High-volume haemofiltration with a new haemofiltration membrane having enhanced adsorption properties in septic pigs

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Abstract

Background. High-volume haemofiltration (HVHF) has been suggested as an adjuvant treatment of septic shock due to its capacities to remove from blood both pro- and anti-inflammatory mediators involved in the sepsis syndrome. Adsorption properties of some haemofiltration membranes are also interesting with this indication because inflammatory mediators are caught in the membrane itself. The aim of this study was to determine the haemodynamic and immunological effects of a new haemofiltration membrane, which has enhanced adsorption properties due to a special surface treatment, allowing the adsorption of endotoxins.

Methods. We compared this membrane to a standard haemofiltration membrane both in vitro and in 20 sepsis-induced pigs, randomized in two groups. One group was haemofiltered with the treated membrane and the other with the standard haemofiltration membrane during 6-h HVHF sessions.

Results. At the end of the experiment, mean ± SD crystalloids requirements (5937 ± 1588 versus 7587 ± 1456 ml, P = 0.026), colloids requirements (1437 ± 320 versus 1912 ± 538 ml, P = 0.027), lactic acidosis (pH = 7.20 ± 0.11 versus 7.10 ± 0.07, P = 0.026) and pulmonary arterial hypertension (MPAP = 24 ± 7 versus 34 ± 8 mmHg, P = 0.008) were less pronounced when HVHF was performed with the treated membrane. In addition, mean ± SD endotoxins levels were lower in the treated membrane group after 1 hour of HVHF (1.91 ± 1.19 versus 11.07 ± 10.64 EU/ml, P = 0.035). Cytokines levels were not different between groups except for IL-1β, which was slightly lower in the treated membrane group.

Conclusions. The use of a membrane with enhanced adsorption properties during a 6-h HVHF session in septic pigs improves haemodynamics compared to a standard haemofiltration membrane. These results are probably due to an efficient endotoxins and cytokines adsorption. A human study using this membrane is now necessary to confirm these results.

Keywords: adsorption; cytokine; endotoxin; high-volume haemofiltration; septic shock

Introduction

Septic shock is still the leading cause of mortality and morbidity in intensive care units and the improvement of its prognosis remains a major therapeutic challenge [1,2].

Over recent decades, the concept of blood purification based on the humoral theory of sepsis has gained increased interest. A broad-based restoration of humoral homeostasis avoiding excessive inflammation is required. One of the numerous research strategies, in this particular field, aims at limiting the overwhelming systemic overflow of pro- and anti-inflammatory mediators released at the early phase of septic shock, which can lead to generalized endothelial damage, tissue injury and multiple organ failure [3].

High-volume haemofiltration (HVHF) is an extracorporeal blood purification therapy aiming at non-selectively reducing the circulating levels and activity of both pro- and anti-inflammatory mediators. Numerous animal and clinical studies have shown the interest of convective exchanges, demonstrating the removal of these inflammatory mediators and a haemodynamic improvement with HVHF [4–6]. Nevertheless, the impact of HVHF on mortality is still difficult to specify. Large mortality studies are, however, in process at the present time.

Haemofiltration membranes also exhibit some adsorption properties allowing the capturing of high-molecular-weight molecules in the membrane itself [7]. Therefore, during septic shock, the more the haemofiltration membrane has adsorption properties, the more cytokines and inflammatory mediators can be removed from blood.
circulation. Thus, associating convection with adsorption for blood purification seems to be quite attractive.

The aim of our study was to investigate the effects of a new haemofiltration membrane at the early phase of septic shock. This haemofiltration membrane prototype has enhanced adsorption properties since a particular surface treatment has been added in order to greatly adsorb endotoxins and cytokines. This membrane and a reference haemofiltration membrane were compared both in vitro and in vivo, in septic pigs, on the basis of haemodynamic and immunological plans.

