Journal of Critical Care 32 (2016) 16–20

Contents lists available at ScienceDirect

Journal of Critical Care

journal homepage: www.jccjournal.org

Markers of endothelial damage and coagulation impairment in patients with severe sepsis resuscitated with hydroxyethyl starch 130/0.42 vs Ringer acetate

Rasmus Beier Müller, MD [a,*], Sisse Rye Ostrowski, MD, PhD, DMSc [b], Nicolai Haase, MD, PhD [a], Jørn Wetterslev, MD, PhD [c], Anders Perner, MD, PhD [a], Pär Ingemar Johansson, MD, PhD, DMSc [b,d]

[a] Department of Intensive Care, Copenhagen University Hospital–Rigshospitalet, DK-2100 Copenhagen, Denmark
[b] Section for Transfusion Medicine, Copenhagen University Hospital–Rigshospitalet, DK-2100 Copenhagen, Denmark
[c] Copenhagen Trial Unit, Centre for Clinical Intervention Research, Copenhagen University Hospital–Rigshospitalet DK-2100 Copenhagen, Denmark
[d] Department of Surgery, University of Texas Health Medical School, Houston, TX 77030

ARTICLE INFO

Keywords:
Endothelial damage
Severe sepsis
Hydroxyethyl starch
Resuscitation
Colloids

ABSTRACT

Purpose: The Scandinavian Starch for Severe Sepsis/Septic Shock (6S) trial showed increased mortality in patients resuscitated with hydroxyethyl starch 130/0.42 (HES) vs Ringer acetate. Different effects of the fluids on the endothelium may have contributed to the observed outcome. We aimed to investigate associations between HES vs Ringer and changes in plasma biomarkers reflecting endothelial damage and coagulation impairment.
Materials and methods: Six biomarkers, including soluble thrombomodulin (sTM) and plasminogen activator inhibitor 1 (PAI-1), were assessed in a subgroup of 208 patients from the 6S trial. We analyzed differences in plasma concentration in the 2 intervention groups using linear or logistic regression models.
Results: The increase in plasma sTM was lower in the HES group (−1.8 ng/mL; 95% confidence interval, −2.9 to −0.7; $P = .002$). The change was not statistically significant associated with mortality whereas increase in plasminogen activator inhibitor 1 was (odds ratio for 1-unit increase, 1.04; 95% confidence interval, 1.01-1.08; $P = .01$).
Conclusions: Resuscitation with HES vs Ringer decreased early endothelial damage. Although this finding should be interpreted with caution, it indicates that the increased mortality observed with HES in the 6S trial may not be explained by endothelial damage and it emphasizes the challenge of using surrogate markers as outcome.

1. Introduction

Resuscitation with hydroxyethyl starch 130/0.42 (HES) vs Ringer acetate led to increased mortality in patients with severe sepsis in the Scandinavian Starch for Severe Sepsis/Septic Shock (6S) trial [1]. However, the underlying mechanisms of harm are not fully understood. We have previously proposed that both bleeding [2] and acute kidney injury [3] may have contributed to the effect of HES on mortality, but other mechanisms could be involved.

There is emerging evidence that the endothelium plays a pivotal role in the pathogenesis of sepsis due to its comprehensive involvement in vascular homeostasis, the coagulation system, and the immune response [4-6]. Thus, a damaged endothelium has been correlated with both disease severity and mortality in patients with severe sepsis [7-9]. The endothelium may therefore be important for the effects of intravenous fluids, and different types of fluids may impact the endothelium differently, which potentially could affect patient outcome.

Soluble products of vascular degradation and platelet activation can be used as biomarkers to assess endothelial damage and coagulation impairment [6,10,11]. We assessed changes in such biomarkers in a subgroup of patients from the 6S trial resuscitated with HES vs Ringer acetate and their association to mortality, in an attempt to elucidate whether these markers could reveal information about the pathophysiology behind the 6S findings. If the fluids affected the endothelium differently, this may have contributed to the observed differences in the intervention effect, and these biomarkers have the potential of being predictors for outcome if associated with mortality.

