Οι προοπτικές για το μέλλον των μεθόδων αφαίρεσης.

Ιωάννης Γ. Γριβέας

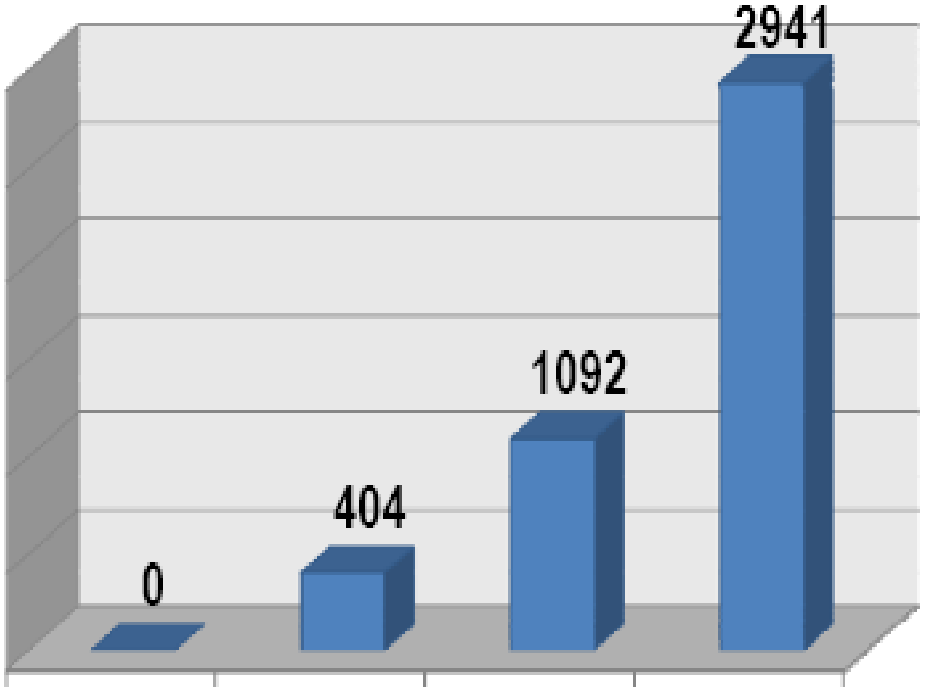
athens-NEPHROLOGY

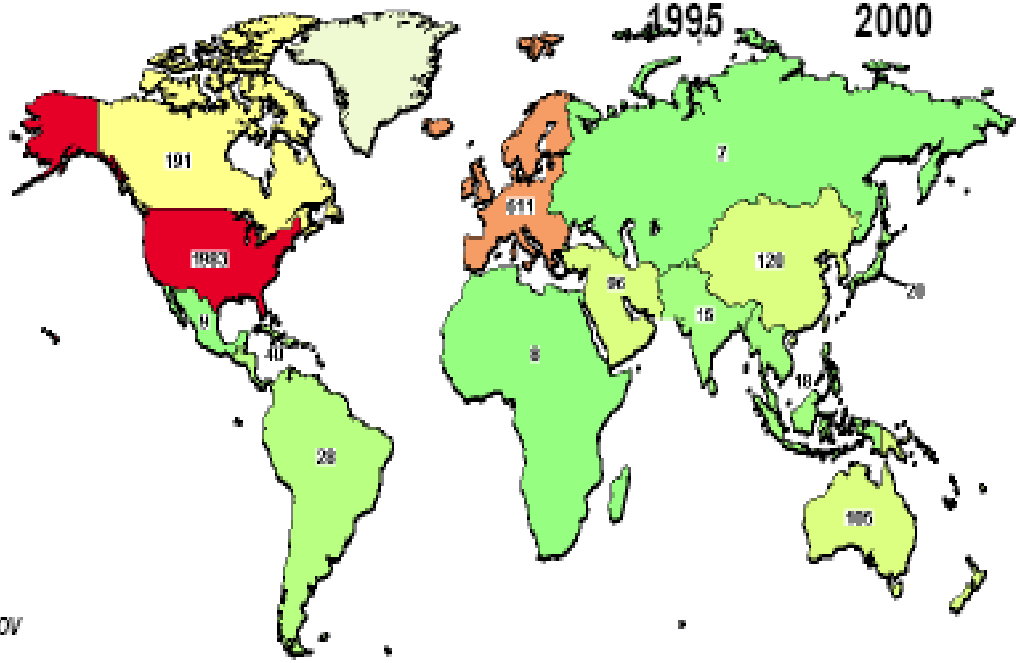
What is Apheresis?

- **Apheresis** is Greek for "to take away" or "subtract"
- **Plasmapheresis** – remove plasma
- **Cytapheresis** – remove cells
  - Leukopheresis – remove white blood cells
  - Erythropheresis – remove red blood cells
  - Plateletpheresis – remove platelets
- Originally performed discontinuously
- Now performed with continuous removal and separation of blood components

Hematopoietic Replacement	▪ Cancer ▪ Genetic diseases	▪ HSC ▪ HPC ▪ MSC ▪ Gene therapy
Immune Modulation	▪ Cancer ▪ Autoimmunity ▪ Infectious diseases	▪ DC ▪ APC ▪ T cell ▪ B cell ▪ NK ▪ HSC ▪ MSC ▪ Macrophages ▪ Gene therapy
Tissue Repair & Regeneration	▪ Cardiovascular ▪ Spinal ▪ Neuronal ▪ Corneal ▪ Orthopedic	▪ HSC ▪ MSC ▪ NSC ▪ Cell matrix implants ▪ Macrophages
Wound Healing	▪ Ulcers ▪ Burns	▪ Artificial skin ▪ Membranes ▪ MSC ▪ Macrophages

- Number of stem cell trials steadily increasing
- Not surprisingly, US and EU centric





Source: Clinilcatrials.gov

Cell therapy companies & their products

~300 therapeutic companies with ~250 cell-based therapies in the market or in some stage of clinical development. These therapies can be roughly broken down into the following stages*:

~110 Phase I
~70 Phase II
~30Phase III
~40 Commercial (marketed in at least one country)

Only ~1/3 of the therapies currently marketed (~13) required and received regulatory approval. In contrast, an estimated 90% of the therapies in development are "products" requiring pre-market approval.

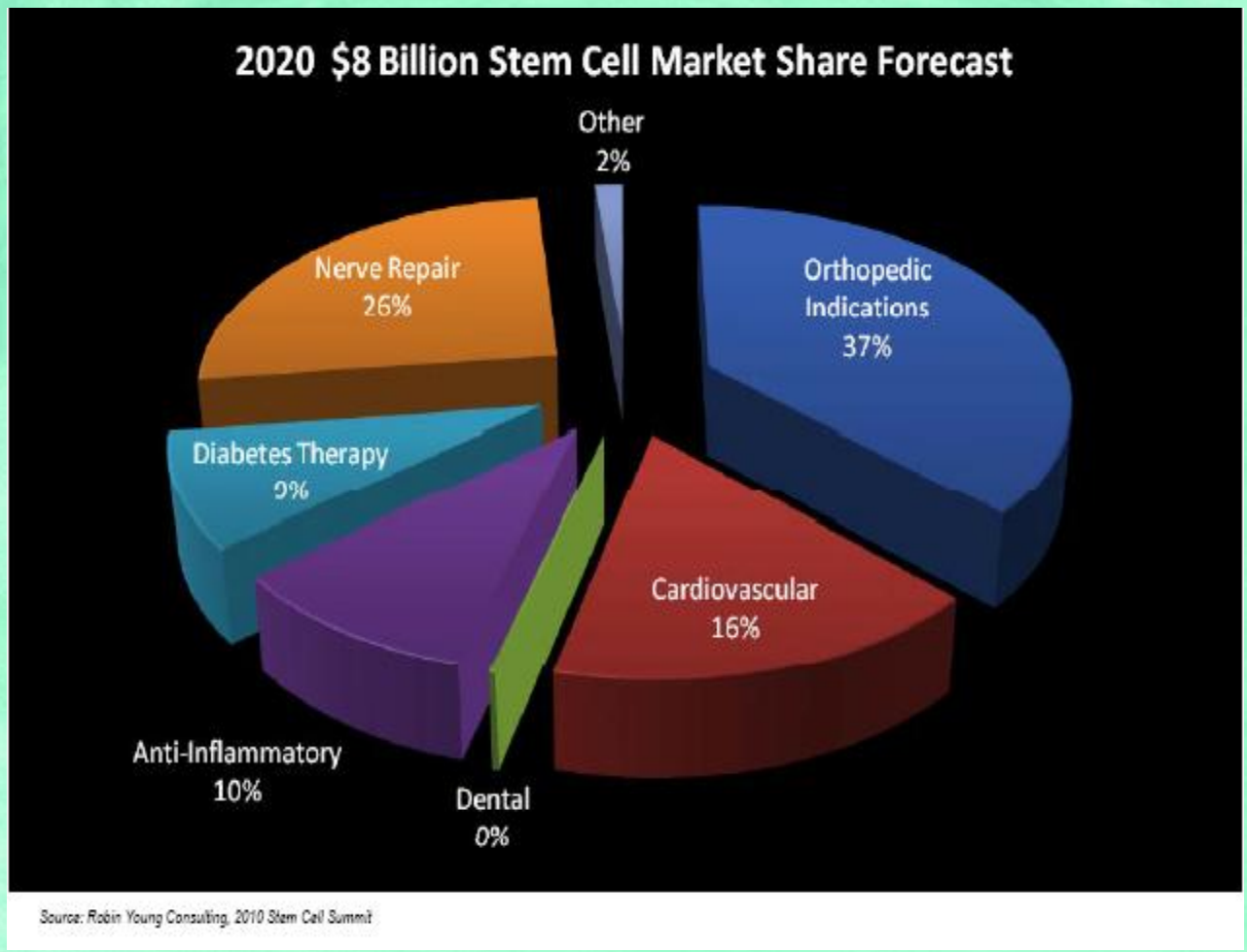
* Note that these numbers are limited to industry-sponsored trials and may not capture fully products in early-stage trials where industry "sponsorship" is less than transparent.

Have the fundamentals changed?

- **Cell therapy is here – instances of it being routine clinical practice & commercial**
- There has been incremental success
  - CT is now very much a part of individual, corporate, academic, policy, and financial consciousness
  - CT is now part of routine clinical practice and commercial products
  - Emerging metrics of a maturing industry (e.g., players, orgs, FDA, etc.)
  - On financial sector's radar
  - Now working on second generation (not first generation) products.

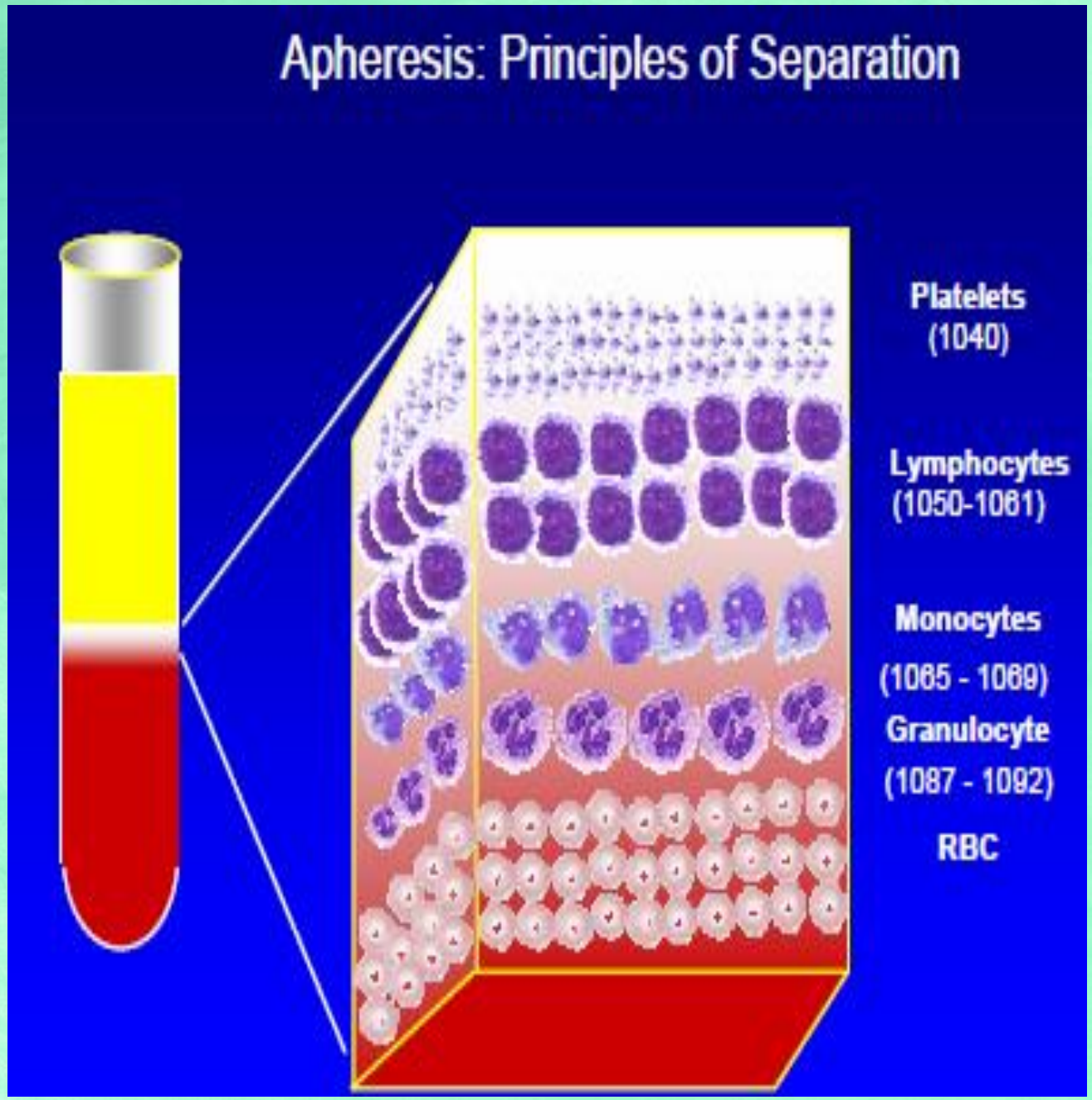
- Very little of this was true 10 year's ago.

Where Might We Go In 10 years?



