# Relationship of Plasma Gelsolin Levels to Outcomes in Critically Ill Surgical Patients

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**Objective:** To examine the relationship between plasma gelsolin levels and mortality following surgery or trauma.

**Background:** Few simple predictive diagnostic tests are available to predict mortality following surgery or trauma. We hypothesize that plasma concentrations of gelsolin, a protein that responds to injured tissue, might be a predictor of patient outcomes.

**Methods:** We conducted a prospective, observational study in the surgical intensive units (ICU) at a tertiary care teaching hospital. A total of 31 patients were enrolled in the study. Chart abstraction was used to gather data about patient demographics, clinical characteristics, and clinical outcomes. Plasma gelsolin concentrations were assessed serially on day 0 through day 5.

**Results:** Low plasma gelsolin levels were associated with increased risk of death occurring in the ICU. Plasma gelsolin levels lower than 61 mg/L predicted longer ICU stay, prolonged ventilator dependence, and increased overall in-hospital mortality.

**Conclusion:** Plasma gelsolin is a potential prognostic biomarker for critically ill surgical patients. Plasma gelsolin replacement may have therapeutic application.

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Despite modern surgical and medical care, mortality and healthcare costs of surgical patients admitted to intensive care units (ICU) remain unacceptably high.[1] As physicians, moreover, we are often limited in providing prognosis for patients and their families following critical illness. Several scoring systems of illness severity provide some population-based risk assessments but offer limited prognostic value for the individual patient.[2]

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Plasma gelsolin is an actin-binding plasma protein that is part of an "actin-scavenging" system that buffers potentially harmful actin molecules released from injured tissues.[3] Low circulating levels of plasma gelsolin may indicate significant depletion of this actin-scavenger system and may serve as a marker of injury severity in critically ill patients, consistent with the report of Mounzer et al that patients admitted to the hospital with severe trauma had lower admission plasma gelsolin levels than patients with lesser injuries.[4]

Since low circulating levels of plasma gelsolin have been documented in humans subjected to severe trauma, sepsis, myonecrosis, conditioning regimens for stem cell transplantation, acute respiratory distress syndrome (ARDS), and liver necrosis,[4–9] and partial restoration of depressed plasma gelsolin levels ameliorated pulmonary injury in rodents exposed to hyperoxia or full-thickness burns[6,10] and decreased the lethality of endotoxin or cecal ligation puncture in mice (abstract, in press), the administration of plasma gelsolin to patients with low plasma gelsolin levels and at risk for critical care complications appears worthy of consideration. We therefore measured daily plasma gelsolin levels of critically ill patients admitted to a surgical ICU (SICU) following burns, trauma, or major surgery to determine the stability or lability of plasma gelsolin levels and the predictive value of these measurements on the clinical outcome of a broader group of critically ill surgical patients.

## MATERIALS AND METHODS

### Patient Selection

Between January 2002 and April 2004, subjects 18 years or older with Injury Severity Scores (ISS)[11] $> 15$ admitted to the SICU at Brigham and Women's Hospital because of trauma, burns or who had undergone major surgery (defined as open-chest or abdominal surgeries), or their designee, were approached for consent. The study was approved by the Hospital's Committee for the Protection of Human Subjects.

### Plasma Collection

Venous blood (3 mL) was collected in EDTA, and plasma harvested. Samples were then frozen at −80°C until analysis. Samples were collected whenever possible at 12 hours, 24 hours, day 2, day 3, day 4, and day 5 after admission.

## Clinical Data Collection and Analysis

In addition to demographic information, body mass indexes [BMI = weight (kg)/height (m)$^2$], Acute Physiology and Chronic Health Evaluation II (APACHE II)[12] scores were recorded for all patients. ISSs were calculated as previously reported[11] for trauma and burn patients only. All enrolled patients were followed until hospital discharge. Deaths occurring in the surgical ICU were designated as ICU mortality and overall in-hospital deaths as hospital mortality. A diagnosis of ARDS was based on the criteria of the American-European consensus conference.[13] Ventilator-free hours and ICU-free days were compiled for intervals within 30 days of admission to the surgical ICU.