2. Methods

Patients enrolled in the 6S trial at 2 Danish intensive care units (ICUs) from March 2009 to November 2011 were eligible for this post hoc analysis (n = 226). The subgroup comprises 208 of those patients (106 in the HES group and 102 in the Ringer group) who had blood sampled either at baseline or day 2.

In the 6S trial, the investigators randomized adult patients with severe sepsis who were in the ICU and in need of fluid to either masked 6% HES 130/0.42 in Ringer acetate (Tetraspan 6%; B Braun, Melsungen, Germany) (n = 398) or Ringer acetate alone (Sterofundin ISO; B Braun) (n = 400). The exclusion criteria included administration of more than 1000 mL of synthetic colloid within 24 hours before

* Corresponding author.
E-mail address: rasmus.muller@regionh.dk (R.B. Müller).

http://dx.doi.org/10.1016/j.jcrc.2015.11.025
0883-9441/

randomization, life-threatening bleeding, or use of renal replacement therapy. Trial fluid administration was at the discretion of the clinician to a maximum daily dose of 33 mL/kg ideal body weight. If patients needed additional fluid for resuscitation, open-label Ringer acetate was administered. The intervention period lasted for the entire ICU stay to a maximum of 90 days.

The present study was approved as a protocol amendment to the 6S trial by the Ethics Committee of the Capital Region of Denmark and the Danish Medicines Agency. Informed consent was obtained from all participants or their legal substitute before enrollment. Previously, publications of the 6S trial includes the trial protocol [12], the primary results [1], long-term outcome [13], subgroup analyses [14], analyses of acute kidney injury [3], bleeding [2], hypocoagulability [15,16], and sympathoadrenal activation [17].

2.1. Plasma sampling and biomarker analyses

Patients enrolled in the present study had blood sampled at baseline before randomization and again on the morning of the second day, which was median (min-max) 38 (24-48) hours after randomization.

In the samples, we measured 6 different soluble vascular-derived biomarkers to give a diverse overview of the interventions group's endothelial and coagulative function. The markers were specifically chosen according to their ability to reflect endothelial damage and coagulation impairment as evidenced in earlier trials: endothelial cell damage (soluble thrombomodulin [sTM] [18]), glycocalyx damage (syndecan 1 [19]), platelet activation (soluble CD40 ligand [sCD40L] [20]), induced natural anticoagulation (protein C [6]), and endothelial cell activation (tissue-type plasminogen activator [tPA] and plasminogen activator inhibitor 1 [PAI-1] [21]). The biomarkers were measured by enzyme-linked immunosorbent assay according to the manufacturer's recommendations (syndecan 1 [Diaclone SAS; Besancon, France; LLD, 4.94 ng/mL] and sTM [Nordic Biosite, Copenhagen, Denmark; lower limit of detection (LLD), 0.31 ng/mL] were analyzed in serum, sCD40L [Quantikine; R&D Systems Europe, Ltd, Abingdon, UK; LLD, 4.2 pg/mL] in heparin plasma and protein C [Helena Laboratories, Beaumont, TX], tPA [ADI, detects sc-tPA, tc-tPA; LLD, 1 ng/mL], and PAI-1 [Assaypro; LLD, 0.07 ng/mL] were analyzed in citrated plasma). The concentrations of the biomarkers were then calculated from the measured absorbance. In some cases, the patient had 2 biomarker measurements at the same time point in which case the mean absorbance was used to calculate the concentration. If the absorbance was below the lower detection limit for the given enzyme-linked immunosorbent assay kit, the value of the lower detection limit was used in the analyses.

2.2. Statistics

We used χ^2 test and Wilcoxon rank sum test to assess potential differences in baseline values between the 2 intervention groups. We assessed the association between the delta concentration from baseline to day 2 of the biomarker levels and the intervention using a linear regression model. We calculated odds ratios using logistic regression for the correlation between the delta biomarker levels and 90-day mortality.

We performed multiple imputations of missing data under the missing at random criterion using 25 imputed data sets and adjusted the analyses of the association between the delta concentrations and intervention and mortality for the stratification variables, university hospital, hematologic malignancy and shock at randomization, and the following baseline characteristics: Simplified Acute Physiology Score (SAPS) II [22] and administration of fresh frozen plasma (FFP) in the 24 hours before randomization (dichotomized yes/no).

