Extracorporeal Approaches to Immunomodulation in Septic Conditions

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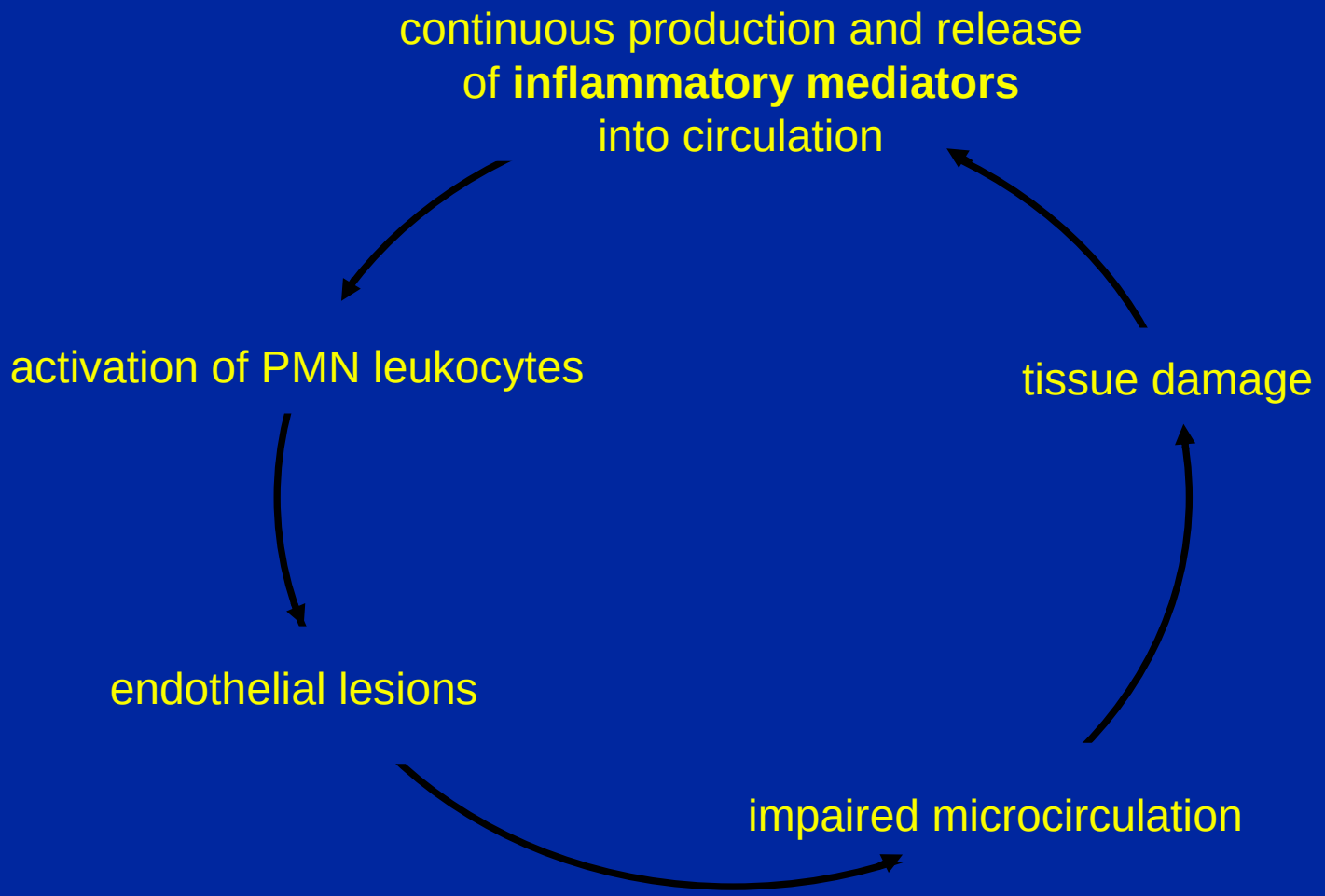
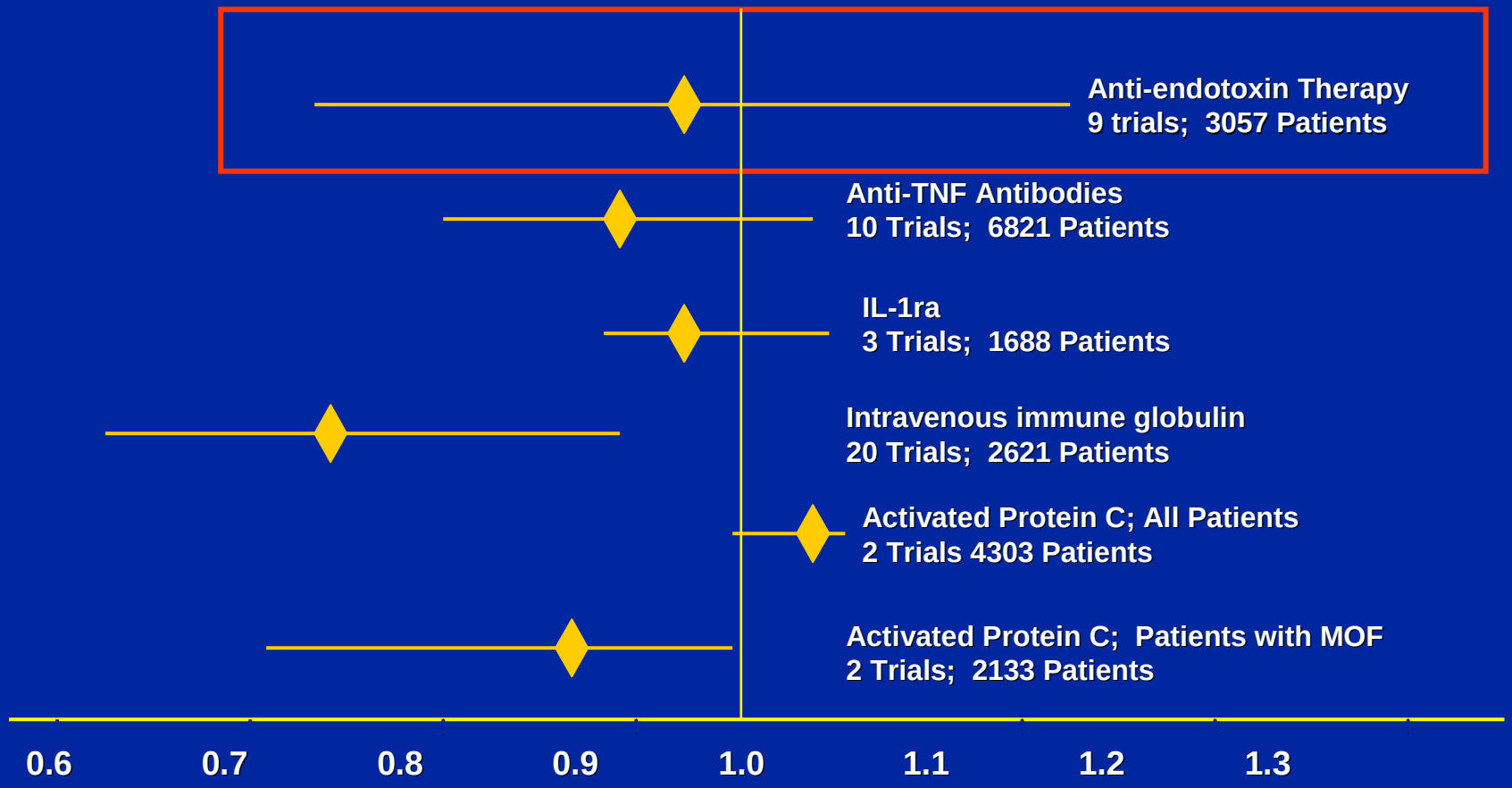
Outline

- Fundamental principles of extracorporeal therapy
- Effect of “standard” CRRT on outcome in septic AKI
- Extracorporeal endotoxin removal: biologic and clinical rationale
- Adapted CRRT techniques for septic acute kidney injury (AKI) conditions
- High cut-off (HCO) membrane-based and AN69 membrane-based approaches

Inflammatory Mediators in Sepsis



Adjuvant Therapy in Sepsis



- Marshall, *J Leukoc Biol* 83:471, 2008

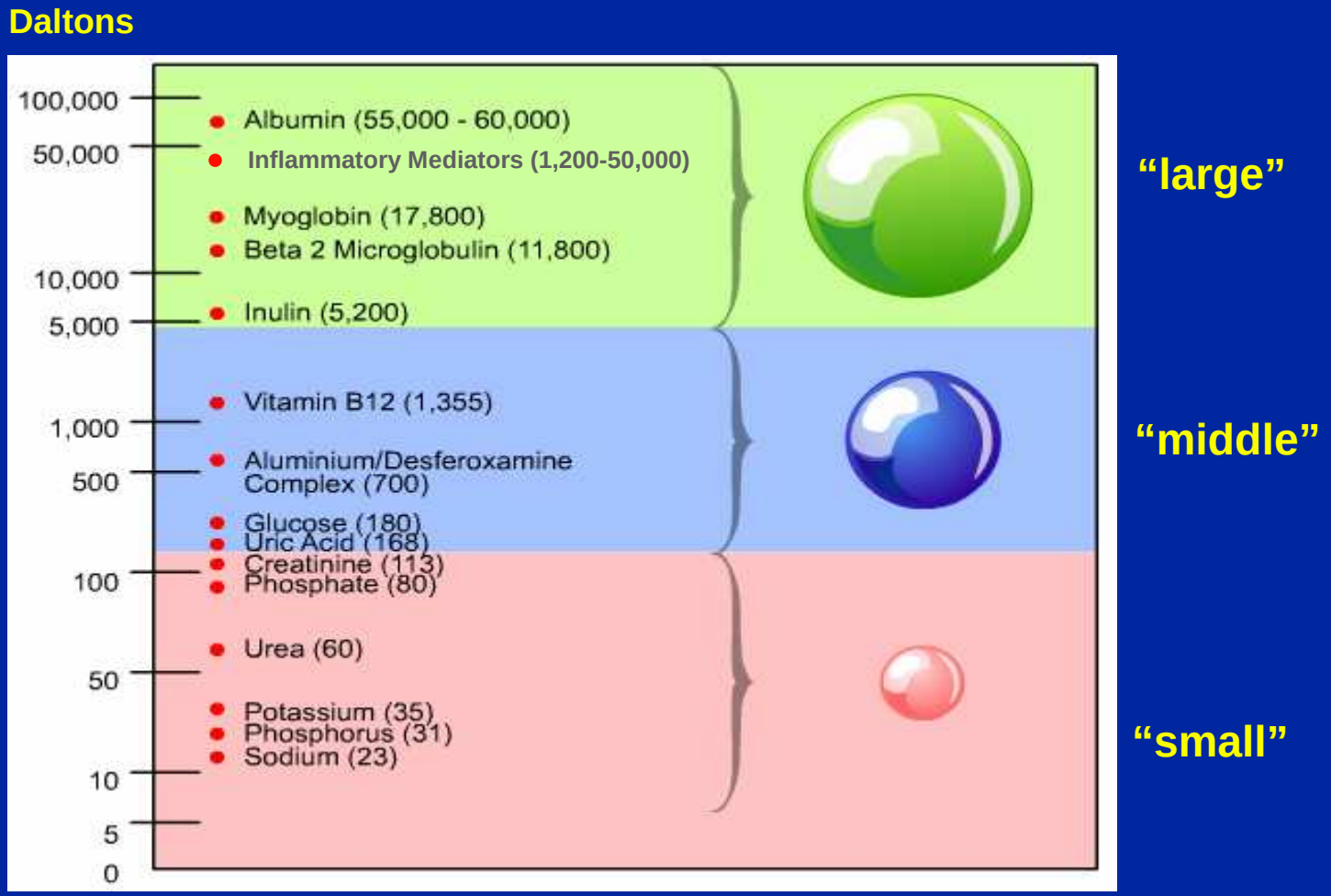
Options for Removing/ Antagonizing Endotoxin

Inhibition of LPS synthesis or immune clearance	Lipid A synthesis inhibitors LPS vaccines Anti-LPS-antibodies
Removal of LPS (or other microbial antigens) from the blood or other body fluids	Removal of LPS or other microbial constituents by filtration or absorption (columns, polymyxin B)
	LPS-binding protei (BPI) Phospholipi binders (high-dens c phospholipid
Inhibition of LPS/CD14 interactions Inhibition of cell signal transduction	TLR receptor blockade E5564 NF-κB inhibition

Non-specific removal (Ex: oXiris)

Specific removal

Solute Classes by Molecular Weight



Mediators: Physical Properties

	Protein Binding	MW
C3a desArg	no	2500
C5a desArg	no	2800
Il-1a	no	17000
Il-1Ra	no	14000
TNFα	Partly	17,500 x 3
sTNFRI	yes	55000
sTNFRII	yes	75000
Il-6	no	25000
Il-8	no	9000
Il-10	no	18000
P A F	Partly	450
Factor D	yes	23000

