CYTOKINES ADSORBENTS :

NEW WEAPONS AGAINST CYTOKINES STORM IN SEPSIS

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Introduction

Sepsis is characterized by a **complex systemic inflammatory response** to a microbial pathogen.

The presence **Endotoxin** (also known as lipopolysaccharide, is a component of gram-negative bacteria) causes an innate immune response characterized by the stimulation of monocytes and release of proinflammatory cytokines and the activation different immune pathways.

The recognition of **endotoxin by immune cells is important in the pathogenesis of septic shock**

Conventional therapy such as **antibiotics and surgical procedures** to remove the source of infection is crucial for treating sepsis, but **these approaches cannot reverse the effects of the bacterial toxins already released into blood or of the endogenous mediators** produced by the host in response to bacteria.

Blood purification techniques including **hemoperfusion, plasma exchange, and hemofiltration with hemoperfusion** are associated with lower mortality in patients with sepsis as it has been demonstrated in a recent meta-analysis .

Removing endotoxin would be an **effective adjunctive approach** in the management of severe sepsis.

Devices to remove endotoxin or inflammatory cytokines have been designed as a strategy to reduce the morbidity and mortality associated with sepsis, especially with **sepsis due to gram-negative bacteria.**

These devices have also been successfully used in patients with sepsis due to gram-positive microorganisms and in **patients with acute respiratory distress syndrome (ARDS), suggesting that they could have an immunomodulating action in addition to endotoxin elimination.**

Table 1: Devices designed to remove endotoxin and cytokines in patients with septic shock.

Device	Company	Composition	Mechanism	Substance eliminated
Toraymyxin 20R	Toray Industries, Japan	Polymyxin B covalently bound to polypropylene-polystyrene fibers fabric	Adsorption	Endotoxin
LPS adsorber	Alteco Medical, Sweden	Synthetic polypeptide bound to porous polyethylene discs	Adsorption	Endotoxin
oXiris	Gambro-Hospal, France	AN69-based membrane, surface treated with a polyethyleneimine (PEI) and grafted with heparin	Adsorption Convection	Endotoxin Cytokines
MATISSE	Fresenius SE, Germany	Human serum albumin immobilised on polymethacrylate beads	Adsorption	Endotoxin
CPFA	Bellco, Italy	Polyethersulfone Plasma filter with adsorption on an unselective hydrophobicresin cartridge, and a synthetic high-permeability polyethersulfone hemofilter for continuous hemofiltration	Adsorption Plasma filtration	Cytokines
Cytosorb	Cytosorbents, USA	Polystyrenedivinyl benzene copolymer beads with a biocompatible polyvinylpyrrolidone coating.	Adsorption Convection	Cytokines

Polymyxin B-Immobilized Cartridge (Toraymyxin 20-R, Toray Industries, Japan)

- Polymyxin B is a cationic polypeptide antibiotic with activity against gram-negative bacteria and **a high affinity to endotoxin,** but its intravenous use has been limited due to nephrotoxicity and neurotoxicity .

- Since 1994, Polymyxin B has been fixed and immobilized with polystyrene fiber in a **hemoperfusion column- Polymyxin B-immobilized cartridge (PMX) that allows endotoxin removal without the toxic effects of this antibiotic.**

- This treatment has been widely used in **Japan for septic shock due to gram-negative bacteria**, and its use was authorized in Europe in 1998. Recent studies support the safety and efficacy of this treatment

LPS Adsorber (Alteco Medical AB, Sweden)

- This medical device designed for **extracorporeal use contains a series of porous polyethylene plates coated with a peptide specific to endotoxin and has a high adsorption capacity.** It has been used in patients with septic shock .

- Yaroustovsky et al. compared LPS adsorber and PMX hemoperfusion in **a small sample of patients** with gram-negative sepsis. **The authors did not find differences in** outcome.

Oxiris (Gambro-Hospal, France)

This AN-69 (polysulfone and polyacrylonitrile) based membrane **adsorbs a large spectrum of plasma inflammatory mediators such as endotoxin and cytokines** .

To date, clinical experience with this device is limited, but **two trials are underway in septic patients and** the results of these two trials are crucial to determine its usefulness compared with the current standard of care.

MATISSE-Fresenius System (Fresenius SE, Germany)

Based on the **endotoxin-binding abilities of human albumin**, this adsorber contains human serum albumin immobilized on polymethacrylate beads.
Although in vitro experiments were promising, phase 2 study results have been disappointing .

