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Elevated Procalcitonin and C-Reactive Protein as Potential Biomarkers of Sepsis in a Subpopulation of Thrombotic Microangiopathy Patients

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Abstract

Thrombotic microangiopathy (TMA) comprises a group of microvascular thrombosis syndromes associated with multiple pathogenic factors. Deficient activity of ADAMTS13 is a pathogenic factor in a subset of TMA patients that provides strong rationale for plasma exchange treatment. However, the subset of TMA patients with normal ADAMTS13 activity remains a heterogeneous group of patients in which the appropriate treatment is not well understood. In addition to the common forms of TMA thrombotic thrombocytopenic purpura and the hemolytic uremic syndrome, the differential diagnosis of TMA may include sepsis, autoimmune disorders, and disseminated intravascular coagulation. Optimal treatment of TMA depends on timely recognition of treatable pathogenic factors. We hypothesized that sepsis is a rapidly identifiable pathogenic factor in a subset of TMA patients. To test this hypothesis, we retrospectively measured the rapid biomarkers of sepsis C-reactive protein (CRP) and procalcitonin (PCT), in a repository of pre-treatment plasma samples from 61 TMA patients treated with plasma exchange. Levels were analyzed in 31 severely ADAMTS13-deficient and 30 ADAMTS13-normal patients. None of the 31 patients with severe deficiency of ADAMTS13 had elevated PCT. However, 11 of 30 (37%) non-ADAMTS13-deficient patient samples were strongly positive for PCT. These patient samples also had a >10-fold higher median CRP level than patients with normal PCT. We conclude that rapid assays may help identify sepsis in a subset of TMA patients.

Keywords

Thrombotic microangiopathy (TMA); sepsis; C-reactive protein (CRP); procalcitonin (PCT); ADAMTS13

Introduction

Thrombotic microangiopathy (TMA) includes a heterogeneous group of syndromes characterized by microvascular thrombosis, thrombocytopenia, microangiopathic hemolytic anemia, and organ failure. Common forms of TMA include thrombotic thrombocytopenic purpura (TTP) and the hemolytic uremic syndrome (HUS). ADAMTS13 deficiency is a dominant pathogenic factor that provides strong rationale for plasma exchange treatment in

a subset of TMA patients. However, the utility of plasma exchange in TMA without ADAMTS13 deficiency is uncertain [1]. Improving treatment in this group of TMA patients will require a better understanding of underlying pathogenic factors.

Procalcitonin (PCT) is the prohormone of calcitonin [2]. During sepsis, bacterial endotoxins, exotoxins, and cytokines stimulate the release of PCT from extrathyroidal sources, including monocytes and macrophages [2]. PCT is characteristically elevated in bacterial sepsis and severe systemic parasitic or fungal infections [2–4]. PCT is minimally elevated in viral infections and non-infectious inflammatory conditions [2,4]. PCT levels increase within 2 to 3 hours of the onset of sepsis. Levels above 2 ng/ml have >90% specificity for sepsis or progression to sepsis in most clinical settings [2]. PCT assays that can be completed within two hours have been developed for rapid detection of sepsis. PCT assays have been commonly used in Europe for many years, and were recently approved by Food and Drug Administration for use in the United States.

C-reactive protein (CRP) is an acute phase reactant released by hepatocytes in response to stimulation by inflammatory cytokines [2,5,6]. CRP induction is sensitive to low levels of inflammation and is elevated in a variety of conditions, including infection, surgery, neoplastic disease, cardiovascular disease, and transplant rejection [5,7]. While modest elevations of CRP are less specific for sepsis than elevated PCT, higher levels have been shown to be relatively specific for sepsis [2,7]. Compared to PCT, the rise in the level of CRP in sepsis is delayed (>12 hours), and elevated levels persist longer [2].

Thirty to seventy percent of patients diagnosed with TMA do not have severe ADAMTS13 deficiency [8–11]. The pathogenesis of TMA in this subset of patients is often uncertain. The presence of an acute, febrile illness suggests that unrecognized sepsis could be an associated factor in some patients. Because the clinical manifestations of sepsis and TMA may overlap, and the recognition of sepsis may help optimize patient management, we tested the hypothesis that rapidly measurable biomarkers of sepsis could be identified in a subset of TMA patients treated with plasma exchange.