Limited Effect of "Standard" CRRT on Plasma Inflammatory Mediator Levels

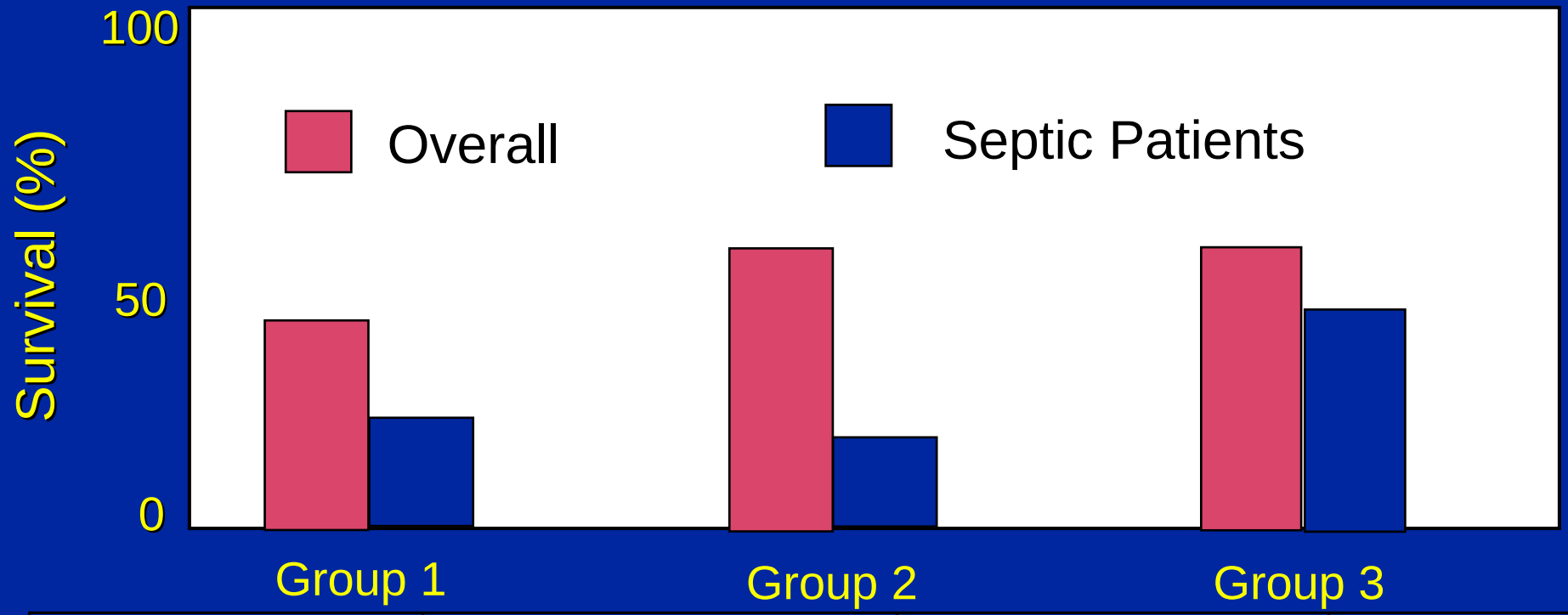
Herring et al, Kidney Blood Press Res 2003

Study (first author)	Patients n/disease	Therapy	Mediator	Elimination in the ultrafiltrate	Effect on plasma levels	Clinical data
Heering [10]	33 sepsis/ cardiac failure	CVVH	TNF-α, IL-1β, IL-6, IL-8, IL-2, IL-10, TNF-RII, IL-11ra, IL-2R, IL-6R	Detection of cytokines in the ultrafiltrate	No reduction	Improvement of cardiovascular hemodynamics
Hoffmann [11]	16 MOF	CVVH	TNF-α, IL-6, IL-1β, IL-8, C5a, C3, C3a	Detection of IL-8	Complement ↓	Improvement of cardiovascular hemodynamics
Kellum [23]	13 ARF, SIRS	CVVH/D	TNF-α, IL-6, Selectin, Etox	Minimal	No reduction IL-6, IL-10; reduction of TNF-α	n.s.
Van Bommel [34]	9 SIRS	CVVH	TNF-α, sTNFr I/II, IL-1ra	IL-1ra	No reduction	No effect
De Vriese [24]	15 sepsis	CVVH	TNF-α, IL-1β, IL-6, IL-1ra, sTNFr-I/II, IL-10	TNF-α, IL-1β, IL-6, IL-1ra, sTNFr-I/II	Reduction	Improvement of cardiovascular hemodynamics
Sanchez [25]	30 trauma ARF	15 CVVH 15 controls	TNF-α, IL-6	Elimination TNF-α, IL-6	No reduction	n.s.
Sander [31]	28 SIRS	CVVH, controls	TNF-α, IL-6	IL-6	No reduction	No improvement of hemodynamics
Schumacher [32]	Sepsis	CVVH	NO_2/NO_3	n.s.	Reduction	n.s.
Cole [4]	11 sepsis/ MOF	CVVH/ HV-HF	Anaphylatoxins C3a, C5a	Minimal	Reduction	Greater reduction of vasopressors by HV-HF
Cole [1]	24 early sepsis	CVVH vs. no CVVH	IL-6, IL-8, IL-10 and C3a, C5b	n.s.	No difference	No clinical improvement

ARF: Acute renal failure; MOF: multiorgan failure; SIRS: systemic inflammatory response syndrome; HV-HF: high-volume hemofiltration; HF: hemofiltration; CAVH: continuous arteriovenous hemofiltration; CVVH: continuous venovenous hemofiltration; CVVHD: continuous venovenous hemodiafiltration; ns: not studied.

"Sepsis Dose" in AKI?

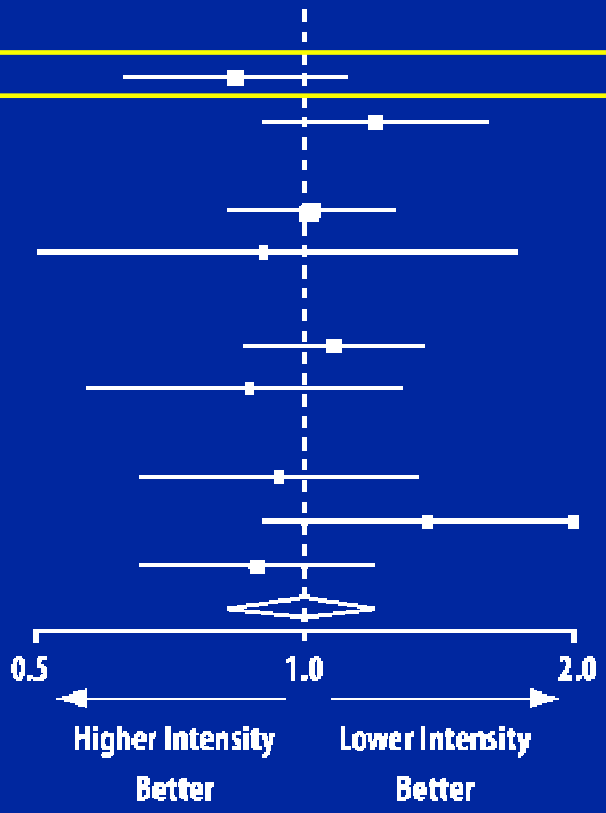
Ronco et al., Lancet 2000



Trial Group	No Sepsis (%)	Sepsis (%)	p-value
Group1	55/126 (44%)	5/20 (25 %)	0.90
Group 2	76/122 (62 %)	3/17 (18 %)	0.001
Group 3	74/125 (59 %)	7/15 (47 %)	0.256

Mortality Outcomes in RENAL

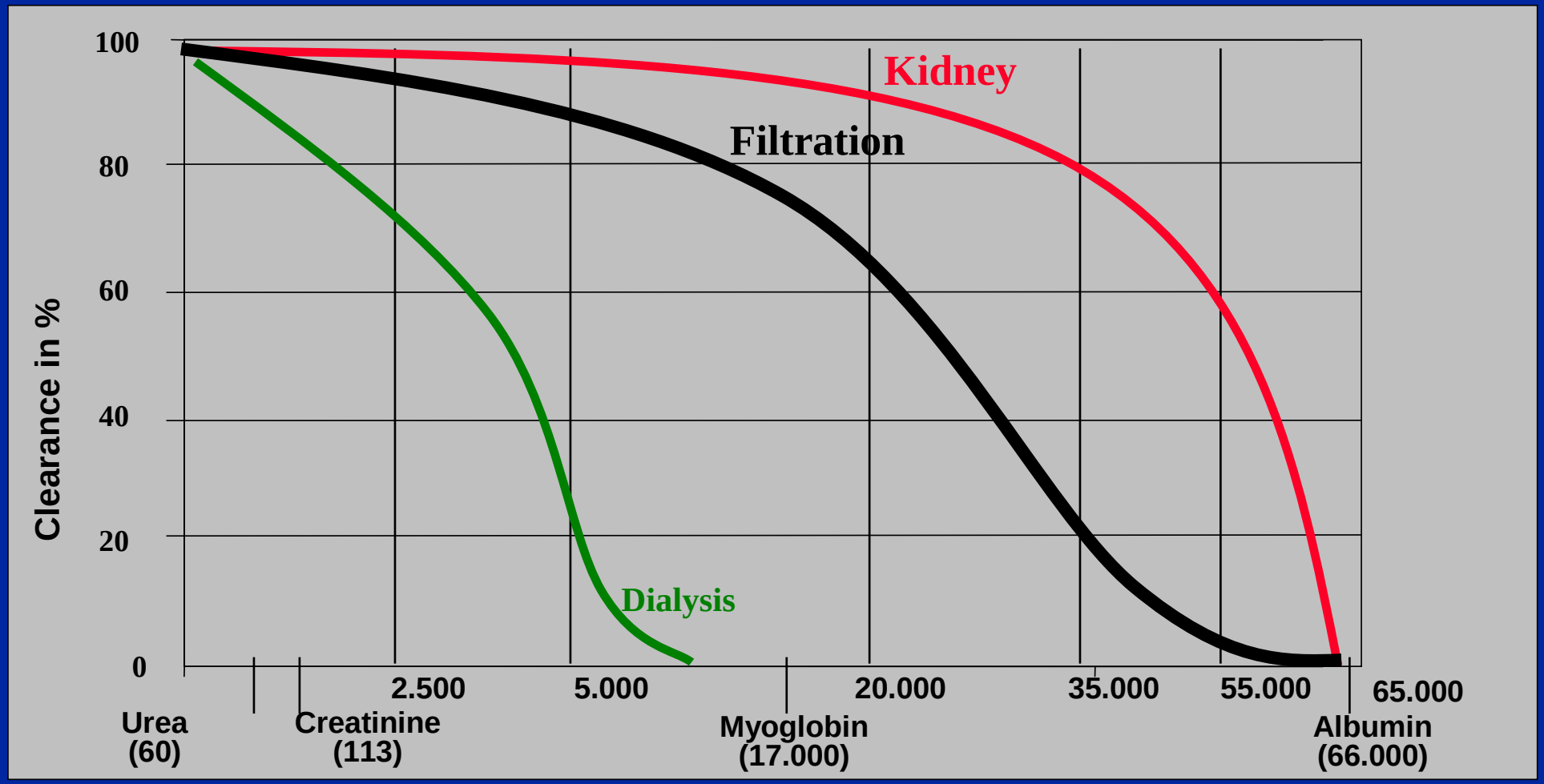
Prespecified Subgroup	Higher Intensity (N=723) no. of deaths/no. of patients (%)	Lower Intensity (N=743)	Odds Ratio (95% CI)
Patients with criteria for sepsis			
Yes	168/139 (45.8)	186/163 (31.2)	0.84 (0.62 - 1.12)
No	134/162 (42.3)	145/179 (?)	1.19 (0.89 - 1.60)
Patients with at least one nonrenal organ failure			
Yes	299/623 (47.6)	306/649 (47.2)	1.02 (0.82 - 1.27)
No	23/93 (24.7)	25/93 (26.9)	0.89 (0.46 - 1.72)
Patients with SOFA cardiovascular score of 3 or 4			
Yes	247/310 (48.4)	254/346 (45.5)	1.08 (0.85 - 1.37)
No	74/210 (35.2)	75/194 (33.7)	0.86 (0.58 - 1.29)
Patients with eGFR=60ml/min.			
Yes	114/250 (45.6)	105/222 (47.3)	0.93 (0.65 - 1.34)
No	81/157 (51.6)	83/185 (43.5)	1.17 (0.89 - 2.10)
Missing	122/114 (?)	146/116 (43.5)	0.88 (0.65 - 1.21)
Death from any cause by day 90	122/221 (44.7)	112/743 (44.7)	1.00 (0.83 - 1.25)



Adapted Extracorporeal Approaches for Enhanced Inflammatory Mediator Removal

- Increased rate of fluid exchange (“high-volume hemofiltration”)
- Membrane modifications
  - Permeability (HCO; septeX)
  - **Surface properties (oXiris)**

Small vs. Large Molecule Clearance



High-Volume Hemofiltration: Pilot Study

Honore et al, Crit Care Med 2002

1° Hemodynamic status	
- MAP	- < 55 mmHg
- Inotropic support : after failure of maximal dosages of dopamine and dobutamine-norepinephrine	- Epinephrine not for more than 2 hours
- Cardiac index	- < 2 l/min/m^2
- Wedge pressure	- > 14 < 18 mm Hg
2° Acid-base balance	
- Arterial pH	- < 7.15
- Serum lactate	- > 5 mmol/L
3° Septic status	
- SIRS criteria (ACCP/SCCM criteria)	- 3 out of 4
- Objective source of sepsis	- Always present
4° Respiratory support	
- Mechanical ventilation	- All the patients
- paO_2/FIO_2 ratio	- < 100
5° Renal status	No incidence at study inclusion