Coupled Plasma Filtration Adsorption (CPFA)Bellco, Italy

This extracorporeal treatment is based on **adsorption of cytokines onto a specially designed resin cartridge, coupled with hemofiltration.**
This device does **not adsorb endotoxin**.

Promising therapy but although further studies are necessary to determine its usefulness in septic patients.

One clinical trial, COMPACT 2, is underway to clarify whether adding high doses of CPFA to current clinical practice can reduce hospital mortality in septic shock patients (ClinicalTrials.gov number NCT01639664).

CytoSorb (Cytosorbents Inc., USA)

This extracorporeal device removes cytokines through adsorption to a high-surface-area biocompatible porous polymer sorbent. This device does not target endotoxin, but it does rapidly eliminate several key cytokines by adsorption in both in vitro and in vivo experiments [38, 39]. This device is very promising, but more studies in septic patients are needed.

SOME THING MORE ABOUT….

Polymyxin B-Immobilized Cartridge

Immunological Mechanisms Described for Polymyxin B-Immobilized Cartridge

1. Endotoxin removal
2 . Immunomodulation

Endotoxin Removal

Endotoxin is a major component of the outer membrane of gram-negative microorganisms . Immune cells recognize endotoxin and other bacterial compounds through the TLR(toll like receptors), a group of transmembrane proteins that **play crucial roles in the host defense against invading pathogens**

During a gram-negative infection, TLR-4 recognizes endotoxin and originates a systemic inflammatory response in sepsis with potentially fatal effects in hosts.

As a consequence, proinflammatory molecules such as **interleukin-1 (IL-1) and tumor necrosis factor alpha (TNFα)** are released and generate other cell responses in the inflammatory cascade

This increase in **cytokines** is followed by a major expression of tissue factor, which **activates coagulation**, and by an increase in **nitric oxide synthesis, which induces vasodilation .**

Endotoxin levels are **high in septic patients** , but they are also **high in critical patients without sepsis, such as** patients undergoing cardiopulmonary bypass and those with chronic heart failure, chronic kidney disease, and other medical conditions.

In critical patients without gram-negative infection, **elevated endotoxin levels are related to translocation of gut bacterial antigens and endotoxin into the bloodstream due to gut barrier dysfunction**

Effect on "Immunoparalysis"

The human body undergoes a biphasic immunological reaction in sepsis. A **proinflammatory reaction** takes place, marked by the release of proinflammatory cytokines like TNFα, as a reaction to the bacterial toxins.

On the other hand, a **counter regulatory anti-inflammatory** reaction arises. This phase acts as negative feedback on the inflammation by inhibiting the proinflammatory cytokines

