Endotoxin removal in septic shock with the Alteco® LPS Adsorber was safe but showed no benefit compared to placebo in the double-blind randomized controlled trial – The ASSET study

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Competing interests

The authors have disclosed that they do not have any conflicts of interest other than that stated below. MSC was Medical Advisor for Alteco AB and received reimbursement for her advice from 2014-2017. After this, she participated as clinical investigator. Moreover, Alteco AB financed the study and MSC, SR, ML, MSC, TIT, SP were reimbursed for travel expenses from the company.

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Running head

Endotoxin elimination in sepsis

Abstract

Purpose

Lipopolysaccharides (LPS) are presumed to contribute to the inflammatory response in sepsis. We investigated if extracorporeal Alteco® LPS Adsorber for LPS removal in early Gram-negative septic shock was feasible and safe. Also, effect on endotoxin level, inflammatory response and organ function were assessed.

Methods

A pilot, double-blinded, randomized, Phase IIa, feasibility clinical investigation was undertaken in six Scandinavian intensive care units aiming to allocate thirty-two septic shock patients with abdominal or urogenital focus to LPS Adsorber therapy or a Sham Adsorber, therapy without active LPS-binding. The study treatment was initiated within 12 hours of inclusion and given for six hours daily on first two days. LPS was measured in all patients.

Results

The investigation was terminated after 527 days with eight patients included in the LPS Adsorber group and seven in the Sham group. Twenty-one adverse effects, judged not to be related to the device, were reported in three patients in the LPS Adsorber group and two in the Sham group. Two patients in the Sham group and no patients in the LPS Adsorber group died within 28 days. Plasma LPS levels were low without groups differences during or after adsorber therapy. The changes in inflammatory markers and organ function were similar in the groups.

Conclusions

In a small cohort of patients with presumed Gram-negative septic shock, levels of circulating endotoxin were low and no adverse effects within 28 days after LPS adsorber-treatment were observed. No benefit compared to a sham device was seen when using a LPS adsorber in addition to standard care.

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Keywords: Septic shock, Endotoxins, Hemoperfusion, Gram-Negative Bacteria

Introduction

Septic shock is a life-threatening condition with profound systemic inflammatory activation triggered by infection (1). Despite management with antimicrobial therapy, source control and support of failing organs, mortality rates remain high (2). Therefore, the development of new treatment options for sepsis and septic shock is crucial for medical, humanitarian and health-economic reasons.

Gram-negative bacteria are a common cause of septic shock. Lipopolysaccharides (LPS), a group of endotoxins, are molecules found in the outer membrane of these bacteria. They are potent activators of the inflammatory system through the innate immune system and have been considered as one of the key triggers of the systemic inflammatory response (3-5). Hence, reducing the levels of LPS seems to be a logical and desirable strategy in the treatment of sepsis and septic shock. It is reasonable to hypothesize that using LPS adsorption membranes for the treatment of selected patients in the early phase of Gram-negative septic shock, within a strictly defined time-frame after the onset of clinical symptoms, may offer therapeutic benefits. Several extracorporeal endotoxin removal devices, based on various endotoxin-binding mechanisms, have been investigated with diverging results (6-10).

Case reports from patients with septic shock suggest that endotoxin removal may be achieved in clinical practice with an LPS adsorber system (Alteco Medical AB, Lund, Sweden) with a high LPS affinity peptide binding mechanism, hereafter referred to as LPS Adsorber (11, 12). However, no randomized controlled trial has investigated the potential benefits of this LPS Adsorber.

We postulated that in patients with Gram-negative infections, early and extensive removal of LPS would limit the inflammatory response that characterizes septic shock. Unlike other device trials, the LPS Adsorber was compared to an identical Adsorber cartridge without active LPS-binding peptide. Thus, a double-blinded randomized sham -controlled pilot feasibility trial was performed.

The primary endpoint was characterization of all reported adverse effects reported as Serious Adverse Device Effects (SADE) and Adverse Device Effects (ADE) as well as Serious Adverse Effects (SAE) and Adverse Effects (AE). Secondary endpoints were: changes in LPS levels during LPS Adsorber therapy; changes in the extent of organ failure; intensive care unit (ICU) mortality and 28-day mortality, ICU and hospital length-of-stay (LOS). We also measured plasma markers of the innate immune system, i.e. cytokines and complement activation as they are regarded as the first-line defense after bacterial infection.

Methods

This was a pilot multicenter, stratified, parallel group, double-blinded, randomized, Phase IIa, feasibility clinical investigation reported according the CONSORT and the SPIRIT guidelines (13, 14). The protocol has been reported previously (15).