Dopamine : up to 20 mg/Kg/min

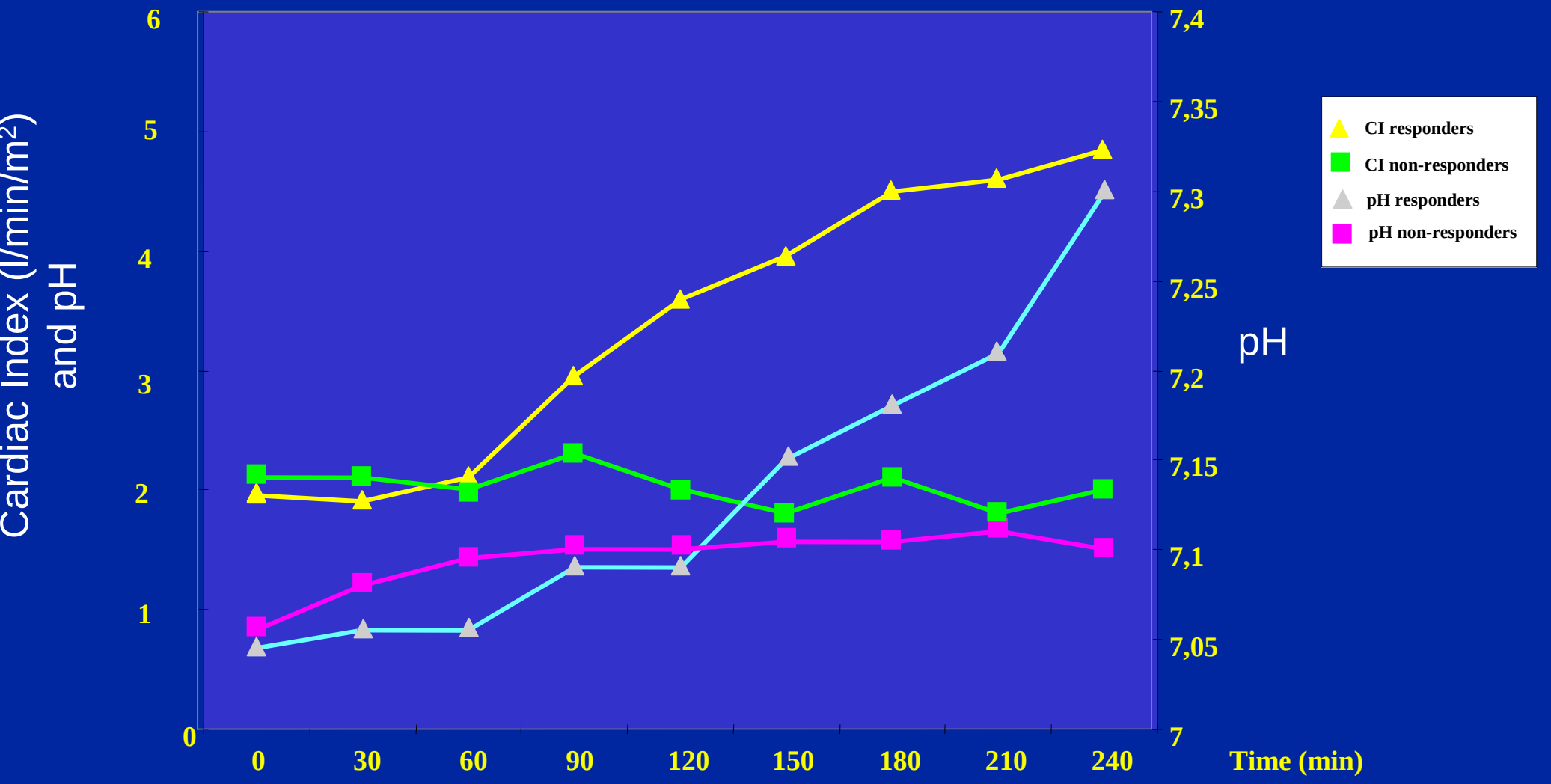
Norepinephrine : up to 2 mg/Kg/min

Dobutamine : up to 14 mg/Kg/min

Epinephrine : up to 0.5 mg/Kg/min

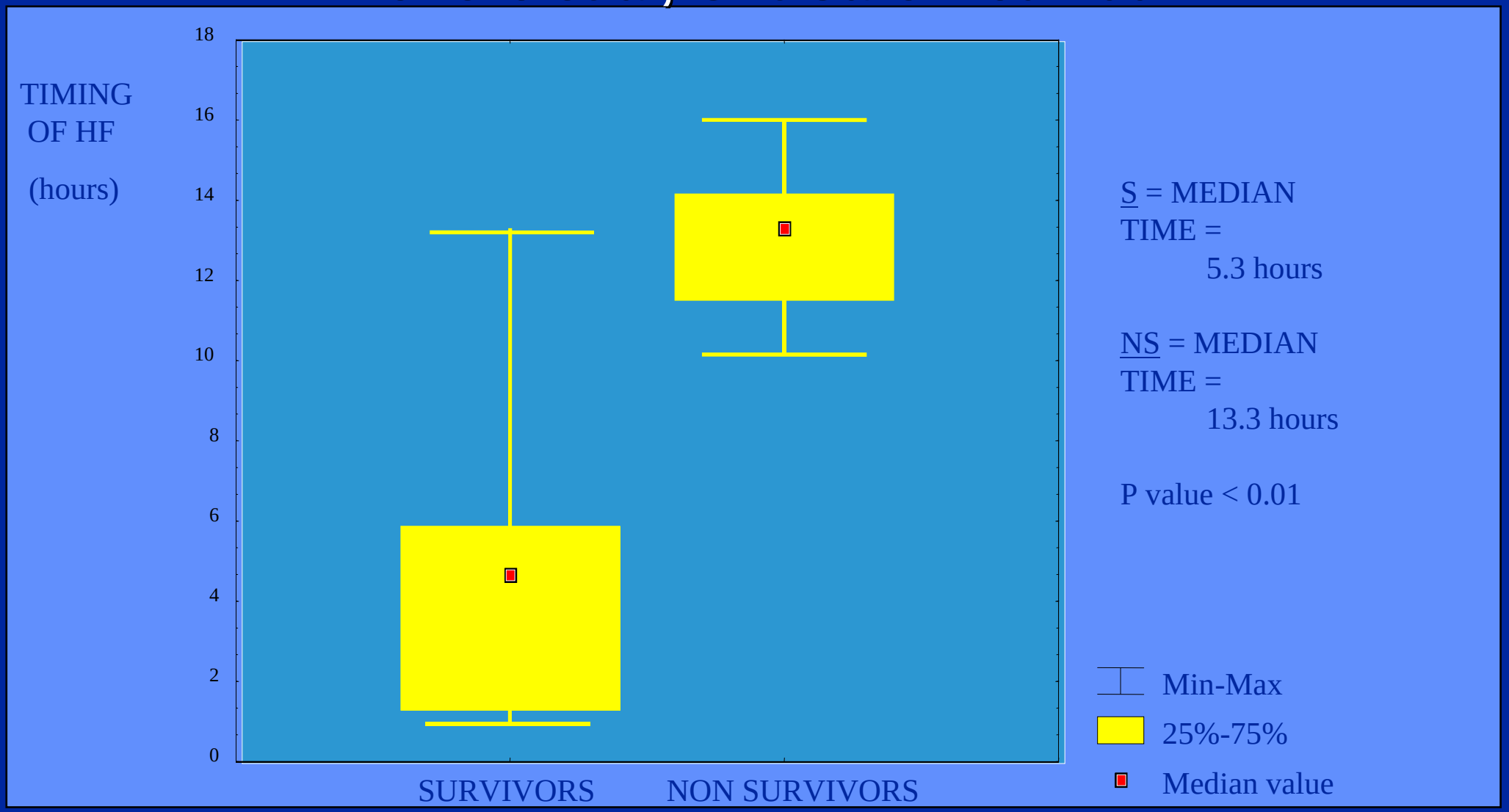
High-Volume Hemofiltration: Pilot Study

Honore et al, Crit Care Med 2002



High-Volume Hemofiltration: Pilot Study

Honore et al, Crit Care Med 2004

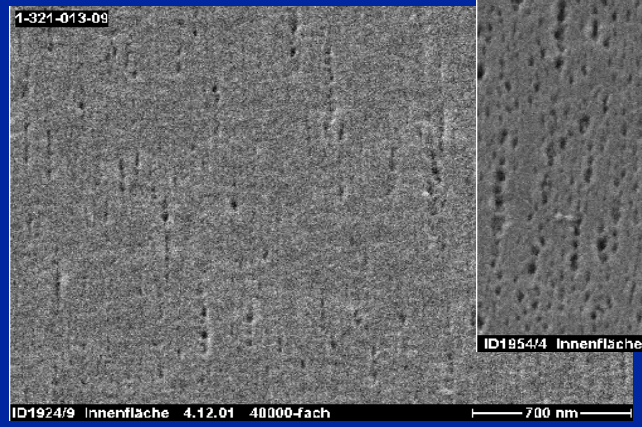


Variation of membrane pore sizes

Electron micrographs of inner membrane surface

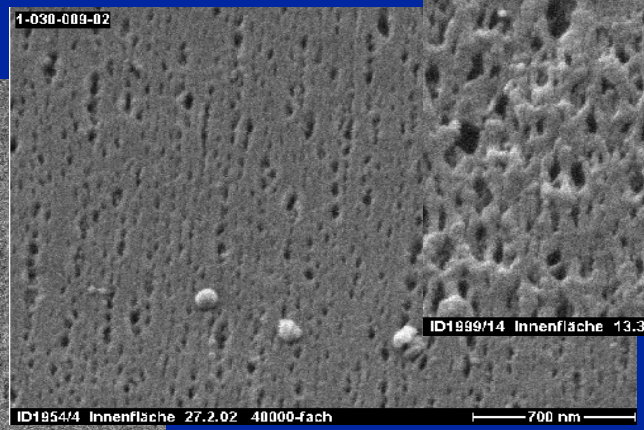
∅: pore diameter

∅ < 0,01 µm



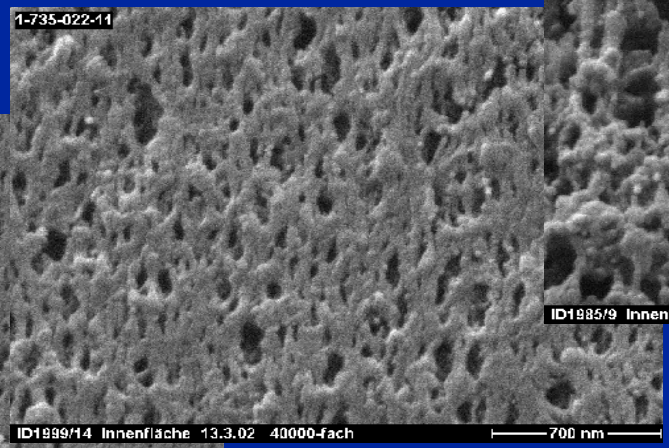
high flux

∅ < 0,02 µm



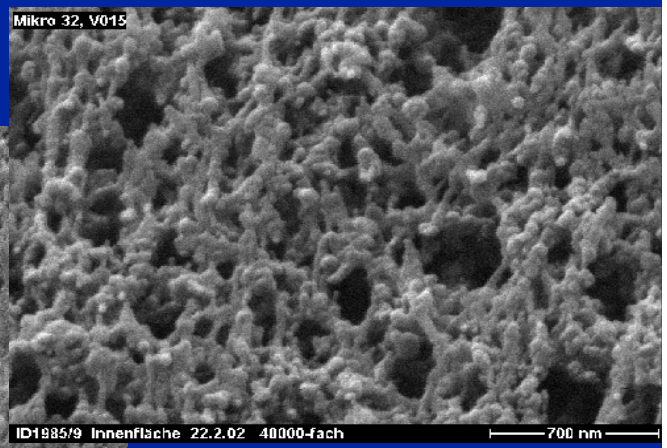
*high cut-off**

∅ ~ 0,09 µm



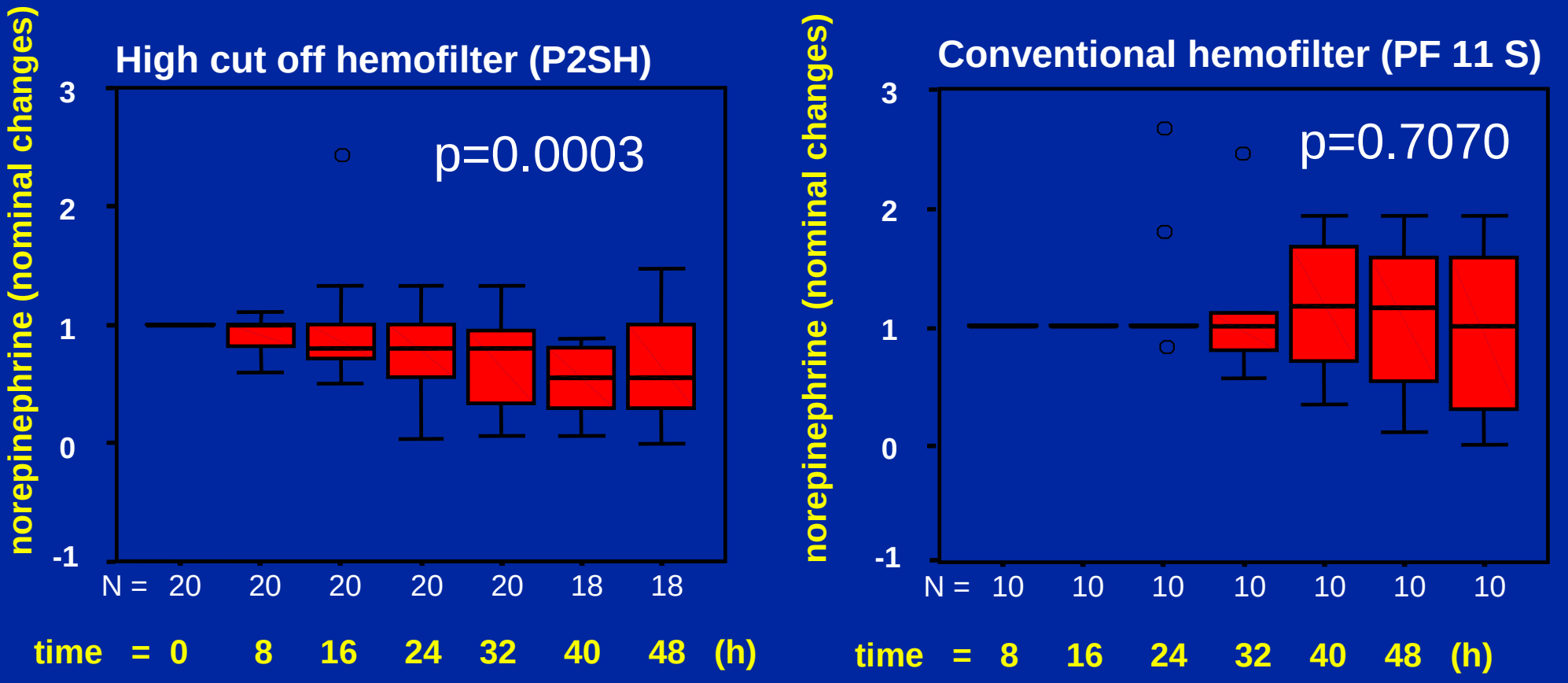
protein separation membrane

∅ ~ 0,30 µm



plasma separation membrane

Effect of High Permeability Hemofiltration on Norepinephrine Requirements