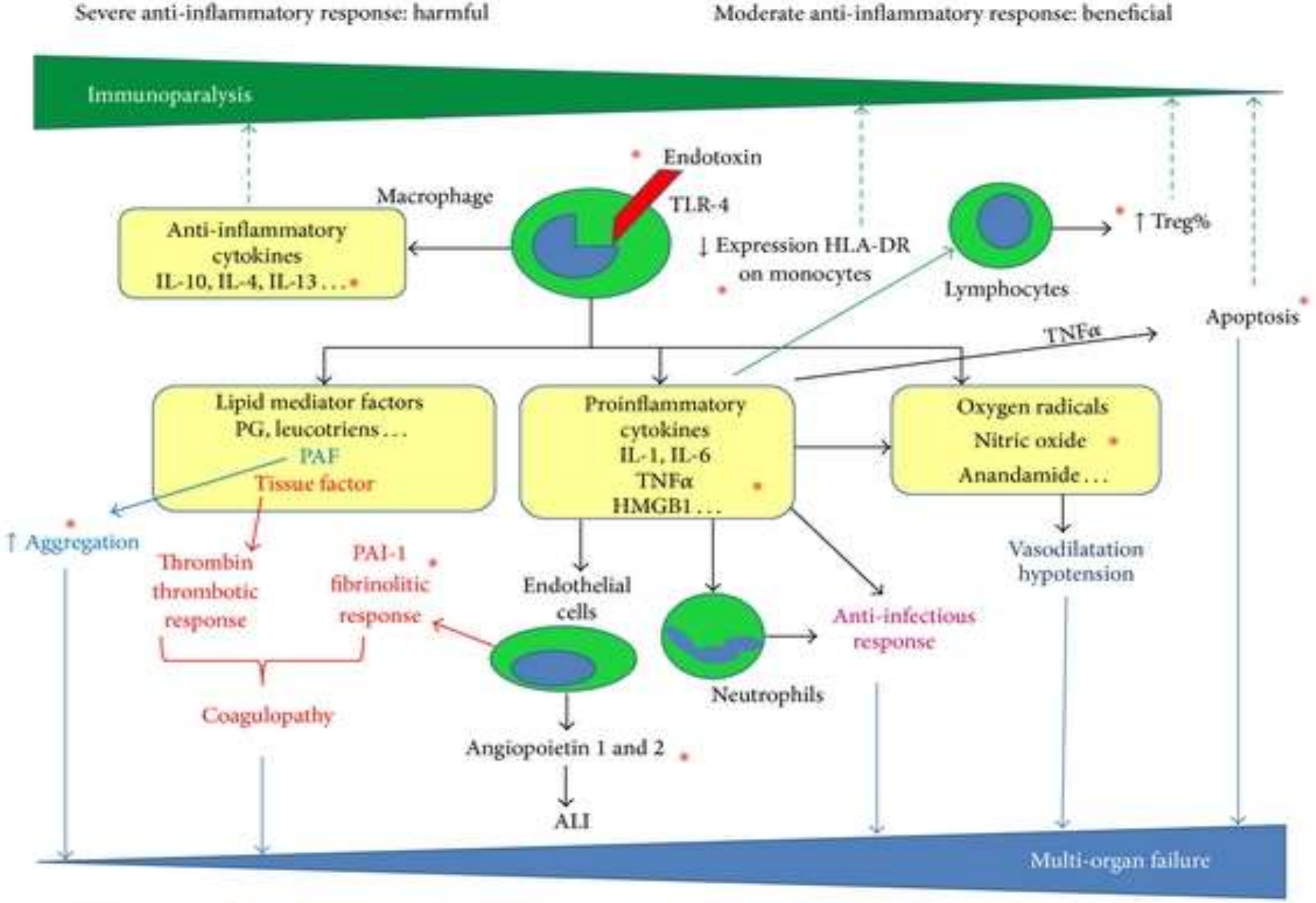
The persistence of a marked compensatory anti-inflammatory response is called "immunoparalysis"
This pronounced immunosuppressive state adversely affects immune function, making the patient vulnerable to opportunistic infections

These two phases of sepsis may occur simultaneously with a lasting anti-inflammatory response in later phases

Most septic patients survive the initial proinflammatory phase, but they die during this second stage.

Recently, **Ono et al.** [56] study concluded that PMX hemoperfusion can be useful in patients **without endotoxemia or in those with gram-positive sepsis and also suggest that PMX hemoperfusion treatment might provide additional benefits for recovery from immunoparalysis**.

This study **sheds new light on the benefits of treating septic patients with PMX hemoperfusion beyond endotoxin removal.**


Severe anti-inflammatory response: harmful
Moderate anti-inflammatory response: beneficial
Immunoparalysis
Endotoxin
Macrophage
TLR-4
Anti-inflammatory cytokines IL-10, IL-4, IL-13 . . .
↓ Expression HLA-DR on monocytes
Lymphocytes
↑ Treg%
Apoptosis
TNFα
Lipid mediator factors PG, leucotriens . . .
PAF
Tissue factor
Proinflammatory cytokines IL-1, IL-6 TNFα HMGB1 . . .
Oxygen radicals Nitric oxide Anandamide . . .
↑ Aggregation
Thrombin thrombotic response
PAI-1 fibrinolitic response
Endothelial cells
Neutrophils
Anti-infectious response
Vasodilatation hypotension
Coagulopathy
Angiopoietin 1 and 2
ALI
Multi-organ failure
Moderate pro-inflammatory response: beneficial
Severe pro-inflammatory response: septic shock

Study : reduction of Cytokines and better outcome:

In patients with severe sepsis**, Tani et al.** found **reductions in endotoxin, TNFα, IL-6, IL-10, and plasminogen activator inhibitor-1 (PAI-1) activities** after PMX hemoperfusion.

In patients with ARDS, **Kushi et al.** found a **reduction in blood levels of PAI-1, neutrophil elastase (NE), and IL-8** after PMX hemoperfusion. NE is a protease that hydrolyzes lung elastin. In these patients, PaO2/FiO2 increased significantly after the treatment, and the authors related this increase to the elimination of IL-8 and NE(neutrophil elastase). In another study, the same group reported a decrease in NE in 20 septic patients treated with PMX hemoperfusion.

In 12 patients with septic shock receiving conventional treatment plus two sessions of PMX hemoperfusion**, Zagli et al.** found a **decrease in IL6, IL10, and TNFα** in patients' serum after the treatment, especially in survivors.