The clinical investigation was conducted in compliance with applicable international standards (ISO 14155:2011), as well as with the ethical principles of the Declaration of Helsinki as adopted by the World Medical Association. The study was approved by local ethical review boards (Uppsala, Sweden; 2014/370, ALT C1-01; Norway, 2014/1059/REK vest; Tampere, Finland R14130).

The final protocol (6.0) was amended during the study on the 12th of December, 2016.

Six general intensive care units (ICU) in Uppsala and Linköping, Sweden, Bergen and Oslo, Norway, and Tampere and Kuopio, Finland participated. We aimed at including 32 patients admitted to the ICU with confirmed septic shock after informed consent from the patient or legal representative. Recruitment of patients started in September 2015. Patients were stratified according to the origin of their suspected endotoxemia: 20 septic shock patients with abdominal focus (Stratum A) and 12 patients with urogenital focus (Stratum B). Patients were randomly allocated to standard care + LPS Adsorber therapy (LPS Adsorber group), or standard care + identical adsorber cartridge without active LPS-binding peptide (Sham group).

All inclusion and exclusion criteria are listed in the Supplemental Digital Content (SDC). A concise version is listed below. Upon enrolment (i.e. pre-treatment phase), patients admitted to the ICU with suspected endotoxemia were screened for fulfilment of the “Illness Severity Criteria” confirming early stage severe sepsis. Within six hours of enrolment, patients who fulfilled the “Treatment Criteria” confirming septic shock were randomized.

Illness Severity Criteria

1) Patients must have suspected infection of abdominal or urogenital origin for which the patient is receiving intravenous antimicrobial therapy
2) Patients must have systemic inflammatory response syndrome (SIRS)(16)
3) At least one of the following criteria during the six hours prior to clinical investigation entry:
    a) Metabolic acidosis
    b) Acute oliguria/renal injury
    c) Acute hepatic dysfunction
    d) Thrombocytopenia

Treatment Criteria

4) SOFA score of 10 or higher (17), AND a Simplified Acute Physiology Score (SAPS II) of 58 or higher (18).
5) Patients must have received ≥ 30 mL/kg of intravenous fluid within the six hours prior to randomization.
6) Vasopressor support for at least two hours prior to randomization to maintain mean arterial pressure (MAP) > 65 mmHg or systolic arterial pressure > 90 mmHg.
7) The clinical investigation intervention initiated within 12 hours of fulfilment of the illness severity criteria.

Randomization

Patients were randomized with 1:1 ratio in blocks of four stratified by Stratum to either LPS Adsorber, or Sham groups by being treated with a randomly numbered blinded cartridge with the lowest serial number on site. Each site received therefore at least four blinded device packages per shipment. Study investigators, laboratory and research staff were blinded to treatment allocation and data until the analyses for the final report. Stratum A and Stratum B patients were kept apart by different serial numbers.

Intervention

Figure S1 (SDC) summarizes the timeline of the clinical investigation. Treatment with LPS Adsorber or Sham adsorber initiated within six hours (Day 1) following fulfilment of the "Treatment Criteria" and given for six hours. Dalteparin or tinzaparin as bolus was used as anticoagulation during each treatment session. A second device treatment was performed 24 hours after the end of the first device treatment on Day 2. Treatment on Day 2 was not given if septic shock had resolved (e.g. no need for vasopressor support, new limitations of care). At least one treatment session with two hours of treatment was required to fulfill the treatment protocol. Patients were followed to 28 days after enrolment. Apart from the protocol for LPS or Sham adsorber therapy, management of the patients was at the discretion of the attend physician.

Endotoxin analysis

Endotoxin was analyzed with limulus amebocyte lysate assay (LAL; Pierce LAL Chromogenic Endotoxin Quantitation Kit, Thermo Scientific, Waltham, MA) measuring endotoxin activity as well as by high-performance liquid chromatography coupled with mass spectrometry (HPLC/MS) quantifying esterized 3-hydroxymyristate (3OH), the most abundant hydroxylated fatty acid of the lipid A moiety of endotoxin (19).

Complement activation and Cytokines

Complement activation was measured by the plasma terminal C5b-9 complement complex (TCC). Also, ten cytokines were measured: Interleukin (IL)-6, IL-8, IL-10, interferon gamma-induced protein 10 (IP-10), monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein 1 beta (MIP-1β), tumor necrosis factor (TNF), IL-1 receptor antagonist (IL-1RA), Regulated on Activation, Normal T Cell Expressed and Secreted (RANTES) and eotaxin. See also the SDC.