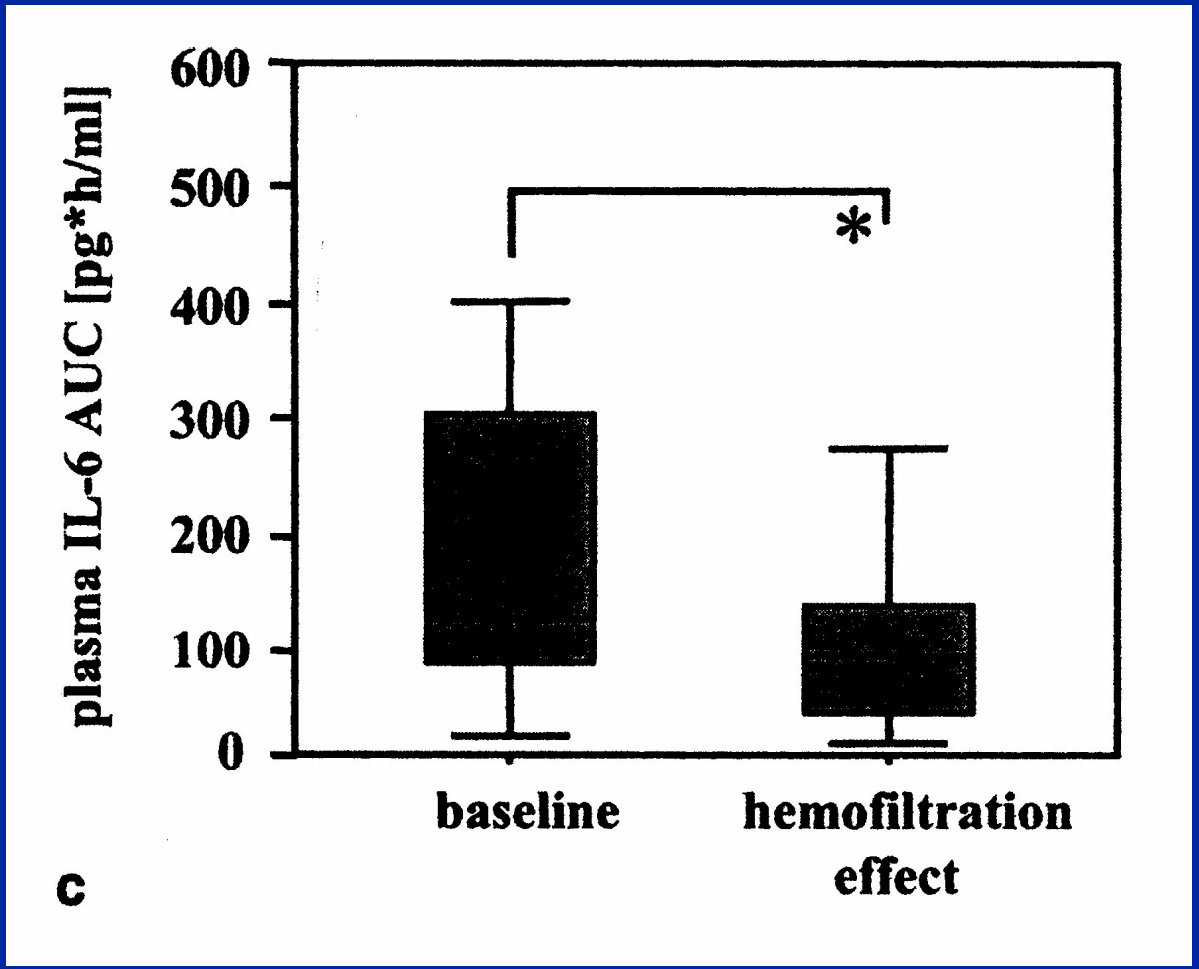


p=0.0303 between P2SH and PF11S

Morgera et al,
Intensive Care Med 2003

Effect of High Permeability Hemofiltration on Plasma IL-6 AUC

Morgera et al, Intensive Care Med 2003

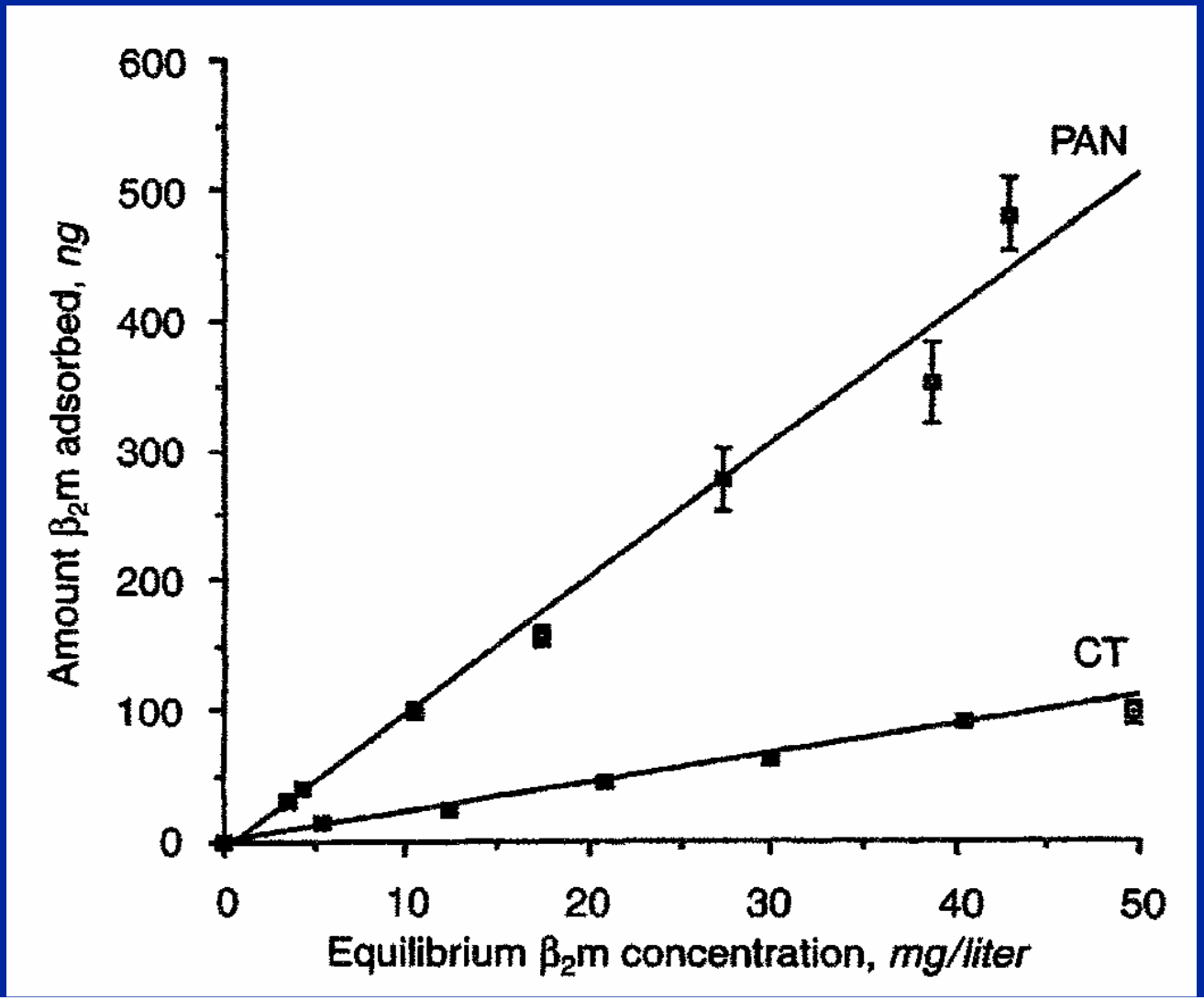


AN69: Historical Perspective

- Copolymer of acrylonitrile and an anionic sulfonate group
- AN69 was first commercially available synthetic membrane (mid 1970's)
- With respect to complement activation, AN69 was recognized as the clinical reference against which other membranes were compared
  - Partially explained by membrane's unique capability to adsorb complement activation products
- "Surface-treated" filter (AN69ST) developed for chronic HD application in 2000 and for CRRT application in 2003

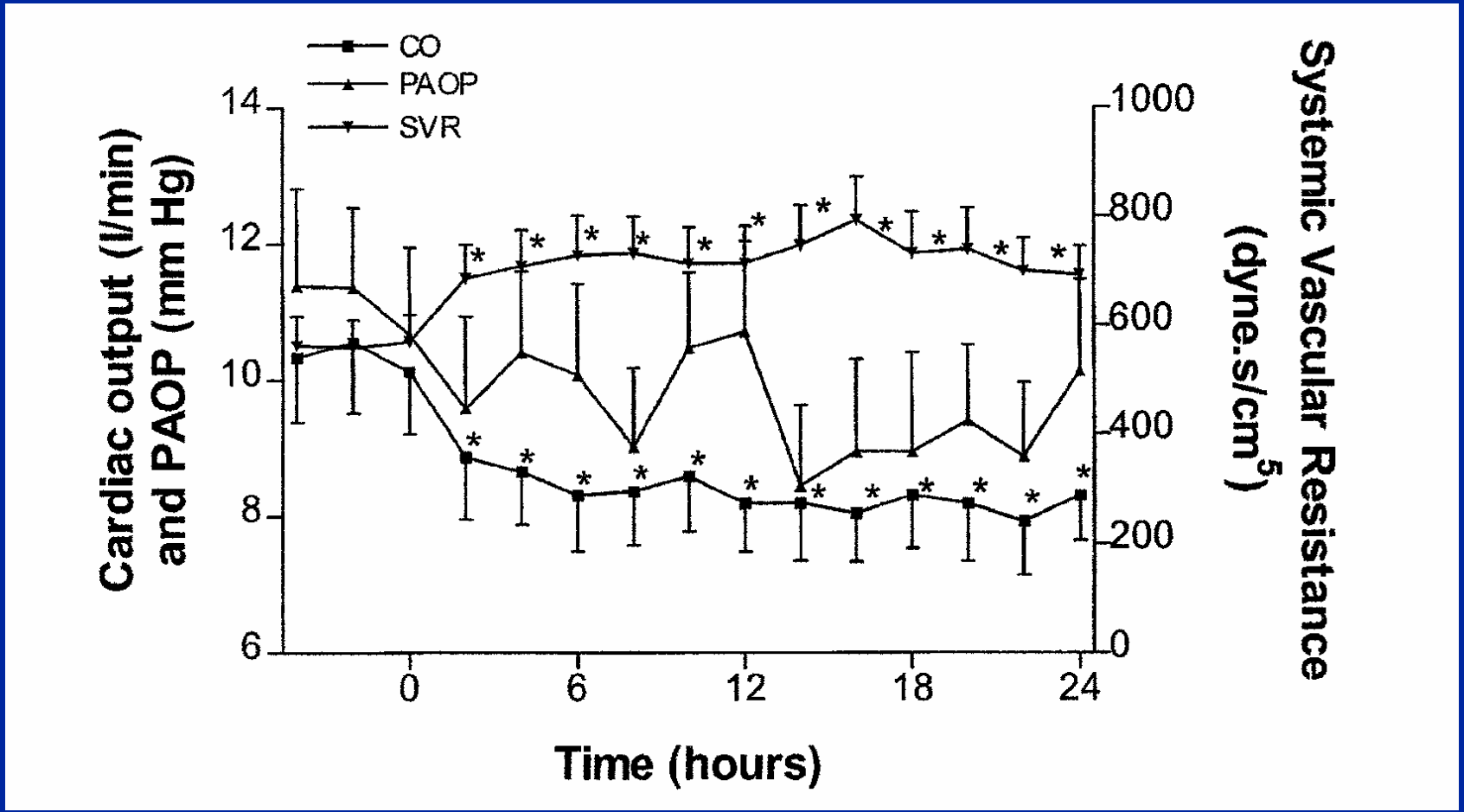
Adsorptive β2-Microglobulin Removal by AN69 vs Cellulose Triacetate