Vascular and Coagulation Proteins

PMX hemoperfusion may have a role in modulating fibrinolysis and inhibiting the development of ischemic organ dysfunction in sepsis.

Angiopoietin-1 is a positive regulator of blood vessel development, remodeling, and maturation.

Angiopoietin-2 is a competitive inhibitor of angiopoietin-1.

Angiopoietin-1 and -2 play a contributory role in the pathogenesis of acute lung injury (ALI) in septic patients.

Angiopoietin-1 reduces pulmonary inflammation and permeability. **Angiopoietin-2 interferes with angiopoietin-1, resulting in pulmonary inflammation and increased permeability.**

Ebihara et al. reported that PMX hemoperfusion could ameliorate the angiopoietin balance in septic patients with ALI.

Oishi et al. recently studied nine patients with acute exacerbation of idiopathic pulmonary fibrosis treated with conventional therapy and PMX hemoperfusion 6 hours/day on two successive days

This is **the first study** to demonstrate **that cytokines and Vascular endothelial growth factor (VEGF) can be directly adsorbed by PMX hemoperfusion independently from endotoxin removal.**

The authors suggest that cytokines can bind to PMX hemoperfusion fibers **directly through ionic/hydrophobic interactions like endotoxin**.

Removal of Cells and Phenotype Change

Nishibori et al. examined the PMX hemoperfusion filters after treating 4 patients with sepsis; PMX hemoperfusion **bound monocytes from the peripheral blood leucocytes. PMX hemoperfusion could produce a beneficial effect by reducing the interaction between monocytes and endothelial cells.**

The inflammatory response in sepsis involves activation of platelets. **High levels of platelet activator factor (PAF) have been observed in sepsis.** **Nakamura et al.** studied the effect of PMX hemoperfusion on platelet activation, comparing 30 patients treated with conventional therapy plus PMX hemoperfusion and 28 patients with conventional therapy alone**. Survival was 60% in the group that received PMX hemoperfusion and 30% in the group that received only conventional treatment.**

Septic patients had increased PAF (platelet activator factor (PAF)), and PMX hemoperfusion reduced the levels of PAF.

Usefulness in Acute Respiratory Failure

Several studies have found that PMX hemoperfusion has beneficial effects on oxygenation in patients with sepsis.

Moreover, PMX hemoperfusion has been successful in patients **with influenza A infection , ARDS in drug-induced injury , interstitial pneumonia and idiopathic fibrosis**

Mechanisms other than endotoxin removal could explain the beneficial effects of PMX hemoperfusion in patients with respiratory failure.

Kushi et al. found decreases **neutrophil elastase (NE), and IL-8 in ARDS after PMX hemoperfusion.**

Abe et al. studied in a retrospective multicenter study of **160 patients with acute exacerbation of idiopathic pulmonary fibrosis or interstitial pneumonia**, they found that the PaO2/FiO2 ratio significantly increased after PMX hemoperfusion . They concluded that PMX hemoperfusion might be an effective adjunctive therapy .

Hara et al, like wise reported that PMX hemoperfusion resulted in improved PaO2/FiO2 ratio 72 hours and 1 week after treatment in **33 patients with acute exacerbation of interstitial pneumonia.**

Tsushima et al treated 20 patients with ARDS with PMX hemoperfusion and compared the outcomes with a historical control group. They found improved PaO2/FiO2 ratio and survival; **however, the methodology of the study limits its power to draw conclusions.**

conclusion

In recent years, many studies have shown that PMX hemoperfusion is a promising strategy **for immunomodulation in septic shock, and ongoing clinical trials will be key in determining its usefulnes**s.

Although most studies have focused on the removal of endotoxin as the principal mechanism through which PMX hemoperfusion improves outcome in sepsis, other studies have revealed mechanisms involving **diverse immunological pathways through which PMX hemoperfusion could improve outcome not only in sepsis but also in non-septic respiratory failure.**

It is interesting to note that the **elimination of endotoxin brings about a reduction in many inflammatory molecules and cells involved in the inflammatory cascade**.

Endotoxin removal devices act at the onset of this complex cascade, and their benefits in terms of immunomodulation are encouraging.

However, these studies are **limited by their small samples**, their observational and in some cases retrospective design, and the lack of control groups in many cases.

Well-designed clinical investigations with larger samples are needed to confirm these findings.