Overall, we had missing data for 498 (20%) of 2496 biomarker measurements distributed on 99 patients (48%). Of those, 41 patients (41%) had no blood sampled on day 2, 12 (12%) of these due to discharge, 9 (9%) because they had died (5 in the HES group and 4 in the Ringer group; these were not imputed), and 20 (20%) had no reason documented.

We used SAS 9.1 (SAS Institute, Inc, Cary, NC) for the analyses and P values less than .05 as the level of statistical significant.

3. Results

At baseline, the 2 intervention groups were comparable regarding general demographics, severity of illness, and biomarker levels (Table 1). Trial fluid was administered in comparable volumes in the intervention groups within the first 2 days (median [min-max], 2250 mL [0-4500 mL] in the HES group vs 2500 mL [0-6000 mL] in the Ringer group [$P = .73$]). Nineteen patients (18%) received renal replacement therapy in the HES group and 20 patients (20%) in the Ringer group ($P = .76$), and at day 90, 54 patients (51%) in the HES group and 56 patients (55%) in the Ringer group had died ($P = .57$).

3.1. Markers of endothelial damage

From baseline to day 2, median sTM increased in both the HES and Ringer group (Fig. 1). However, patients assigned to HES had a lower increase (mean difference in change of sTM, −1.8 ng/mL; 95% confidence interval [CI], −2.9 to −0.7; $P = .002$) as compared with those in the Ringer group. However, the change in sTM was not associated with 90-day mortality (Fig. 2).

The median syndecan 1 concentration also increased from baseline to day 2 in both the HES and Ringer group (Fig. 1), but there was no difference in syndecan 1 delta values between the intervention groups (Table 2) and delta syndecan 1 was not associated with 90-day mortality (Fig. 2).

Table 1
Baseline characteristics

	HES (n = 106)	Ringer acetate (n = 102)	P
Age, median (min-max)	66 (37-87)	66 (23-88)	.69
Male sex, n (%)	63 (59)	55 (54)	.48
Surgery before randomization, n (%)	41 (39)	46 (45)	.35
Emergency	32 (30)	35 (34)	
Elective	11 (10)	14 (14)	
Source of sepsis, n (%)			
Lungs	59 (56)	68 (67)	.12
Abdomen	28 (26)	35 (34)	.23
Urinary tract	9 (9)	3 (3)	.14
Soft tissue	20 (19)	15 (15)	.46
Colloids before randomization, n (%)[a]	43 (43)	48 (47)	.52
SAPS II, median (min-max)	52 (20-99)	55 (26-99)	.73
SOFA score ex. GCS, median (min-max)	8 (2-14)	8 (1-18)	.23
Shock, n (%)	89 (84)	87 (85)	.79
Lactate (mmol/L), median (min-max)	1.9 (0.7-9.9)	2.1 (0.5-15)	.56
Biomarkers, median (min-max)			
Syndecan 1 (ng/mL)	93.2 (12.8-363.5)	102.2 (11.6-379.5)	.31
sTM (ng/mL)	7.9 (1.3-18.8)	7.4 (1.7-22.1)	.46
sCD40L (pg/mL)	252.2 (14.3-1658.1)	262.7 (8.1-1355.7)	.80
Protein C (%)	49.5 (5.6-121.9)	43.5 (1.6-229.7)	.97
tPA (ng/mL)	14.9 (3.6-139.4)	19.4 (3.5-159.1)	.19
PAI-1 (ng/mL)	27.1 (7.4-45.3)	34.3 (5.5-46.2)	.20

SOFA indicates Sepsis-related Organ Failure Assessment; GCS, Glasgow Coma Scale.
[a] Colloids refer to hydroxyethyl starch, gelatine, dextran, or albumin. We had missing data on 5 patients in the HES group. *Shock* was defined as a mean arterial pressure of less than 70 mm Hg, the need of continuous treatment with vasopressor or inotropic agents, or a plasma lactate level of more than 4.0 mmol/L in the hour before randomization. The SAPS II [22] and Sequential Organ Failure Assessment scores [23] were obtained in the 24 hours before randomization. The Sequential Organ Failure Assessment score was modified by excluding the Glasgow Coma Scale score. Lactate was the highest arterial or venous lactate registered within 2 hours before randomization.