Clark et al, Kidney Int 1995



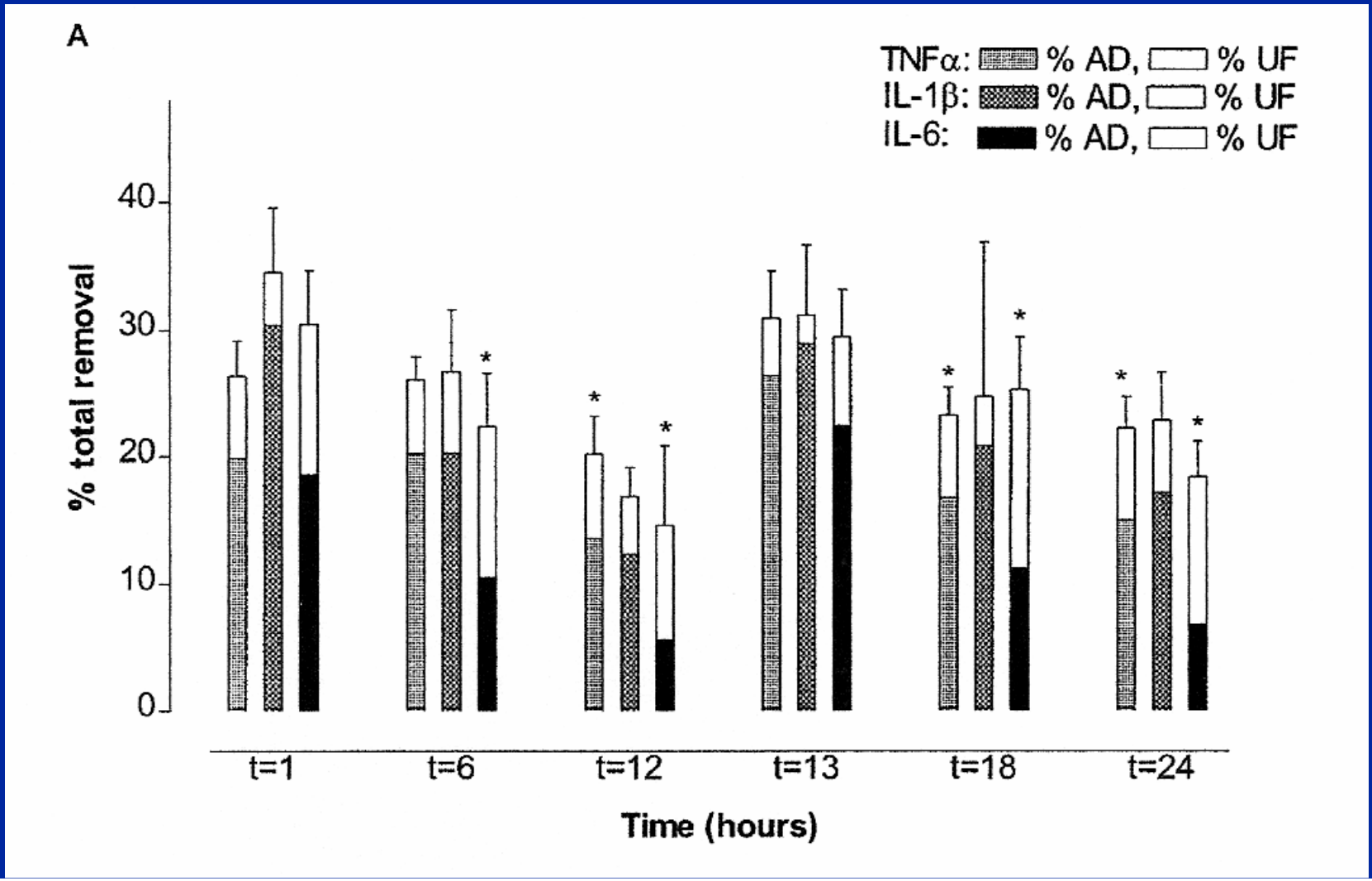
Effect of CVVH with an AN69 Filter on Hemodynamic Parameters in Septic AKI Patients

De Vriese et al., JASN 1999



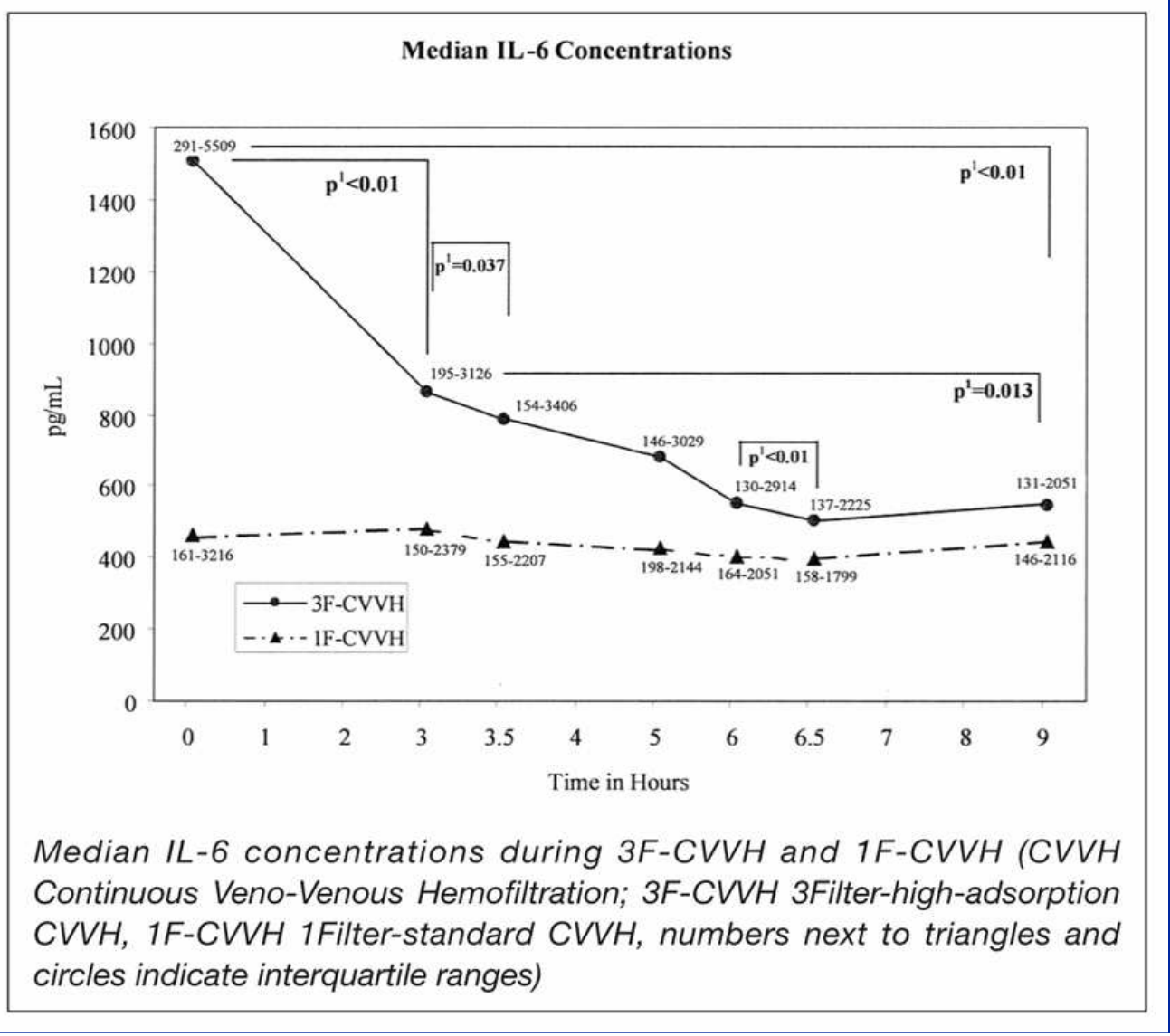
Cytokine Removal in "Standard" CRRT for AKI (AN69 Filter)

De Vriese et al, JASN 1999



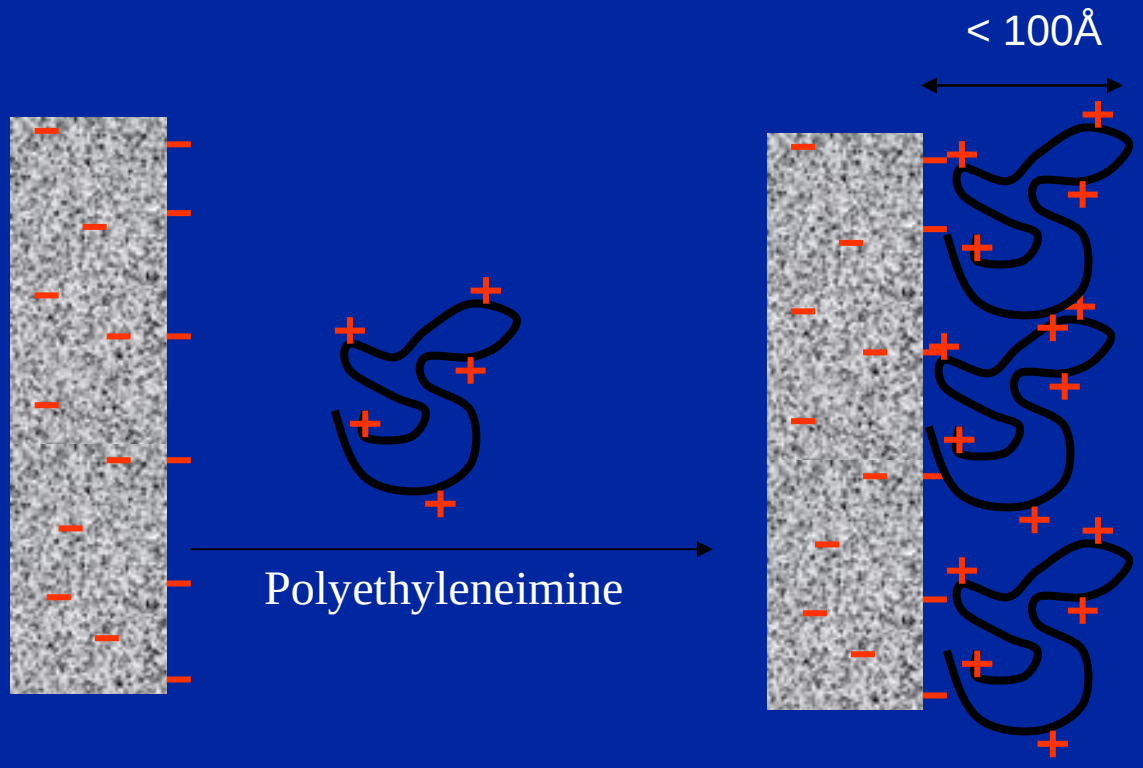
IL-6 Concentration Changes – AN69

Haase et Al., Int J Artif Organs 2007



Median IL-6 concentrations during 3F-CVVH and 1F-CVVH (CVVH Continuous Veno-Venous Hemofiltration; 3F-CVVH 3Filter-high-adsorption CVVH, 1F-CVVH 1Filter-standard CVVH, numbers next to triangles and circles indicate interquartile ranges)