Materials and Methods

Patient samples

Study subjects were from a cohort of patients referred to BloodCenter of Wisconsin (Milwaukee, WI) for plasma exchange during the acute phase of TMA, as previously reported [8,12,13]. In this study, TMA refers to disorders potentially warranting treatment by plasma exchange, typically referred to as TTP, HUS, or TTP/HUS. Diagnostic criteria for TMA included thrombocytopenia, microangiopathic hemolytic anemia, elevated lactate dehydrogenase, and minimal evidence of disseminated intravascular coagulation. A BloodCenter of Wisconsin physician evaluated each patient at the time of presentation to confirm the diagnosis of TMA. The study was approved by the human research subject committees at BloodCenter of Wisconsin and The University of Iowa. Plasma samples were collected from the first 200 ml in waste bags during initial plasma exchange treatment. The archived plasma samples were stored at -20°C until use. The patients in this study were selected from the BloodCenter of Wisconsin TMA database and sample repository with the goal of sampling similar numbers of known severely ADAMTS13-deficient and non-severely ADAMTS13-deficient patients. Because of our interest in studying time to recovery in this and other studies, patients were selected who survived and achieved remission lasting at least two weeks. The patients were otherwise selected for availability of plasma samples and clinical data. CRP and PCT were analyzed in a total of 64 plasma samples obtained at the initiation of plasma exchange treatment. Three samples were eliminated from analysis for equivocal or non-interpretable PCT results, as explained below.

Of the 61 samples included in the statistical analyses, 30 were from patients previously determined to have nominally normal levels of ADAMTS13 activity, and 31 from patients with known severely deficient ADAMTS13 levels.

De-identified plasma samples from patients with a clinical diagnosis of sepsis and positive blood cultures were used for positive control samples for PCT assays. Aliquots of control plasma were kept frozen at -20°C until use.

ADAMTS13 assay

The ADAMTS13 activity levels were determined in previous studies, as reported [8,12]. Briefly, 98 μL of undiluted plasma mixed with 50 μL of purified VWF (final concentration 3U/mL), 10 mmol/L Pefabloc SC (Boehringer, Mannheim, Germany), and 8 μL of 10 mmol/L BaCl_2 , was dialyzed (1.5 mol/L urea, 0.005 mol/L Tris, pH 8.0) in a hydrophilic filter membrane (VSWP, 47-mm diameter; Milipore, Bedford, MA) for 15 hours at 37°C . The reaction was quenched with 10 μL of 0.2 mol/L EDTA, pH 7.4. Products of VWF proteolysis were electrophoresed in to 0.65% agarose in the presence of SDS, and the mobility of VWF multimers was detected with a mixture of ^{125}I -labeled VWF MoAbs recognizing multiple VWF subunit domains. Patient ADAMTS13 activity was determined by comparing the electrophoretic range of substrate VWF remaining after digestion by patient plasma to the electrophoretic range of undigested VWF in the same gel. The interpretation scheme divided patients into two groups: 1) non-severely deficient activity (ADAMTS13-normal)—the remaining VWF substrate electrophoretic pattern revealed disappearance of more than 15% of the largest VWF multimers; and 2) severely deficient activity (ADAMTS13-deficient)—the remaining VWF substrate electrophoretic pattern revealed disappearance of less than 15% of the largest VWF multimers.

PCT assay

PCT determinations were performed using PCT-Q, a semiquantitative solid-phase immunoassay (B.R.A.H.M.S. PCT-Q, B.R.A.H.M.S.-Diagnostica GmbH, Hennigsdorf, Germany). The assay was performed according to the manufacturer's recommendations using 200 μL of freshly thawed plasma. PCT is a stable analyte in vitro retaining 96% detectability after 24 hours storage at 4°C [2]. The PCT-Q is an immunochromatographic assay in which an immune complex sandwich is formed generating a colorimetric endpoint. The endpoint is determined by visual inspection of tests cards compared to a standard card. PCT concentrations are discriminated categorically as <0.5 ng/ml (normal), ≥ 0.5 ng/ml, ≥ 2 ng/ml, or ≥ 10 ng/ml. Because studies have shown that PCT levels ≥ 2 ng/ml have $>90\%$ specificity for sepsis or progression to sepsis, whereas levels <0.5 ng/ml are not associated with sepsis [2,14], we categorized results of <0.5 ng/ml as PCT negative for sepsis, and results ≥ 2 ng/ml as PCT positive for sepsis. Two of the 63 results were in the intermediate range of >0.5 ng/ml and <2.0 ng/ml, and were determined to be equivocal for sepsis and eliminated from analyses. Another sample did not have a clearly interpretable result and was eliminated from analyses. All three eliminated samples were in the subgroup of ADAMTS13-normal TMA patients. The total number of samples used for statistical analyses was 61: 31 samples in the ADAMTS13-deficient subgroup and 30 in the ADAMTS13-normal subgroup.

