sequential organ failure assessment, VEGF = vascular endothelial growth factor, WBC = white blood cell. Values are expressed as mean ± SEM or numbers (%).

Area Under the ROC Curve for Day 1 Soluble Factor Levels, SOFA Score and APACHE II Scores in Predicting Outcomes

Areas under the ROC curve for plasma Ang-1, Ang-2, CRP, and lactate levels, and SOFA and APACHE II scores on

| Variables       | Odds Ratio | 95% CI | P value |
|-----------------|------------|--------|---------|
| Ang-1 < median  | 2.57       | 1.12–5.90 | 0.025   |
| Ang-2 > median  | 1.72       | 0.76–3.92 | 0.189   |
| APACHE II score | 1.05       | 0.98–1.12 | 0.128   |
| SOFA score      | 0.99       | 0.85–1.15 | 0.857   |
| Lactate         | 1.01       | 0.99–1.03 | 0.243   |
| CRP             | 1.00       | 0.99–1.01 | 0.401   |

Ang = angiopoiotin, APACHE = acute physiology and chronic health evaluation, CI = confidence interval, CRP = C-reactive protein, SOFA = sequential organ failure assessment.

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day 1 were compared for predicting clinical outcomes (Table 3). Values for areas under the ROC curves showed that day 1 plasma Ang-1 levels had good discriminative power in predicting MODS (0.743) and mortality (0.898). In addition, APACHE II score also had good discriminative power for MODS (0.674) and mortality (0.740). The areas under the ROC curve for day 1 plasma Ang-2 (0.717), CRP (0.617), and lactate (0.716) levels and SOFA scores (0.883) were adequate only in predicting mortality but not MODS.

Plasma Ang-1 and Organ Dysfunction and Illness Severity

To further understand the relationship between Ang-1 and organ dysfunction, Ang-1 levels were compared to several variables of illness severity and laboratory findings associated with organ dysfunction (Table 4). Ang-1 negatively correlated with APACHE II ($r = -0.255, P = 0.012$) and SOFA scores ($r = -0.565, P < 0.0001$), number of organs affected by failure ($r = -0.580, P < 0.0001$), and serum CRP ($r = -0.241, P = 0.018$) and lactate ($r = -0.206, P = 0.044$) levels. In contrast, the plasma Ang-1 levels positively correlated with platelet count ($r = 0.780, P < 0.0001$) such that the platelet count of patients with plasma Ang-1 higher than the median level ($n = 48$) ($259.6 ± 16.5$ $1000/\text{mm}^3$) was higher than those with Ang-1 level lower than the median value ($n = 48$) ($106.4 ± 11.4$ $1000/\text{mm}^3, P < 0.0001$) (Figure 4).

DISCUSSION

This study reveals that plasma Ang-1 levels changes and are decreased with the evolution of MODS in patients with severe sepsis. Concentrations of Ang-1 increase in patients with resolved MODS but remain low in patients with persistent MODS. Moreover, Ang-1 levels on day 1 of sepsis
Angiopoietin-1 Correlates With MODS and Mortality in Sepsis

**TABLE 3.** Comparison of Areas Under the Receiver Operating Characteristic (ROC) Curves for Variables on Day 1 of Severe Sepsis (n = 96)

| MODS       | Area (95% CI) | P value | Mortality | Area (95% CI) | P value |
|------------|--------------|---------|-----------|--------------|---------|
| Ang-1      | 0.812 (0.726–0.898) | <0.001  | 0.743 (0.726–0.847) | 0.031 |
| Ang-2      | 0.717 (0.610–0.824)  | <0.001  | 0.632 (0.515–0.750) | 0.060 |
| APACHE II  | 0.740 (0.642–0.838)  | <0.001  | 0.674 (0.563–0.784) | 0.005 |
| SOFA score | 0.883 (0.816–0.951)  | <0.001  | 0.716 (0.609–0.823) | <0.001 |
| Lactate    | 0.716 (0.612–0.820)  | <0.001  | 0.691 (0.581–0.800) | 0.056 |
| CRP        | 0.617 (0.504–0.730)  | 0.049   | 0.605 (0.485–0.725) | 0.061 |

Ang = angiopoietin, APACHE = acute physiology and chronic health evaluation, CI = confidence interval, CRP = C-reactive protein, MODS = multiple organ dysfunction syndrome, SOFA = sequential organ failure assessment.

TABLE 4. Bivariate Correlation Between Angiopoietin-1 and Clinical and Biologic Variables in Patients With Sepsis

| Variables                  | Correlation (r) | P value |
|----------------------------|-----------------|---------|
| APACHE II                  | -0.255          | 0.012   |
| SOFA score                 | -0.565          | <0.0001 |
| Number of organ failure    | -0.580          | <0.0001 |
| Platelet number            | 0.780           | <0.0001 |
| pH value                   | -0.165          | 0.107   |
| PaO2/FiO2                  | 0.046           | 0.347   |
| Leukocyte count            | 0.166           | 0.107   |
| Lactate                    | -0.206          | 0.044   |
| CRP                        | -0.241          | 0.018   |

APACHE = acute physiology and chronic health evaluation, CRP = C-reactive protein, FiO2 = fraction of inspired oxygen, PaO2 = partial pressure of oxygen in arterial blood, SOFA = sequential organ failure assessment.

**FIGURE 4.** The platelet number in patients with plasma angiopoietin-1 (Ang-1) concentrations lower and higher than the median value. Open bars, patients with plasma Ang-1 concentrations lower than the median value; solid bars, patients with plasma Ang-1 concentrations higher than the median value; *P < 0.05 compared to patients with plasma Ang-1 concentrations lower than the median value. Data were expressed as mean ± SEM.
In conclusion, plasma Ang-1 levels are repressed in sepsis-induced MODS and mortality. Plasma concentrations of this marker increase concomitantly with the resolution of MODS and independently predict mortality in patients with severe sepsis. These may suggest that the inadequate amount of Ang-1 plays a critical role in progression from MODS to mortality. Thus, plasma Ang-1 levels may be used for monitoring MODS status in patients with severe sepsis, particularly day 1 plasma Ang-1 levels, which may be used as an early predictor of mortality.

ACKNOWLEDGEMENT

The authors thank National Science Council of Taiwan and research grants from Chang Gung Memorial Hospital, Taiwan for funding.

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