Subjects and methods

In vitro study

Before testing the membrane in vivo, it was decided to assess its in vitro adsorption capacities. Bovine plasmatic serum 500 ml contaminated with 40 EU/ml of endotoxins E. coli O55:B5 Biowhittaker® circulated in a closed circuit, in contact with the membrane, for 60 min at a flow rate of 250 ml/min. The samples were withheld at T0, T10, T30 and T60 min in order to establish the endotoxins adsorption kinetic profile. The experiment was repeated five times for each membrane (AN69 M100 membrane and the treated membrane).

Moreover, human serum 500 ml, contaminated with cytokines in pathologic concentrations (TNF-α = 400 pg/ml, IL-1β = 200 pg/ml, IL-1ra = 2000 pg/ml, IL-10 = 3000 pg/ml), circulated in a closed circuit, in contact with the membrane, for 3 h at a flow rate of 150 ml/min. Each experiment was performed with only one cytokine, three times for each membrane (PSHF Gold Baxter® membrane, AN69 M100 membrane and the treated membrane). The samples were withheld at T0, T120 and T180 min in order to establish the cytokines adsorption kinetic profile.

Septic shock model

Following the approval of the local animal research review committee, a total of 20 healthy young pigs were studied. All animals were female, issued from the Landras Pietrain race, and were 3 months old with an average weight of 35 kg. The principles of laboratory animal care were followed during the study. The animals were anaesthetized with 3 mg/kg of intravenous propofol (AstraZeneca Laboratories, Rueil-Malmaison, France). Afterwards, intubation was performed with a 6.5 mm intraoral tube and the pigs were placed on mechanical ventilation using a 50% fraction of inspired oxygen. The tidal volume and the respiratory frequency were adjusted to produce an EtCO2 of 40 mmHg during the investigation. The maintenance of anaesthesia was performed by using sevoflurane (Abbott Laboratories, Rungis, France) at a minimal alveolar concentration of 1 and 10 µg/h sufentanil (Janssen-Cilag Laboratories, Issy-les-Moulineaux, France) [8]. A warming blanket maintained the animal body temperature at 37 ± 0.5°C.

An arterial catheter was introduced into the right internal carotid artery to monitor the systemic arterial pressure, to determine arterial blood gas and to withdraw blood samples. A pulmonary arterial catheter was inserted via the right external jugular vein to measure pulmonary arterial pressure, pulmonary capillary wedge pressure (PCWP), central venous pressure (CVP) and thermodilution cardiac output (CO). A 12-Fr triple-lumen dialysis catheter was also introduced through the left external jugular vein to perform haemofiltration and to infuse the pigs.

Sepsis was induced with an intravenous infusion of live Pseudomonas aeruginosa American Type Culture Collection 15442 through the central venous access (5 × 10⁸ Colony Forming Units/ml at 10 ml/h) [9–11]. All the pigs were infused with this bacterial strain in order to avoid any variation of virulence. During the infusion of the P. aeruginosa suspension, the systolic pulmonary arterial pressure (SPAP) was monitored. When the SPAP reached 45 mmHg, the infusion was stopped to limit the increase of the right ventricular afterload in order to obtain a reproducible hemodynamic state without any right heart dysfunction, as previously described [11].

The beginning and the end of the P. aeruginosa infusion were respectively named Tb and Tb’. High-volume haemofiltration was started 2 h after Tb. The beginning of HVHF was named T0 and each successive hour was given a number from T1 to T6.

Treatment groups

Before the beginning of HVHF, the septic pigs were randomized in two groups of 10. The first group was haemofiltered with the new haemofiltration membrane (Gambro industries, Meyzieu, France) and the other was haemofiltered with the standard AN69® M100 haemofiltration membrane (Gambro industries, Meyzieu, France). The treated membrane is a polycrylonitrile haemofiltration membrane prototype, which has in vitro enhanced adsorption properties because of a surface treatment (modification of the surface polarity), conferring the possibility of strongly adsorbing high-molecular-weight molecules such as inflammatory mediators and endotoxins. The modification of the surface polarity is effective by the addition of a polycation on the membrane surface. This polycation is a positive charge allowing the catching via surface adsorption of endotoxins that are considered as negative charges. The surface area was 0.9 m² and the cut-off point was 40 kDa for both membranes, so the convective capacities were the same for both groups. Sieving coefficients were also the same for both membranes (Table 1). In both groups, the blood flow rate was 150 ml/min, the ultrafiltration rate was 50 ml/kg/h, the duration of the HVHF session was 6 h, and after 3 h of treatment, the haemofiltration membrane was replaced by the same one in order to preserve and optimize the membrane adsorption properties. No weight loss was