## Plasma Gelsolin and Albumin Measurements

Plasma gelsolin levels were measured by a functional assay based on gelsolin's ability to promote actin monomer nucleation to accelerate polymerization.[14] This assay is rapid and reproducible and accurately reflects gelsolin protein concentration in the presence of gelsolin binding molecules including actin and phospholipids.[4] Plasma samples were diluted 1:3 in 0.1 mol/L KCl, 0.2 mmol/L $MgCl_2$, 1 mmol/L EGTA, 0.5 mmol/L ATP, 0.5 mmol/L $\beta$-mercaptoethanol, and 10 mmol/L Tris-HCl buffer, pH 7.4 (Buffer B). Of the diluted plasma sample, 5 $\mu$L was added to 280 $\mu$L Buffer B in 6 × 50 mm borosilicate culture tubes. The actin polymerization reaction was initiated by adding 15 $\mu$L 20 $\mu$mol/L pyrene actin in 0.5 mmol/L ATP, 0.2 mmol/L $MgCl_2$, 5 mmol/L $\beta$-mercaptoethanol, 0.2 mmol/L $CaCl_2$, 0.2 mmol/L Tris-HCl buffer, pH 7.4 (Buffer A). Actin polymerization was monitored for 200 seconds in a FluoroMax-2 Jobin Yvon-Spex spectrofluorimeter at excitation and emission wavelengths of 366 and 386 nm, respectively, and the slope of the linear phase of actin polymerization was recorded. Gelsolin concentrations were estimated from a standard curve using recombinant human plasma gelsolin. Stock pyrene actin for these assays, prepared by the method of Kouyama and Mihashi,[15] was stored at −80°C in lots, thawed, and diluted 10× with Buffer A, centrifuged at 250,000*g* for 30 minutes after standing overnight. For each sample, gelsolin level was assayed blindly and in triplicate.

Plasma albumin levels were measured colorimetrically using a commercial kit (Stanbio, Boerne, TX).

**TABLE 1.** Patient Demographics and Clinical Characteristics

| Clinical Characteristic | Ratio or Median (Range) |
|---|---|
| Male:female | 24:7 |
| Age (yr) | 37 (18–87) |
| BMI (kg/m$^2$) | 50 (17–75) |
| APACHE II | 20 (11–40) |
| ISS (N = 26) | 50 (17–75) |
| **Diagnosis and Outcome** | **No. of Cases** |
| Trauma | 20 (65) |
| Burn | 6 (19) |
| Postoperative | 5 (16) |
| ARDS | 8 (26) |
| ICU mortality | 3 (10) |
| Hospital mortality | 6 (19) |

## Statistical Methods

Data are presented as mean ± SD or as medians (range) when appropriate. The Mann-Whitney *U* test and Fisher exact test were used for comparisons of continuous and dichotomous variables, respectively. Logistic regression analysis was performed to analyze factors contributing to hospital death, using the software Statview 5.0.1 (SAS Institute Inc., Cary, NC). A receiver operating curve was generated using MedCalc (MedCalc Software, Belgium). A *P* value less than 0.05 was considered statistically significant.

# RESULTS

The demographic characteristics and admission diagnoses of the 31 patients enrolled are displayed in Table 1. Most cases had trauma or burns (26 of 31); 5 cases had undergone major surgery. The overall mortality rate was 19% (6 of 31); of the deaths, 3 (50%) occurred in the ICU.

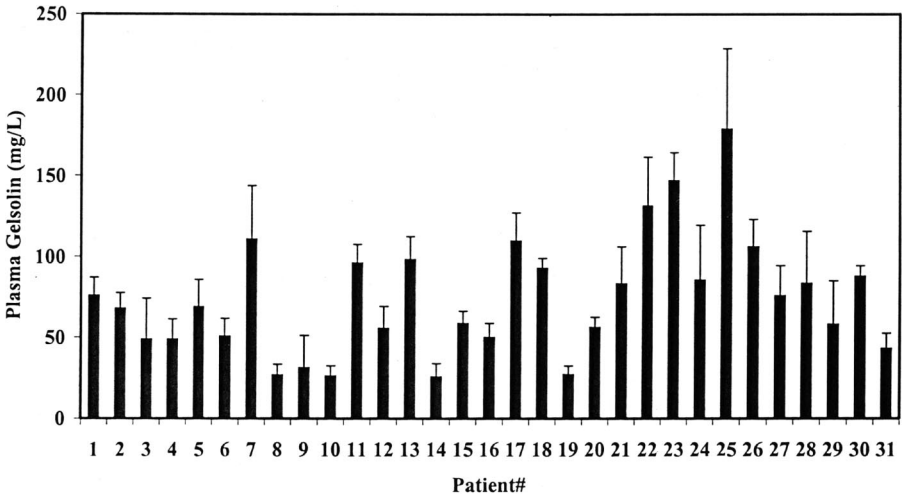


**FIGURE 1.** The average plasma gelsolin level of each patient in the study period varied over a wide range. But for each patient, plasma gelsolin levels were relatively stable as indicated by the relatively small standard deviation of each patient's plasma gelsolin concentration over the study period.

