Clinica Chimica Acta 440 (2015) 87–92

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Novel markers predict death and organ failure following hemorrhagic shock☆

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ARTICLE INFO

Article history:
Received 11 February 2014
Received in revised form 31 October 2014
Accepted 31 October 2014
Available online 7 November 2014

Keywords:
Hemorrhagic shock
Multi-organ failure
Hemorrhagic shock markers
ADAMTS13
HSP27
sP-Selectin

ABSTRACT

Background: ADAMTS 13, sP-Selectin and HSP27 have been investigated as potential prognostic markers in patients with hemorrhagic shock.
Methods: This study was part of a double-blind, randomized, parallel-group, controlled trial and included seventeen trauma patients presented to ED with severe hemorrhagic. The sera for testing were collected from these patients at the time of admission. Investigators and laboratory personnel performing testing were blinded to the patients' identity and clinical course.
Results: The prognostic value of ADAMTS13, sP-Selectin, and HSP27 was compared to prognostic value of systolic blood pressure (SBP), base deficit estimation (BD), heart rate (HR), shock index (SI) and tissue oxygen saturation (StO2) by constructing the receiver operation characteristics (ROC). The area under the curve (AUC) of the ROC for HSP27 (0.92) was greater than for SBP (0.45), BD (0.89), HR (0.61), SI (0.45) and StO2 (0.46). AUC for sP-Selectin (0.86) and for ADAMTS13 antigen (0.74) were comparable with BD one, but greater than for the rest of currently used tests.
Conclusion: Serum concentrations of ADAMTS13, HSP27 and sP-Selectin measured during the admission, appear to be comparable to or better than SBP, BD, SI, HR and StO2 in predicting MODS and death after hemorrhage from trauma. These potential new markers deserve further investigation.

1. Introduction

Estimation of the depth of hemorrhagic shock (the level of hypoperfusion) as well as identification of the site of occult bleeding can both be quite difficult. Accurate evaluation of occult bleeding requires substantial clinical experience augmented by expensive and time consuming imaging studies [1]. Assessing the depth of shock with current measures, such as systolic blood pressure (SBP), tissue oxygen saturation (StO2), heart rate (HR), base deficit estimation (BD) and shock index (SI), represents complex diagnostic problems [2,3] because these parameters give only an indirect estimation of the degree of hypoperfusion and thus require cautious interpretation. It has been shown that approximately 20% of patients with hemorrhagic shock have normal SBP, BD, and StO2 [2]. In many cases, the estimation of peripheral perfusion by base deficit estimation alone leads to equivocal results [4–6]. Vital signs are also clearly insufficient [3] for assessing the depth of shock. In addition, there are no currently established guidelines for the safe termination of resuscitation of patients with hemorrhagic shock [7,8].

To enhance clinical decision support at the point of care in trauma patients, we investigated Disintegrin and Metalloproteinase with a Thrombospondin Type 1 Motif, Member 13 (ADAMTS13), soluble P-Selectin (sP-Selectin), and Heat Shock Protein 27 (HSP27) for their usefulness as prognostic markers of poor outcomes after hemorrhagic shock. ADAMTS13 and sP-Selectin were chosen based on their involvement in both coagulation and systemic inflammation [9–11] – 2 cascades activated during hemorrhagic shock [12]. Heat Shock Protein 27 was selected based on its intra-cellular cytoprotective properties and its appearance in the circulation during stressful conditions [13,14]. We compared the ability of our novel markers to predict death or development of Multi Organ Dysfunction Syndrome (MODS) with the performance of SBP, BD, HR, StO2, and SI which are widely used today. We postulated that our HSP27, ADAMTS13, and sP-Selectin would be useful and could possibly outperform conventional indicators for predicting morbidity and mortality after massive bleeding.

2. Methods

2.1. Study design

This study was part of a prospective, double-blind, randomized, parallel-group, controlled trial assessing vasopressin efficacy in

☆ This paper has not been presented, but partial results were presented as an abstract [20].

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http://dx.doi.org/10.1016/j.cca.2014.10.045
0009-8981/

resuscitation of patients with hemorrhagic shock. The patients were enrolled at the University Hospital in San Antonio, Texas, over a period of 2 y (2008–2009). During this time, the sera from 17 patients in severe hemorrhagic shock were collected before administration of any treatment (from convenience samples at the time of admission) and sent for testing to the proteomics laboratory in the Pathology Department at the UTHSC in San Antonio, Texas.

2.2. Patient selection

Seventeen patients were enrolled in the study: three female and fourteen male, all were >18 y with an average age (SD, min–max) of 44.4 (18.6, 18–84). All patients were victims of blunt trauma and exhibited clinical evidence of hemorrhagic shock with a systolic blood pressure <90 mmHg and a shock index ranging from 0.81 to 2.33. Five of these 17 patients died within 24 h and 1 developed MODS.