<table>
<thead>
<tr>
<th>Urea</th>
<th>Creatinine</th>
<th>Vitamin B12</th>
<th>Inulin</th>
<th>Myoglobin</th>
<th>Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0.95</td>
<td>0.95</td>
<td>0.55</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Data obtained with the following conditions: bovine plasma, protein concentration = 60 g/l, temperature = 37°C, blood flow rate = 100 ml/min and ultrafiltration flow rate = 20 ml/min.
scheduled so the substitution fluid (Hemosol B0®) was infused with 100% post-dilution at the ultrafiltration rate (50 ml/kg/h). Potassium chloride was added to Hemosol B0® in order to obtain a KCl concentration of 4 mmol/l. In both groups, anticoagulation was performed with heparin 2500 IU directly administered intravenously 5 min before the beginning of HVHF. The maintenance of anticoagulation was performed during the HVHF session with a continuous infusion of heparin 500 IU/h in both groups, from T0 to T6.

Throughout the experiment, a mean arterial pressure (MAP) of 65 mmHg and a PCWP of 10 mmHg were maintained by a continuous infusion of epinephrine and fluid loading in order to get a normotensive septic shock. Fluid loading was composed of both an isotonic saline solution and hydroxyethylstarch added as soon as a major relative hypovolaemia was observed. Heart rate (HR), MAP, pulmonary arterial catheter parameters such as PCWP, arterial pressure waveform and lung auscultation were the main parameters used for the estimation of volaemia and the haemodynamic management.

Measured variables

Haemodynamic parameters such as HR, MAP, CVP, SPAP, mean pulmonary arterial pressure (MPAP), PCWP and CO were recorded at regular intervals for 8 h, from Tb to T6. Systemic arterial resistances (SAR) and pulmonary arterial resistances (PAR) were calculated from standard formulae [12]. Epinephrine and fluid loading requirements were also recorded each hour after the beginning of the P. aeruginosa infusion over a period of 8 h, from Tb to T6. Blood gas analysis and lactate level withdrawn from peripheral blood through the arterial catheter were regularly recorded. Finally, endotoxins levels were measured at T0, T1 and T6 and cytokines levels (tumour necrosis factor alpha (TNF-α) and interleukins (IL) such as IL-1β, IL-1ra, IL-6) were measured at T0 and T6 (Quantikine®-colorimetric ELISA kits, R&D Systems, Lille, France). At the end of the investigation, the animals were killed with an intravenous injection of potassium chloride 2 g.

Statistical analysis

Continuous variables were expressed as mean values ± SD or median (interquartile range), as appropriate. Haemodynamic and biochemical parameters were analysed using the Student t-test after testing the normality of the values with a Kolmogorov–Smirnov test. Endotoxins levels were analysed using ANOVA for repeated measurements followed by a Duncan post hoc test. Cytokines levels were analysed using the Mann–Whitney U-test. A P value < 0.05 was considered statistically significant (Statistica® 7.0, StatSoft Inc., Tulsa, OK, USA).

Results

Concerning the in vitro results, endotoxins and cytokines adsorption was clearly more important with the treated membrane. Indeed, 66% of the endotoxins were adsorbed after 60 min in the in vitro conditions described above. Cytokines adsorption was also greater with the treated membrane for all the studied cytokines (TNF-α, IL-1β, IL-6, IL-1ra). The in vitro endotoxins and cytokines adsorption kinetics are shown in Figures 1 and 2.