SOMETHING MORE ABOUT …

Cytosorb

Early report: The use of Cytosorb haemabsorption column as an adjunct in managing severe sepsis: initial experiences, review and recommendations

Craig Morris
Lewis Gray
Marco Giovannell

JICS.
JOURNAL OF INTENSIVE CARE SOCIETY
2015 VOL.16(3),257-264.


Sepsis
Trauma
Burns
Influenza
Surgery
ARDS
Pancreatitis
Life Support
Liver Failure

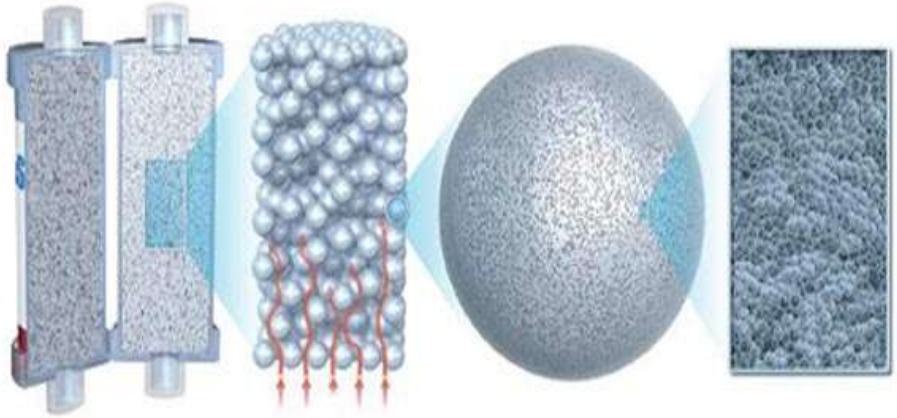
A novel synthetic haemabsorption column (Cytosorb) has recently become commercially available and useful in sepsis

Cytosorb haemabsorption appears effective in reducing inflammatory cytokines during sepsis,BUT,,much of the basic science and clinical benefits remain unclear.

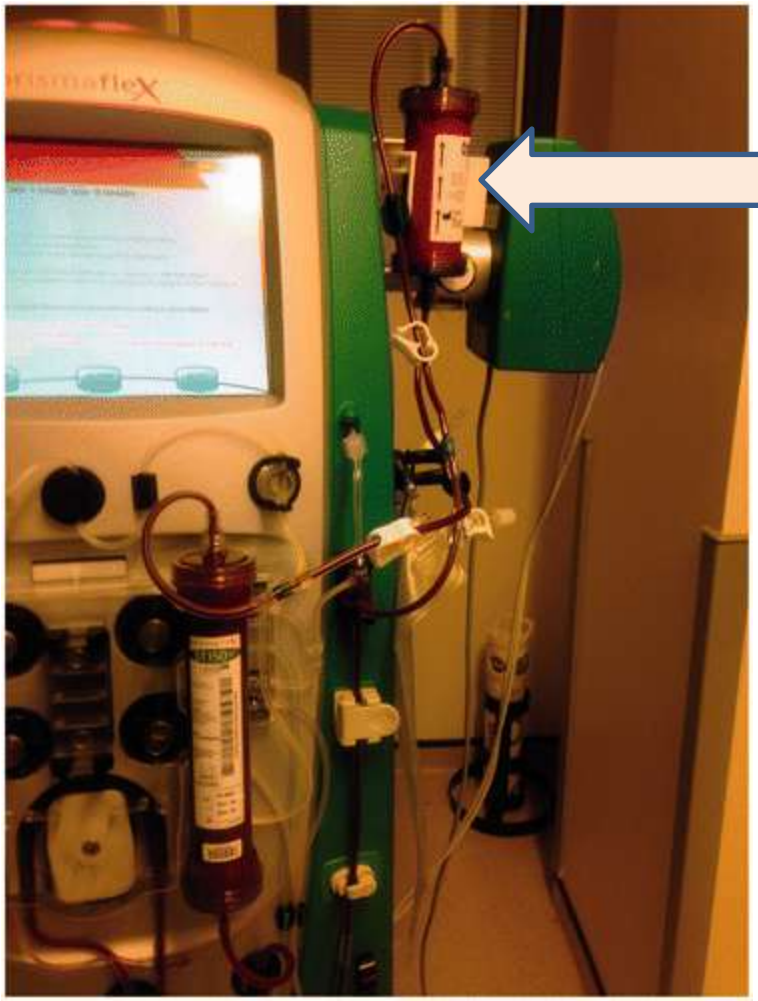
Significant interactions including removal of antibiotics may be harmful.

Some case senario..