Phase III Clinical Trials

- Refractory angina and chronic myocardial ischemia
- Renal cell carcinoma
- Multiple sclerosis
- Prostate cancer
  - Similar to Provenge but with some important variations


Apheresis: Principles of Separation
Platelets
(1040)
Lymphocytes
(1050-1061)
Monocytes
(1065 - 1069)
Granulocyte
(1087 - 1092)
RBC

Mononuclear Cell Collection

- Patient assessment
  - History and physical examination by physician
  - Nursing evaluation for each collection procedure
    - Dyspnea
    - Fever >38 C
    - Hypotension/hypertension
    - Change in mental status
- COBE Spectra
  - MNC program, 1.5-2.0 TBV target
- Manufacturer specifications
  - Total product volume: 180-360 mL
    - Autologous plasma: 150 mL
    - Mononuclear cell volume: 30-210 mL

Removal of pathogenic macromolecules from the bloodstream:

- Autoantibody
- Probable autoantibody
- Antigen-Antibody complexes (circulating immune complexes)
- Alloantibody
- Paraproteins (light chains, monoclonal cryoglobulins, etc.)
- Non-immunoglobulin proteins
- Endogenous toxins
- Exogenous poisons

Table 1. Ideal target molecule characteristics for Therapeutic Plasma Exchange

Identified etiologic agent or toxic substance
High molecular weight ($\geq$ 15000 Da)
Slow rate of formation
Low turnover
Low volume of distribution

Examples of pathogenic target molecules for therapeutic plasma exchange in kidney disease

Kidney disease	Target molecule
Anti-GBM disease	Autoantibody reactive with type IV collagen – Rapid decline in anti-GBM antibodies with TPE
Thrombotic Thrombocytopenic Purpura	Acquired autoantibody reactive with ADAMTS13 enzyme
Pauci-Immune RPGN	Autoantibodies against components of the cytoplasm of neutrophils-sequential ANCA levels have not been performed
Multiple myeloma	Free kappa and lambda light chains
Cryoglobulinemia	Immunoglobulin M anti-IgG antibody, immune complexes
Recurrent Focal segmental glomerulosclerosis	Circulating glomerular permeability factor, suPAR:)-Clinical remission correlated with reduction in suPAR levels below about 2000pg/ml (Wei Nature Medicine 2011; 17: 952)
Atypical HUS	Complement regulatory components or auto-antibodies- not specifically shown
Kidney transplantation	Alloantibodies reactive with HLA antigens-)-)-DSAs can be removed from plasma by TPE

Disease	TA Modality	Category
Hyperviscosity in monoclonal gammopathies: - Symptomatic, - Prophylaxis for rituximab	TPE	I
Immune thrombocytopenia (ITP): Refractory	TPE IA	IV III
Post-transfusion purpura	TPE	III
Red cell alloimmunization in pregnancy	TPE	III
SLE: - Severe - Nephritis	TPE	II IV
TMA, Drug associated: - Ticlopidine - Clopidogrel, Cyclosporine, Tacrolimus	TPE TPE	I III
TMA, HSCT associated, Refractory	TPE	III
TTP	TPE	I
ABO incompatible transplantation: - Organ - Major HPC Marrow or Apheresis	TPE TPE	I - III II

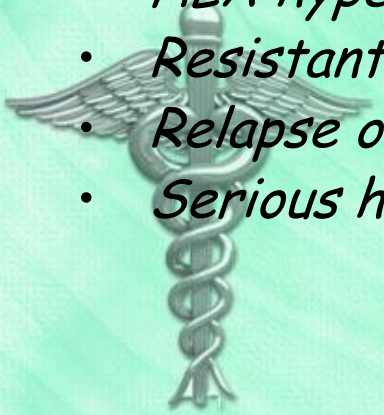
Therapeutic Apheresis of Immune diseases in Nephrology Department

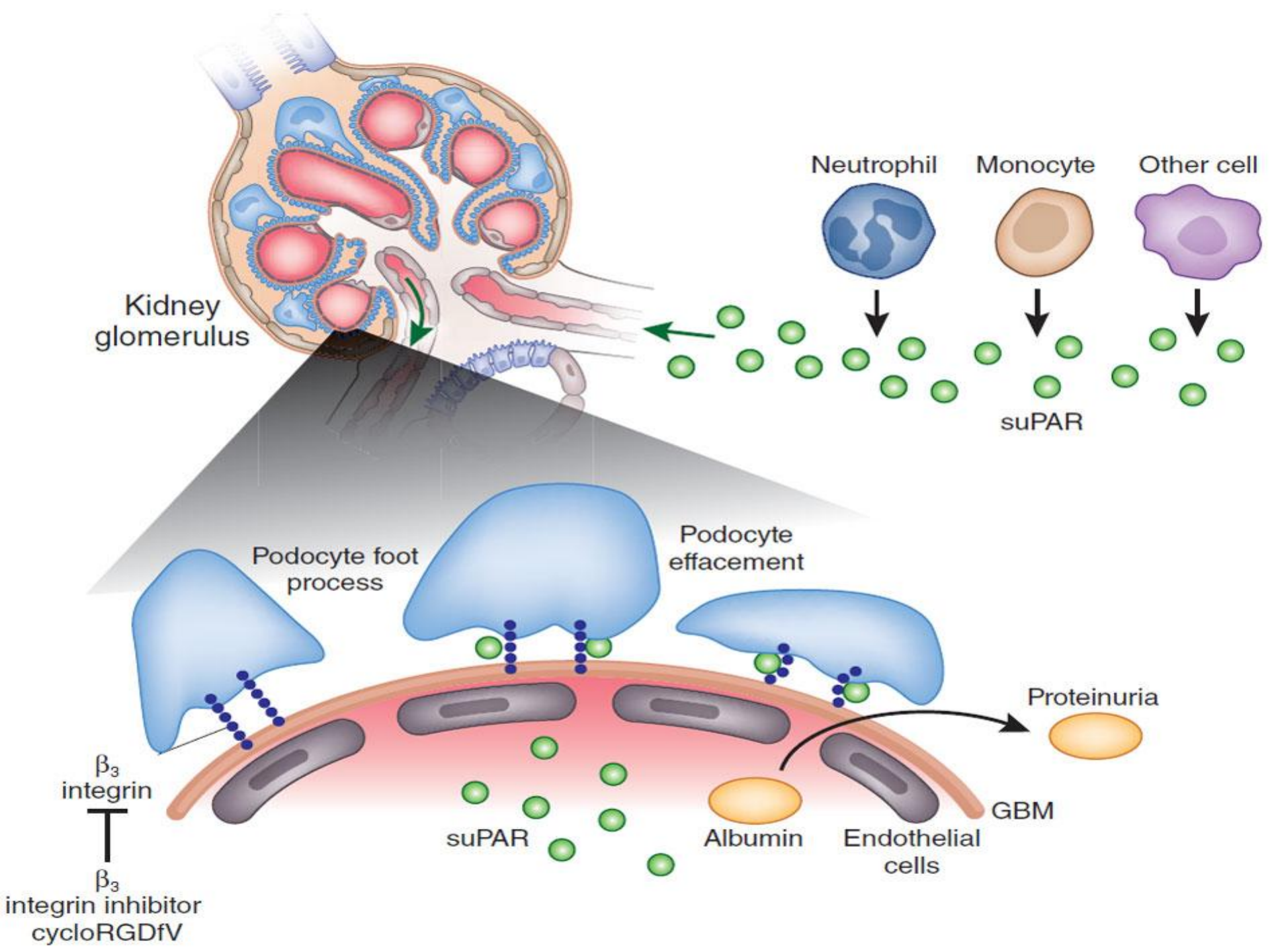
Visvardis G., Manou E., Griveas I., Meimaridou D., Mitsopoulos E., Kyriklidou P., Papadopoulou D., Ginikopoulou E., Rottstein L., Sakellariou G.

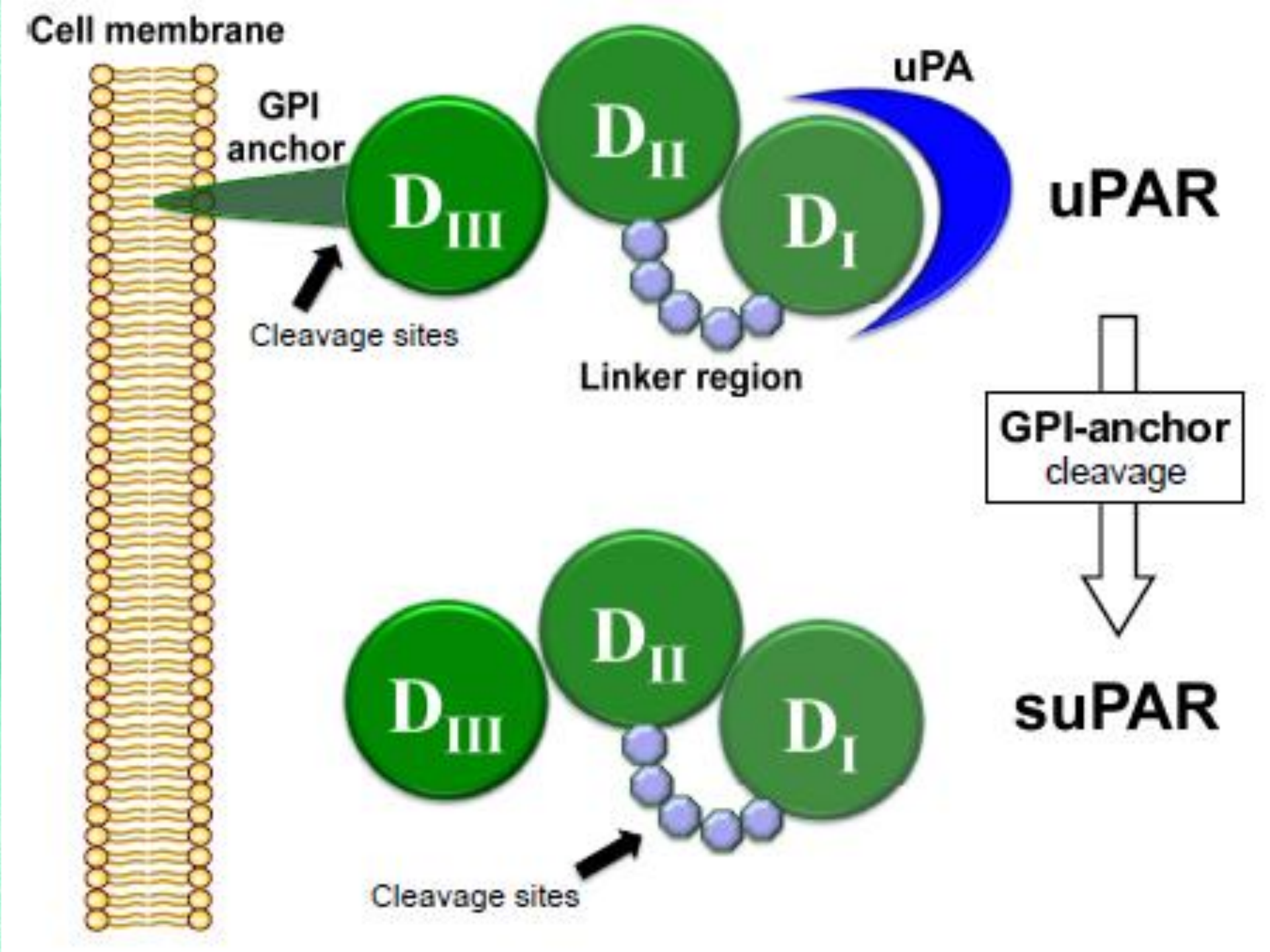
Department of Nephrology, Papageorgiou General Hospital, Thessaloniki, Greece

Primary disorders

- *Thrombotic thrombocytopenic purpura: 7*
- *Macroglobulinemia Walldestrom: 2*
- *Multiple myeloma: 1*
- *Cryoglobulinemia: 1*
- *Guillen Barre: 4*
- *Stiff man syndrome: 1*
- *Vasculitis: 4*
- *Systemic lupus erythematosus: 3*
- *Lyell syndrome: 3*
- *Myasthenia Gravis: 1*
- *HLA hyperimmunisation before renal transplantation: 1*
- *Resistant nephritic syndrome: 1*
- *Relapse of FSGS after renal transplantation: 1*
- *Serious hyperlipidemia in a patient under hemodialysis: 1*




Neutrophil
Monocyte
Other cell
Kidney glomerulus
suPAR
Podocyte foot process
Podocyte effacement
Proteinuria
β3 integrin
suPAR
Albumin
Endothelial cells
GBM
β3 integrin inhibitor cycloRGDfV


Cell membrane
GPI anchor
D_{III}
D_{II}
D_I
uPA
uPAR
Cleavage sites
Linker region
GPI-anchor cleavage
D_{III}
D_{II}
D_I
suPAR
Cleavage sites

Διαλυτός Υποδοχέας Πλασμινογόνου Ουροκινάσης (suPAR): ένας υποσχόμενος δείκτης φλεγμονής

Ι. Γριβέας, Χ. Ανδριόπουλος, Ν. Μπακιρτζή, Α. Δράκου
Μονάδα Χρόνιας Αιμοκάθαρσης "Νεφροιατρική"

athens-NEPHROLOGY

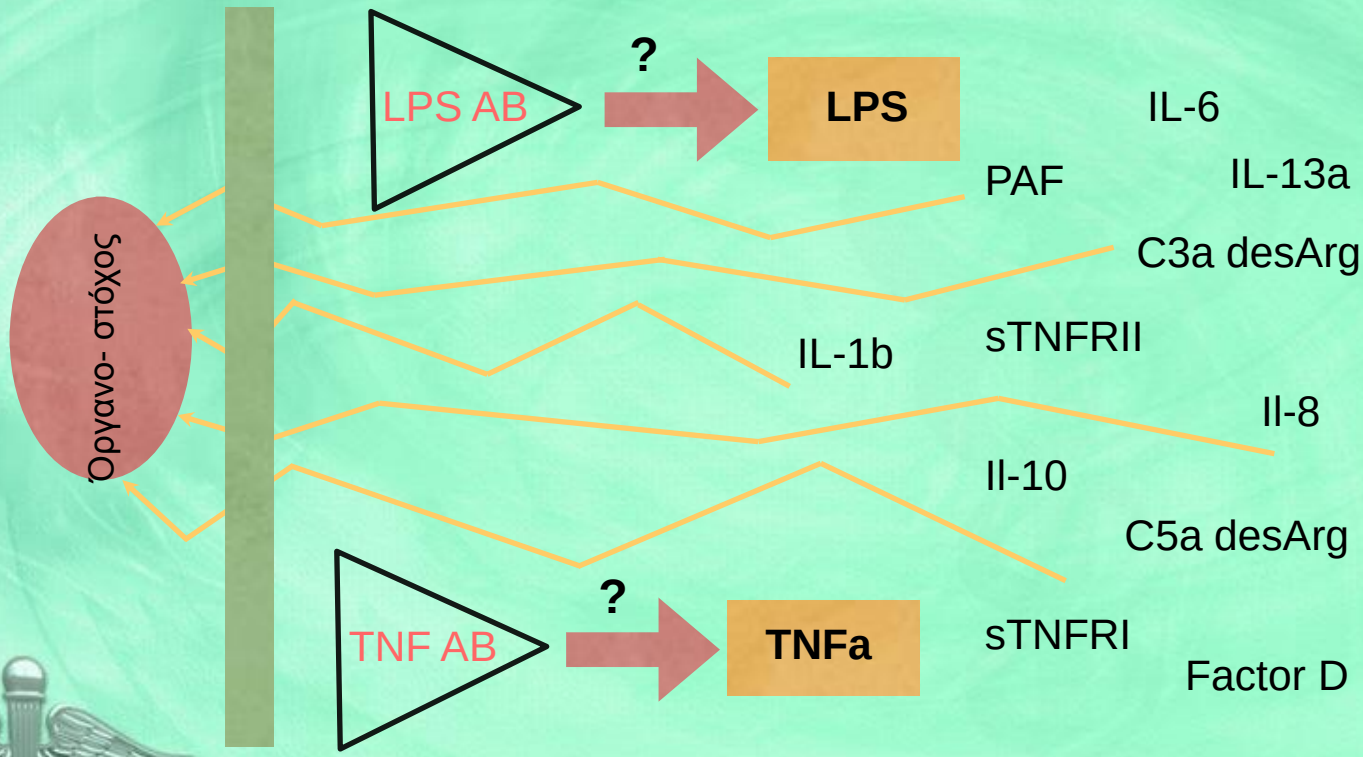
Sepsis and MODS

- Extremely complex nature of inflammatory response in sepsis.
- Unlikely a single agent (e.g. anticytokine Rx) would work.
- Ronco proposed that '…unspecific removal of soluble mediators, be they pro or antiinflammatory, without completely eliminating their effect, may be the most logical and adequate approach to a complex and long-running process such as sepsis.'

