Osteopontin predicts survival in critically ill patients with acute kidney injury

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Abstract

Background. The cytokine osteopontin is involved in the pathophysiology of experimental acute kidney injury. We have tested the hypothesis that osteopontin levels might serve as a biomarker predicting outcome in critically ill patients requiring renal replacement therapy after acute kidney injury.

Methods. We measured circulating plasma osteopontin levels in 109 critically ill patients with acute kidney injury at inception of renal replacement therapy and 4 weeks thereafter. Critically ill patients without acute kidney injury served as controls. Osteopontin was measured with ELISA.

Results. Baseline osteopontin levels in patients with acute kidney injury were significantly higher compared with controls (P < 0.0001). Baseline osteopontin levels in patients recovering from acute kidney injury were significantly elevated compared with patients with permanent loss of kidney function after acute kidney injury (P = 0.01). In addition, in patients recovering from acute kidney injury without further need for renal replacement therapy, osteopontin levels were significantly lower 4 weeks after initiation of renal replacement therapy (P = 0.0005). Moreover, multivariate Cox analysis revealed osteopontin levels at renal replacement therapy inception as an independent and powerful predictor of mortality (P < 0.0001). In the ROC-curve analysis, an osteopontin cut-off value of 577 ng/mL separated survivors from non-survivors with a sensitivity of 100% and a specificity of 61% (AUC 0.82; 95% confidence interval: 0.74–0.89; P < 0.0001).

Conclusions. Osteopontin may serve as a novel biomarker for both, overall survival and renal outcome in critically ill patients with acute kidney injury, that require renal replacement therapy.

Keywords: acute kidney injury; mortality; osteopontin; renal replacement therapy

Introduction

Acute kidney injury (AKI) in critically ill patients has been identified as an independent risk factor for increased mortality [1]. Survival of patients with AKI in the intensive care unit (ICU) is still unacceptably low despite significant advances in supportive care [2]. A recent multinational, multi-centre study of 29 000 critically ill patients including 1700 with AKI revealed that the in-hospital mortality is high, exceeding 60% [3]. Thus, detection of patients at particular risk for both death and prolonged kidney failure after AKI in the setting of intensive care medicine and renal replacement therapy (RRT) remains an area of utmost interest.

Osteopontin (OPN) is a cytokine that is broadly expressed and upregulated during inflammation and various
other conditions [4–6]. OPN has an arginine–glutamate–aspartic acid cell binding sequence, and via this sequence, it interacts with a variety of cell surface receptors [7–9]. Secreted OPN binds to the αβ3 integrin-receptor and subsequently induces phosphoinositide-3-kinase/Akt-dependent NF-κB activation [4–6]. It is involved in the recruitment and retention of macrophages and T cells to sites of inflammation. Classical mediators of acute inflammation such as tumour necrosis factor α and interleukin-1β strongly induce OPN expression [10–12], while other mediators that can induce OPN are angiotensin II, transforming growth factor β, and hypoxia [13–18]. Circulating OPN has therefore been proposed to be a mediator in the pathogenesis of systemic inflammatory response syndrome (SIRS) and sepsis [19]. Moreover, recent experimental studies have highlighted a role for OPN in various models of AKI [20–28].

In the present study, we tested the hypothesis that OPN might serve as a biomarker predicting survival and renal outcome in critically ill patients with AKI requiring RRT. In the initial stage, OPN might reflect the level of renal injury.