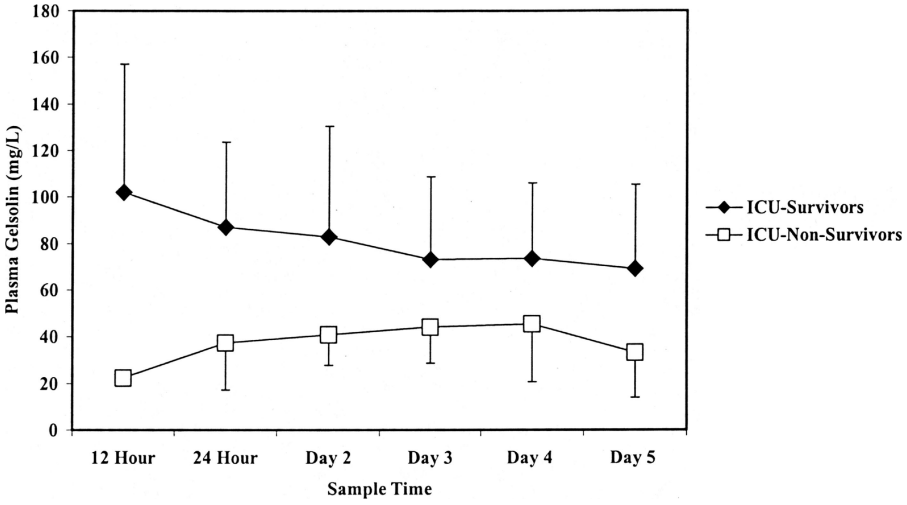


**FIGURE 2.** Plasma gelsolin levels at various time points stratified by ICU mortality.

The average gelsolin levels of the study patients varied over a wide range (25.68–179.24 mg/L). For each patient, gelsolin levels did not vary as widely over the study period, as shown in Figure 1. Figure 2 shows the plasma gelsolin levels of all study patients from 12 hours following ICU admission through day 5, stratified by ICU survival. Normal plasma gelsolin levels measured by various techniques, including the method used in this study, are between 150 and 300 mg/L.[3,5,7,8,14,16] The mean plasma gelsolin levels of all study patients were below the normal values and stratified into 2 groups: a higher mean for ICU survivors and a lower mean for nonsurvivors. Despite a greater than 2-fold difference in average plasma gelsolin concentration between these groups, the levels remained relatively stable over the observation interval. Therefore, plasma gelsolin levels closest to 24 hours of admission for each patient were chosen for further analyses, testing gelsolin as a prognostic indicator for clinical outcomes compared with the established APACHE II system.

Table 2 shows that ICU survivors had significantly higher levels of plasma gelsolin compared with ICU nonsurvivors. In contrast, plasma albumin levels were not significantly different between the 2 groups, suggesting that depletion of plasma gelsolin in these patients is not nonspecific. APACHE II scores were also significantly different between the survivors and nonsurvivors, consistent with APACHE II as a known predictor of patient outcomes in surgical ICUs.[17] ISS scores were not analyzed here because only 2 of the ICU nonsurvivors were trauma or burn patients.

**TABLE 2.** ICU Survivors vs. ICU Nonsurvivors

| Clinical Factor | ICU Survivors | ICU Nonsurvivors | *P* |
|---|---|---|---|
| Plasma gelsolin (mg/L) | 81.30 (19.89–180.83) | 26.32 (25.22–60.47) | **0.02** |
| Albumin (g/dL) | 3.13 (1.63–4.55) | 4.13 (2.47–4.62) | 0.5 |
| APACHE II | 19 (11–34) | 29 (26–40) | **0.02** |
| BMI (kg/m$^2$) | 25.4 (18.9–45.9) | 25.8 (20.7–26.6) | 0.69 |

Data are median (range).