Εφαρμογή θεραπευτικής αφαίρεσης στη σήψη
Μια ιδέα που έφτασε η ώρα της εφαρμογής της

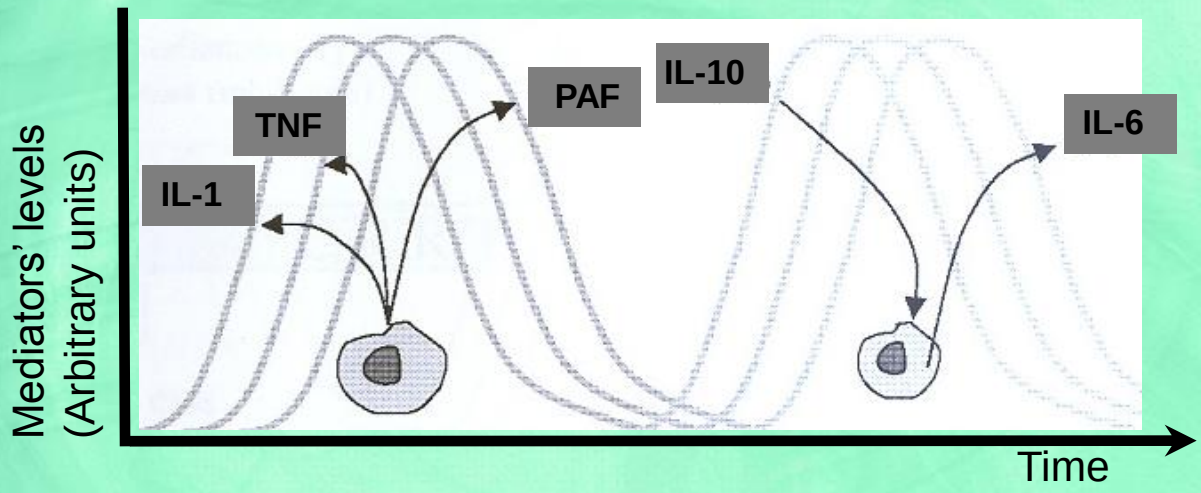
- Καλύτερη κατανόηση της σήψης και γνώση όλο και περισσότερων μεσολαβητών - παραγόντων που συμμετέχουν ενεργά στην παθοφυσιολογία του συνδρόμου
- Ποικίλλες θεραπευτικές προσεγγίσεις: "Μαγική σφαίρα" εναντίον συγκεκριμένου παράγοντα ή "μαγική ασπίδα" που προστατεύει από πολλαπλές ουσίες που ενέχονται στην εκδήλωση του συνδρόμου
- Η ιδέα της "αποτοξίνωσης" του αίματος σε ασθενείς με σήψη έχει ήδη συμπληρώσει μια δεκαετία

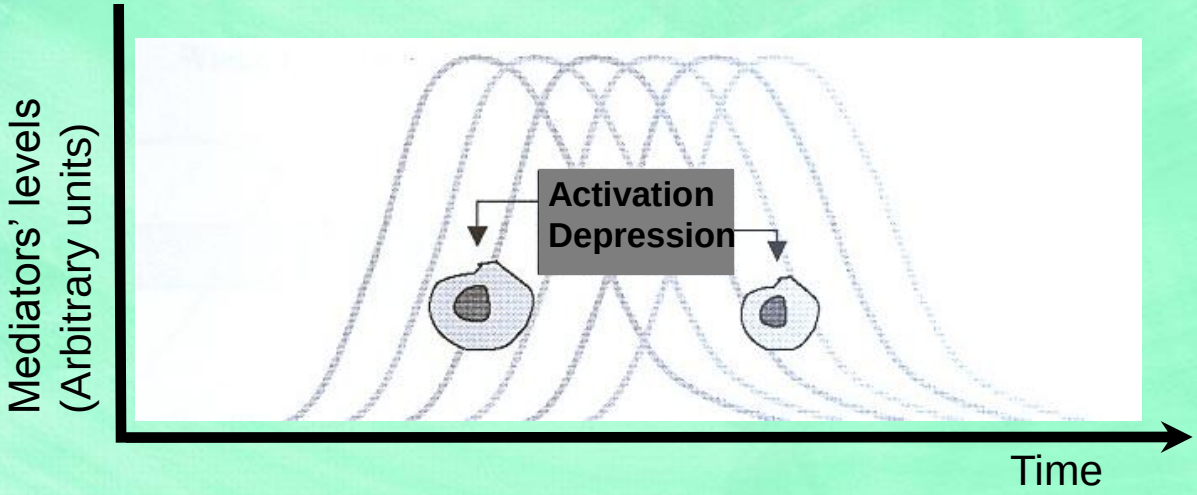
"Καταιγίδα κυτοκινών" στη διαδικασία της σήψης: "μαγική σφαίρα" ή "μαγική ασπίδα"



Ciro Tetta et al, Ther Apher, 2002

The Peak Concentration Hypothesis

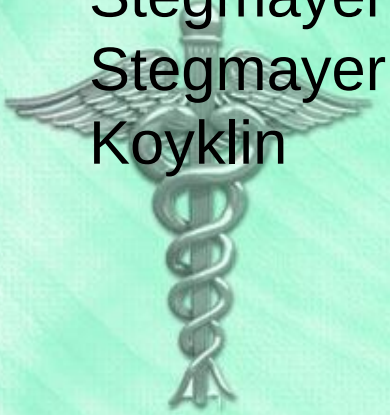




C. Ronco et al, Artif organs, 2003

Πλασμαφαίρεση στη θεραπεία της σήψης

		Αρ. ασθενών	% επιβίωση
Bjorvatn	1984	4	100
Brandtzaeg	1985	8	75
Graf	1987	2	100
Hauser	1987	4	0
Stegmayer	1987	4	100
Asanuma	1989	19	69
Stegmayer	1990	13	69
McClelland	1990	2	100
van Deuren	1992	15	60
Stegmayer	1995	27	81
Stegmayer	1998	56	79
Koyklin	1998	100 (rand.)	66



kazuyoshi Hanasawa, Ther Apher, 2002

Η χρήση των προσροφητών στις θεραπείες εξωσωματικής κάθαρσης

1980s

Ανακύκλωση διαλύματος

Θεραπεία δηλητηριάσεων

"επικαλυμένοι"

Προσροφητές

Αναγέννηση υπερδιηθήματος

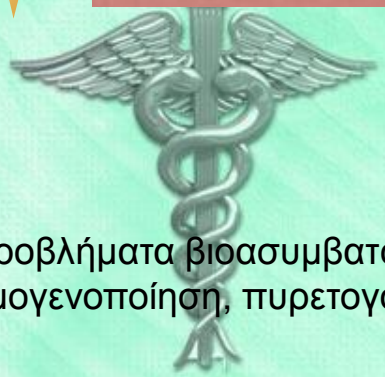
1990s

Αναγέννηση πλάσματος

"ακάλυπτοι"

Αιμοδιαδιήθηση

Νεότερες τεχνικές συνεχούς εξωνεφρικής κάθαρσης (HF,HDF,CPFA)



Προβλήματα βιοασυμβατότητας, απελευθέρωση μετάλλων, έκλυση μικροσωματιδίων, χαμηλή ομογενοποίηση, πυρετογόνες αντιδράσεις, ηλεκτρολυτικές δχ, αιμόλυση

Ronco C et al, Kid Int, 2000

Αιμοπροσρόφηση με τη χρήση πολυμυξίνης Β (PL-B)

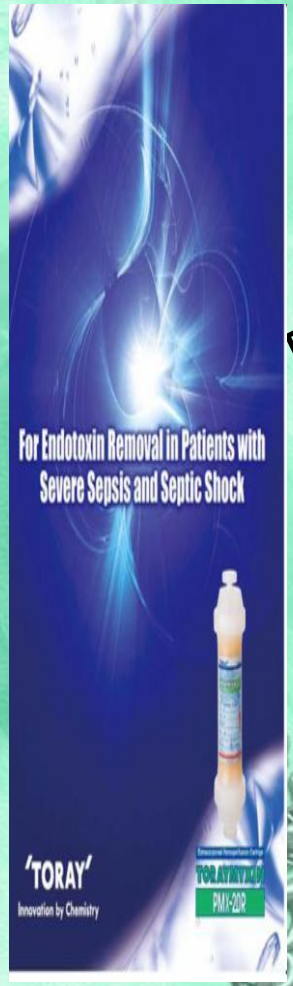
- Πολυκατιονικό αντιβιοτικό που συνδέει την ενδοτοξίνη και την αδρανοποιεί. Αντεδείκνυται η ενδοφλέβια χορήγηση λόγω νεφρο- και νευροτοξικότητας

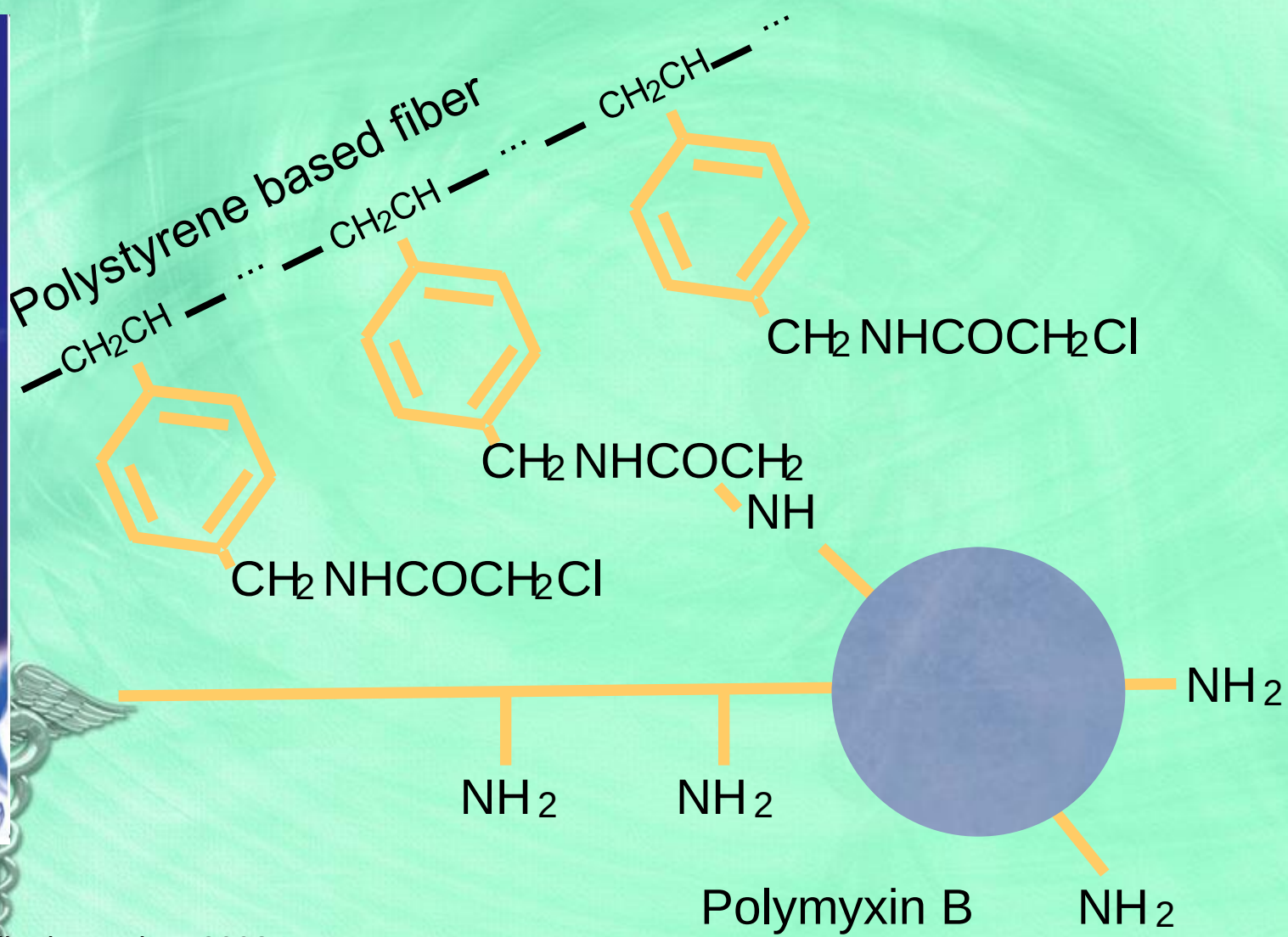
- Συνδεδεμένα ινίδια πολυστερένιου και πολυπροπυλένιου: υλικό - φορέας που συνδέει και ακινητοποίει την PL-B προσδίδοντας μεγαλύτερη σταθερότητα

- 30.000 ασθενείς έχουν υποβληθεί σε αιμοπροσρόφηση με PL-B από το 1994 στην Ιαπωνία

- Χωρίς ιδιαίτερες παρενέργειες εκτός από υποτασικά επεισόδια και θρομβοπενία

Πολυμυξίνη Β συνδεδεμένη ομοιοπολικά με το πολυστερένιο σε μορφή ινιδίων





Hisataka Shoji, Ther Apher, 2002

Polymyxin B Hemoperfusion
EUPHAS Clinical Trial

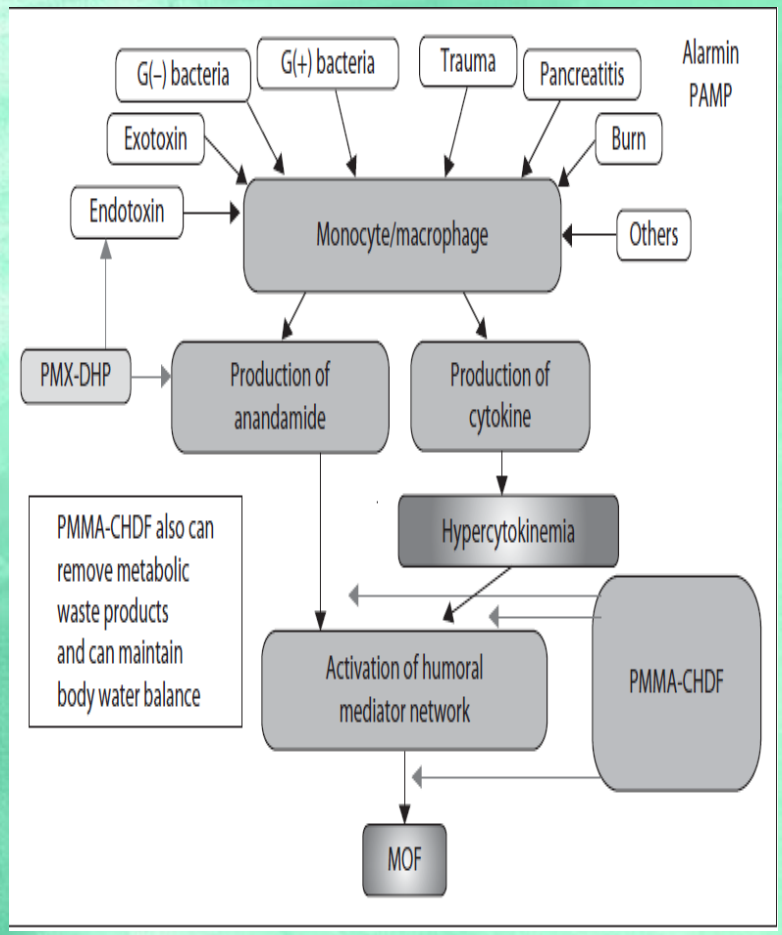
- Early Use of Polymyxin B Hemoperfusion in Abdominal Sepsis (EUPHAS)
  - Randomized 64 patients @ 10 Italian tertiary care ICUs
  - Significant improvements:
    - Cardiac index; Left ventricular stroke index; Oxygen delivery index
  - Shorter hospital stay and better 28-day survival (32% in the hemo-adsorption group compared with 53% in the control group ($P = 0.03$)).
  - Not different:
    - Endotoxin and IL-6 levels pre-post treatment
    - Organ dysfunction (SOFA) between control and treatment group
  - The study was prematurely stopped because
    - It was judged to be unethical to deprive patients of hemoadsorption
    - Inability to blind treating physicians

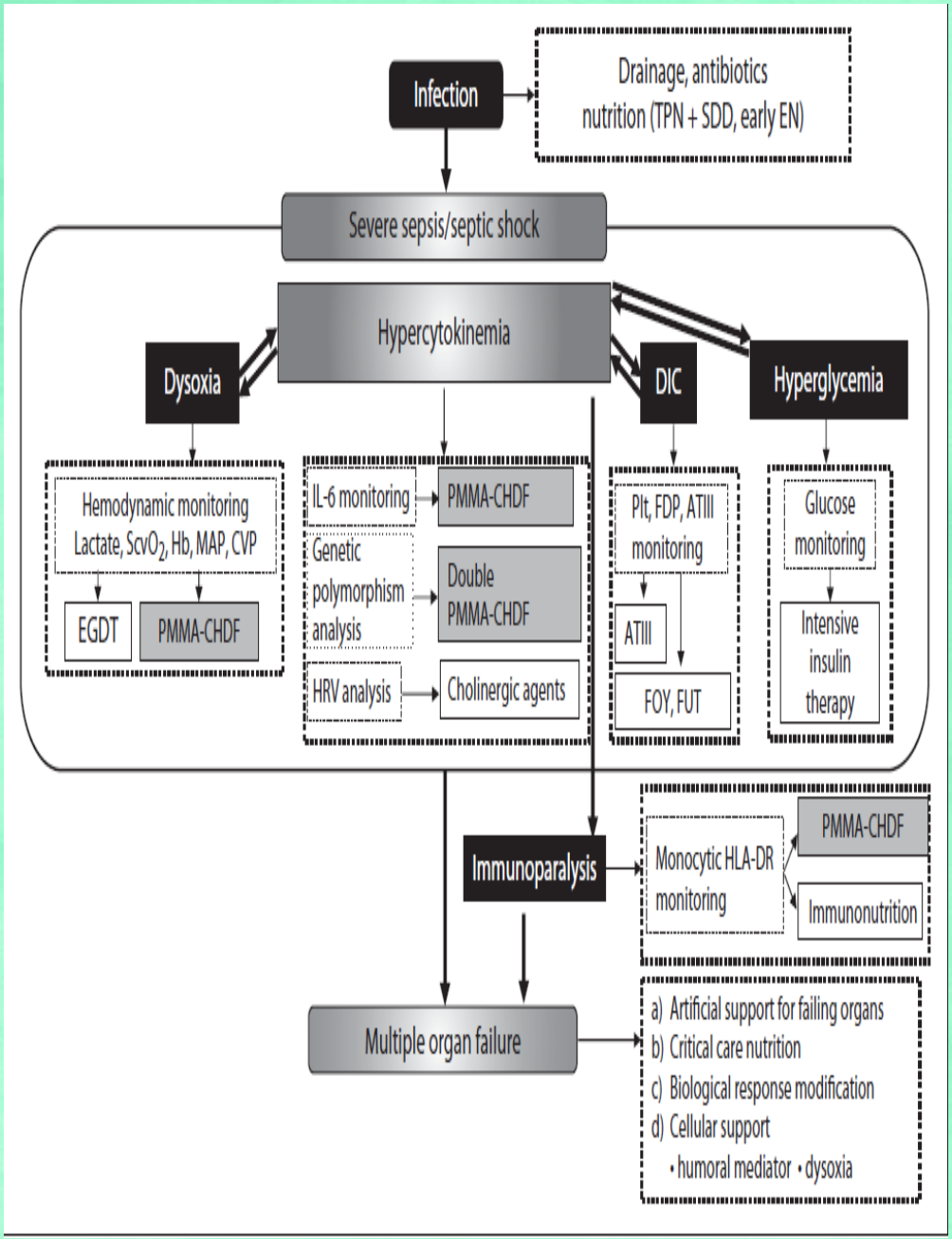
Polymyxin B Hemoperfusion
EUPHRATES Clinical Trial

- Evaluating the Use of Polymyxin B in Randomized controlled trial of Adults Treated for Endotoxemia and Septic Shock (EUPHRATES)
  - 360 patients in 15 centers in the United States
  - primary end point of 28-day mortality

Conclusions re Hemoperfusion with Polymyxin-B:

- "No large-scale randomized trials have been completed and lower mortality has not yet been sufficiently demonstrated." (JC Schefold).
- 3 authors conclude that single LPS adsorption did not improve morbidity or organ dysfunction (Amoureaux et al; Staubach et al; Cruz et al).