Surface Treatment: Principle

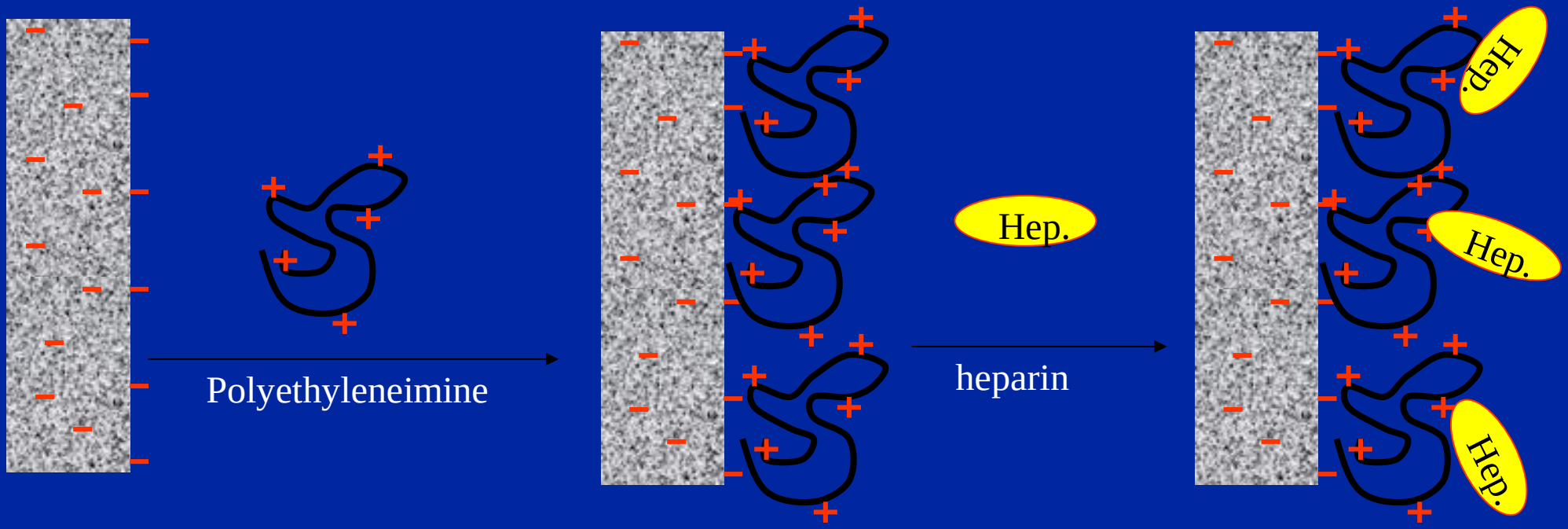


About 10 mg/m²

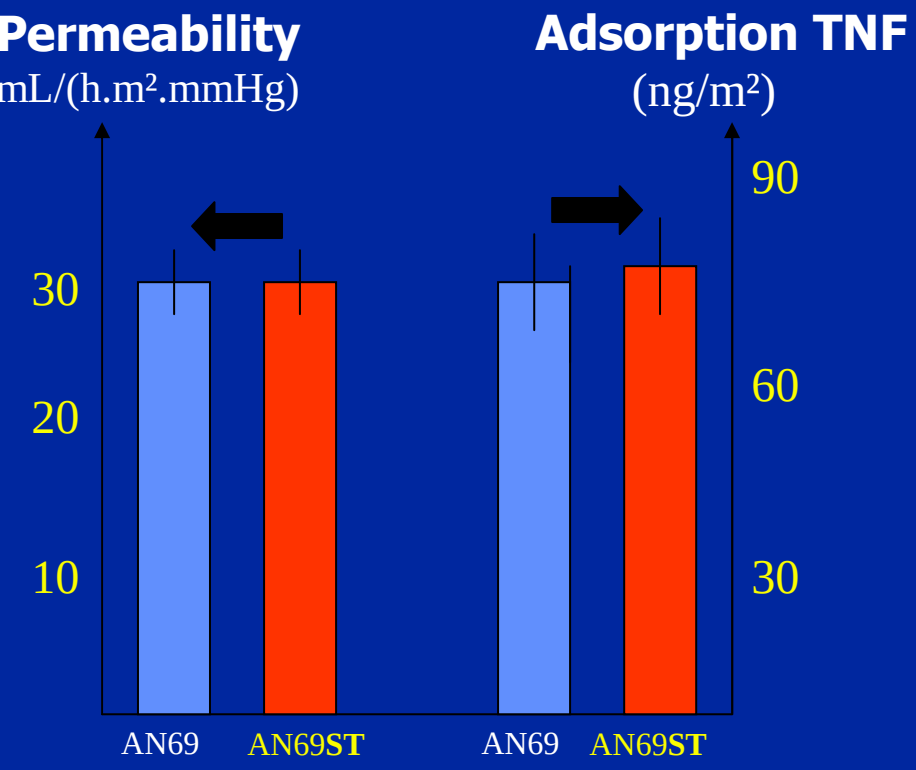
AN69 membrane

AN69 ST membrane*

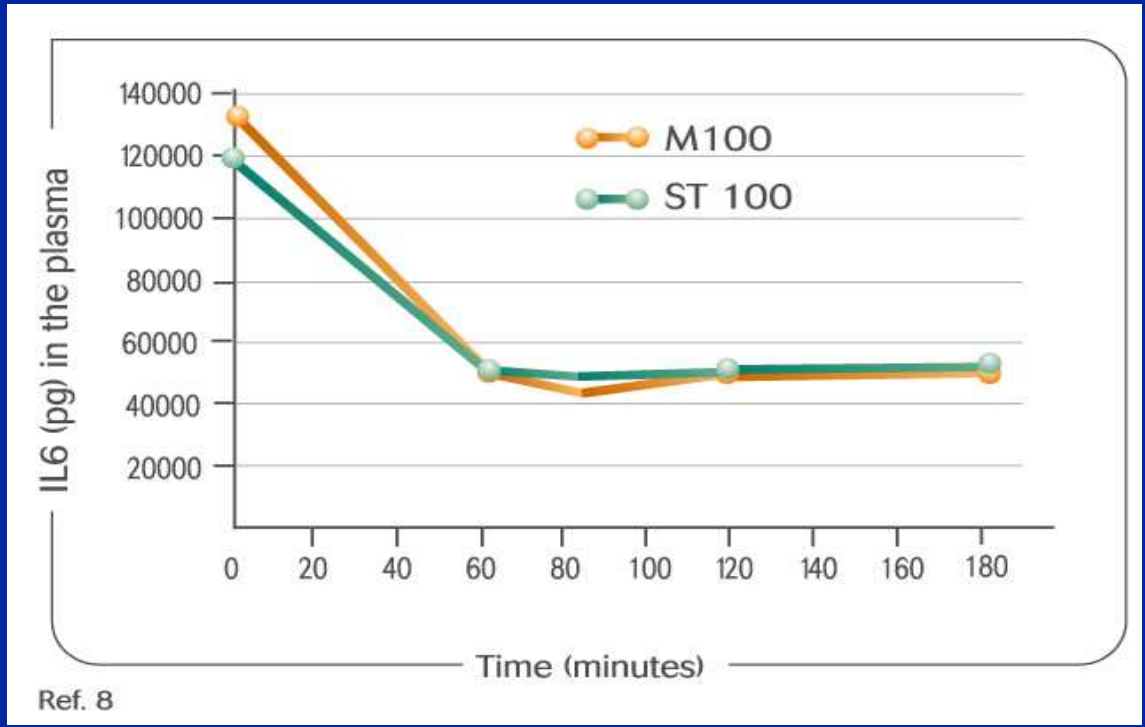
AN69 ST* and Heparin Adsorption



Effect of Surface Modification on Membrane Properties of AN69ST

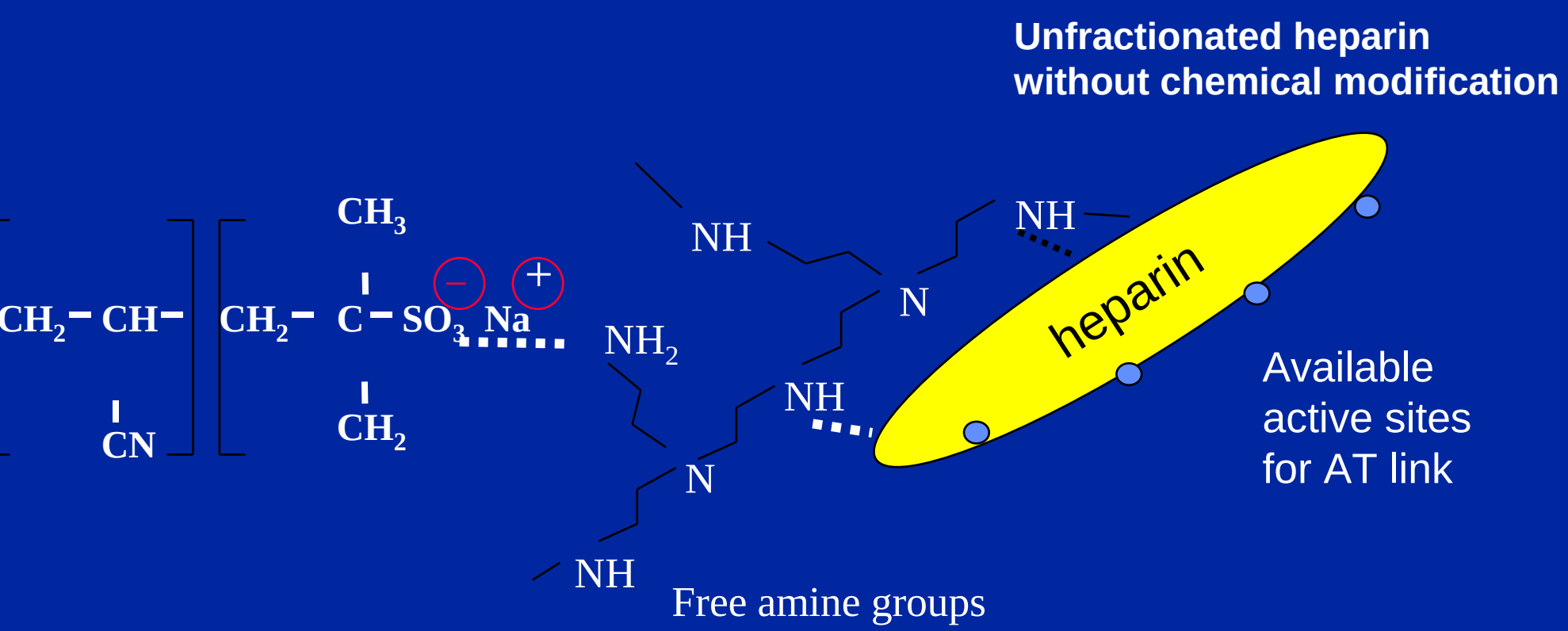


Adsorption kinetics of IL-6 in plasma



Ref. 8

oXiris: Grafting Mechanism (Heparin)

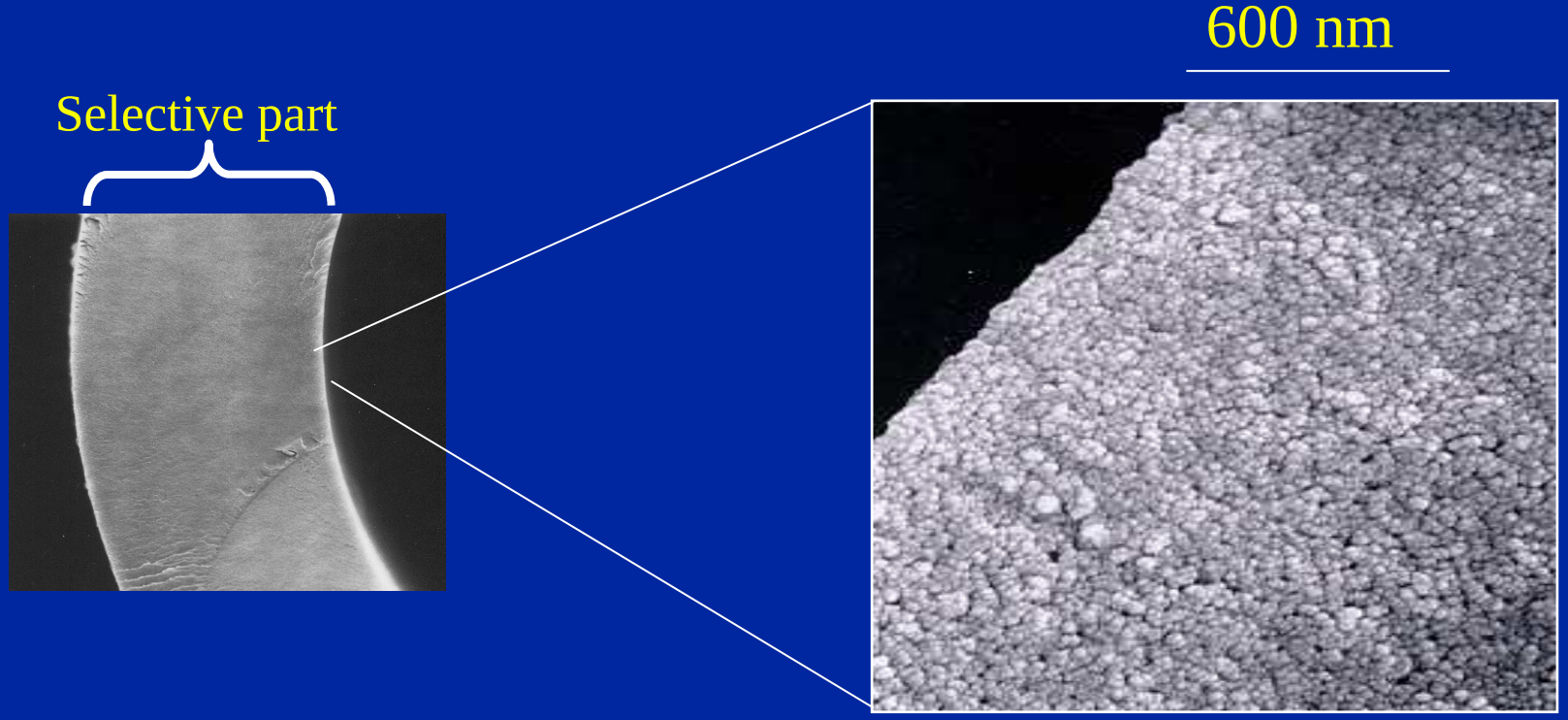


oXiris: Adsorption of Low-Molecular Weight Proteins

AN69 membrane:

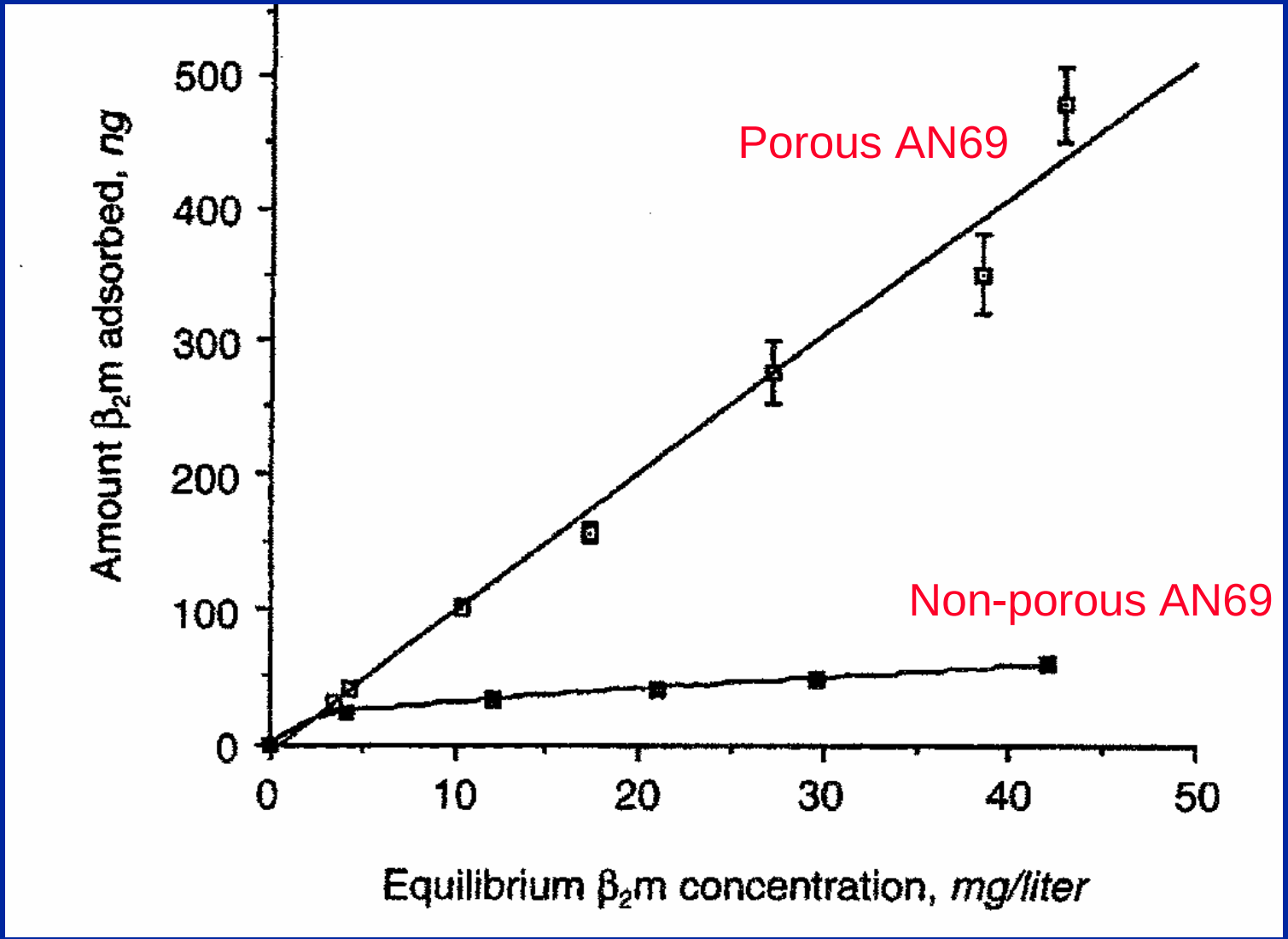
- **symetrical / dense microstructure**
- **homogeneous distribution of sulphonate groups**
- **high sieving coefficient for low molecular weight proteins (< 40 000Da)**