Although high BMI has been reported to be a risk factor of mortality in medical ICU patients,[18] we did not find BMI to be significantly different between ICU survivors and nonsurvivors. This is most likely explained by the differences between surgical and medical patients, since in the surgical ICU, BMI has not consistently been found to be a risk factor for poor outcomes.[19,20]

A receiver operating curve (ROC) analysis using plasma gelsolin and ICU mortality data was then performed, which yielded an area under the ROC curve (AUC) of 0.905 (Fig. 3). Additional analysis was performed using 61 mg/L as a cutoff point because this cutoff point produced 100% sensitivity while maximizing specificity at 78.6%.

Table 3 shows patient characteristics and outcomes stratified by plasma gelsolin levels. Age, gender, and BMI were not significantly different between patients with plasma gelsolin levels less than or greater than 61 mg/L. On the other hand, APACHE II scores were significantly higher in those with plasma gelsolin levels lower than 61 mg/L, which implies that the plasma gelsolin level of an individual patient depends more on the severity of illness than on age, gender, or body habitus of the individual patient. ISS was not significantly

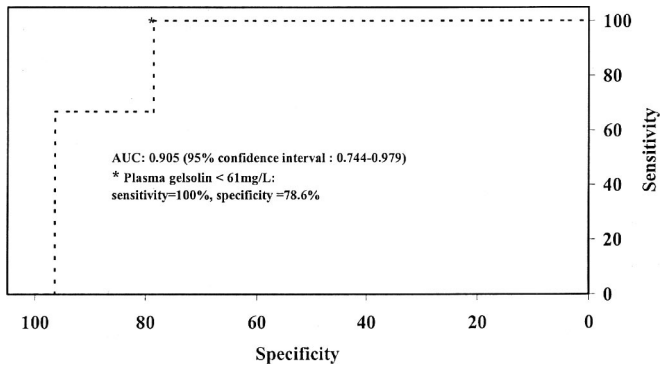


**FIGURE 3.** Receiver operating characteristic curve (ROC) for various cutoff levels of plasma gelsolin in identifying death occurring in the ICU.

**TABLE 3.** Patients Stratified by Plasma Gelsolin Levels of ≥61 mg/L or <61 mg/L

| Clinical Characteristic or Outcome | Plasma Gelsolin Level | | |
|---|---|---|---|
| | ≥61 mg/L | <61 mg/L | *P* |
| APACHE II | 17 (11–34) | 26.5 (24–40) | **<0.01** |
| ISS | 38 (17–66) | 50 (50–75) | 0.18 |
| BMI (kg/m$^2$) | 25.4 (18.9–45.9) | 25.55 (20.7–31.2) | 0.94 |
| Age (yr) | 32 (12–81) | 52 (19–87) | 0.08 |
| No. male/total | 20/23 | 4/8 | >0.05 |
| Albumin (g/dL) | 3.18 (2.51–4.55) | 2.71 (1.63–4.62) | 0.28 |
| No. ARDS/total | 6/23 | 2/8 | >0.99 |
| ICU-Free days | 22 (0–28) | 0 (0–20) | **<0.01** |
| Ventilator-free hours | 650 (0–707) | 10.5 (0–648) | **<0.01** |

Data are median (range) or no. of cases/total cases.

different likely because of the small number of patients included in the analysis. Moreover, plasma gelsolin levels can be used to identify patients at risk for increased morbidity as those with gelsolin levels lower than 61 mg/L had significantly shorter ICU-free days and ventilator-free hours.

Since only 3 ICU deaths occurred, we tested a second endpoint, hospital mortality, by performing logistic regression analyses with hospital mortality as the outcome of interest. In univariate analyses, older age, greater APACHE II score, and a plasma gelsolin below 61 mg/L predicted hospital death. In multivariate analyses (Table 4), only plasma gelsolin remained an independent predictor of death (OR = 36.89, $P = 0.04$).

Because a low gelsolin level was not associated with the development of ARDS, we tested whether ARDS is associated with ICU or hospital mortality and found that the development of ARDS was associated with neither ICU nor hospital mortality (Table 5), consistent with a recent study reporting a lack of association between ARDS and mortality in trauma patients.[21]

## DISCUSSION

We found that low plasma gelsolin levels are associated with increased death occurring in the surgical ICU, confirming the finding of Mounzer et al that low plasma gelsolin levels are predictive of poor outcomes in patients with trauma.[4] Our study extends that correlation to patients with severe burns and recovering from major surgical procedures and shows that plasma gelsolin levels can predict clinical outcomes among such patients. Furthermore, we found that a plasma gelsolin level lower than 61 mg/L, about 25% of normal

**TABLE 4.** Multivariate Logistic Regression Analysis of Factors Contributing to Hospital Mortality

| Clinical Factor | OR | 95% Confidence Interval | *P* |
|---|---|---|---|
| Age (yr) | 1.07 | 0.97–1.17 | 0.17 |
| APACHE II | 0.95 | 0.71–1.27 | 0.73 |
| Plasma gelsolin < 61 mg/L | 36.89 | 1.13–1206.00 | **0.04** |

OR indicates odds ratio.