G(–) bacteria
G(+) bacteria
Trauma
Pancreatitis
Alarmin
PAMP
Exotoxin
Burn
Endotoxin
Monocyte/macrophage
Others
PMX-DHP
Production of anandamide
Production of cytokine
PMMA-CHDF also can remove metabolic waste products and can maintain body water balance
Hypercytokinemia
Activation of humoral mediator network
PMMA-CHDF
MOF


Infection
Drainage, antibiotics
nutrition (TPN + SDD, early EN)
Severe sepsis/septic shock
Hypercytokinemia
Dysoxia
DIC
Hyperglycemia
Hemodynamic monitoring
Lactate, ScvO2, Hb, MAP, CVP
EGDT
PMMA-CHDF
IL-6 monitoring
PMMA-CHDF
Genetic polymorphism analysis
Double PMMA-CHDF
HRV analysis
Cholinergic agents
Plt, FDP, ATIII monitoring
ATIII
FOY, FUT
Glucose monitoring
Intensive insulin therapy
Immunoparalysis
Monocytic HLA-DR monitoring
PMMA-CHDF
Immunonutrition
Multiple organ failure
a) Artificial support for failing organs
b) Critical care nutrition
c) Biological response modification
d) Cellular support
• humoral mediator • dysoxia

Blood purification technique	Indication
Continuous hemofiltration/ continuous hemodiafiltration	Acute kidney injury Severe sepsis/septic shock Severe acute pancreatitis Fulminant hepatic failure
Plasma exchange	Fulminant hepatic failure Thrombotic thrombocytopenic purpura Toxic epidermal necrolysis
Direct hemoperfusion	Drug intoxication (charcoal DHP) Endotoxic shock (PMX-DHP)

Table 2. Blood purification techniques for cytokine removal in non-renal indications

PMMA-CHDF
Plasma diafiltration
High-flow-volume CHDF
Online CHDF
Cytokine adsorbing column

Table 1. Structures, adsorption rates of cytokines in vitro, and animal study in cytokine adsorbing columns

	Column				
	CytoSorb	CYT-860-DHP	Lixelle	CTR-001	MPCF-X
Structure	polystyrene divinyl co-polymer beads	polystyrene-based conjugated fiber	porous cellulose beads	porous cellulose beads	cellulose beads
Adsorption rate					
Methods	in vitro circuit (1 h)	batchwise (2 h)	batchwise (2 h)	batchwise (2 h)	batchwise (1 h)
TNF-α	<50%	20%	31.2%	53%	100%
IL-1β		97%	98.5%	98%	
IL-6	<50%	92%	82.9%	80%	98.9%
IL-8		99%	99.9%	80%	70%
Animal study					
Aminal	rat		rat	rat	
Methods	endotoxin injection, cecal ligation and puncture		endotoxin injection	endotoxin injection	
Time	240 min		120 and 180 min	120 min	

Cytokine Adsorbing Columns

(Non-selective)

Company	Cytosorbents	Toray	Kaneka	Kaneka
Product	Cytosorb	Cyt-860-DHP	Lixelle	CTR-001
Structure	Polystyrene divinyl co-polymer beads	Polystyrene conjugated fiber	Porous cellulose beads	Porous cellulose beads
Methods	In vitro circuit	Batch	Batch	Batch
TNF-α	<50%	20%	31.2%	53%
IL-1β		97%	98.5%	98%
IL-6	<50%	92%	82.9%	80%
IL-8		99%	99.9%	80%
Animal	Rat		Rat	Rat
Method	Endotoxin injection, CLP		Endotoxin injection	Endotoxin injection

CytoSorb Cytokine Extractor

CytoSorbent's products are comprised of porous, adsorbent polymer beads that target molecules up to 50,000 Daltons, such as pro-inflammatory and anti-inflammatory cytokines i.e. IL-1, IL-6, TNF and Il-10, which are associated with sepsis.

The beads contain pores that are large enough to allow toxins to enter the beads and adhere to the bead through hydrophobic interactions while allowing large proteins to pass around the beads, back into the patient.

Altrichter et al. Critical Care 2011, 15:R82
http://ccforum.com/content/15/2/R82

CRITICAL CARE

RESEARCH Open Access

Extracorporeal cell therapy of septic shock patients with donor granulocytes: a pilot study

Jens Altrichter[1], Martin Sauer[2], Katharina Kaftan[1], Thomas Birken[2], Doris Gloger[3], Martin Gloger[4], Jörg Henschel[4], Heiko Hickstein[1], Ernst Klar[5], Sebastian Koball[1], Annette Pertschy[5], Gabriele Nöldge-Schomburg[2], Dierk A Vagts[2] and Steffen R Mitzner[1*]

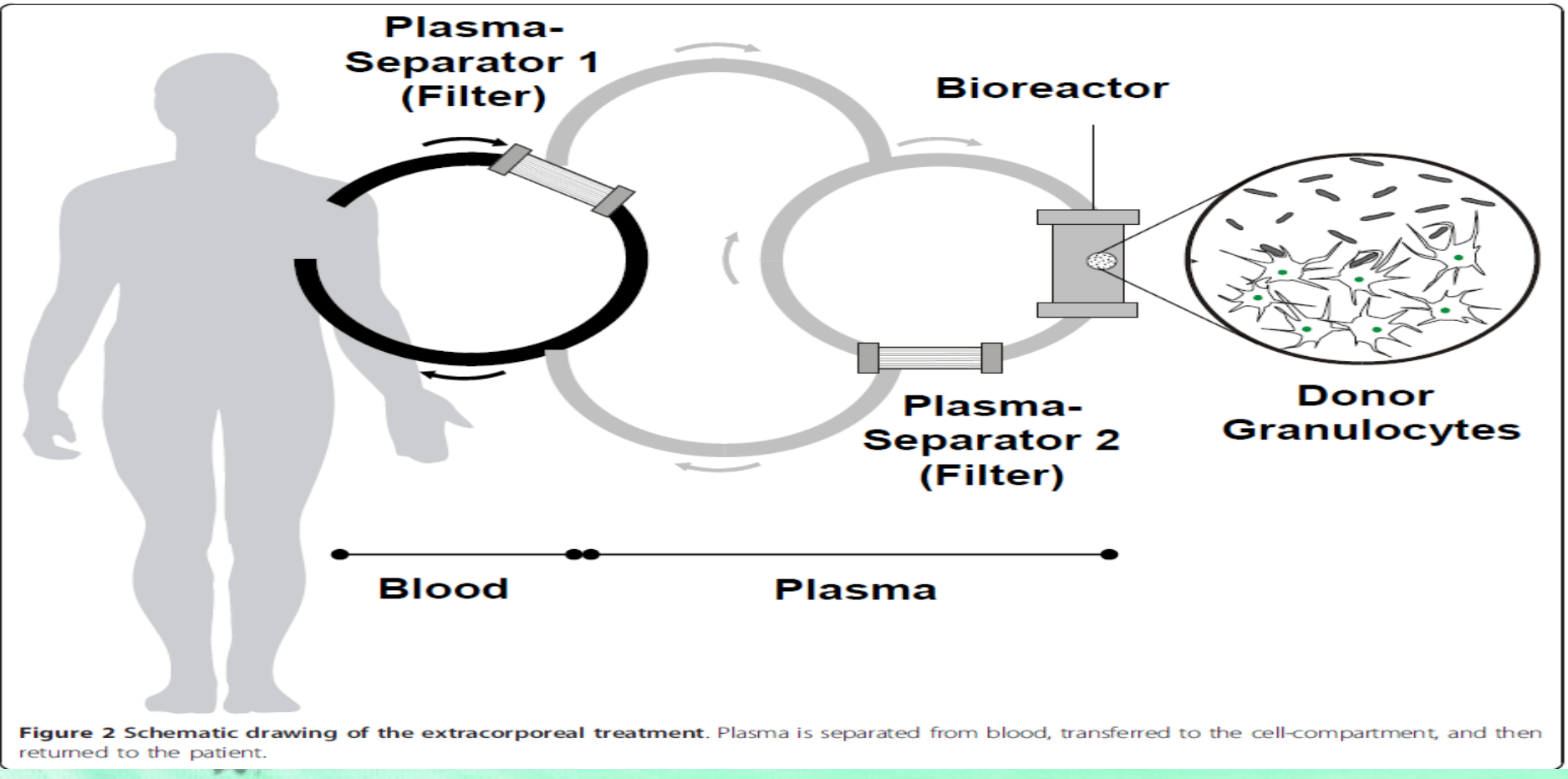


Figure 2 Schematic drawing of the extracorporeal treatment. Plasma is separated from blood, transferred to the cell-compartment, and then returned to the patient.


15TH INTERNATIONAL CONFERENCE ON DIALYSIS
ADVANCES IN CKD 2013
RENAL RESEARCH INSTITUTE
January 23-25, 2013
Wyndham Rio Mar
Rio Grande, Puerto Rico

Leukocyte Modulation Hypothesis

Univ. of Michigan; CytoPherx, Inc.

- A biomimetic membrane device (the SCD) *preferentially* binds activated leukocytes
- Regional citrate anticoagulation promotes a lower systemic leukocyte activation profile due to a decline in iCa which inhibits key leukocyte activation processes

The effects of a novel therapeutic device on acute kidney injury outcomes in the intensive care unit: a pilot study.

Ding F, et al. ASAIO Juournal 2011 Sep-Oct;57(5):426-32.

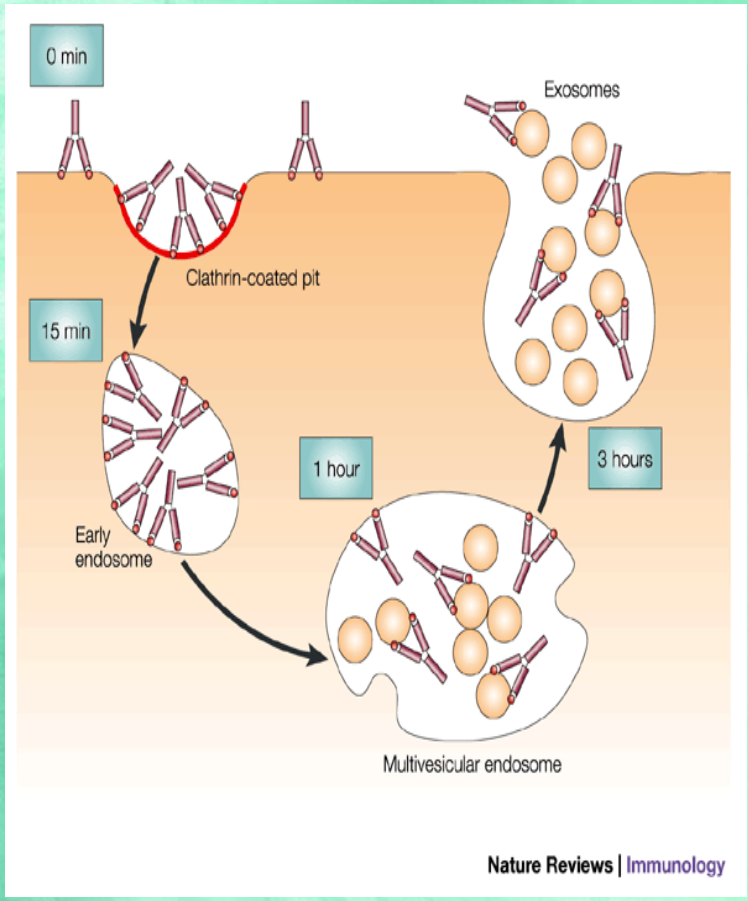
Division of nephrology, Huashan Hospital, Fudan University, Shanghai, China.

- A prospective, single-arm, single-center study designed to evaluate the SCD device on clinical outcomes in AKI in the ICU.
- The mortality for the case-matched controls was 77.78%, whereas the mortality in the SCD treatment group was 22.22% ($p = 0.027$).

The Effect of the Selective Cytopheretic Device on Acute Kidney Injury Outcomes in the Intensive Care Unit: A Multicenter Pilot Study

James A. Tumlin,* Lakhmir Chawla,† Ashita J. Tolwani,‡ Ravindra Mehta,§ John Dillon,– Kevin W. Finkel,** J. Ricardo DaSilva,†† Brad C. Astor,§§ Alexander S. Yevzlin§§ and H. David Humes.

- A single-arm, open-label, multicenter pilot study to assess the safety and effi-cacy of a selective cytopheretic device (SCD) in patients with AKI.
- 60-day mortality rate of 31.4%.
- Among the 22 patients who survived to day 60 in, 100% recovered kidney function and did not need dialysis.
- A pivotal trial of SCD therapy is underway in the US.

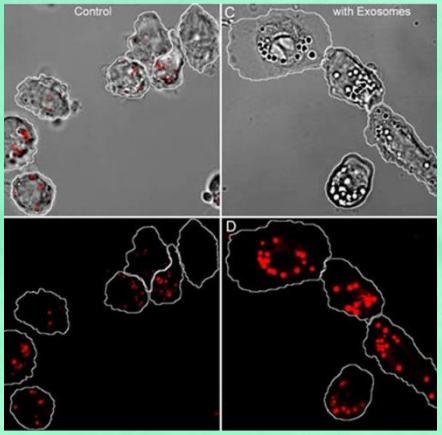


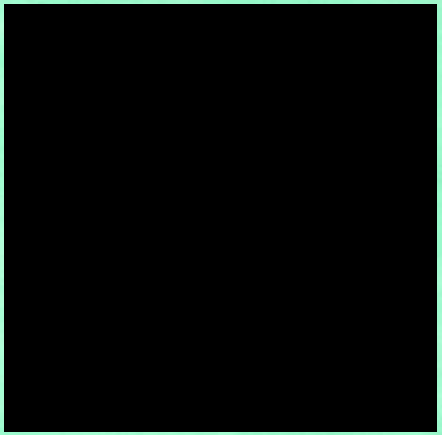
Exosomes

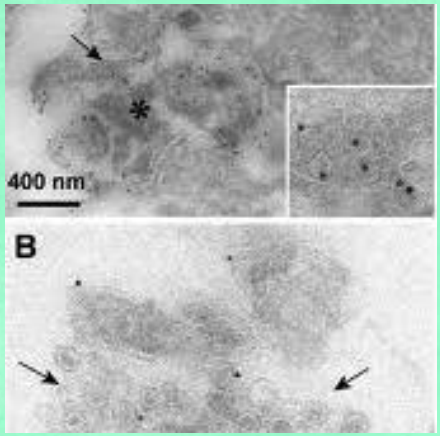
- A specific subset of membrane-bounded vesicles formed intracellularly within vesicular endosomes.
- Released into the extracellular environment by many cells from different tissues and organs.
- Exist in a wide range of biological fluids, including blood and urine.
- Between 30 and 100 nm in diameter.