Organ failure

Sequential Organ Failure Assessment (SOFA) score, renal function, liver function, circulatory support and respiratory support data were collected as reported in the results.

Data

Data was collected by local research staff. Sites were monitored regularly by the clinical research organization (CRO; TFS AB, Lund, Sweden). Training, assessment of collected data, data storage and management were performed by the CRO.

Statistics

The statistical analysis plan was published prior to commencing the study (15). Since this was a pilot study and with no evaluation of primary performance variables, the sample size was chosen for practical reasons. The Full Analysis Set consists of all randomized patients who were randomized and was analyzed by intention to treat. Differences in parametric data were assessed by t-tests or mixed linear models, while nonparametric data were assessed by Mann-Whitney, Wilcoxon paired or Friedmans ANOVA tests as appropriate. Correlations were assessed with Spearman-Rank correlations. A $p<0.05$ was considered significant. Statistica® 13.2 software (Statsoft, Tulsa, OK) and GraphPad Prism were used. Data are presented as median (interquartile range; IQR) unless stated otherwise.

Results

The study was terminated prior to reaching the pre-specified number of patients due to a low inclusion rate after 527 days, when 15 patients had been enrolled, of whom eight randomized to the LPS Adsorber and seven to the Sham group. Eight patients completed the full treatment protocol with two treatments. The inclusion of patients is depicted in Figure 1.

Patient characteristics are presented in Table 1. Six of 15 patients had growth of Gram negative bacteria in blood. The microbiological cultures are presented in Table S1 (SDC). The most common verified source of infection was colon perforation followed by cholangitis in septic shock patients with abdominal focus.

Safety and feasibility

Including SAEs (SDC, Table S2), there were a total of 21 AEs in five patients (33.3%): 3 patients in the LPS Adsorber group and 2 patients in the Sham group. No AEs were judged to be related to device treatment i.e. none were SADE or ADE.

Four patients in the LPS adsorber group and three patients in the Sham group had adsorber devices replaced due to clotting. One patient in each group discontinued the clinical investigation because of repeated clotting of the device. One patient in the LPS Adsorber group discontinued because a lack of need for further ICU care after the first treatment.

Endotoxin levels in plasma

Endotoxin (LPS) levels measured with the LAL assay were below the detection limit in almost all samples (data not shown). Quantification of endotoxin by measuring 3OH showed very low levels of endotoxin and no difference in endotoxin levels between patients treated with LPS and sham absorbers at 2 and 6 hours after the start of LPS adsorber therapy on day 1 and 2 (Figure 2).

Inflammatory response

The complement activation marker, TCC, increased in a biphasic fashion during the time periods corresponding to the LPS and sham adsorber treatments without differences between the groups, consistent with an equal activation of complement by the two devices (Figure 2). The activation by the devices contributed more to the complement activation than the sepsis per se.

The 10 cytokines showed a marked inter-individual variation with no statistical significance between the groups (Figure 2). They showed distinctly different patterns during the observation period. TNF, IL-6, IL-1ra, IL-8 and MIP-1β were increased from start, and gradually declined to baseline levels. RANTES showed a similar pattern, except for a peak in the LPS adsorber group at 24 hours. IP-10 increased markedly from start to 24 hours and then declined. Eotaxin declined after start and then showed a patter similar to TCC with two peaks corresponding to the adsorption periods. MCP-1 and IL-10 stayed low during the whole observation period.

Organ function

The median pre-treatment SOFA score was 12 in both groups prior to randomization, that were similar between the groups during the study (Table 2). The extent of organ failure expressed as Urine output per day, Cystatin C estimated glomerular filtration rate ($eGFR_{Cyst}$), Plasma bilirubin, Vasopressor dependency index, Arterial Lactate levels, PaO_2/FiO_2 ratio, and Blood platelet count during the first two days of the study were also similar between the groups. Although most of these variables improved during the first two days, platelets decreased in both groups during this period ($p<0.05$). Renal replacement therapy free days were 23 (12-28) *vs.* 12 (1-26), vasopressor free days 24 (20-26) *vs.* 24 (21-26), and ventilator free days (up to 28 days) were 21 (20-27) *vs.* 23 (19-25), for the LPS Adsorber group *vs.* Sham group, respectively (p=n.s. for all). 3OH did not correlate to SAPS, SOFA score, PaO2/FiO2 ratio, Vasopressor dependency index, Arterial Lactate, Blood platelet count, Plasma bilirubin, or Cystatin C $eGFR_{Cyst}$ at the start of treatment 1.