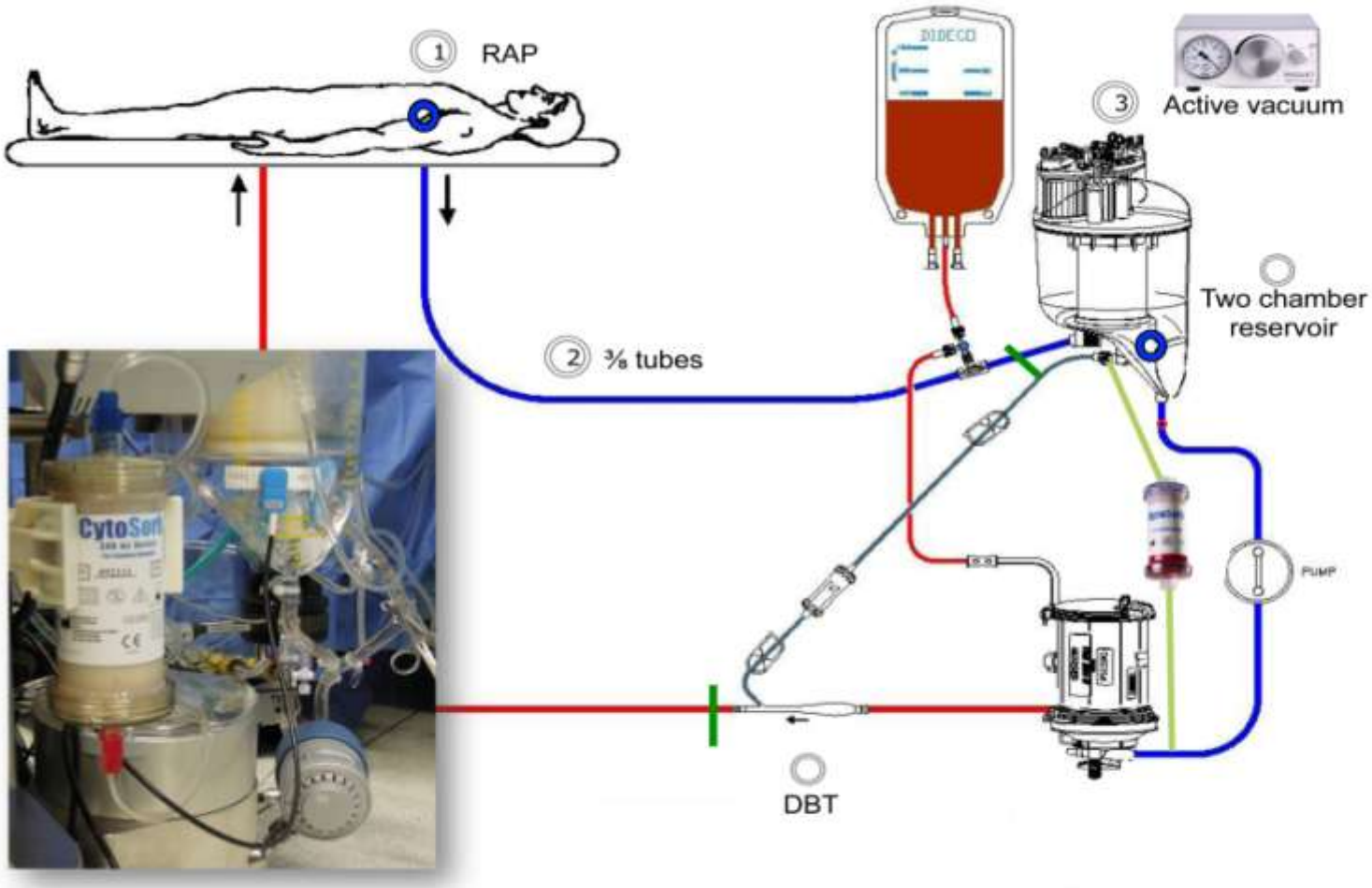
A previously healthy patient in the 40 s was admitted with **acute septic shock and multiple organ failure. Clinically and radiographically the cause was severe community acquired pneumonia** with a lobar consolidative pattern and positive urine antigen testing for *Streptococcus pneumoniae*.
The patient developed rapidly worsening **multiple organ** (cardiovascular, respiratory and renal) failure with **prominent haemodynamic instability** requiring high-dose infusions of norepinephrine, dobutamine and vasopressin and still only achieving systolic arterial pressures of 60–70 mm Hg. Bedside echocardiography demonstrated severe, global left ventricular dilatation and systolic failure with ejection fraction approximately 20%.
The patient was receiving maximal supportive care including continuous veno-venous haemodiafiltration (CVVHDF) for oligo-anuric acute renal failure and, in the face of refractory septic shock, the decision was made **to add the Cytotosorb™ haemabsorption column into the return limb of the circuit** .
He received standard 1.8 l h^{-1} (approximately 25 ml kg^{-1} h^{-1}) CVVHF(continuous veno-venous hemo filtration) combined with blood pump speeds 200–300 ml min^{-1} as vascular access tolerated.