Exosomes

- Have a molecular envelope structure that is remarkably similar to that of viruses.
- Have a hydrophilic core containing proteins, mRNAs and microRNAs (miRNA).
- Originally thought to be “cellular trash bags”
- Now, widely believed to be involved with intercellular communications.







Exosomes

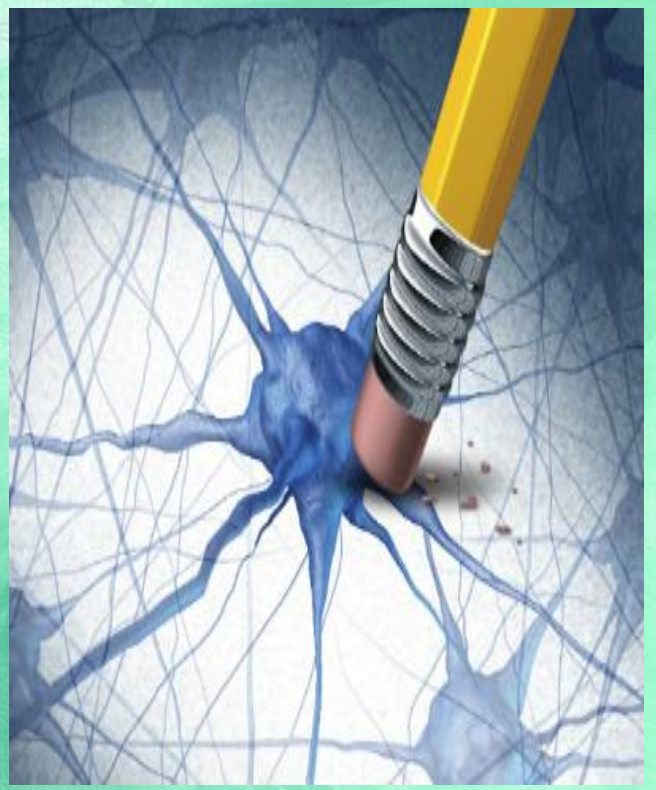
- Both mRNAs and miRNAs present in the exosomal fraction maintain their function when transferred to other cells demonstrating that exosomal RNA transfer may be an important route for epigenetic signaling between cells.
- Transferred RNAs can affect protein production and gene expression in target cells.
- The dissemination of pro-cancer cargo by exosomes has been identified as promoting several critical aspects of cancer pathogenesis, including:
  - signaling for tumor growth,
  - metastasis,
  - angiogenesis, and
  - resistance to chemo- and immunotherapeutic agents.

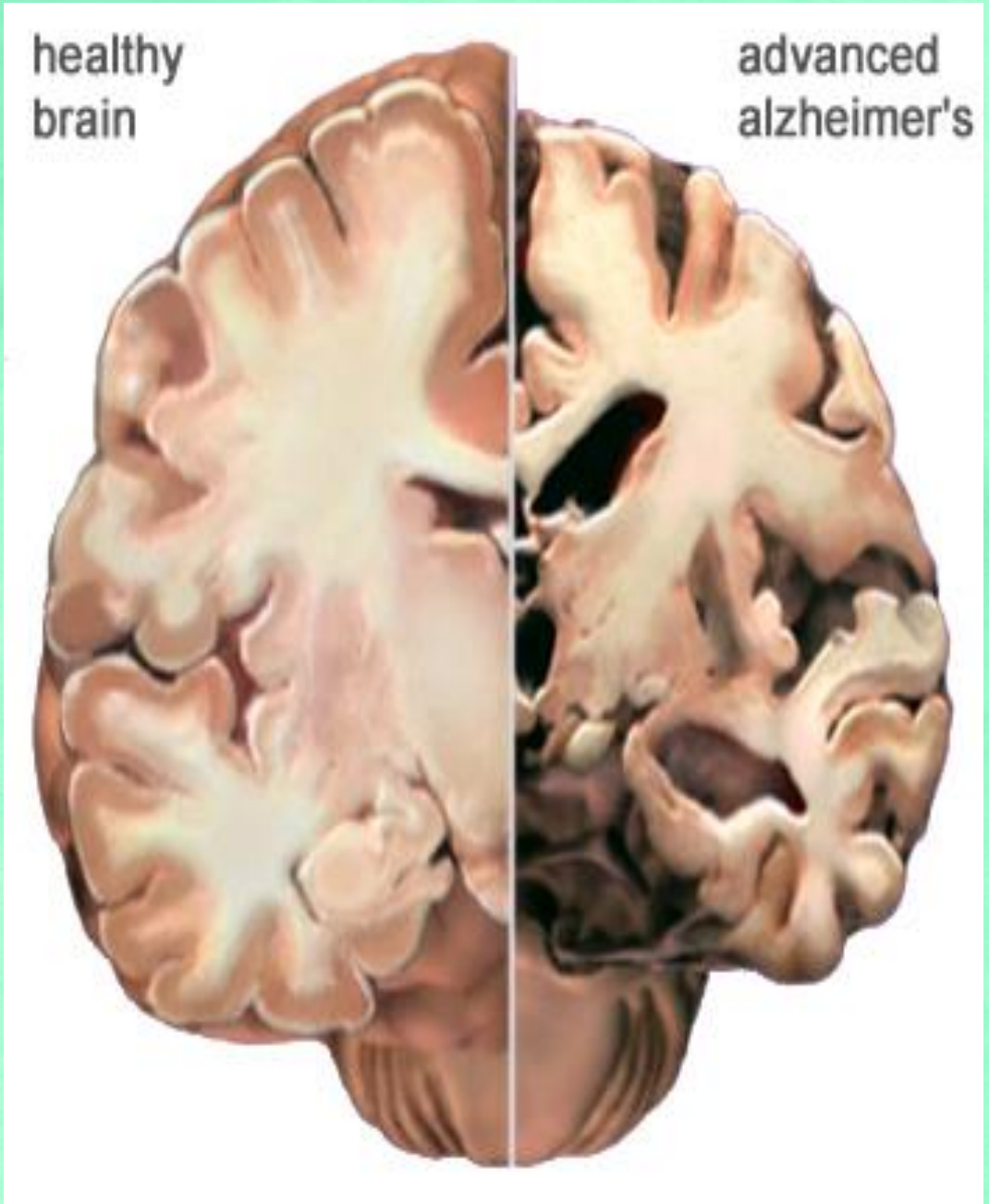
Exosomes

- The generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), plays a major role in endothelial activation and vascular failure.
- Two main enzymatic sources of ROS/RNS (NADPH oxidase & NO synthase) are expressed by platelets.
- Exosomes generated from platelets exposed to LPS are quite similar to those found in septic patients w/r/t protein content, phosphatidylserine exposure, and redox activity.
- Platelet-derived exosomes from septic patients induce vascular cell apoptosis and contain inducible nitric oxide synthase (iNOS).

Current Military-funded Anti-sepsis Project

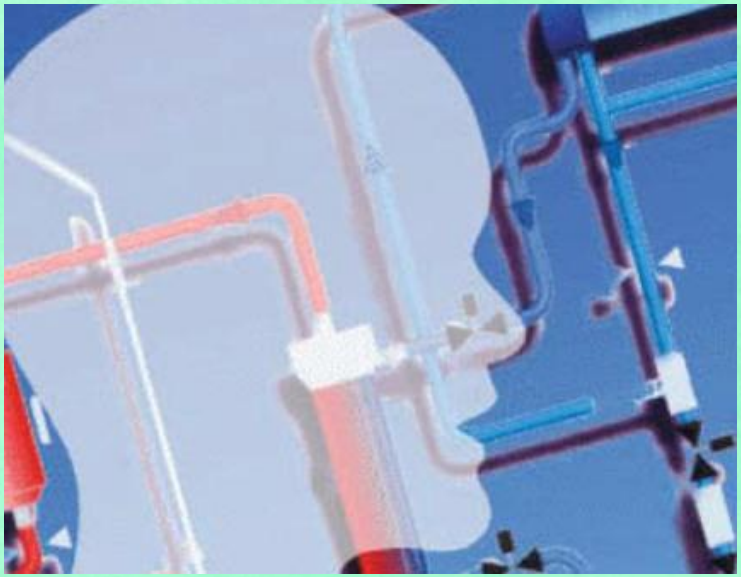
- Goal: Reduce sepsis-related mortality in wounded warfighters.
- Approach: Extracorporeal Blood Purification (Dialysis-like Therapeutic)
- Multi-company consortium




healthy
brain
advanced
alzheimer's

Phase III Clinical Trials

- Glioblastoma
- Recurrent glioblastoma
- Alzheimer's disease
  - Utilize plasma exchange for treatment



ANTI-AGING MEDICINE

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Review Article

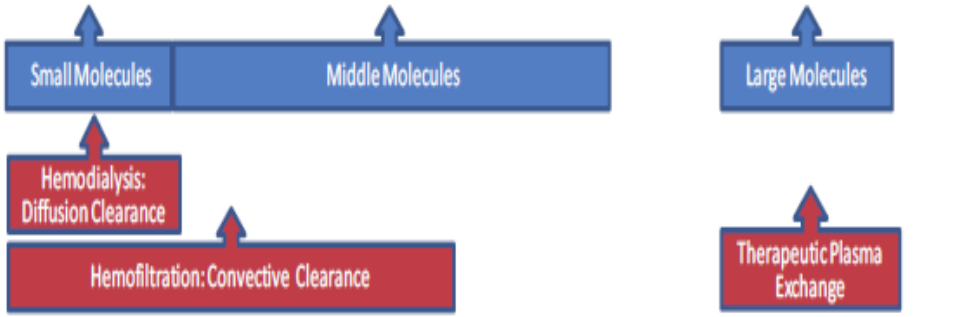
Can an Apheresis Therapy become an Effective Method for Anti-Aging Medicine?

Hiroshi Miyamoto, Yukihiko Nose

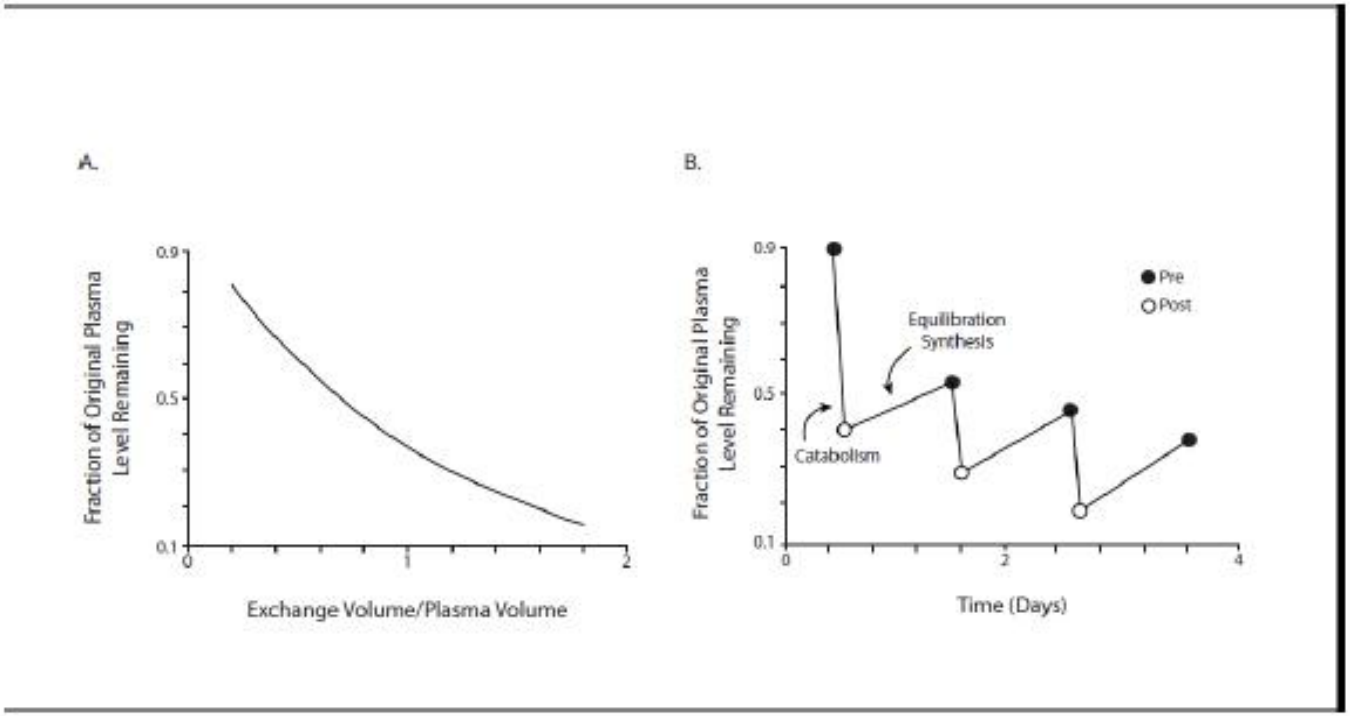
Michael E. DeBakey Department of Surgery, Baylor College of Medicine

Molecular Size and Clearance Modality

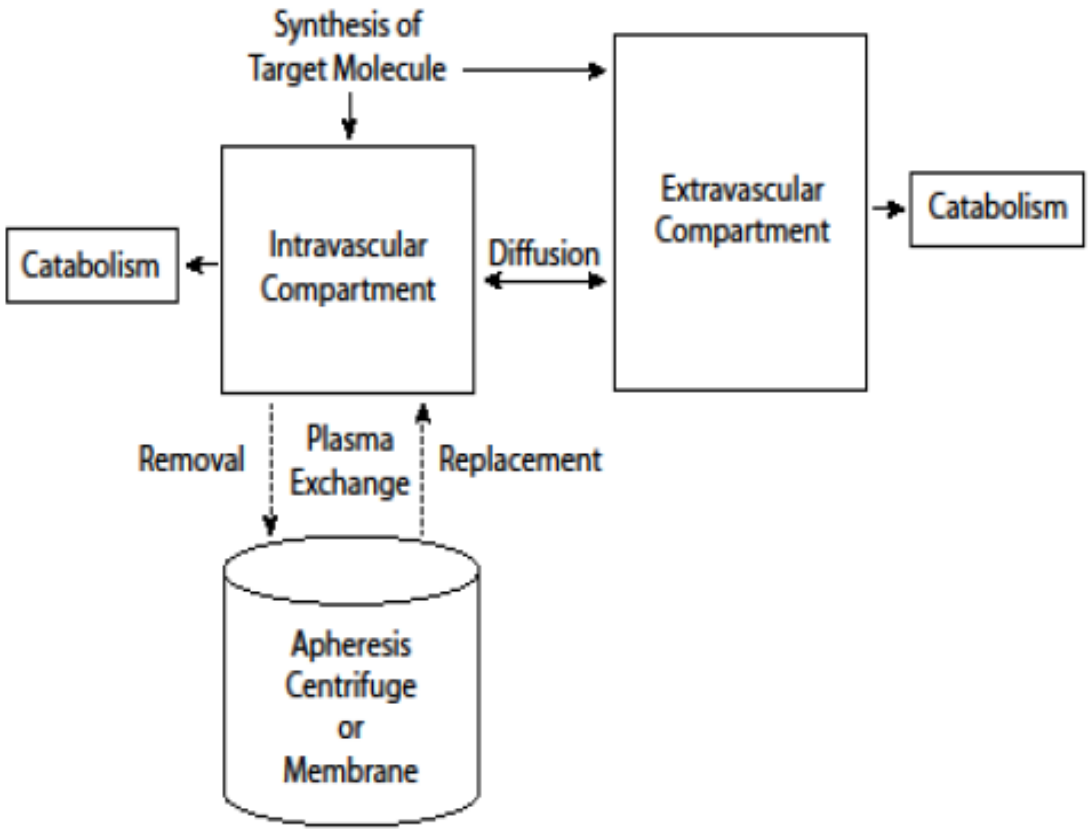
BUN	Creatin	VitB12	β2-microglobulin	K Light Chain	λ Light Chain	Albumin	IgG	IgM
0.06	0.113	1.355	11.8	25	50	66	160	950



Molecular Clearance



Internal and External Balance



ASFA Guidelines: Indications

TABLE I. Indications for Therapeutic Apheresis–ASFA 2013 Categories [1]