Adsorption mechanism based on ionic binding in bulk



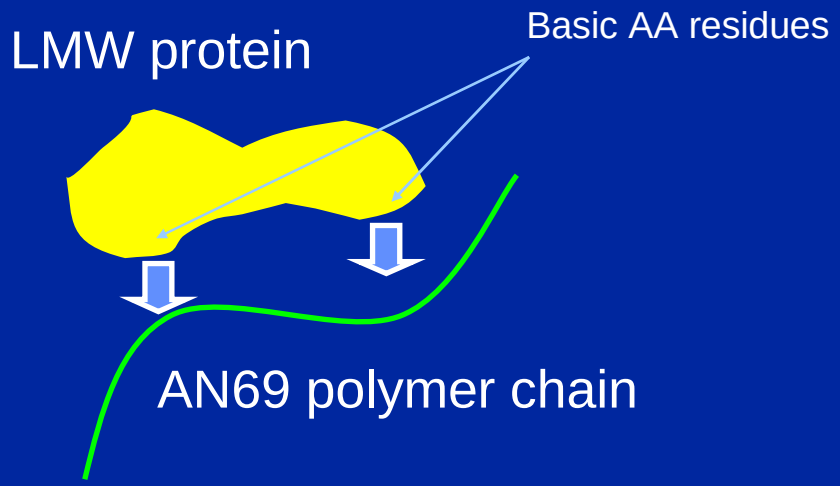
B2M Adsorption Isotherms

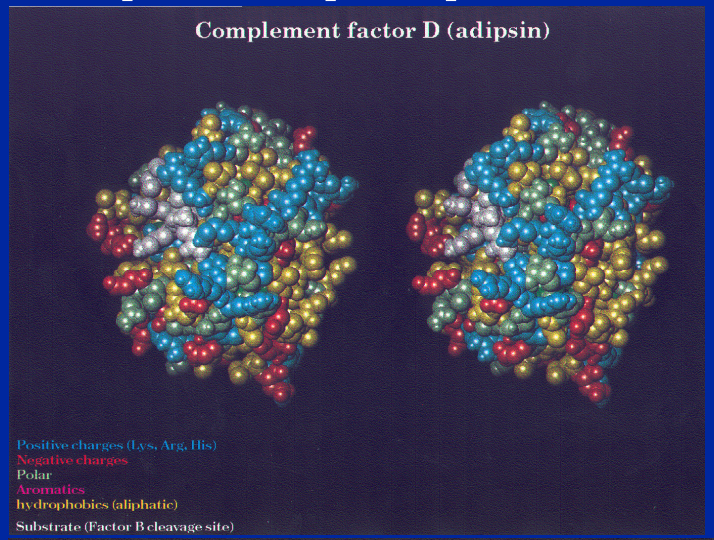
Clark et al, Kidney Int 1994

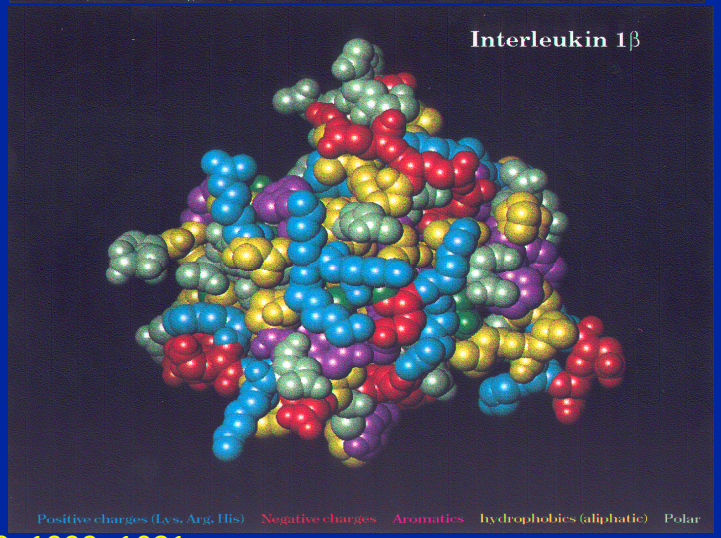


oXiris: Action Mode

Low molecular weight protein adsorption properties







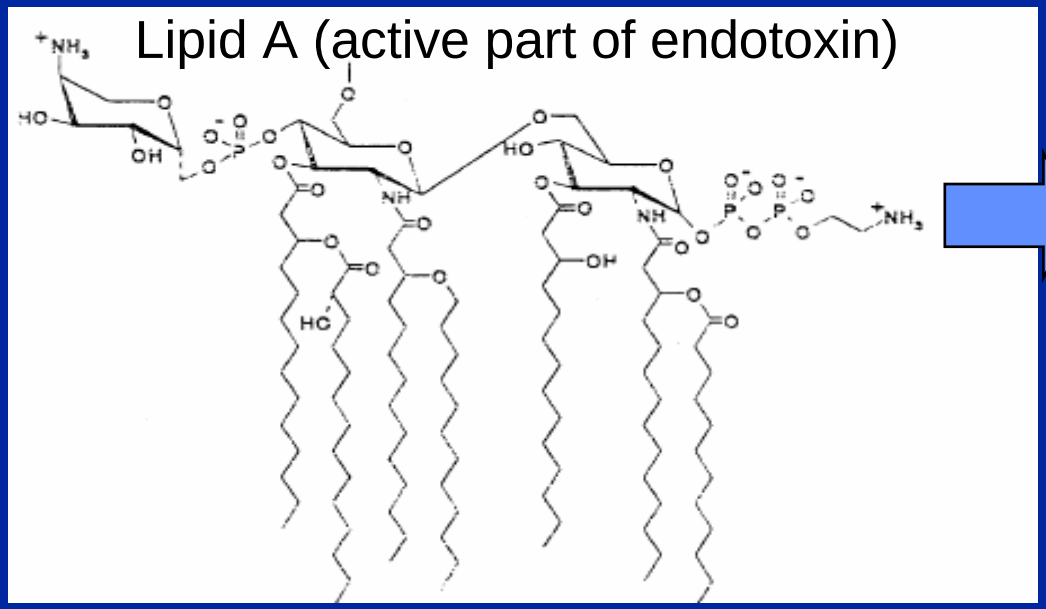
- *Adsorption of low molecular weigt proteins to hemodialysis membranes: experimental results and simulations* - P. Valette, M. Thomas, P. Déjardin Biomaterials, 20, 1999, 1621
- *Influence of the charge of low molecular weight proteins on their efficiency of filtration and / or adsorption on dialysis membranes with different intrinsic properties* - N. Moachon, M. Thomas, G. Quash Biomaterials, 23, 2002, 651

oXiris: Action Mode
Endotoxin adsorption

Endotoxin:

- large MW molecule (100 000 to 5M)
- major component of gram – bacteria
- chemical composition: polysaccharide, carbohydrate and lipid A (LPS)

Lipid A (active part of endotoxin)



Ionic bonding with free amine groups of PEI

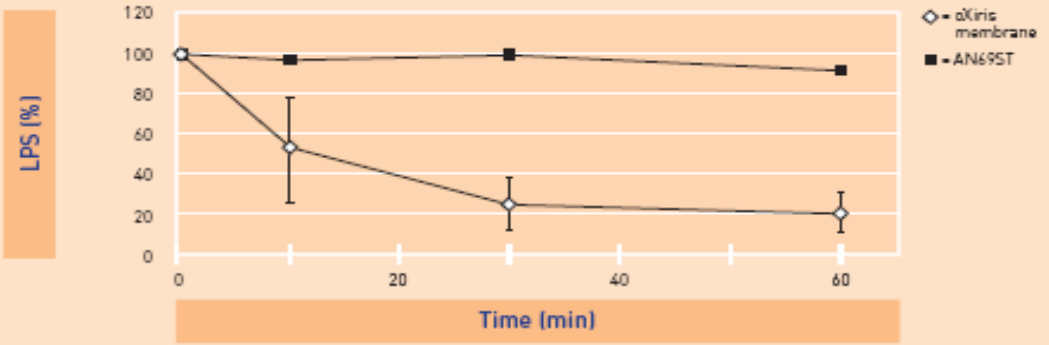
** S. Morimoto et al, Polymer journal, vol.27, 8, 1995, 831
S. Mitzner et al, Artificial organs, 17 (9), 1993, 775

oXiris Filter: Adsorption

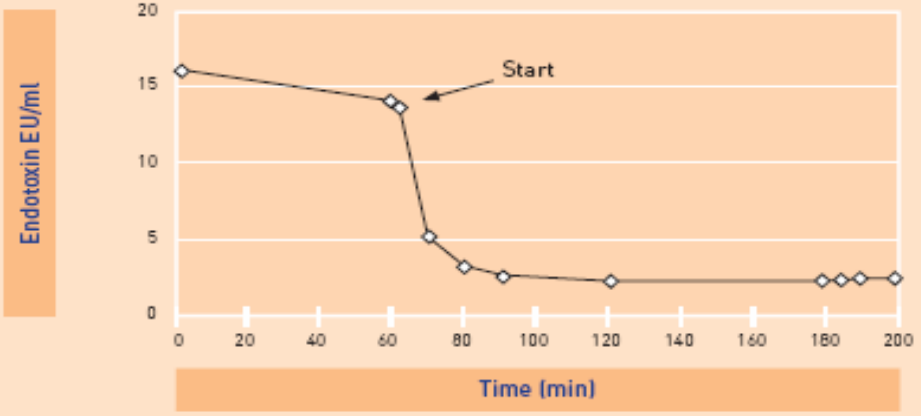
- **Cytokine adsorption : adsorption takes place in the membrane bulk mainly on the sulfonic groups (negligible influence of the surface treatment)**
- **Endotoxin adsorption: adsorption only due to the surface treatment (interaction between amine groups of PEI and phosphate groups of lipid A).**

oXiris – LPS Adsorption

LPS adsorption on AN69ST and oXiris membranes –
Endotoxin E.C. 055B5, bovine blood



LPS adsorption on oXiris surface area 1.6 m²,
oXiris closed circuit, 500 ml, bovine blood