CytoSorb
For Cytokine Removal
CE




CytoSorb
300 mL Device
For Cytokine Removal
LOT
051514
SN
CE
0344


1 RAP
2 ⅜ tubes
3 Active vacuum
Two chamber reservoir
Pump
DBT
CytoSorb

Progress of patient after use of CYTOSORB…

There was little clinical evidence of improvement for the first 24 h, but the **vasoactive doses reduced** and by day three was no longer supported with vasoactives and at day five the ejection fraction had returned to over 60%.

The profile of his inflammatory parameters is detailed below:

ICU timing	IL-6	CRP	WCC	Neutrophils
Pre-Cytosorb™	> 5000	263	0.4	0.3
6 h Cytosorb™	> 5000	308	0.6	0.4
37 h Cytosorb™	3264	340	5.4	5.0
60 h Cytosorb™	1198	257	19.8	18.5

IL-6 Interleukin-6 (normal range 07 $pgml^{-1}$) Our laboratory does not provide a numerical value if > 5000, CRP C reactive protein mg l^{-1}, WCC white cell count micro l^{-1}

At 60 h, the patient was no longer uraemic and CVVHDF was stopped as the filter clotted.

Considering the marked improvement in haemodynamic condition and Cytosorb™,therapy was discontinued and subsequently patient was discharged to the ward and ultimately home in good health.

Another case to discuss..

A young patient in 20s was admitted with severe community acquired pneumonia.

The patient was managed supportively with mechanical ventilation and antibiotics but progressed to septic shock and acute renal failure requiring CVVHDF to control rising potassium. Vasoactive support was progressively increasing so the decision was made to include the Cytosorb™ in treatment.

Over the course of the next 6 h the clinical condition improved rapidly with reducing vasoactive infusions and ultimately the patient's trachea was extubated at 72 h.

The patient recovered well after surgery and has been discharged home.

ICU timing	IL-6	CRP	WCC	Neutrophils
Pre-Cytosorb™	312	227	35.1	24.3
38 h Cytosorb™	253	140	23.9	21.6

(A planned interim IL-6 sample was not collected and at 38 h the patient was no longer uraemic and substantially improved, so Cytosorb™ and CVVHDF were discontinued).

Discussion

These case reports appear to be among the first published uses of Cytosorb™ as an **adjunct in managing sepsis.**

As Cytosorb™ is now commercially available in Europe, its introduction into clinical practice must occur in a controlled fashion, informed by clinical and surrogate outcomes.

Cytosorb (CytoSorbents Corporation; Monmouth Junction, NJ) is a novel synthetic haemabsorption column, which received CE(European Comformity) approval in 2011 for the management of inflammatory conditions with elevated cytokine levels[1] and is **currently the only CE-approved extracorporeal device marketed for inflammatory mediator removal**.

Cytosorb is currently marketed across for **the spectrum of inflammation including sepsis, cardiopulmonary bypass, pancreatitis and burns.**

There is currently limited published data on the basic science and clinical experience of the device although CE marking centered upon (unpublished) findings from a **small European trial of patients with acute respiratory distress syndrome (ARDS) complicating sepsis,** where interleukin 6 (IL-6) blood concentrations in Cytosorb-treated patients were almost halved (49.1%) vs. standard care.

To date, no clinical outcomes from this study have been published although promotional data suggest Cystosorb-treated patients had **less deaths** (0 vs. 62.5% control) and **fewer patients required mechanical ventilation** (33 vs. 88%) at 28 days.

Manufacturer data also suggest over 300 patient treatments to date with good tolerability and safety.

The Cytosorb device is a relatively simple haemabsorption column consisting of a suspended column of beads of highly porous resin (polystyrene divinylbenzene PSDVB) covered with a biocompatible polyvinylpyrrolidone coating.[4]

The beads are 300–600 µm in diameter with density 1.02 g cm^{-3} and porosity 67.7%.