Category	Description
I	**Disorders for which apheresis is accepted as first-line therapy, either as a primary standalone treatment or in conjunction with other modes of treatment.** *Example: plasma exchange in Guillain-Barre syndrome as 1st-line standalone therapy; plasma exchange in myasthenia gravis as 1st-line in conjunction with immunosuppression and cholinesterase inhibition*
II	**Disorders for which apheresis is accepted as second-line therapy, either as a standalone treatment or in conjunction with other modes of treatment.** *Example: plasma exchange as standalone secondary treatment for acute disseminated encephalomyelitis after high-dose IV corticosteroid failure; extracorporeal photopheresis added to corticosteroids for unresponsive chronic graft-versus-host disease*
III	**Optimum role of apheresis therapy is not established. Decision making should be individualized.** *Example:extracorporeal photopheresis for nephrogenic systemic fibrosis; plasma exchange in patients with sepsis and multi-organ failure*
IV	**Disorders in which published evidence demonstrates or suggests apheresis to be ineffective or harmful. IRB approval is desirable if apheresis treatment is undertaken in these circumstances.** *Example: plasma exchange for active rheumatoid arthritis*

ASFA Guidelines:

Recommendation Grades

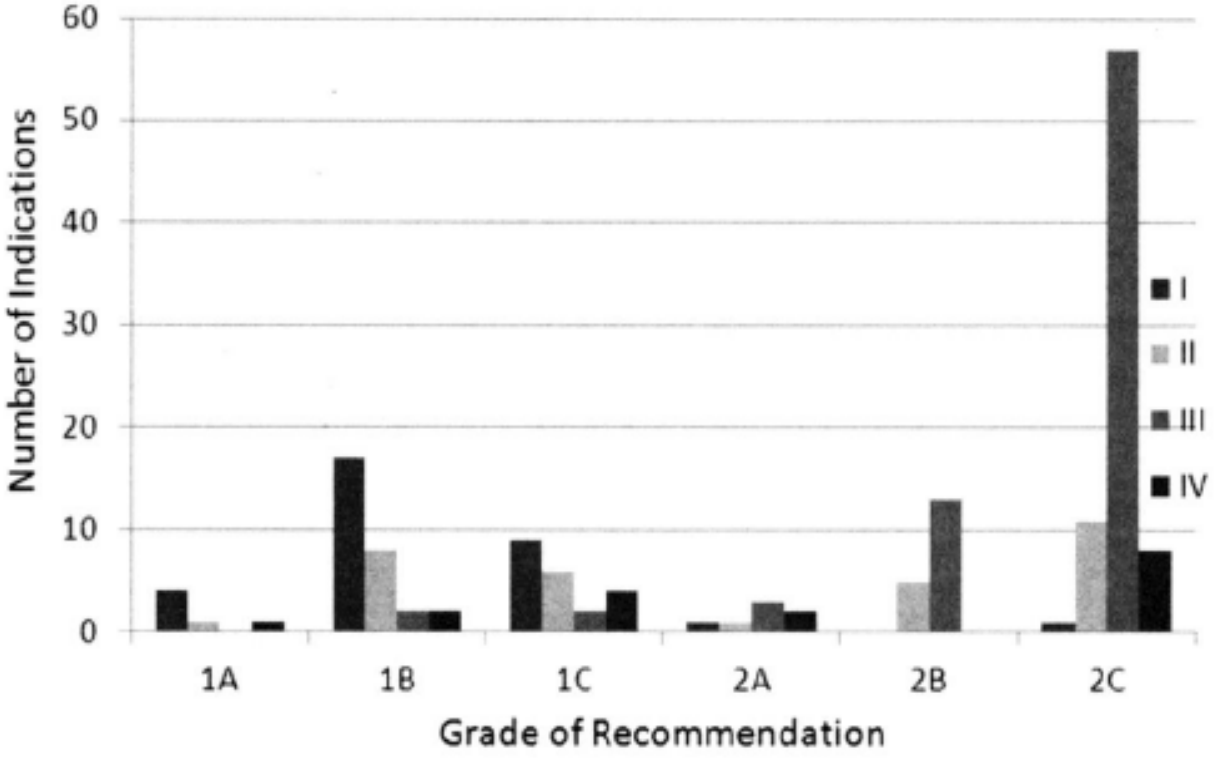
TABLE III. Grading Recommendations Adopted from Guyatt et al. [13]

Recommendation	Description	Methodological quality of supporting evidence	Implications
Grade 1A	Strong recommendation, high-quality evidence	RCTs without important limitations or overwhelming evidence from observational studies	Strong recommendation, can apply to most patients in most circumstances without reservation
Grade 1B	Strong recommendation, moderate quality evidence	RCTs with important limitations (inconsistent results, methodological flaws, indirect, or imprecise) or exceptionally strong evidence from observational studies	Strong recommendation, can apply to most patients in most circumstances without reservation
Grade 1C	Strong recommendation, low-quality or very low-quality evidence	Observational studies or case series	Strong recommendation but may change when higher quality evidence becomes available
Grade 2A	Weak recommendation, high quality evidence	RCTs without important limitations or overwhelming evidence from observational studies	Weak recommendation, best action may differ depending on circumstances or patients' or societal values
Grade 2B	Weak recommendation, moderate-quality evidence	RCTs with important limitations (inconsistent results, methodological flaws, indirect, or imprecise) or exceptionally strong evidence from observational studies	Weak recommendation, best action may differ depending on circumstances or patients' or societal values
Grade 2C	Weak recommendation, low-quality or very low-quality evidence	Observational studies or case series	Very weak recommendations; other alternatives may be equally reasonable

RCT: randomized controlled trial

Guyatt G, et al. Grading strength of recommendations and quality of evidence in clinical guidelines: report from an American College of Chest Physicians task force. *Chest*

ASFA Guidelines: Grade of Recommendation



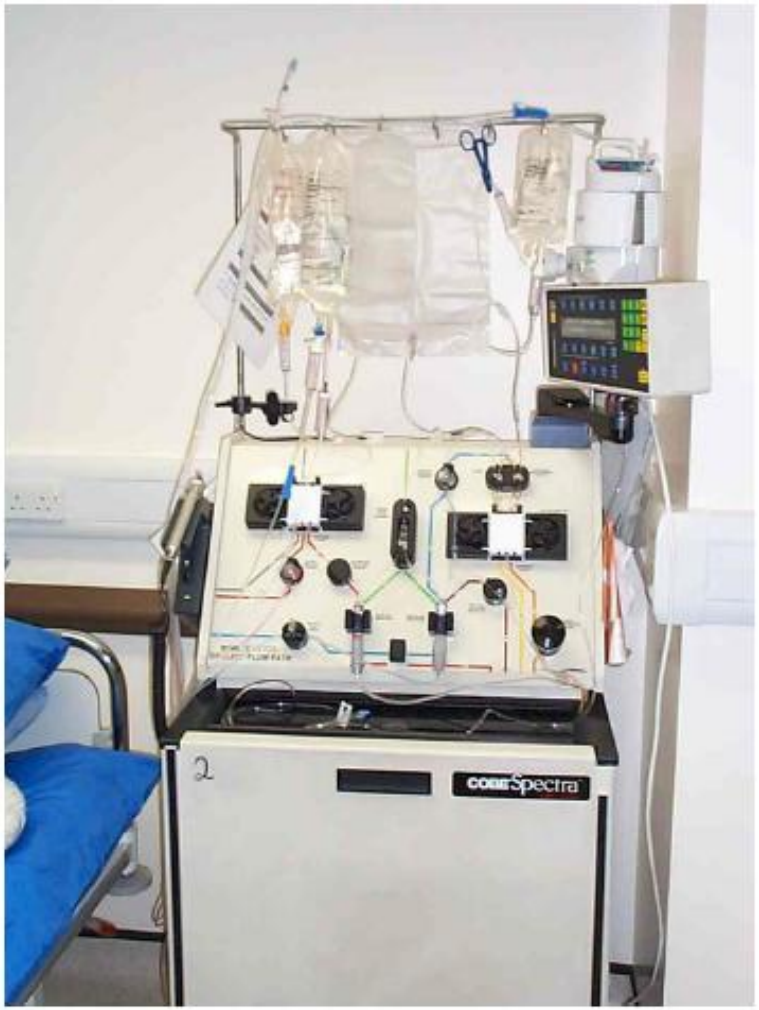
ASFA Category 1 Renal Indications

1. ANCA-Associated RPGN – dialysis dependence or diffuse alveolar hemorrhage (8)
2. Anti-GBM disease – diffuse alveolar hemorrhage or dialysis independence (1)
3. Cryoglobulinemia – symptomatic, severe (0*)
4. Focal segmental glomerulosclerosis (0)
5. Atypical hemolytic uremic syndrome – Factor H antibodies (0)
6. Kidney transplant, ABO compatible – Antibody-mediated rejection (3) or desensitization, living donor with positive crossmatch (0)
7. Kidney transplant, ABO-incompatible – Desensitization, live donor (0)
8. Thrombotic thrombocytopenic purpura (7)
9. Thrombotic microangiopathy drug-associated – Ticlopidine (0)

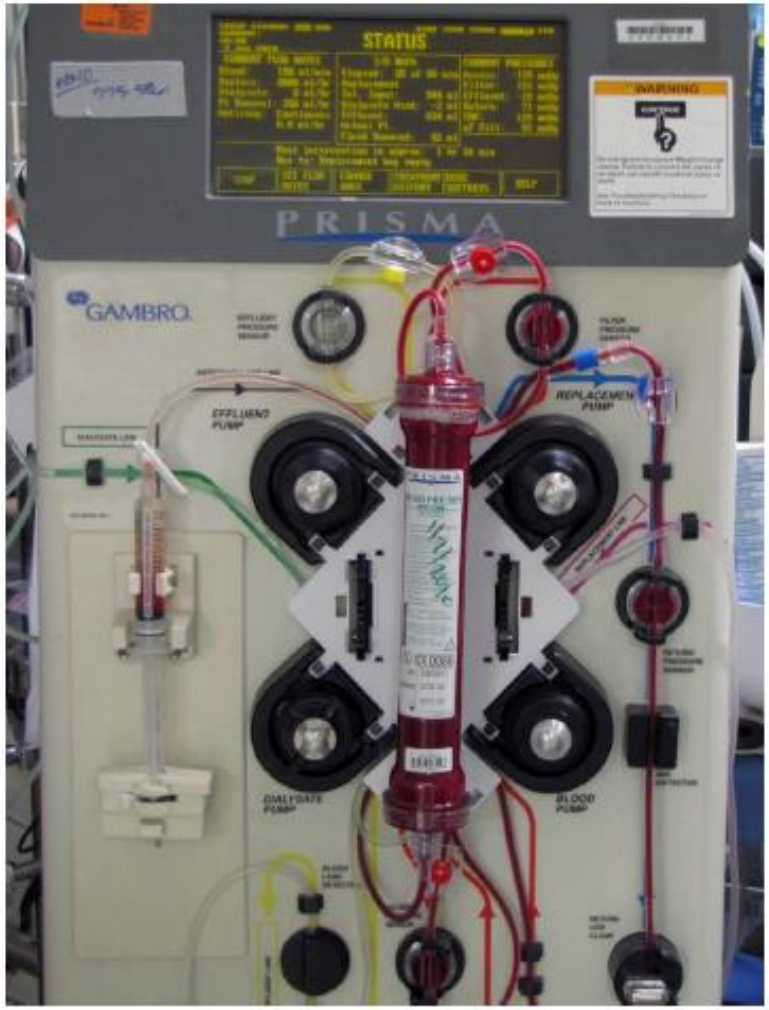
*- One randomized clinical trial with immunoadsorption apheresis

Challenge 1: The Apheresis Modality

Cobe Spectra

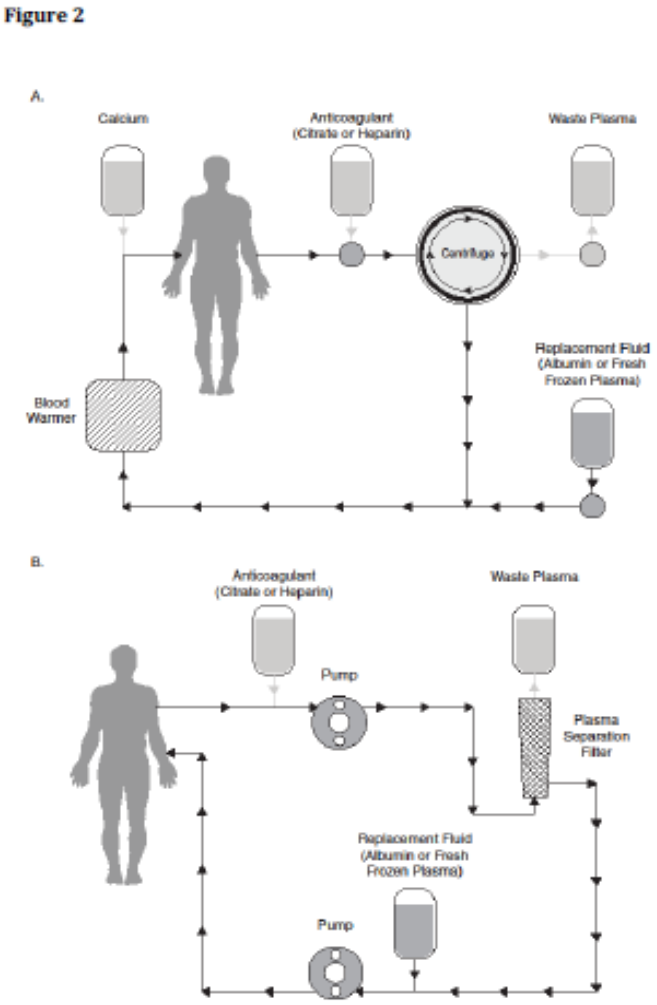


Gambro Prisma



Apheresis Technology

- Centrifuge
- Membrane



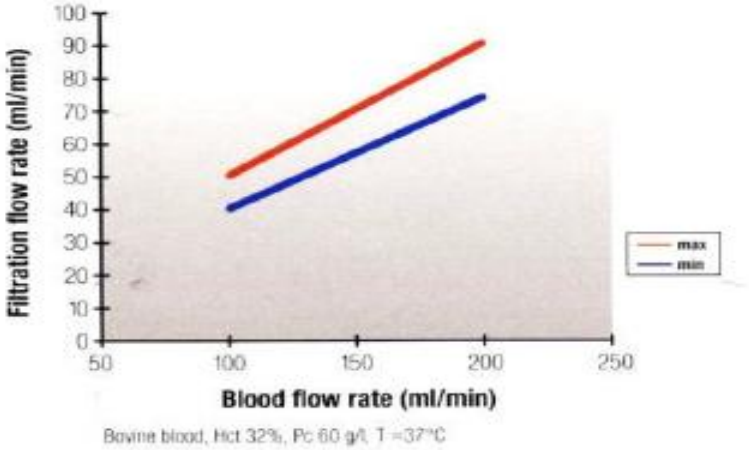
- Latex Free
- Hollow fiber plasmafilter
- Fluid circuitry already attached to the filter:

Blood side
- access line with injection segment for gravity infusion of saline + 2-liter collection bag
- return line
- replacement line (with vented spike for bottles) connected in post-dilution mode

Effluent side
- effluent line + 5-liter effluent bag

Filter Performances

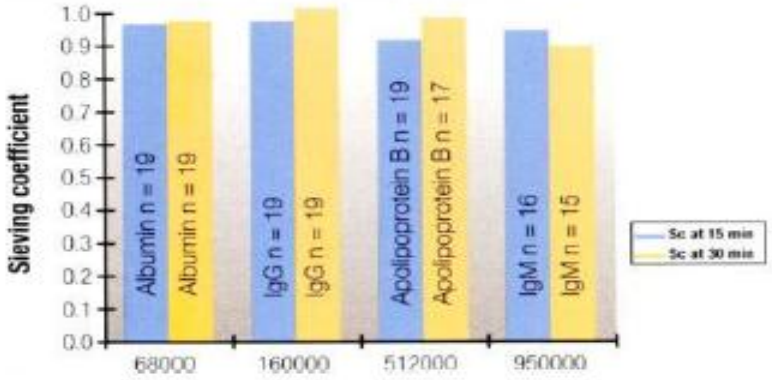
Filter flow rate at TMP = 80 mmHg



Bovine blood, Hct 32%, Pc 60 g/l, T =37°C

Blood pressure drop < 35 mmHg at blood flow rate = 100 ml/min

Sieving coefficient: total protein ≤ 0.95



Membrane Technology

- Gambro Prisma

Performance Comparison

(S. Conrad, MD)