Concerning the in vivo results, the mean ± SD volume of P. aeruginosa suspension and duration of P. aeruginosa infusion were similar between groups (11 ± 2 ml in the AN69 group versus 12 ± 2 ml in the treated membrane group and 66 ± 12 min for the AN69 group versus 72 ± 12 min for the treated membrane group). No pig died before the end of the experiment. In both groups, no treatment was prematurely discontinued because of technical problems. The haemodynamic state of the pigs rapidly decreased (tachycardia, epinephrine requirement and fluid expansion requirement to maintain MAP and PCWP). A hyperkinetic profile of septic shock was observed in both groups with increased CO and decreased SAR. The mean ± SD HR increased from 98 ± 15 beats/min at Tb to 148 ± 16 beats/min at T6 (treated membrane group) and from 95 ± 16 beats/min at Tb to 138 ± 20 beats/min at T6 (AN69 group). The mean ± SD CO increased from 2.5 ± 1.8 l/min at Tb to 5.5 ± 2.8 l/min at T6 (treated membrane group) and from 2.5 ± 1.2 l/min at Tb to 6.9 ± 4.8 l/min at T6 (AN69 group). The mean ± SD SAR decreased from 2092 ± 920 dyn/s/cm² at Tb to 797 ± 346 dyn/s/cm² at T6 (treated membrane group) and from 2050 ± 859 dyn/s/cm² at Tb to 672 ± 205 dyn/s/cm² at T6 (AN69 group). All the pigs were resuscitated in a similar way since MAP and PCWP were respectively maintained at 65 mmHg and 10 mmHg in both groups during all the experiment (Figure 3).

At the end of the experiment, mean ± SD crystalloids requirements (5937 ± 1588 versus 7587 ± 1456 ml, P = 0.026), colloids requirements (1437 ± 320 versus 1912 ± 538 ml, P = 0.027), lactic acidosis (pH = 7.20 ± 0.11 versus 7.10 ± 0.07, P = 0.026) and pulmonary arterial hypertension (SPAP = 30 ± 8 versus 39 ± 9 mmHg, P = 0.029 and MPAP = 24 ± 7 versus 34 ± 8 mmHg, P = 0.008) were less pronounced in the treated membrane group than in the AN69 group. Concerning epinephrine requirements, no statistical difference was observed between
both groups at T0 and T6. Haemodynamic and biochemical parameters are summarized in Tables 2 and 3.

Moreover, mean ± SD endotoxins levels were lower in the treated membrane group after 1 h of HVHF (1.91 ± 1.19 versus 11.07 ± 10.64 EU/ml, \( P = 0.035 \)). Serum endotoxins levels during the experiment are reported in Table 4. Median (interquartile range) cytokines levels were not different between groups except for IL-1β, which was slightly lower in the treated membrane group at T6 [98 (49–205) versus 230 (126–350) pg/ml, \( P = 0.04 \)]. Cytokines levels at T0 and T6 are shown in Table 5.

**Discussion**

This is an animal study showing that a modified haemofiltration membrane with enhanced adsorption properties may be of interest in the removal of cytokines and
**Table 2.** Mean ± SD haemodynamic and biochemical parameters just before the start of HVHF at T0