Outcome

Two patients, one male and one female, died within 28 days in the Sham group (after eight and 10 days), whereas no patients died in the LPS Adsober group. ICU LOS was 11 (6-14) days in the LPS Adsorber group and 11 (7-27) in the Sham group. One from the LPS Adsorber group (24 days) and one from the Sham group (22 days) were discharged from hospital before 28-days.

Discussion

The aim of this double-blinded randomized pilot study was to investigate the safety, feasibility, and potential biological and clinical effects of the specific, high affinity, high capacity LPS Adsorber system (11, 12). The investigation was terminated prematurely due to low inclusion rate, with less than half of the pre-set number of patients included. LPS Adsorber treatment was safe in this small cohort of patients, however technical problems, specifically clotting, was very common leading to abrogated adsorber treatments. Plasma endotoxin levels were low both before and after treatment, and were not different in the LPS adsorber and the Sham groups. The extent of inflammatory reaction, organ dysfunction and outcome were similar in the LPS adsorber and the Sham groups.

Given that this is a pilot study and the pre-set number of patients was not reached, the conclusions from the study are limited. Yet, several findings are of interest for further studies using the LPS Adsorber and for studies in other anti-LPS strategies. Importantly, the study shows that double-blinded device studies are feasible in the critical care setting.

Patients undergoing LPS adsorber therapy did not experience more adverse effects compared to the Sham group. However, our data suggests that the adsorption procedure induced complement activation as measured by TCC, platelet consumption and eotaxin increase in both groups.

Although LPS levels in plasma have been reported to be increased during the course of sepsis (6), both *in vitro* and clinical data suggests that endotoxin levels are highest in early phase of septic shock (20, 21). This study was thus designed assuming that LPS adsorption would be most beneficial

immediately after the initial dose of antibiotics was administered. Yet, we found low LPS levels despite inclusion criteria aiming at identifying patients with high endotoxin levels. Endotoxin levels were below the detection limit with LAL and low with HPLC/MS method (22). A reason for this could be that endotoxin levels decreased after the initial dose of antibiotics prior to LPS adsorber treatment similarly to a previous report (21). Alternatively, given the low mortality, despite the preset SAPS II and SOFA requirements, the endotoxin levels may have been low due to low degree of acute illness. A breakdown of LPS in the samples cannot be excluded. However, blood samples were handled according to our standard laboratory procedures, and we have previously reported detectable endotoxin levels from experimental research with these methods (23, 24).

The levels of endotoxin estimated by 3OH were low and did not decrease during LPS Adsorber therapy. This raises the question if extracorporeal LPS-adsorber therapies add substantially to endogenous endotoxin elimination. Although there are reports that imply decreased endotoxin levels with the endotoxin adsorber used in our study (11, 12), experimental and *in vitro* data have challenged all currently used LPS-adsorber therapies (23, 25).

Previous large randomized controlled studies on extracorporeal endotoxin elimination (6-10) included patient cohorts with variable illness severity. We aimed to include patients with high severity of illness with a predicted 28 day mortality over 40% setting high SAPS II and SOFA requirements for inclusion (26, 27). However, despite reaching the preset illness severity scores the mortality in our study was 13% at 28 days. Both patients who died received sham adsorber treatment while no patients died in the LPS Adsorber group. The occurrence of death to the Sham group does not yield a statistical difference and given the low number of patients in the study, even if there was difference the fragility index of this investigation would be very low (28).

Another aim of the study was to start LPS removal as early as possible after identifying an eligible patient. We experienced substantial difficulties in finding patients with a relatively specific group of pathologies. Specifically, capturing patients not responding to initial resuscitation (thus presumed to

have high mortality), obtaining informed consent in these patients, then initializing a complex therapy within a short timeframe were logistical challenges. This problem is underlined by the decrease of most inflammatory mediators in our study irrespective of LPS Adsorber or sham treatment was given, suggesting that any intervention aimed at removing endotoxin or inflammatory mediators would need to be instituted very early during the course of septic shock, preferably with first dose of antibiotics. Alternatively, endotoxin removal therapies should be directed at a group with already high pre-treatment endotoxin levels, that we were unable to identify using clinical criteria alone.

Strengths and Limitations

Unlike previous studies (6-10), as far as we know, this is the first double-blinded extracorporeal endotoxin elimination study, which was made possible with a sham cartridge without LPS adsorber properties. Other obvious strengths of the study are the extensive clinical and laboratory data collected, including the analysis of inflammatory markers as well as the follow-up to 28 days.