Materials and methods

Patients and methods

This study is a post hoc measurement of prospectively collected blood samples from the HANDOUT trial [30]. The study protocol was approved by the Hannover Medical School Ethics Committee (project/approval no. 2905) and was conducted in accordance with the Declaration of Helsinki and German Federal Guidelines. Patients in seven ICUs of the tertiary care centre at the Hannover Medical School suffering from AKI were evaluated for inclusion. Patients with non-obstructive, RRT-dependent AKI were included. The inclusion criteria were loss of kidney function of >30% calculated estimated glomerular filtration rate (eGFR) with either the MDRD or Cockcroft–Gault equation and/or cystatin C-GFR within 48 h prior to inclusion and oliguria/anuria (<30 mL/h >6 h prior to inclusion) or hyperkalaemia (≥6.5 mmol/L) or severe metabolic acidosis (pH <7.15 and bicarbonate <12). Exclusion criteria were pre-existing chronic kidney disease as defined by eGFR <60 mL/min or a serum creatinine concentration >1.7 mg/dL more than 10 days prior to initiation of the first RRT. Further exclusion criteria were participation in another study, consent denial or withdrawal, and need for extracorporeal membrane oxygenation therapy. The enrolment was performed by attending nephrologists after obtaining written informed consent from a patient or his/her legal representatives. If the patient was recovering and able to communicate, he/she was informed of the study purpose, and consent was required to further maintain his/her legal status as a study participant.

After inclusion, the specific medical condition leading to RRT initiation was documented out of a list of four possible causes requiring immediate RRT. All patients received a nutritional intake of at least 25–30 kcal/kg/day, preferentially delivered as enteral nutrition. The prescribed protein intake was >1.2 g/kg/day. RRT in all patients was performed in a slow extended dialysis (SLED) mode using the GENIUS™ dialysis system (Fresenius Medical Care, Bad Homburg, Germany) as described in detail elsewhere [20]. The dose of the RRT was tailored according to the patient individual need, starting with at least one treatment daily. RRT was discontinued in patients meeting the following criteria for renal recovery: urine output >1000 mL/day and/or increased solute clearance, i.e. decline in pre-treatment serum creatinine concentration with eGFR >15 mL/min (by MDRD, Cockcroft–Gault equation and/or cystatin C-GFR), Serum creatinine C, serum creatinine and serum C reactive protein (CRP) levels were determined by routine laboratory methods.

Table 1. Demographic, clinical and laboratory characteristics of patients

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Total</th>
<th>Survivors</th>
<th>Non-survivors</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (n, %)</td>
<td>64 (59)</td>
<td>40</td>
<td>25</td>
<td>0.64</td>
</tr>
<tr>
<td>Female (n, %)</td>
<td>45 (41)</td>
<td>29</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Discipline of ICU admission</td>
<td></td>
<td></td>
<td></td>
<td>0.39</td>
</tr>
<tr>
<td>Medicine (n, %)</td>
<td>46 (42)</td>
<td>27 (39)</td>
<td>19 (48)</td>
<td></td>
</tr>
<tr>
<td>General surgery (n, %)</td>
<td>27 (25)</td>
<td>16 (23)</td>
<td>11 (28)</td>
<td></td>
</tr>
<tr>
<td>Cardiac surgery (n, %)</td>
<td>36 (33)</td>
<td>26 (38)</td>
<td>10 (24)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>52 (40–63)</td>
<td>52 (44–63)</td>
<td>51 (37–63)</td>
<td>0.77</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25 (22–28)</td>
<td>25.4 (22–28)</td>
<td>24.7 (22–28)</td>
<td>0.62</td>
</tr>
<tr>
<td>SOFA score</td>
<td>13 (10–15)</td>
<td>14 (11–15.5)</td>
<td>13 (10–15)</td>
<td>0.68</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>113 (56–197)</td>
<td>82 (46–191)</td>
<td>145.5 (67–211.5)</td>
<td>0.16</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>75.5 (67–90)</td>
<td>77 (70–93)</td>
<td>74 (64–86)</td>
<td>0.27</td>
</tr>
<tr>
<td>Osteopontin (ng/mL)</td>
<td>616 (432–638)</td>
<td>451 (408–625)</td>
<td>631 (619–649)</td>
<td>&lt;0.0001 *</td>
</tr>
</tbody>
</table>

*P < 0.05.