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AN69 mb (n = 10)</th>
<th>Treated mb (n = 10)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td>126 ± 24</td>
<td>121 ± 22</td>
<td>0.63</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>72 ± 12</td>
<td>68 ± 10</td>
<td>0.43</td>
</tr>
<tr>
<td>SPAP (mmHg)</td>
<td>33 ± 7</td>
<td>29 ± 6</td>
<td>0.19</td>
</tr>
<tr>
<td>MPAP (mmHg)</td>
<td>29 ± 6</td>
<td>25 ± 6</td>
<td>0.15</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>12 ± 2</td>
<td>11 ± 2</td>
<td>0.28</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>4.3 ± 1.8</td>
<td>3.8 ± 1.5</td>
<td>0.54</td>
</tr>
<tr>
<td>SAR (dyn/s/cm²)</td>
<td>1210 ± 534</td>
<td>1242 ± 353</td>
<td>0.88</td>
</tr>
<tr>
<td>PAR (dyn/s/cm²)</td>
<td>356 ± 236</td>
<td>295 ± 124</td>
<td>0.48</td>
</tr>
<tr>
<td>Epinephrine (mg)</td>
<td>0.21 ± 0.32</td>
<td>0.29 ± 0.47</td>
<td>0.66</td>
</tr>
<tr>
<td>Crystalloids (ml)</td>
<td>2250 ± 719</td>
<td>2125 ± 646</td>
<td>0.69</td>
</tr>
<tr>
<td>Hydroxyethylstarch (ml)</td>
<td>513 ± 300</td>
<td>419 ± 245</td>
<td>0.45</td>
</tr>
<tr>
<td>pH</td>
<td>7.37 ± 0.05</td>
<td>7.39 ± 0.06</td>
<td>0.43</td>
</tr>
<tr>
<td>Lactate (mmol/l)</td>
<td>3.80 ± 1.54</td>
<td>2.95 ± 0.22</td>
<td>0.22</td>
</tr>
</tbody>
</table>

**Table 3.** Mean ± SD haemodynamic and biochemical parameters after a 6-h HVHF session, at T6

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AN69 mb (n = 10)</th>
<th>Treated mb (n = 10)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td>138 ± 20</td>
<td>148 ± 16</td>
<td>0.23</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>64 ± 6</td>
<td>59 ± 8</td>
<td>0.13</td>
</tr>
<tr>
<td>SPAP (mmHg)</td>
<td>39 ± 9</td>
<td>30 ± 8</td>
<td>0.029</td>
</tr>
<tr>
<td>MPAP (mmHg)</td>
<td>34 ± 8</td>
<td>24 ± 7</td>
<td>0.008</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>12 ± 3</td>
<td>11 ± 4</td>
<td>0.53</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>6.9 ± 4.8</td>
<td>5.5 ± 2.8</td>
<td>0.44</td>
</tr>
<tr>
<td>SAR (dyn/s/cm²)</td>
<td>672 ± 205</td>
<td>797 ± 346</td>
<td>0.34</td>
</tr>
<tr>
<td>PAR (dyn/s/cm²)</td>
<td>325 ± 186</td>
<td>234 ± 148</td>
<td>0.24</td>
</tr>
<tr>
<td>Epinephrine (mg)</td>
<td>3.27 ± 3.02</td>
<td>2.11 ± 0.05</td>
<td>0.27</td>
</tr>
<tr>
<td>Crystalloids (ml)</td>
<td>7587 ± 1456</td>
<td>5937 ± 1588</td>
<td>0.026</td>
</tr>
<tr>
<td>Hydroxyethylstarch (ml)</td>
<td>1912 ± 538</td>
<td>1437 ± 320</td>
<td>0.027</td>
</tr>
<tr>
<td>pH</td>
<td>7.10 ± 0.07</td>
<td>7.20 ± 0.11</td>
<td>0.026</td>
</tr>
<tr>
<td>Lactate (mmol/l)</td>
<td>14.11 ± 3.36</td>
<td>9.61 ± 4.47</td>
<td>0.022</td>
</tr>
</tbody>
</table>

**Table 4.** Mean ± SD serum endotoxins levels (EU/ml)

<table>
<thead>
<tr>
<th>Time</th>
<th>AN69 mb (n = 10)</th>
<th>Treated mb (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>3.98 ± 3.31</td>
<td>4.26 ± 7.68</td>
</tr>
<tr>
<td>T1</td>
<td>11.07 ± 10.64</td>
<td>1.91 ± 1.19</td>
</tr>
<tr>
<td>T6</td>
<td>2.96 ± 2.75</td>
<td>2.26 ± 2.39</td>
</tr>
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</table>

(endotoxins during the early phase of an experimental septic shock.