Patient weight: 56kg Patient Hct: 33% # of plasma exchanges: 1

	Centrifuge		Membrane	
Effluent Rate (plasma replacement rate*)	1500 ml/h	1750 ml/h	1500 ml/h	2500 ml/h
Blood Flow Rate	50 ml/min	50 ml/min	200 ml/min	200 ml/min
Treatment time	105 min	90 min	105 min	63 min
Filtration Fraction %** (plasma removal 'efficiency')	65%	76%	19%	31%
Post Filter Hematocrit % (HCTpost)	66%	79%	38%	42%

* assumes no patient plasma loss prescribed

** not referred to as 'filtration' fraction with centrifuge

Effect of Plasma Volume Exchanged

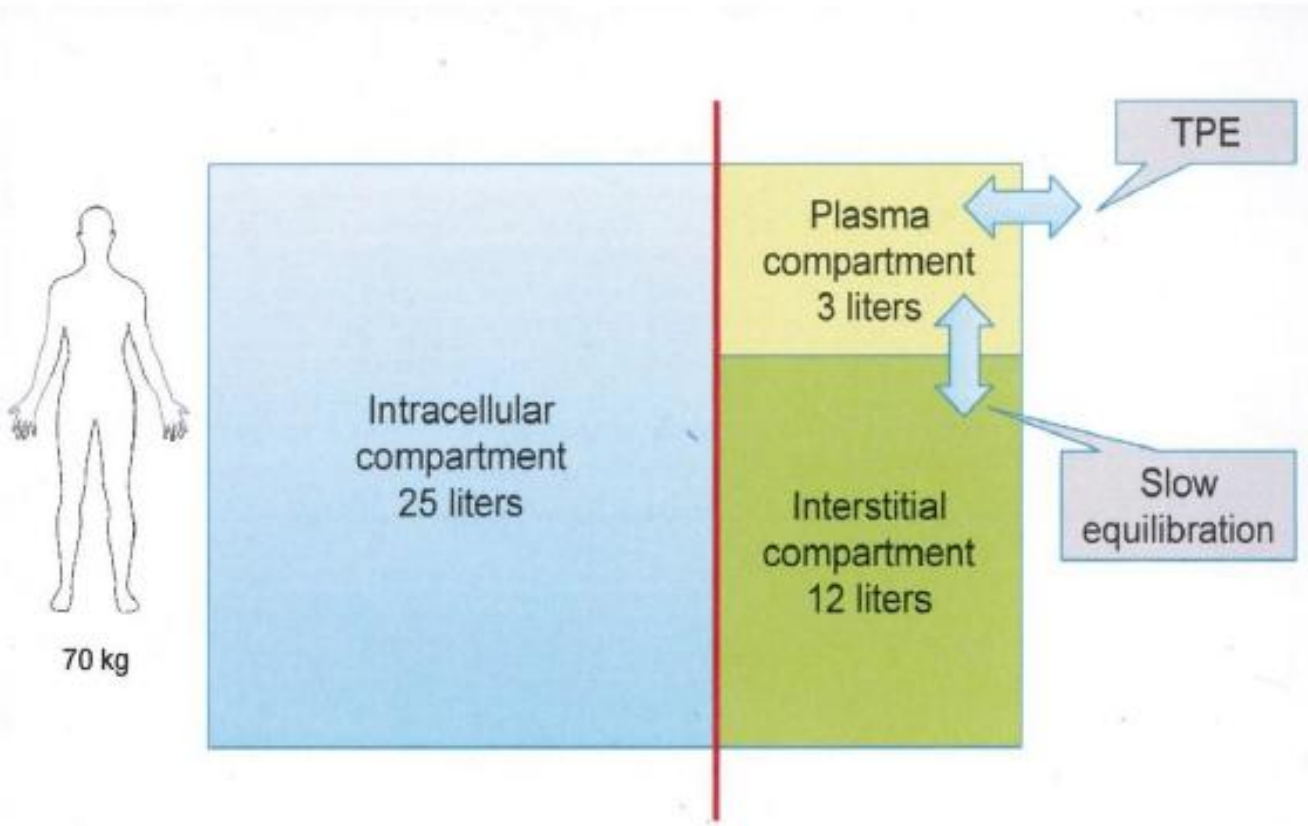
(S. Conrad, MD)

Plasma volume exchanged	Amount of substances removed	Relative incremental amount removed
0.5	39%	39%
1.0	63%	24%
1.5	78%	15%
2.0	86%	8%
2.5	92%	6%

Adapted from Daugirdas, Handbook of Dialysis 4th edition ,2006

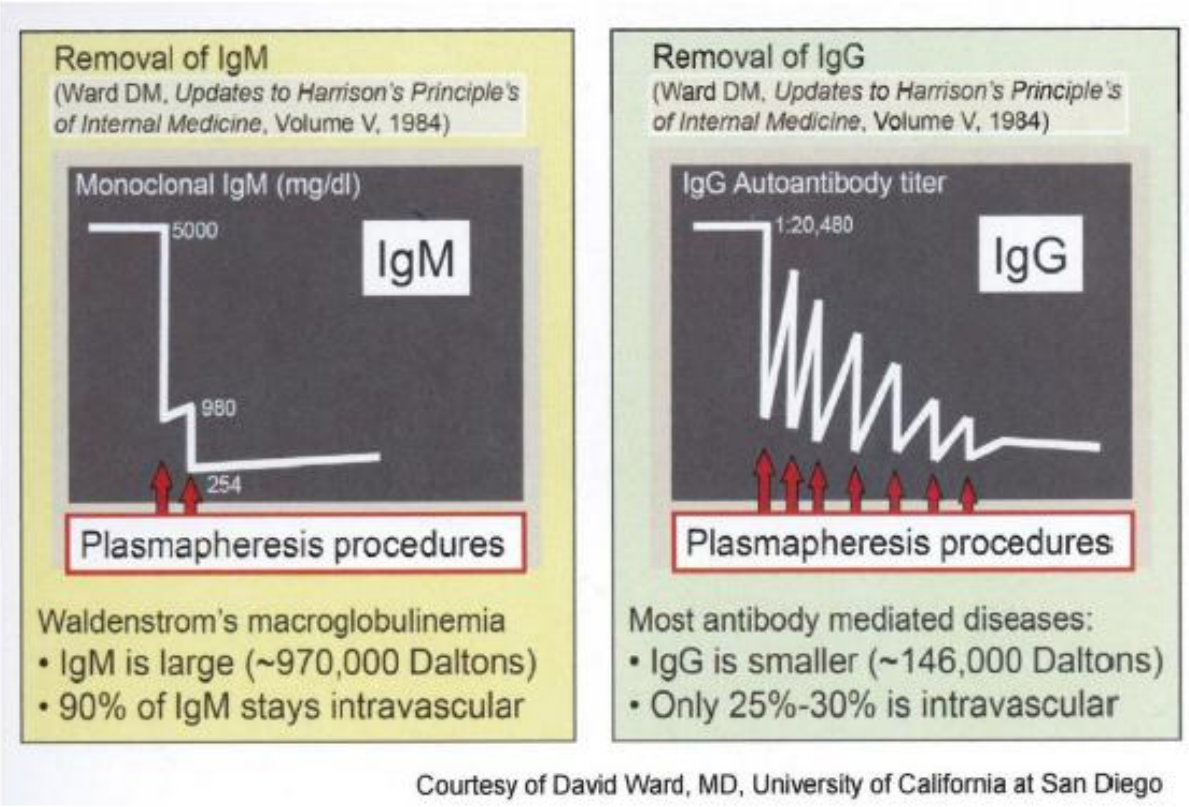
Body Compartments

(S. Conrad MD)



Number of Exchanges Needed Depends on Volume of Distribution

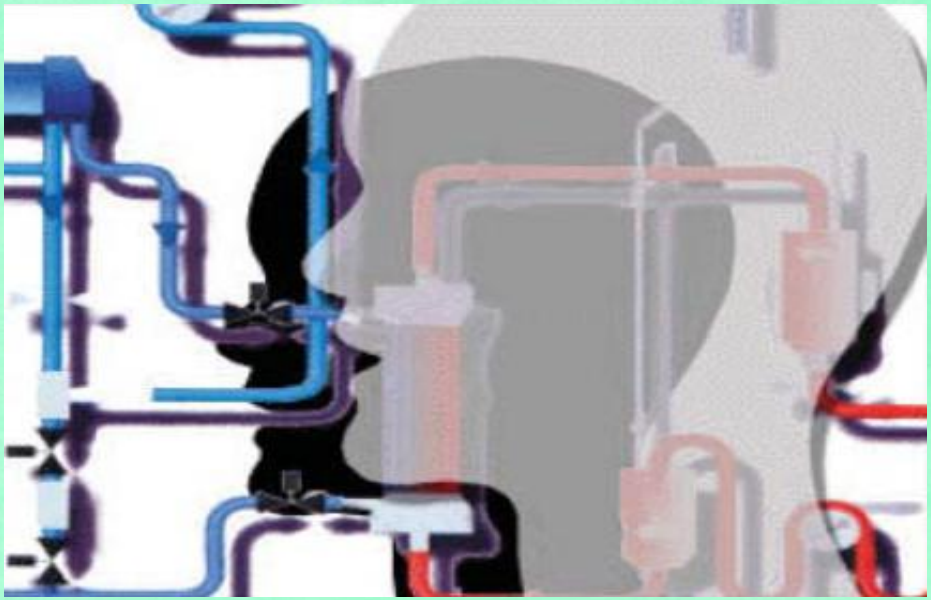
(S. Conrad MD)



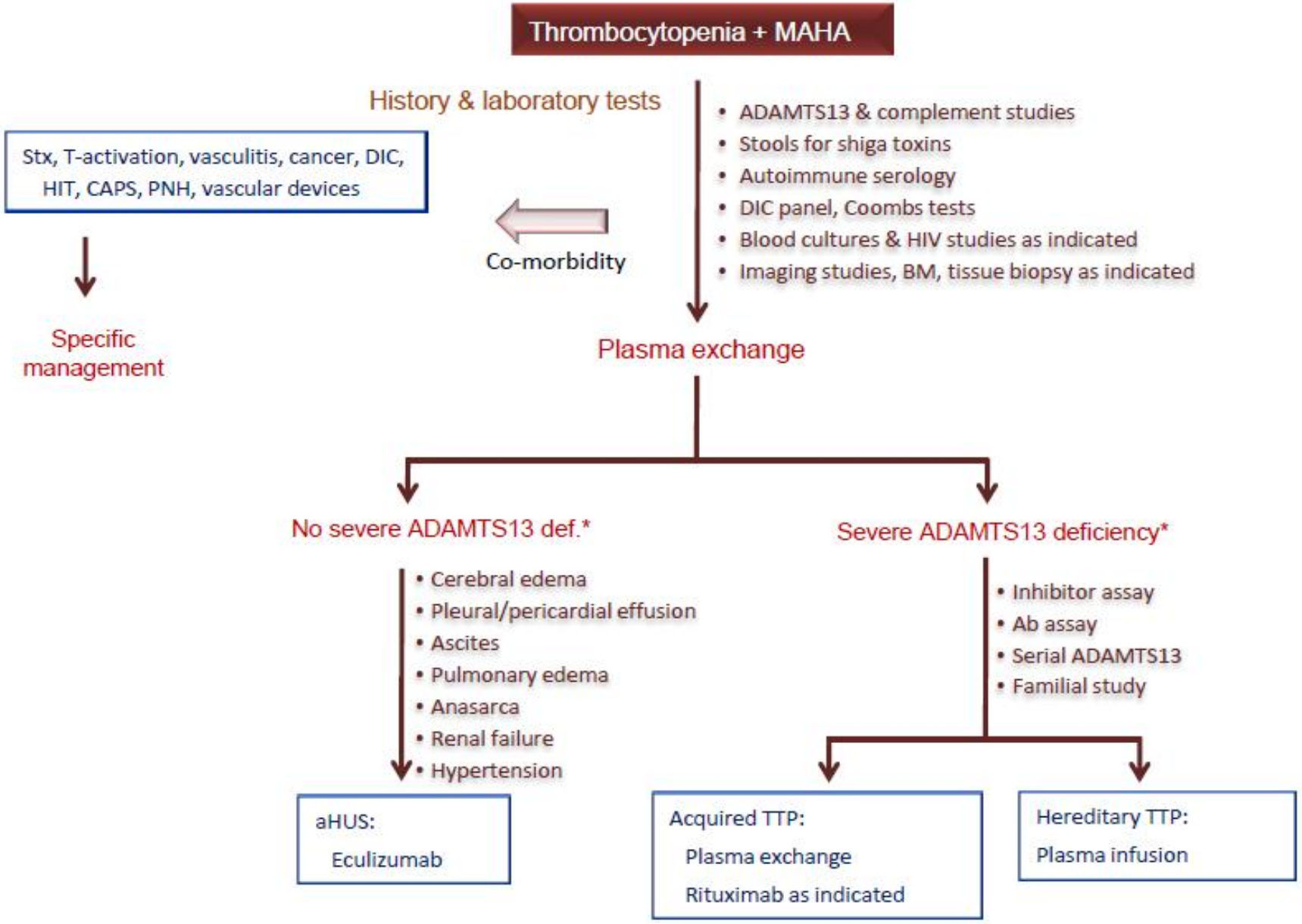
Challenge 2: Role of ADAMTS13 Removal in Thrombotic Thrombocytopenic Purpura

THROMBOTIC THROMBOCYTOPENIC PURPURA

Incidence: 0.37/100,000/year (US)	**Procedure** TPE	**Recommendation** Grade 1A	**Category** I
# of reported patients*: >300			
RCT	**CT**	**CS**	**CR**
7 (301)	2 (133)	26 (980)	46 (83)



A Practical Approach to Thrombocytopenia/MAHA



Challenge 3: Defining the Role of Therapeutic Plasma Exchange in Atypical Hemolytic Uremic Syndrome Treated with Eculizumab

Differential diagnosis for TMAs: aHUS, TTP, and STEC-HUS

Thrombocytopenia[1,7]
Platelet Count <150,000
or >25% Decrease
From Baseline[1]

AND

Microangiopathic Hemolysis[2,7]
Schistocytes[2,7] *and/or*
Elevated LDH[7] *and/or*
Decreased Haptoglobin[7] *and/or*
Decreased Hemoglobin[7]

Plus one or more of the following:

Neurological Symptoms[3,4,9,14]
Confusion[3,4] *and/or*
Seizures[9,12] *and/or*
Other Cerebral Abnormalities[4]

Renal Impairment[5,6,7]
Elevated Creatinine[6] *and/or*
Decreased eGFR[6,7] *and/or*
Abnormal Urinalysis[5]

Gastrointestinal Symptoms[7,8,9]
Diarrhea +/- Blood[8] *and/or*
Nausea/Vomiting[9] *and/or*
Abdominal Pain[9] *and/or*
Gastroenteritis[7,8]

Evaluate ADAMTS13 Activity and Shiga-toxin/EHEC* Test[10,11,12,13]

≤5% ADAMTS13 Activity[10,11,12]	>5% ADAMTS13 Activity[10]	Shiga-toxin/EHEC Positive[13]
TTP	**aHUS**	STEC-HUS*

*Shiga-toxin/EHEC test is warranted in history/presence of GI symptoms.

High clinical suspicion of aHUS is required in all patients presenting with any sign or symptom of systemic, complement-mediated thrombotic microangiopathy[1]

TMA = thrombotic microangiopathy.
aHUS = atypical hemolytic uremic syndrome.
TTP = thrombotic thrombocytopenic purpura.
STEC-HUS = Shiga-toxin–producing *E coli* hemolytic uremic syndrome.
LDH = lactate dehydrogenase.
eGFR = estimated glomerular filtration rate.
EHEC = enterohemorrhagic *E coli*.