CRP assay

CRP levels were determined by the Clinical Chemistry Laboratory of the Department of Pathology at University of Iowa Hospitals and Clinics using an automated clinical chemistry analyzer (Roche Diagnostics, Indianapolis, IN). The normal reference range for the assay is <0.5 mg/dl. Because the validated lower limit of detection of this assay is 0.5 mg/dl, all values <0.5 mg/dl were assigned a value of 0.49 mg/dl for statistical and graphical purposes.

In the ADAMTS13-deficient subgroup there were 10 values <0.5 mg/dl that were assigned a value of 0.49 mg/dl, and 3 in the normal ADAMTS13 subgroup.

Statistics

The rates of normal and elevated PCT levels were compared between groups using Fisher's exact test. Levels of CRP were compared between subgroups using the nonparametric Peto-Prentice score test [15] (R software, NADA library). Numbers of plasma exchange procedures were compared between groups using the Mann-Whitney rank sum test.

Results

PCT results for sepsis were negative (<0.5 ng/ml) in all of the 31 samples from ADAMTS13-deficient patients. By contrast, 11 of the 30 (37%) ADAMTS13-normal patient samples were PCT positive for sepsis (≥ 0.2 ng/ml) ($p < 0.001$) (Table I).

CRP values were elevated (≥ 0.5 mg/dl) in 21 of 31 ADAMTS13-deficient patients and 27 of 30 ADAMTS13-normal patients ($p < 0.05$). The median CRP level in the subgroup of ADAMTS13 deficient patients (1.0 mg/dl) was significantly lower than the median CRP level in the subgroup of ADAMTS13-normal patients (2.65 mg/dl) ($p < 0.01$) (Figure 1A). Among the 50 total patients that were PCT negative for sepsis, there was no significant difference in CRP levels between the subgroup of 31 patients with ADAMTS13 deficiency (median = 1.0 mg/dl), and the subgroup of 19 patients with normal ADAMTS13 activity (median = 0.9 mg/dl) ($p = 0.77$). By contrast, the median CRP level in the 11 (ADAMTS13-normal) patients that were PCT positive for sepsis was significantly higher (10.3 mg/dl) than the median CRP level of the 50 patients that were PCT negative for sepsis (1.0 mg/dl) ($p < 0.001$) (Figure 1B). These results suggested that CRP levels were significantly higher in patients that were PCT positive for sepsis, irrespective of ADAMTS13 activity levels.

To determine if elevated PCT was associated with the length of treatment, the number of plasma exchange treatments in the subpopulation of 11 patients with elevated PCT was compared to the 19 patients with normal ADAMTS13 activity and normal PCT. There was no significant difference in the number of plasma exchange procedures required to achieve remission in the two subgroups (median = 6, versus 7, respectively; $p = 0.5$, Mann-Whitney rank sum test).

Discussion

This retrospective study revealed that among a group of TMA patients who were treated with plasma exchange and reached remission, one in six had biochemical evidence of sepsis in initial blood samples. Interestingly, all of the patients with evidence of sepsis were among the subpopulation with normal ADAMTS13 activity.

Multiple studies in a wide range of patient populations have reported that PCT levels ≥ 2 ng/ml have between 90% and 100% specificity for the diagnosis of bacterial sepsis or impending sepsis [2–4,7,14,16–19]. In the 11 patients in this study with PCT levels ≥ 2 ng/ml, the range of elevation of CRP (4.4 to 25.3 mg/dl) was also consistent with sepsis [4,7,16,19,20]. These results suggest that future studies of TMA patients should include biomarkers of sepsis such as PCT and CRP. If future studies confirm that PCT and CRP are reliable early markers of sepsis in TMA patients, these biomarkers could help guide management of TMA patients.