ICU, intensive care unit; BMI, body mass index; RRT, renal replacement therapy; eGFR, estimated glomerular filtration rate; SOFA, Sequential Organ Failure Assessment; APACHE II, Acute Physiology and Chronic Health Evaluation II; MAP, mean arterial blood pressure; CRP, C-reactive protein.
Patient characteristics and OPN levels at baseline

Patients were grouped as survivors and non-survivors at 4 weeks after initiation of RRT. Patient groups were comparable with respect to baseline demographics, indications for RRT and the proportion of RIFLE categories (Table 1). Differences in the renal SOFA subscore and OPN levels in survivors vs. non-survivors at baseline were highly significant (P = 0.02 and P < 0.0001, respectively). Baseline OPN levels in critically ill patients with AKI (n = 109; 616 (432–638) ng/mL) were elevated 2-fold compared with critically ill non-AKI patients (n = 15; 226 (179–344) ng/mL; P < 0.0001). Patients with sepsis had higher SOFA and Apache II scores that resulted from more severe cardiovascular and respiratory impairment compared with patients with either non-SIRS/Sepsis or SIRS, respectively (data not shown). Consistently, septic patients had poorer 28-day survival [53% vs. 76% (non-SIRS/Sepsis) and 70% (SIRS)]. Differences in OPN levels in patients with non-SIRS/Sepsis, SIRS or Sepsis did not reach statistical significance (P = 0.09).

Circulating OPN levels in critically ill patients

We did not find an association between OPN and APACHE II or SOFA score. However, OPN levels correlated significantly with the renal SOFA subscore (r = 0.219, P = 0.022), while there was no association with any other subgroup. Furthermore, OPN levels in patients in the RIFLE failure group were significantly elevated compared with the RIFLE risk group (P < 0.01). We found no significant differences in OPN levels in patients with or without sepsis (P = 0.07), diabetes (P = 0.78), surgery (P = 0.27) or shock (P = 0.21). Similarly, OPN levels did not differ between patients on a medical, general surgical or cardiac surgical ICU as assessed by ANOVA (P = 0.75). Baseline OPN levels did not correlate with serum cystatin C levels (P = 0.64) or serum creatinine levels (P = 0.4).

Circulating OPN levels in patients with AKI and renal recovery

Twenty-four of 69 surviving patients (35%) were still dependent upon RRT at 4 weeks after initiation of therapy. OPN levels at initiation of RRT predicted renal recovery since baseline OPN levels in patients recovering from AKI were significantly higher compared with patients still dependent upon RRT at 4 weeks after start of therapy.
In patients recovering from AKI after initiation of RRT, plasma OPN levels decreased significantly (P = 0.0005), while in patients still requiring dialysis 4 weeks after initiation of treatment, OPN levels did not change (P = 0.19). Importantly, we did not detect OPN in spent dialysate (n = 8), indicating that it is not removed by the treatment procedure. Moreover, OPN levels were not associated with urinary output, indicating that increased OPN levels are not a matter of impaired urinary excretion (r = 0.04, P = 0.7). Dependence upon RRT was significantly associated with baseline OPN levels (HR 0.995, 95% confidence interval 0.990–0.999, P = 0.024).

Circulating OPN predicts 28-day mortality in the ICU in critically ill patients with AKI

A total of 40 patients died in our cohort. OPN levels in non-survivors were significantly elevated compared with survivors [631 (619–649) vs. 451 (408–625) ng/mL, P < 0.0001] (Figure 1). To determine the relationship between OPN levels at initiation of RRT and mortality, we initially performed univariate Cox proportional hazards analyses. In our cohort of 109 critically ill patients with AKI, age, gender, body weight, height or body mass index were not significantly associated with survival (Table 2). The same was true for heart rate, the presence of diabetes, major surgery, serum C-reactive protein and serum creatinine levels prior admission to the ICU. RIFLE criteria were also not of prognostic value. Among the variables tested OPN levels, APACHE II and SOFA scores, sepsis, and the presence of non-septic shock displayed prognostic significance at a 10% level and were subsequently subjected to multivariate Cox regression analysis (Table 2). Only OPN levels (P < 0.0001) and Apache II score (P = 0.049) remained independent predictors of survival. In fact, OPN levels were identified as the strongest independent prognostic factor for survival in our cohort, with an area under the ROC curve (AUC) value of 0.82 (standard error of the mean: 0.04; 95% confidence interval: 0.74–0.89; P < 0.0001) (Figure 2). For comparison, the SOFA score yielded an AUC value of 0.57 (standard error of the mean:

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
</tr>
<tr>
<td>OPN (ng/mL)</td>
<td>3.891</td>
<td>2.326–6.509</td>
</tr>
<tr>
<td>Age</td>
<td>0.991</td>
<td>0.971–1.012</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>0.839</td>
<td>0.442–1.592</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.996</td>
<td>0.935–1.061</td>
</tr>
<tr>
<td>Diabetes (yes/no)</td>
<td>2.222</td>
<td>1.194–4.134</td>
</tr>
<tr>
<td>Surgery (yes/no)</td>
<td>0.635</td>
<td>0.337–1.195</td>
</tr>
<tr>
<td>Shock (yes/no)</td>
<td>2.618</td>
<td>0.928–7.382</td>
</tr>
<tr>
<td>S-creatinine</td>
<td>0.998</td>
<td>0.987–1.010</td>
</tr>
<tr>
<td>RIFLE criteria</td>
<td>1.578</td>
<td>0.855–2.912</td>
</tr>
<tr>
<td>SOFA score</td>
<td>1.061</td>
<td>0.960–1.172</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>1.038</td>
<td>0.994–1.084</td>
</tr>
<tr>
<td>CRP</td>
<td>1.001</td>
<td>0.998–1.003</td>
</tr>
<tr>
<td>Heart rate</td>
<td>1.003</td>
<td>0.989–1.016</td>
</tr>
</tbody>
</table>

*P < 0.05.

OPN, osteopontin; SOFA, Sequential Organ Failure Assessment; APACHE II, Acute Physiology and Chronic Health Evaluation II; CRP, C-reactive protein; CI, confidence interval; HR, hazard ratio.

Fig. 2. Receiver-operating curve (ROC) analysis of circulating osteopontin (OPN) levels for the prediction of survival 4 weeks after initiation of renal replacement therapy [area under the curve: 0.82; standard error of the mean (SEM): 0.04; 95% confidence interval: 0.74–0.89; P < 0.0001].

Fig. 3. Kaplan–Meier curves of survival stratified to circulating osteopontin (OPN) above and below a cut-point of 577 ng/mL. Log-rank test confirmed statistical significance for OPN (P < 0.0001).
illustrates the Kaplan–Meier cut-point of 577 ng/mL. Log-rank test confirmed statistical significance for OPN (P < 0.0001). We also analysed the patients’ survival status 3.5 years after inclusion into the Handout trial concerning the association with baseline OPN levels. In the group of survivors, 36 patients were lost to follow-up, 6 patients died during the follow-up period and 27 patients were still alive. Baseline OPN levels were not predictive concerning long-term survival (P = 0.6).

Discussion

Our study is the first clinical evaluation of circulating OPN levels in critically ill patients with AKI requiring RRT. The results are as follows: (i) baseline OPN levels in patients with AKI are significantly elevated compared with control subjects. (ii) OPN levels in patients in the RIFLE failure group were significantly higher compared with the RIFLE risk group, (iii) OPN levels in patients recovering from AKI were significantly elevated compared with patients with complete and permanent loss of kidney function and (iv) OPN levels decrease in patients recovering from AKI, while they remain high in patients still dependent upon RRT 4 weeks after initiation of RRT. (v) OPN levels in non-survivors are significantly higher than in survivors, (vi) OPN levels were identified as the strongest independent prognostic factor for 28-day survival in the multivariate Cox proportional hazards regression analysis, and (vii) an OPN cut-off value of 577 ng/mL separates survivors from non-survivors with a sensitivity of 100%. (viii) Baseline OPN levels were not predictive concerning long-term survival.