The bead pores are 8–50 Angstrom units allowing **adsorption for smaller molecules (< 50 kDa) and excluding larger proteins, e.g. albumin (70 kDa) or fibrinogen (340 kDa).**

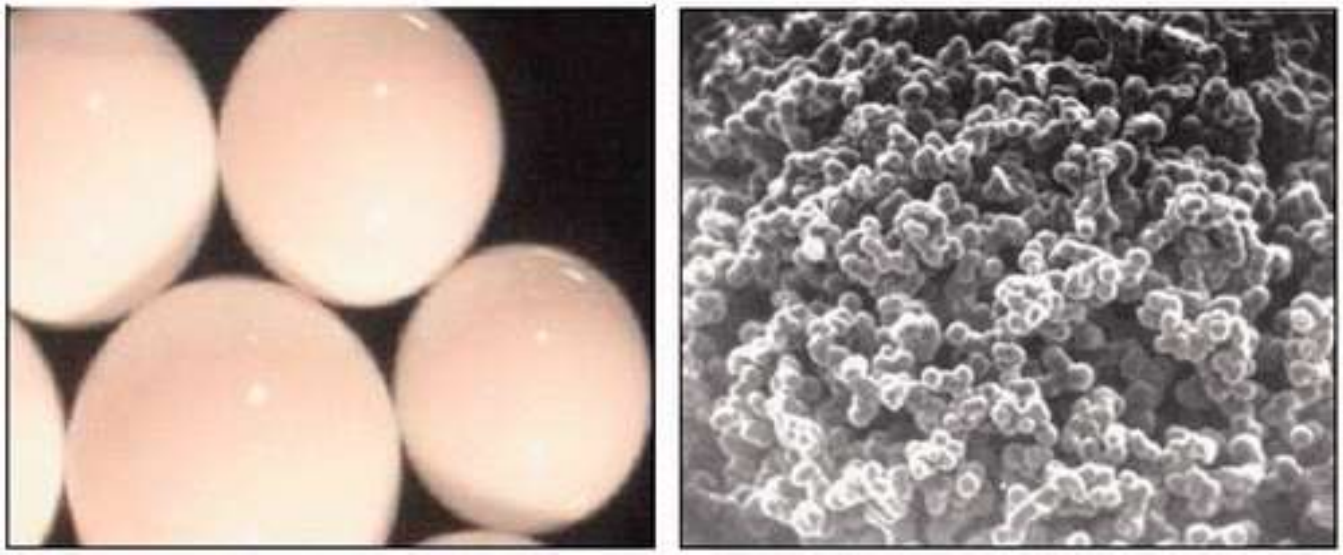


Figure 3. Macroscopic appearance of Cytosorb beads and electron microscopy of heavily pore covered structure.

??? BUT….Fundamental issues

Indications, contraindications for use ?

It remains unclear whether elevated **biomarkers** (e.g. IL-6) are sufficient to identify likely responders or whether **clinical features** or **disease acuity** (e.g. APACHE II) are better. Conversely, whether benefit is related to absolute IL-6 level is unclear, e.g. does peak level > 5000 suggest better therapeutic efficacy than 300?

How is therapeutic efficacy defined?

It is not adequate to simply demonstrate reduction in biomarkers and this **must be linked to robust clinical endpoints from other sepsis studies including mortality, organ support requirements, length of stay.**

When should Cytosorb therapy stop?

It is unknown whether a falling biomarker indicates success in stopping inflammation and the improvement may be sustained by ongoing care (e.g. antibiotics) or **whether markers (e.g. IL-6) need reducing to very low levels.** The latter approach might be harmful in inducing **immunosuppression and possible later adverse events (e.g. secondary infection). C**linical endpoints are used e.g. is success falling vasoactive infusions or their discontinuation ARE MORE HELPFUL

Should Cytosorb be used as an independent extracorporeal therapy in the absence of a need for such a therapy?

All treated patients in Derby have also had AKI requiring CVVHDF; BUT is it ANY beneficialresults to commence **independent extracorporeal therapy** and indeed could Cytosorb prevent renal failure?

Unintended consequences.

Cystosorb™ therapy has many implications common to an extracorporeal circuit but also affects **drug kinetics** (especially antibiotics) and will affect **albumin-bound substances, e.g. hormones or toxins (e.g. bilirubin)** in largely unstudied ways.

This may demonstrate unwanted effects, e.g. **sub-therapeutic antibiotic concentrations** but could also **open the way to novel applications, e.g. support in liver failure or treating drug toxicity demands further evaluation.**

Well-designed clinical investigations with larger samples are needed

Thank you