On the other hand, early termination significantly limits the power of this study. However, one aim of this pilot study was to test the protocol for a possible phase IIb trial. With the current inclusion rate in six large centers several additional years would have been required to finalize the study. Another limitation of the study was that only one patient with urogenital sepsis was included. Urogenital sepsis is caused in the majority of cases by Gram-negative bacteria (29). Although septic shock is not uncommon in this group of patients, illness severity scores in this group were below inclusion levels. A further consequence of the early termination is that the characteristics of the patients at inclusion differed in some aspects, limiting the validity of findings that are based on group differences.

The study was designed to identify patients with Gram-negative sepsis, but not all patients presented with these bacteria according to culture results. Although the aim of the study was to decrease high levels of endotoxin in the blood related to Gram-negative infection, endotoxemia is present in sepsis

of other etiologies too (30), suggesting that LPS adsorption therapy could have been of benefit even in patients with other types of severe infections.

Future studies

Based on this and other studies the role of endotoxin removal in sepsis is uncertain (10, 13). Any future study must identify patients with high endotoxin levels. Moreover the endotoxin removal should be started early with high capacity and specific endotoxin adsorbers.

Conclusions

In a small cohort of patients with presumed Gram-negative sepsis, no adverse effects of LPS adsorber-treatment were observed within 28 days after treatment, but it did not offer any clinical benefit compared to a sham device. The low level of circulating endotoxin suggests that anti-endotoxin strategies are unlikely to give the desired benefit in this selected group of patients.

List of abbreviations

AE	Adverse effects
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CIP	Clinical investigation plan
CRO	Clinical research organization
CRRT	Continuous renal replacement therapy
CT	Computed tomography
eCRF	Electronic case report form
$eGFR_{Cyst}$	Estimated glomerular filtration rate based on Plasma Cystatin C
HIT II	Heparin-induced thrombocytopenia II
HPLC-MS	High-performance liquid chromatography coupled with mass spectrometry
ICU	Intensive care unit
IMD	Interventional medical device
LAL	Limulus amebocyte lysate assay
LOS	Length-of-stay
MAP	Mean arterial pressure
PaO_2/FiO_2	Arterial oxygen tension/Fraction of inspired oxygen
RRT	Renal replacement therapy
SAE	Serious adverse effects
SAPS II	Simplified acute physiology score
SIRS	Systemic inflammatory response syndrome
SOFA	Sequential organ failure assessment score

Declarations

Ethical Approval and Consent to participate

The clinical investigation was conducted in compliance with applicable international standards (ISO 14155:2011) and regulatory requirements, as well as with the ethical principles of the latest revision of the Declaration of Helsinki as adopted by the World Medical Association. The study has been approved by Regionala etikprövningsnämnden i Uppsala, Sweden (Regional Ethical Review Board in Uppsala; 2014/370, ALT C1-01), Regionale Komiteer For Medisinsk og Helsefaglig Forskningsetikk, Norway (Regional Committees for Medical and Health Research Ethics; 2014/1059/REK vest) and Tampereen Yliopistollisen Sairaalan Erityisvastuualueen Alueellinen Eettinen Toimikunta, Finland (Regional Ethical Committee of the Tampere University Hospital; R14130). Each patient was consented according to the regulations of the country where the patient is included in the study. The signed informed consent form was obtained by the Investigator prior to inclusion in the study. Should subjects be incapable of giving informed consent (e.g., subjects may be unconscious or obviously disoriented), the Clinical Investigator requested informed consent from the legally acceptable representative(s). As soon as the subject's medical condition allowed, he/she was informed about the clinical trial, and asked to provide informed consent for continued participation. Participation in the clinical investigation is voluntary and subjects (or their legally accepted representatives) could discontinue their participation at any time.

Authors' contributions

ML drafted the manuscript based on the protocol. ML and JT contributed equally to the protocol. AL, SP and TEM planned and conducted the laboratory investigations. All authors contributed to and approved the manuscript. ML, JT, RF, JS and SR designed the study and wrote the protocol with contributions from MSC, RK, TIT, HF, AK and SB. SR is the principal investigator. Site investigators are TIT, HF, AK, LDG and SB.

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Supplemental Digital Content (SDC)

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Figure legends

Figure 1. CONSORT diagram

Figure 2. *LPS levels, complement activation (TCC) and 10 cytokines during LPS Adsorber and sham treatment of septic patients.* Red lines represent LPS adsorber group, green lines represent Sham adsorber group. All values are displayed as median (line in the box), mean (dot) and interquartile range (box) and 10-90th percentile (whiskers). No group differences were seen. Abbreviation of cytokines are as described in Materials and Methods.

Tables

Table 1 and 2 are submitted in separate files.