Experimental in-vitro conditions

- Saline (NaCl 9g/L) and bovine blood (60g/L of protein, Htc 32%)
- Saline or blood volume: 500 mL
- Saline or blood flow rate: 150 mL/min (closed circuit)
- Utrafiltration rate: 2 L/h (recircling)
- Endotoxin from E. Coli (055:B5)
- Initial concentration: 10 EU/mL in saline, 40 EU/mL in blood
- Test duration: 1 or 4h

prismaflex
oXiris

oXiris – Septic Shock Model

An improvement of hemodynamic parameters with oXiris

hemodynamic and biochemical parameters	AN69 membrane (n=10)	Treated membrane (oXiris) (n= 10)	*P*-value	
HR (beats/min)	138 ± 20	148 ± 16	0,23	
MAP (mmHg)	64 ± 6	59 ± 8	0,13	
SPAP (mmHg)	39 ± 9	30 ± 8	0,029	P< 0.03
MPAP (mmHg)	34 ± 8	24 ± 7	0,008	P< 0.03
PCWP (mmHg)	12 ± 3	11 ± 4	0,53	
CO (l/min)	6,9 ± 4,8	5,5 ± 2,8	0,44	
SAR (dyn/s/cm^5)	672 ± 205	797 ± 346	0,34	
PAR (dyn/s/cm^5)	325 ± 186	234 ± 148	0,24	
Epinephrine (mg)	3,27 ± 3,02	2,11 ± 1,05	0,27	
Crystalloids (ml)	7587 ± 1456	5937 ± 1588	0,026	P< 0.03
Hydroxyethylstarch (ml)	1912 ± 538	1437 ± 320	0,027	P< 0.03
pH	7,10 ± 0,07	7,20 ± 0,11	0,026	P< 0.03
Lactate (mmol/l)	14,11 ± 3,36	9,61 ± 4,47	0,02	P< 0.03

prismaflex oXiris

Experimental in-vivo conditions

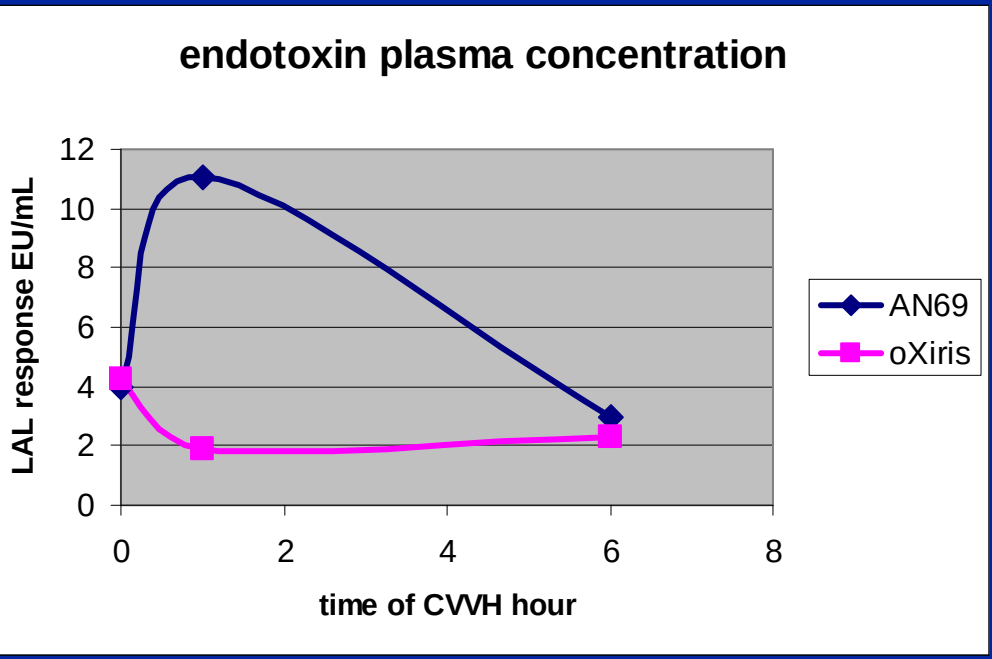
- Pig model 35 kg
- Injection Pseudomonas aeruginosa
- Stabilization 1h
- Arterial and pulmonary arterial catheters
- 2 groups (2x10)
- 1 Group treated by haemofiltration with current M100 device (untreated AN69 membrane), 1m², 6h
- 1 Group treated by oXiris (surface treated, pre-heparinized membrane), 1m², 6h
- Mean arterial pressure maintained at 65 mmHg by continuous injection of crystalloids and colloids.
- Medication with epinephrine.

Endotoxin Removal - oXiris

Rimmele et al., Nephrol Dial Transplant 2008

Endotoxin plasma concentration

	AN69 mb ($n = 10$)	Treated mb ($n = 10$)
T0	3.98 ± 3.31	4.26 ± 7.68
T1	11.07 ± 10.64	1.91 ± 1.19[a]
T6	2.96 ± 2.75	2.26 ± 2.39



	Cytokines	AN69 mb ($n = 10$)	Treated mb ($n = 10$)	P-value
T0	IL-1β	0 (0–0)	0 (0–0)	0.30
	IL-1ra	0 (0–0)	0 (0–0)	0.30
	TNF-α	192 (67–826)	1247 (235–1846)	0.08
	IL-6	0 (0–0)	0 (0–0)	0.30
T6	IL-1β	230 (126–350)	98 (49–205)	0.04
	IL-1ra	1434 (1053–1662)	1254 (803–1490)	0.50
	TNF-α	280 (174–409)	227 (139–302)	0.60
	IL-6	104 (77–412)	66 (60–244)	0.20

T0 = beginning of HVHF, T1 = time after 1 h of HVHF, T6 = time after 6 h of HVHF.
Statistics: ANOVA for repeated measurements followed by a Duncan post test. $P = 0.014$ for the time factor, $P = 0.064$ for the type of membrane. Using a Duncan post test, we found a significant difference at T1 between groups ($P = 0.035$)[a].

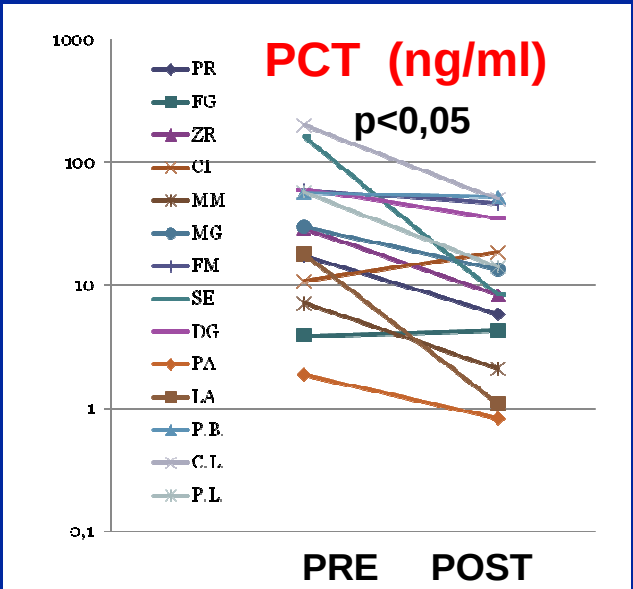
Removal of Endoxins and Cytokines during CRRT

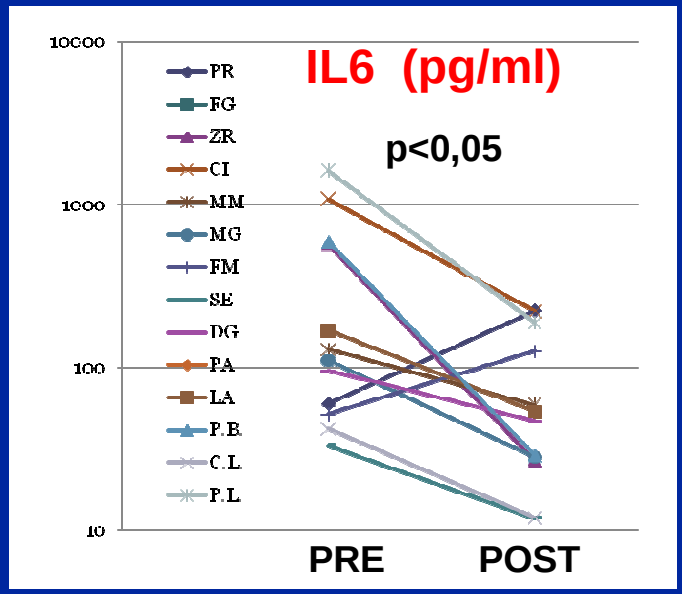
- Oxiris, AN 69 membrane treated with a polyethyleneimmine (PEI) and grafted with heparin with increased absorption of endotoxins
- CVVHDF with effluent dose of 50 ml/kg/h (60% convective)
- 14 patients with AKI having severe sepsis (n=7) or septic shock (n=7) from gram negative bacterial infection

Results:

SOFA from 13.2±2.5 to 8.8±4.3 ($p<0.05$);
MAP from 65±20 to 81±16 mmHg ($p<0.01$),
URINE OUTPUT from 0.7±0.6 to 1±0.6 L/12h ($p<0.05$),
NORADRENALINE from 0.3±0.3 to 0.03±0.04 γ/Kg/h ($p<0.05$)

Outcome: Survival 78% (11/14),
Renal recovery 81% (9/11)





Caravetta et. al – SMART 2010

Polymixin B Column Systematic Review

Cruz et al, Crit Care 2007

- This systematic review of the published literature found positive effects of PMX-F:
  - ↑ MAP
  - ↓ dopamine/dobutamine use
  - ↑ PaO_2/FiO_2 ratio
  - ↓ Mortality
  - ↑ Endotoxin removal
- Limited ability to make conclusions because of the suboptimal quality of included studies
- These putative benefits remain to be determined definitively in a prospective trial

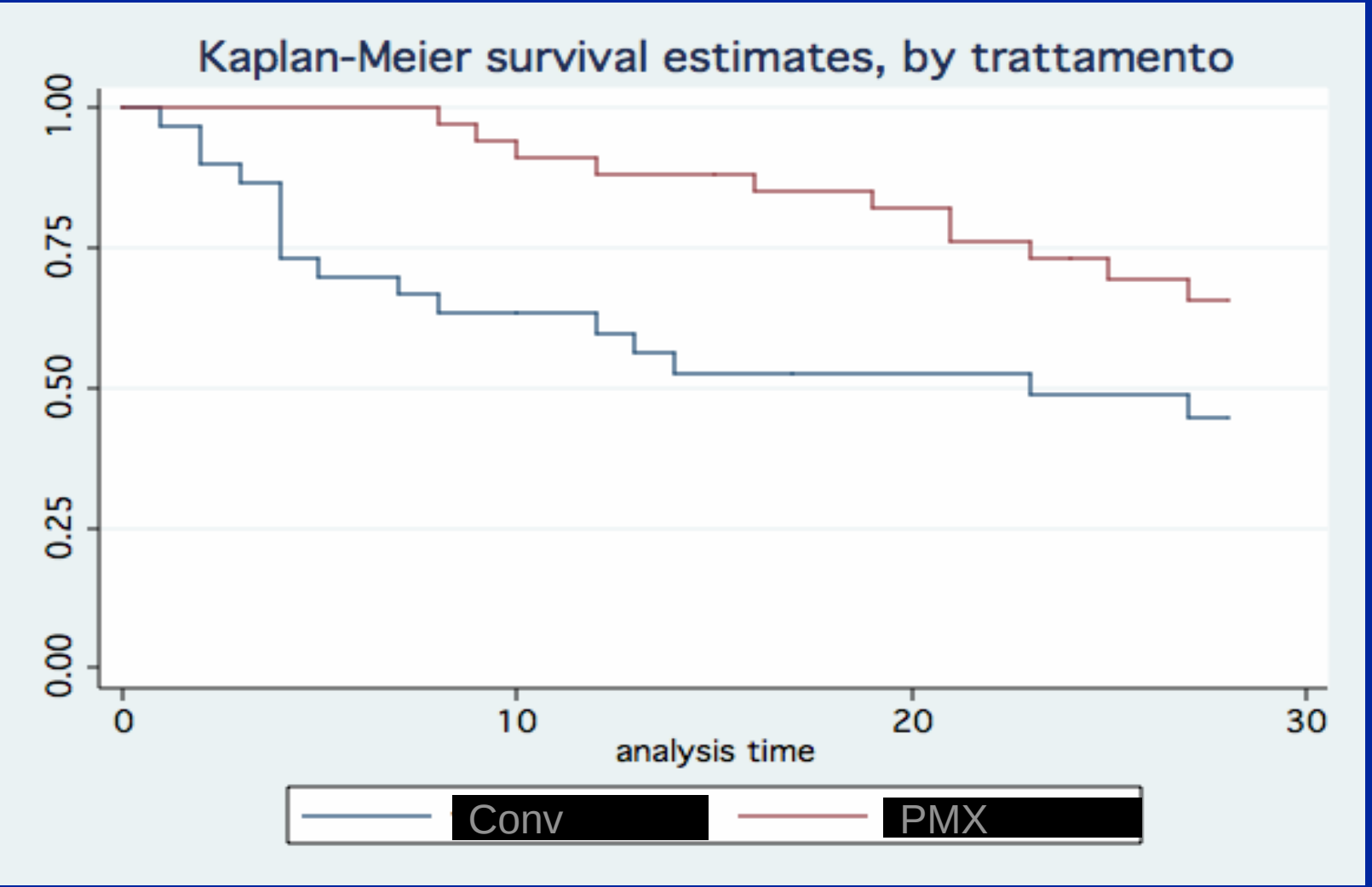
Baseline Characteristics in EUPHAS Trial

Cruz et al, JAMA 2009

	Conventional	PMX	P
N	30	34	
Age (years)	67 ± 15	61 ± 13	0.19
Male (%)	18 (60)	24 (71)	0.53
APACHE II	20 ± 7	21 ± 6	0.86
SOFA	9 ± 4	11 ± 3	0.07
MAP (mmHg)	74 ±11	76 ±11	0.40
Noradrenaline (µg/kg/min)	4.6 ± 4.1	3.1 ± 3.9	0.13
Dopamine	4.6 ± 4.1	3.1± 3.9	0.13
Inotropic score	16.0 (5.0, 37.3)	20.0 (8.0, 47.8)	0.85
WBC (x1000/mm3)	11.0 ± 6.8	13.7± 6.8	0.12
PaO2/FiO2	222 ± 84	235 ± 87	0.53
Diuresis (ml/hr)	87 ± 80	66 ± 60	0.22
Creatinine (µmol/L) (mg/dL)	150 ± 106 1.7 ± 1.2	203 ± 159 2.3 ± 1.8	0.18
RRT	6 (20)	13 (38)	0.17

28-Day Survival in EUPHAS Trial

Cruz et al, JAMA 2009



Summary

- Although not a “stand-alone” panacea, extracorporal immodululation may provide effective adjuvant support in septic AKI patients – techniques include:
  - High-volume hemofiltration
  - Adsorption-based approaches
  - Large pore (high cut-off) membranes
- The potential clinical benefit of an AN69 membrane-based approach (oXiris) is the ability to achieve:
  - Adsorptive removal of both endotoxin and mediators
  - Anticoagulant effects through immobilized heparin
  - Multi-center oXiris RCT involving patients with AKI and septic shock being organized now
- The clinical effects of other approaches (e.g., high-volume hemofiltration, polymixin B column) remain to be further determined (IVOIRE, EUPHAS II, EUPHRATES Trials underway or complete)