In experimental studies using gene profiling, OPN mRNA was found to be enhanced in kidneys of male Sprague–Dawley rats recovering from experimental AKI as a result of ischaemia/reperfusion injury [21]. Such upregulation may represent a beneficial adaptive response because the disruption of the OPN gene in OPN knockout mice results in more severe injury after experimental AKI [22]. OPN expression correlates with macrophage infiltration in various animal models of acute and chronic kidney injury such as glomerulonephritis, hypertensive glomerulosclerosis and cyclosporine nephropathy [23–25]. OPN gene and protein expression is induced in both proximal and distal tubular cells following toxin-induced AKI [26]. Hypoxia is also associated with increased tubular expression of OPN in vitro and in vivo [27,28]. Here, OPN acts as a survival factor and protects cells from entering apoptosis. Despite diminished macrophage infiltration and interstitial fibrosis, the obstructed kidneys of OPN knockout mice exhibit increased levels of tubular cell apoptosis compared with wild-type mice, suggesting that OPN is capable of providing survival signals to tubular epithelial cells in vivo [29]. These experimental findings might explain the differences in OPN values observed in our cohort. We hypothesize that the differences in OPN levels with regard to renal recovery and overall mortality are due to the complex function of OPN in injury and disease. In response to tubular injury, the local expression of OPN might be upregulated. Similar to experimental studies on the role of OPN in AKI [21–29], it is conceivable that OPN also serves as a survival factor within the kidney protecting tubular epithelial cells from entering apoptosis in humans. Once tubular epithelial cells recover from the insult, the tubular expression of OPN decreases since its anti-apoptotic signals are no longer required resulting in lower circulating levels of OPN. There are several factors that indicate that the differences in circulating levels of OPN in patients recovering from AKI as opposed to patients still dependent upon RRT at 4 weeks after initiation of therapy can be attributed to its role as a survival factor within the tubular epithelium rather than removal by treatment procedure or altered urokinetics/urodynamics in patients with incipient diuresis as compared with anuric patients. These include the lack of an association between OPN and markers of renal function (i.e. cystatin C and serum creatinine) as well as urinary output and the lack of a detection of OPN in spent dialysate. The results of our study permit the conclusion that the level of circulating OPN at the time of renal injury predicts the probability of renal recovery.

OPN levels also predicted overall 28-day survival in critically ill patients with AKI. This finding is well in line with other studies pertaining to the prognostic significance of OPN for mortality. OPN was identified as a strong independent predictor of mortality in patients with chronic heart failure, non-small cell lung cancer and breast cancer [35–37].

Osteopontin has been shown to be a mediator of severe inflammation in patients with systemic inflammatory response syndrome and sepsis, possibly by altering the release of IL-6 [19]. We speculate that the level of critical illness and inflammation is reflected by circulating levels of OPN. OPN might serve as a marker and mediator of multi-organ dysfunction through the retention of inflammatory cells and the release of IL-6. Death ensues as a consequence of severe inflammation. These patients present with the highest levels of circulating OPN.

There are certain limitations to our study. Our study represents results from a relatively small, single-centre cohort of adult patients without pre-existing renal impairment. In addition, the overwhelming majority of our patients presented with severe AKI (RIFLE category failure). Thus, our results need validation in larger trials, including patients with pre-existing chronic kidney disease (CKD) and a larger number of patients with less severe AKI. The fact that the vast majority of patients in our cohort presented with severe AKI underlines the prognostic significance of OPN as a predictor of mortality, since more
Conflict of interest statement. No declared.

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Outcomes of cancer and non-cancer patients with AKI

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Abstract

Background. Studies on cancer patients with acute kidney injury (AKI) are restricted to specialized intensive care units (ICUs). The aim of this study was to compare the characteristics and outcomes of cancer and non-cancer patients requiring renal replacement therapy (RRT) for AKI in general ICUs.

Methods. A prospective cohort study was conducted in 14 ICUs from three tertiary care hospitals. A total of 773 patients (68%) (P = 0.042). However, in multivariate analyses, older age, medical admission, poor chronic health status, comorbidities, ICU days until the RRT start and number of associated organ dysfunctions were associated with hospital mortality. The diagnosis of cancer was not independently associated with mortality [odds ratio = 1.54 (95% confidence interval, 0.88–2.62), P = 0.115]. Mortality in cancer patients was mostly dependent on the number of associated organ dysfunctions. Of note, 85% cancer patients recovered renal function at hospital discharge.

Conclusions. In general ICUs, one in six patients requiring RRT has cancer. Despite a relatively higher mortality, the presence of cancer was not independently associated with mortality in the present cohort.

Keywords: acute kidney injury; cancer; intensive care unit; outcome; renal replacement therapy