Once in the blood circulation, lipopolysaccharide or lipotechoic acid activates the immune system leading to a major inflammatory response called systemic inflammatory response syndrome (SIRS) [7]. The inflammatory mediators released during this inflammatory cascade have beneficial effects but are also responsible for organ failures when the inflammatory response is not controlled, therefore resulting in multiple organ failure syndrome that often precedes death. The modulation of this major systemic inflammatory response is now one of the numerous research strategies in the field of sepsis [3]. Lack of clinical success with antiendotoxin or anticytokine therapy has shifted interest to extracorporeal therapies to reduce circulating levels of the mediators of sepsis [13].

Based on the conception principle, HVHF is therefore very interesting at the early phase of septic shock. Numerous in vitro studies have shown that synthetic filters used in haemofiltration can remove a large number of substances and inflammatory mediators involved in the sepsis syndrome including TNF-α, IL-1, IL-6, IL-8, IL-1ra, platelet activating factor (PAF), nitric oxide (NO), leukotrienes, prostaglandins, thromboxanes and complement factors [14–17]. Moreover, many animal studies have shown benefits on survival in endotoxic shock models, and recent human studies have also demonstrated that HVHF improves haemodynamics and tends to improve survival of septic patients [4–6,18–22].

Adsorption is another physicochemical principle allowing the catching of some molecules in the haemofiltration membrane itself after the interaction with variable polarity ionic charges. This process is close to the one used in molecular biology with ion-exchange resins. Haemofiltration membranes have variable adsorption properties depending on the composition of the membrane. Membranes getting important adsorption properties would be able to remove not only additional molecules from blood circulation but also molecules with molecular weight beyond the membrane cutoff. This can be particularly attractive knowing the large scale of pro- and anti-inflammatory mediators’ molecular weights, ranging from 0.5 kDa to 60 kDa. Concerning the haemofiltration membrane composition, the experimental study of Rogiers et al., conducted in an acute canine endotoxin shock model, has mentioned a transient haemodynamic superiority of polyacrylonitrile compared...
to polysulfone, which was explained by a more effective adsorption of inflammatory mediators [23]. The beneficial effects of adsorption in this indication have recently been pointed out by a systematic review of the published literature [7]. Indeed, although the analysed studies were of suboptimal quality, Cruz et al. found positive effects of direct haemoperfusion with a polymyxin B immobilized fibre column on blood pressure, use of vasocative agents, gas exchange and mortality [7]. This has been related to the removal of circulating endotoxins by adsorption, preventing the progression of the biological cascade of sepsis [7].

In our study, the adsorption properties of this polyacrylonitrile haemofiltration membrane have been enhanced by a surface treatment modifying the membrane surface polarity (addition of a polycation which is a positive charge allowing the catching via surface adsorption of endotoxins that are considered as negative charges). Therefore, this membrane can strongly adsorb endotoxins and inflammatory mediators in vitro (Figures 1 and 2). In this porcine model of septic shock, the use of this treated haemofiltration membrane during a 6-h HVHF session decreased the clinical and biological severity of the shock obtained compared to the use of a standard haemofiltration membrane. This was objectivized by a reduction of the amount of clinical intervention necessary to sustain the desired MAP and PCWP levels. Fluid expansion requirement, lactic acidosis and pulmonary arterial hypertension were less pronounced in the treated membrane group than in the AN69 group at the end of the experiment. Regarding immunological parameters, we observed a significant decrease of the endotoxins level after 1 h of HVHF in the treated membrane group (Table 4) and no difference was observed concerning blood cytokines levels except for IL-1β, which was slightly lower in the treated membrane group at T6 (Table 5). These immunological results are similar to numerous previous studies, which did not show any influence on cytokines plasma levels with HVHF, even if cytokine elimination and significant clinical benefits were demonstrated [24–27]. Cytokines from the blood compartment are only representative of a small part of the totality of the cytokines of the body, and cytokine exchanges between the blood compartment side and the interstitial and tissue sides are still unclear [28]. The Honoré concept, also called the threshold immunomodulation hypothesis, suggests that HVHF promotes cytokine flow from tissue and interstitium to the blood compartment, which means an effect of HVHF outside the blood compartment [28]. The measurement of cytokines plasma levels is therefore debatable while tissue levels should preferably be measured whenever possible [29]. Mechanisms governing endotoxins removal are probably as complicated as those of cytokines removal.