The parent study exclusion criteria were: admission to the emergency department >6 h after the traumatic injury occurred; receiving more than four liters of fluid before admission; enrollment in another shock trial; asystole or cardiopulmonary resuscitation before random group assignment; pregnancy or suspicion thereof; a "Do Not Resuscitate" order and religious objections to receiving blood products. Because informed consent for study participation could not be granted at the time of admission due to the clinical situation of the patients being in shock, the enrollment was made under the provisions for "exception from informed consent" (in accordance with regulation 21 CFR 50.24: Exception from Informed Consent Requirements for Emergency Research). The University of Texas Health Science Center San Antonio Institutional Review Board approved this protocol. The control group consists of ten male and ten female patients who came to the emergency room without hemorrhagic shock. The chosen patients matched the ages of the testing group. The salvaged blood obtained for standard testing was used.

2.3. Medical intervention

Utilizing the standard Advanced Trauma Life Support (ATLS) protocol, all patients received routine aggressive critical care for their traumatic injuries. To guide the patients' care, the standard clinical parameters of SBP, HR, BD, SI and StO2 were measured. Saline infusion with vasopressin or placebo was initiated within one hour after the patients' SBP ≤90 mmHg. The blood samples submitted for marker testing were drawn before the study treatment was administered. The primary endpoint for this original study was determining whether the standard of care (saline) plus vasopressin was superior to saline alone and assessed by a 30-day mortality rate. The secondary endpoints were 24-h and 5-day mortality rates and the incidence of MODS through day 30. Our markers were evaluated against the modified secondary endpoint that was the death or MDOS at any time of 30 day observation. Patients who were tested for out markers received standard care treatment with saline alone.

The concentrations of all three markers were measured in the proteomics laboratory at the UTHSC at San Antonio by using GenoLab ELISA kit for sP-Selectin, Technoclone GmbH kit for ADAMTS 13 activity, GenoLab ELISA kit for ADAMTS concentration, and HSP27 ELISA Kit from R&D systems for HSP27. The testing was done according to the instructions from the kit inserts.

2.4. Statistical analysis

The main goal of our project was to evaluate the efficacy of sP-Selectin, ADAMTS13, and HSP27 in identifying trauma patients with hemorrhagic shock who were at high risk to die or develop MODS. In addition, we tested whether there is any difference in concentration of our markers between the group of patients with hemorrhagic shock and the group of control patients who appeared in the emergency room without hemorrhagic shock. The statistical power for this study is 0.80, if it is based on group difference of 1.4 standard deviations and calculated for two-tailed significance of 0.05 for the two groups patients with shock.

Because our data may be non-normally distributed, to compare sP-Selectin, ADAMTS13, and HSP27 concentrations in our three groups (patients without hemorrhagic shock, patients with hemorrhagic shock who lived, and patients with hemorrhagic shock who died or developed MODS), we used the exact non-parametric Wilcoxon for parameters with just 2 groups of patients and an ANOVA using rank transformed data when there were three groups of patients. The difference in the median was tested by the Wilcoxon non-parametric two sample test with a significance set at $p < 0.05$.

The probability of death or MODS based on concentrations of these markers was also tested by logistic regression with a significance set at $p < 0.05$. In addition, the lack of fit was tested with adequate fit set at $p > 0.05$ to support the use of logistic regression. Prediction error based on the leave on procedure was done using logistic regression to help assess how well markers did at predicting outcome of cases not used in the analysis.

2.5. Clinical analysis

The clinical performance of our markers was assessed by calculation of the sensitivities, specificities, and predictive values to forecast death or MODS. In addition, we constructed ROC curves for our markers and compared their areas under the curve (AUC) to the AUC for currently used diagnostic tests such as HR, SBP, SI, StO2, and BD.

ROC curves were compared with SAS ver 9.3 using the non-parametric approach of DeLong, Delong, and Clarke-Pearson (1988). All statistical analysis was performed by SAS, Inc. ver 9.3software and R i386 2.15.3. Specificity and sensitivity of different markers were compared using the McNemar test. The exact binomial confidence intervals were obtained with binom.test from R.

3. Results

Our 3 markers successfully distinguished between patients who lived and those who died or developed MODS.

3.1. Statistical analysis

Comparing the medians between 2 groups of patients with hemorrhagic shock (survivors, $n = 11$; vs. MODS/death, $n = 6$), we found a statistically significant difference in the Wilcoxon non-parametric two sample test (Table 1), on logistic regression analysis, and on lack of fit tests for HSP27 and BD (data not presenting). ADAMTS13 antigen concentration, ADAMTS13 activity/antigen, and sP-Selectin medians were substantially different between these groups, but not statistically significant. ADAMTS13 activity, StO2, SI, HR, and SBP were not effective markers in any of these tests (Table 1). Logistic regression discarded sP-Selectin as a potential marker (data not presenting).

Using the patients with hemorrhagic shock and the control emergency department patients, we found a statistically significant difference between these groups for HSP27, ADAMTS13 antigen concentration, and ADAMTS13 activity/antigen concentration ratio. The sP-Selectin concentration and ADAMTS13 activity were different but not statistically significant.

3.2. Clinical evaluation

To assess diagnostic performance of our markers, we compared them against the standard protocol for monitoring patients with hemorrhagic shock (BD, StO2, SI, HR, and SBP). This was done by constructing a ROC curve and by comparing the areas under the ROC curve (AUC). This curve was constructed by plotting sensitivity on the vertical axis and one-minus-specificity on the horizontal axis.