In addition to indicating the presence of a bacterial infection, our data suggest that elevated PCT in TMA is also associated with a low likelihood of ADAMTS13 deficiency. If future

studies confirm that positive biomarkers of sepsis are strongly associated with normal ADAMTS13 activity, this information may prove to be helpful in patient management..

Important limitations of this study include a lack of information in the database to allow correlations between elevated PCT and other clinical markers of sepsis or other factors influencing clinical course. Specifically, there is no information regarding culture results or other documentation of infection, antibiotic use, or the role of infection treatment in recovery. Future studies of TMA patients in which PCT and CRP can be studied in the context of complete data regarding infections may help establish the clinical utility of these markers.

Although TMA was once regarded as a single entity with a wide spectrum of manifestations [21], recent studies have shown that TMA associated with severe deficiency of ADAMTS13 activity is a clinically distinct entity. Severe ADAMTS13 deficiency in TMA is associated with statistically longer treatment times, greater risk of relapse, and lower initial platelet counts, hematocrit levels, and creatinine levels [8,22]. In the current study, PCT evidence of sepsis was observed exclusively in the ADAMTS13-normal TMA subgroup. This finding further supports the observation that TMA with severe deficiency of ADAMTS13 is clinically and pathologically distinct from TMA with normal ADAMTS13 activity.

There is accumulating evidence that inflammation plays a role in the pathogenesis of some forms of TMA. TMA plasma has been shown to induce microvascular endothelial cell apoptosis [23,24], and elevated levels of interleukin-8 and tumor necrosis factor α have been observed in TMA patients [25]. Inflammatory cytokines have been shown to stimulate the release of VWF from endothelium, which promotes microvascular thrombosis in flow chamber and murine models [26–28]. Our observation of elevated CRP present in nearly 80% of the TMA patients in this study further supports the importance of inflammation in the pathogenesis of many forms of TMA.

The pathogenesis of TMA in patients without severe deficiency of ADAMTS13 or evidence of septic inflammation remains a major challenge. Most of such patients in our study had elevated CRP, but the causes of inflammation and microvascular thrombosis in these patients remain unknown. A better understanding of the causes of elevated CRP in this subgroup of patients may help advance the management of TMA.

This retrospective study revealed that one in six TMA patients referred for plasma exchange had both normal ADAMTS13 activity and biochemical evidence of sepsis at the time of presentation. Rapid assays for PCT that were recently approved by the Food and Drug Administration should be used in future studies to help identify sepsis as a pathogenic factor in a subset of TMA patients.

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Figure 1A

Figure 1B

Figure 1.

Figure 1A: Boxplots of CRP levels in TMA patient subgroups. (A1) ADAMTS13-deficient TMA patients (N=31). (A2) ADAMTS13-normal TMA patients (N=30).

Figure 1B: Boxplots of CRP levels in TMA patient subgroups. (B1) PCT-negative (N=50). (B2) PCT-positive, ADAMTS13-normal patients (N=11).

The boxes represent the 25th and 75th percentiles; the horizontal bars within boxes represent medians; the whisker bars refer to the observations that are 1.5 inter quartile range (IQR) away from the box, where the IQR is the distance between the 25th and 75th percentiles. Observations that are more than 1.5 IQR away from the box are considered as outliers. CRP and PCT were determined in plasma samples obtained at the time of initial plasma exchange treatment for TMA. CRP values < 0.5 mg/dl (the validated limit of detection) were assigned a value of 0.49 mg/dl for graphical and statistical purposes, the location of which is indicated by the horizontal line. CRP values in subgroup A2 and B2 were compared against values in subgroup A1 and B1, respectively, using the nonparametric Peto-Prentice score test.

Table I

PCT assay results in 61 TMA patients

	PCT negative for sepsis	PCT positive for sepsis
ADAMTS13 deficient	31	0
ADAMTS13 normal	19	11

Fisher's exact test $p < 0.001$