All these in vitro and in vivo data make this treated haemofiltration membrane quite attractive, although several limits can be pointed out from this study. First of all, the clinical relevance of this study can be discussed since haemofiltration was initiated very quickly after the bacterial infusion (2 h). In clinical conditions, treatment of septic shock begins much later and haemofiltration is usually started when acute kidney injury has appeared. A clinical study is obviously necessary to complete these data and to evaluate this membrane in clinical conditions. Secondly, pig mortality was not evaluated since all animals were sacrificed at the end of the experiment. Besides, the short observational duration of 6 h can also be opened to criticism as continuous renal replacement therapies are meant to run for 24-h periods. However, what does really matter in blood purification for sepsis is the early start of the therapy rather than its duration since the peak concentration of inflammatory cytokines is reached at the very early phase of septic shock. This is probably the reason why numerous other studies reported in the medical literature were conducted on the same design with a short observational period [4–6,20,21,30]. Moreover, due to the high complexity of the technique, we did not perform any postexperiment in vitro analysis of the filters for the recovery and the measurement of the adsorbed cytokines and endotoxins. This would have permitted potentially direct quantification of the cytokines and endotoxins adsorption. It would have also permitted assessment of whether there was a lactate adsorption, which could have participated in the decrease of the lactate level in the treated membrane group via an increased clearance of lactate by membrane adsorption. Finally, knowing that adsorption is a very short term way for inflammatory mediators removal because of rapid membrane saturation, it seems to be very important to optimize this mechanism as best as possible. Since the smaller the membrane, the faster the saturation occurs, the membrane surface area needs to be more important than 0.9 m², particularly if the membrane is used in humans and not in pigs weighing 35 kg. In addition, to take advantage of the adsorption properties of a haemofiltration membrane, it is recommended to regularly change the membrane due to rapid membrane saturation [25]. These frequent membrane changes can be a source of difficulties in medical clinical practice by increasing significantly the cost and the nursing workload of the haemofiltration sessions. On the other hand, frequent changes allow us to avoid processes of deadsorption, which can occur when devices with adsorption properties are used continuously. More experimental and clinical work has to be done in order to determine how long this treated membrane should be used in clinical practice without any change and to find the best arrangement between frequently changing the membrane and financial considerations. For example, one of the possible answers could be the consideration of those frequent changes only for the very early phase of septic shock, when serum endotoxins and cytokines levels are very important.

In conclusion, the major finding of this study can be summarized as follows: the use of this new haemofiltration membrane having enhanced adsorption properties during a 6-h HVHF session improves the haemodynamic state of a P. aeruginosa porcine model of septic shock. These results are probably due to efficient endotoxins and cytokines adsorption in the membrane itself. Many blood purification strategies derived from standard HVHF are currently proposed as adjuvant treatment for septic shock: very high rates of plasma water exchange, larger pore size haemofiltration membranes [31], haemofiltration membranes with enhanced adsorption properties, pulse HVHF [32] and coupled plasma filtration adsorption [33,34]. The challenge for the coming years will be to find the best compromise...
between theoretical interests and practical limitations among all these extracorporeal treatments. Performing HVHF with membranes getting enhanced adsorption properties can boast a potentially promising response, but more animal and clinical research has to be done.

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Conflict of interest statement. C. Lambert is a scientific employee from Gambro-Hospal Industries Company, the study sponsor. None of the remaining authors declare any conflicts of interest.

(See related article by O. Joannes-Boyau et al. Are the synergistic effects of high-volume haemofiltration and enhanced adsorption the missing key in sepsis modulation? Nephrol Dial Transplant 2009; 24: 354–357.)

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