**TABLE 5.** ICU and Hospital Mortality in ARDS and Non-ARDS Patients

| Clinical Outcome | ARDS | Non-ARDS | *P* |
|---|---|---|---|
| ICU mortality | 1/8 | 2/23 | >0.99 |
| Hospital mortality | 1/8 | 5/23 | >0.99 |

Data are no. of cases/total cases.

value, predicts longer ICU stay, prolonged ventilator dependence, and increased risk of in-hospital death, independent of APACHE II score. Plasma gelsolin levels above 25% of normal may be a critical point for maintaining normal physiology. In experimental models, mice subjected to hyperoxia were found to have a plasma gelsolin level that decreased to 25% of normal. This plasma gelsolin level decrement coincided with neutrophil infiltration in the lungs. In human bone marrow transplant recipients, plasma gelsolin levels dropping to 25% were associated with increased mortality and respiratory complications.[9,22]

Mounzer et al found no correlation between hemoglobin values and plasma gelsolin concentrations but did find plasma levels of albumin correlated with plasma gelsolin levels.[4] However, the extent of plasma gelsolin depletion was much greater than that of albumin, leading Mounzer et al to conclude that plasma gelsolin depletion was specific and not a result of generalized plasma protein loss. In agreement with this conclusion, our study convincingly shows that plasma gelsolin depletion was a specific phenomenon. Albumin levels had no significant prognostic significance in our patient group.

In contrast to the study of Mounzer et al, however, we did not find an association between plasma gelsolin levels and the development of ARDS. Mounzer et al reported that gelsolin levels lower than 2 standard deviations below the normal average was associated with significantly increased risk of ARDS and death in trauma patients.[4] Compared with that study, we applied a more stringent statistical analysis on a more heterogeneous and sicker population of surgical ICU patients. Possibly, the correlative finding from the Mounzer et al study reflects the inclusion of less sick patients who were not admitted to ICU. This approach would predictably lead to a wider divergence of plasma gelsolin levels between ARDS and non-ARDS patients. In addition, our study has established that plasma gelsolin levels are quite stable over 5 days, indicating that plasma gelsolin measurements would be useful in the clinical setting to earmark patients early who could potentially benefit from replacement therapy. Because we did not collect plasma samples beyond the first 6 days of admission, our study does not address if and when plasma gelsolin recovers to normal level in these patients, and whether the timing of plasma gelsolin recovery correlates with clinical improvements. Limited previous work has documented recovery of plasma gelsolin levels depleted by injury given sufficient time.[23] Further study is required to address these questions.

The mechanism of plasma gelsolin's actions is poorly understood. Gelsolin was first discovered as an intracellular protein involved in actin dynamics.[24] Plasma gelsolin was

subsequently identified as a secreted isoform of the cytoplasmic gelsolin.[25] The function ascribed to plasma gelsolin as an actin scavenger is based on the known interaction between cytoplasmic gelsolin and actin. One study reported that infusing a sufficient quantity of actin intravenously caused death in rats.[26] However, it is not clear how or if circulating actin is toxic. Some investigators have found circulating actin in the blood of patients with tissue injury.[7,27] Others have not found actin in the plasma of injured patients.[4] A more likely reason for the beneficial effect of gelsolin replacement in animal models is that gelsolin binds and neutralizes other inflammatory mediators, such as fibrin, fibronectin, amyloid A$\beta$-protein, and lysophosphatidic acid.[14,24,28,29] We have chosen to focus the current study on the utility of plasma gelsolin levels in predicting clinical outcomes, and have not investigated whether circulating actin or gelsolin-actin complexes are present in surgical ICU patients. We plan to conduct further studies to identify binding partners of plasma gelsolin in injured patients, which will help to elucidate the physiologic function of plasma gelsolin.

## CONCLUSION

We have shown that plasma gelsolin levels of critically ill surgical patients can identify patients at increased risk of death and prolonged ventilator and ICU dependence. Plasma gelsolin level can be used as a reliable biomarker in surgical ICU and may be a potential therapeutic target.

## ACKNOWLEDGMENTS

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