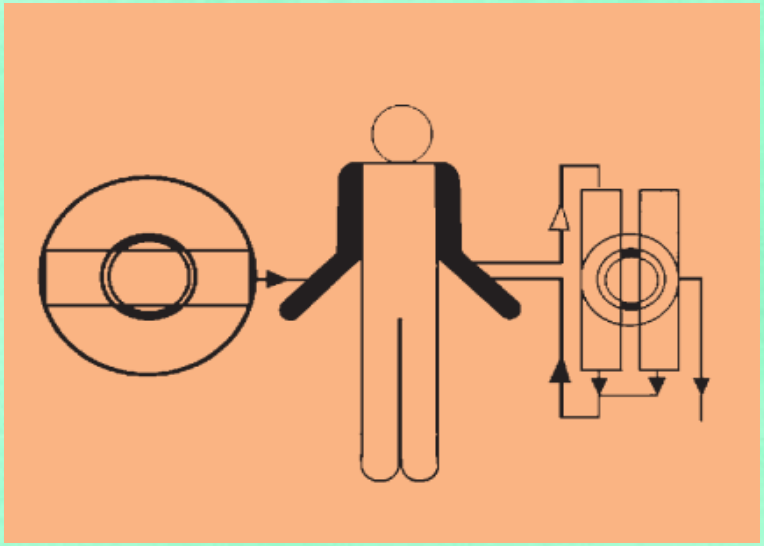
HEMOLYTIC UREMIC SYNDROME, ATYPICAL

Incidence: 3.3/1,000,000/yr (<18 yo); 7/1,000,000/yr (children in European community)	**Condition**	**Procedure**	**Recommendation**	**Category**
	Complement factor gene mutations	TPE	Grade 2C	II
	Factor H autoantibodies	TPE	Grade 2C	I
	MCP mutations	TPE	Grade 1C	IV

# of reported patients*: >300

	RCT	CT	CS	CR
Complement factor gene mutations	0	0	4(23)	21(26)
Factor H autoantibody	0	0	2(6)	2(2)

MCP = membrane cofactor protein



Atypical Hemolytic Uremic Syndrome

- 5-10% of HUS cases
- Incidence of 3-7 per million per year
- Noninfection-related
- Poorer prognosis and outcome
  - 2/3 die, require dialysis, or have permanent kidney injury during the first year
- Sporadic or familial
- About two-thirds are associated with genetic or acquired disorders of regulatory components of the complement system

Atypical HUS: Classification

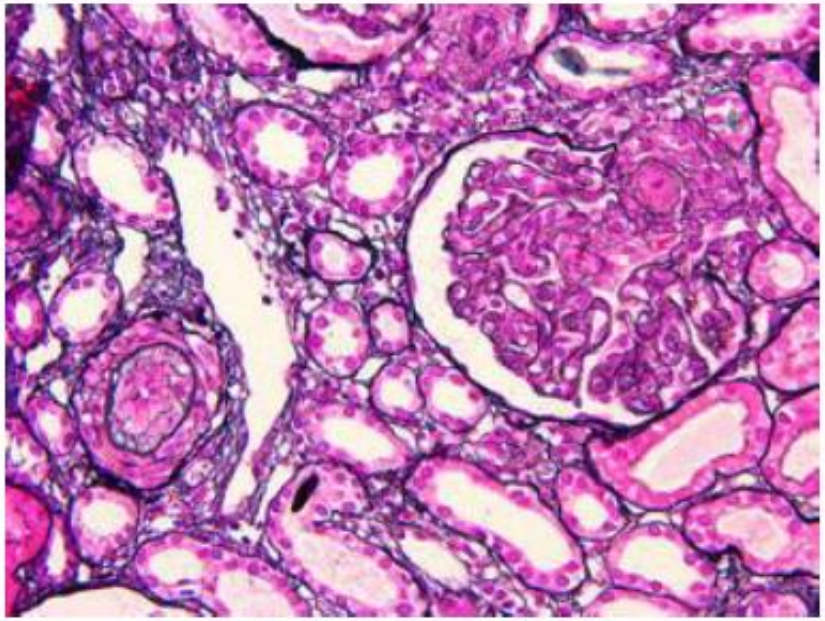
Table 1. **Classification of Atypical Hemolytic–Uremic Syndrome.***

Form of Disease	Complement Abnormalities
Familial	Mutations in *CFH*, 40–45%; in *CFI*, 5–10%; in *C3*, 8–10%; in *MCP*, 7–15%; in *THBD*, 9%; and in *CFB*, 1–2%.
Sporadic	
Idiopathic	Mutations in *CFH*, 15–20%; in *CFI*, 3–6%; in *C3*, 4–6%; in *MCP*, 6–10%; in *THBD*, 2%; and in *CFB*, 2 cases; anti-CFH antibodies: 6–10%
Pregnancy-associated	Mutations in *CFH*, 20%; in *CFI*, 15%
HELLP syndrome	Mutations in *CFH*, 10%; in *CFI*, 20%; and in *MCP*, 10%
Drugs	Rare *CFH* mutations (mostly unknown)
Organ transplantation	Mutations in *CFH*, 15%; in *CFI*, 16%
Human immunodeficiency virus infection	Unknown†
Cancer	Unknown†

* HELLP denotes hemolytic anemia, elevated liver enzymes, and low platelet count.

† There are no published data on the frequency of complement gene mutations or anti-CFH autoantibodies in patients with this condition.

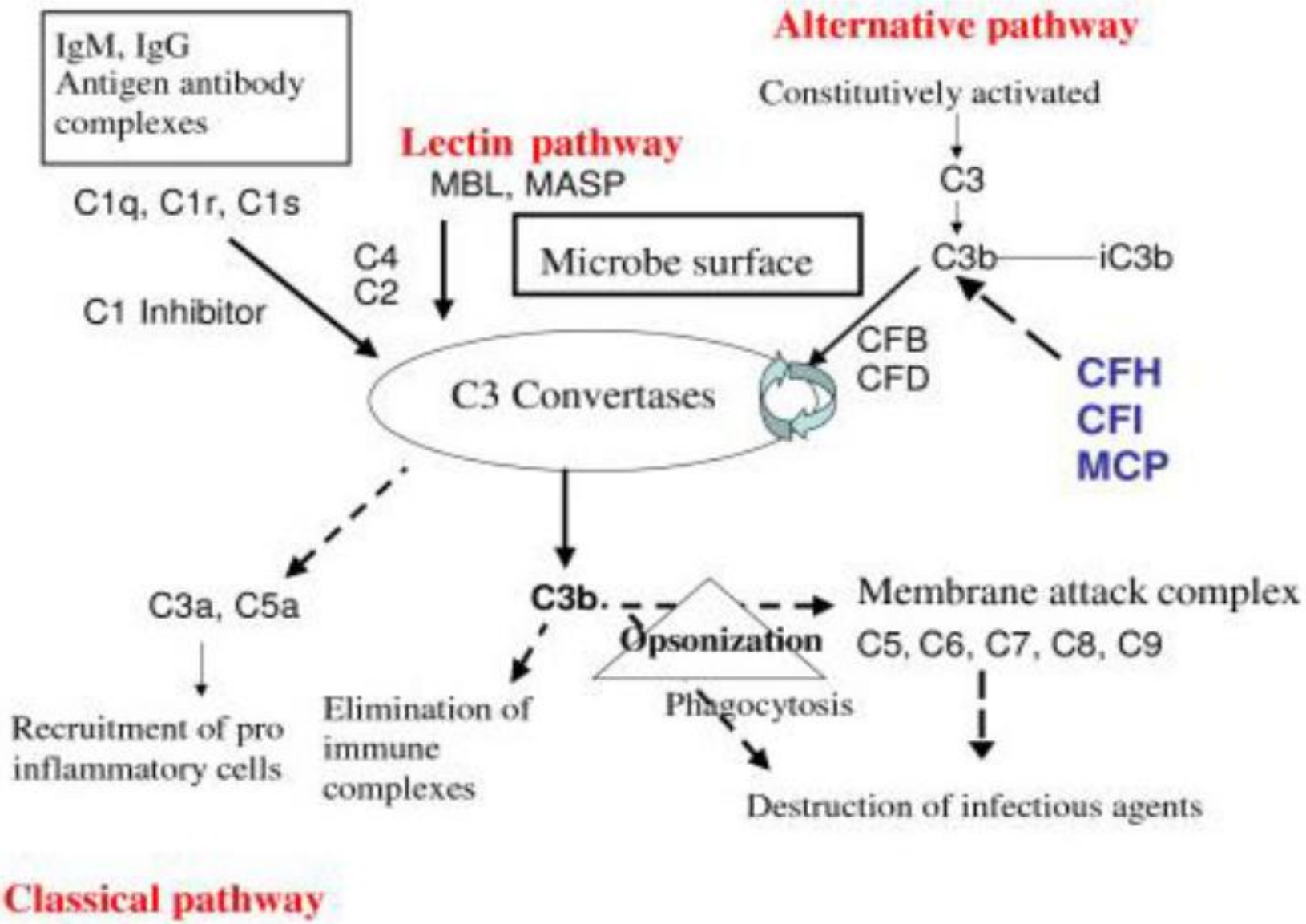
Pathogenesis of aHUS



- PAS section showing obliterated arteriolae, mesangial infiltration, narrowing of the capillary lumina

- Endothelial injury
- Formulation of platelet-fibrin hyaline micro-thrombi
- Occlusion of arterioles and capillaries
- NEW INSIGHTS:
  - Caused by uncontrolled activation of the alternative complement system

Complement Activity and aHUS



Main Complement Regulators Involved in the Alternative Complement Pathway

Table 1 | Main complement regulators involved in the alternative pathway and their function

Complement regulator name	Abbreviation/ alternative names	Function
Factor I	CFI	Plasma serine protease that cleaves C3b, producing inactive iC3b, in the presence of soluble cofactors and/or membrane-bound complement regulators
Factor H	CFH	Plasma molecule that recognizes C3b and cell surfaces through the C-terminus, whereas the N-terminus region mediates cofactor activity for CFI; CFH also directly accelerates the decay of C3-convertase C3b/Bb
Membrane cofactor protein	MCP/CD46	Integral transmembrane protein that binds C3b and C4b and serves as a cofactor for CFI
Thrombomodulin	THBD	Transmembrane protein; in addition to its well-established anticoagulant function, it is involved in the generation of thrombin-activatable fibrinolysis inhibitor, a plasma carboxypeptidase B that cleaves C3a and C5a; thrombomodulin binds to C3b, accelerating its inactivation by CFI in the presence of CFH
Decay accelerating factor	DAF/CD55	Phosphatidylinositol-anchored glycoprotein that prevents the assembly of the C3b/Bb complex or accelerates its decay
Protectin	CD59	Phosphatidylinositol-anchored glycoprotein that binds C5b/C6/C7/C8, preventing C9 from binding and polymerizing

Laboratory Diagnosis of aHUS

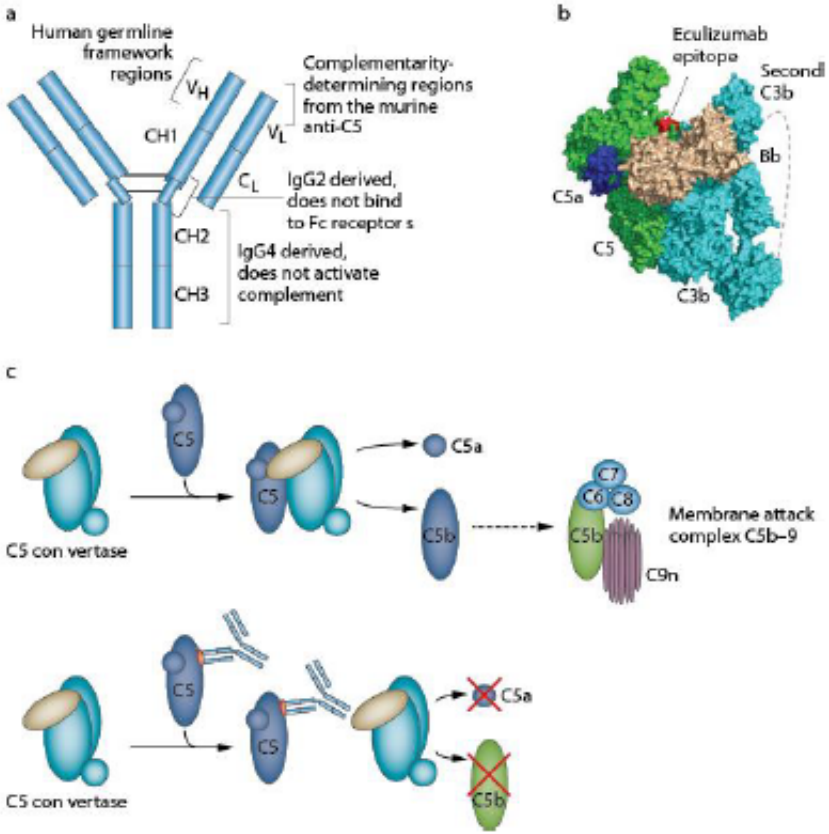
- Conventional complement tests: C3, C4, CH50, AH50
  - Abnormal in 30%
  - Not specific for aHUS
- Plasma CFH and CFI protein concentrations
  - Decreased in 30% of patients with CFH or CFI mutations
- Mutation analysis (CFH, MCP, CFI, CFB, C3, THBD)
  - Abnormal in 40% (sporadic) - 70% (familial)
- CFH antibody
  - 5% - 10%

Role of TPE in atypical HUS

- Therapeutic plasma exchange has been first-line treatment for all aHUS
- Can remove auto-antibody or mutated circulating complement regulators while replacing absent or defective complement regulators
- No prospective trials
- More recently, Eculizumab used in plasma-resistant atypical HUS

Use of Eculizumab for atypical hemolytic uremic syndrome

(Nat Rev Nephrol 2012)



- Humanized monoclonal antibody that functions as a complement inhibitor by blocking cleavage of C5 into C5a and C5b and decreasing formation of membrane attack complex

Clinical Trials of Eculizumab for aHUS

	C08-002	C08-003	C09-001
Design	Prospective	Prospective	Retrospective
Patient age (median, y)	Adolescent-adult (28)	Adolescent-adult (28)	Children-Adult
Number	17	20	30 (19, <18 y)
Genetic mutations	76%	70%	53%
Months from Dx, median (range)	10 (0.26 – 236)	48 (0.66 – 286)	-
Entry status	Active TMA	Maintenance PE/PI	-
Duration, median (range) in mo.	PE/PI ≥4x/week	10 (2.4 – 47)	-
Dosage[1]	900 mg qW x4, 1,200 mg at W5 & q2W		-
Duration, median (range) in wk.	38 (2-64)	40 (26-52)	28 (1-70)
PE/PI or new dialysis	0 (0 – 0.31)	0	0
eGFR ↑, median (range), mL/min/1.73 m^2	+20 (-1, 98)	+5 (-1, 20)	≥15: 47%
Event free survival[2]	15 (88%)	16 (80%)	-
Normal platelet count & LDH	13 (76%)	18 (90%)	17 (89%)

[1]Adult dosage
[2]Event free survival (events: platelet count decrease >25%, PE/PI, new dialysis)

Greenbaum LA et al, Licht C et al, JASN 2011;22:197A

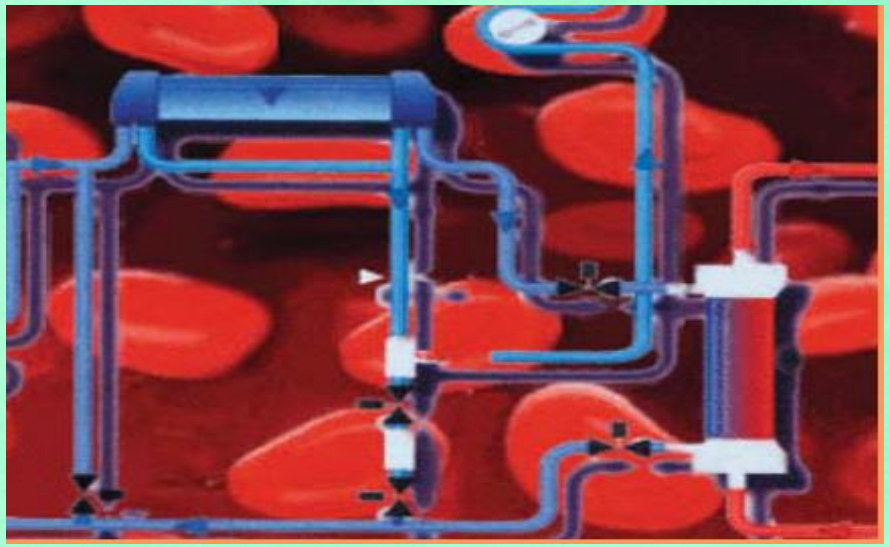
Challenge 4: Role of Therapeutic Plasma Exchange in Multiple Myeloma

MYELOMA CAST NEPHROPATHY

Incidence: 1/100,000/yr	Procedure	Recommendation	Category
	TPE	Grade 2B	II

# of reported patients*: 100–300

RCT	CT	CS	CR
5 (182)	0	8 (102)	7 (10)



Free Light Chains

- By-product of intact immunoglobulin synthesis
- 25-50 KDs
- Normal levels very low
- Physiologically, removed by renal clearance
- Widely distributed in body compartments
- Intravascular compartment may contain only 20%

Serum Free Light Chains: Renal Clearance

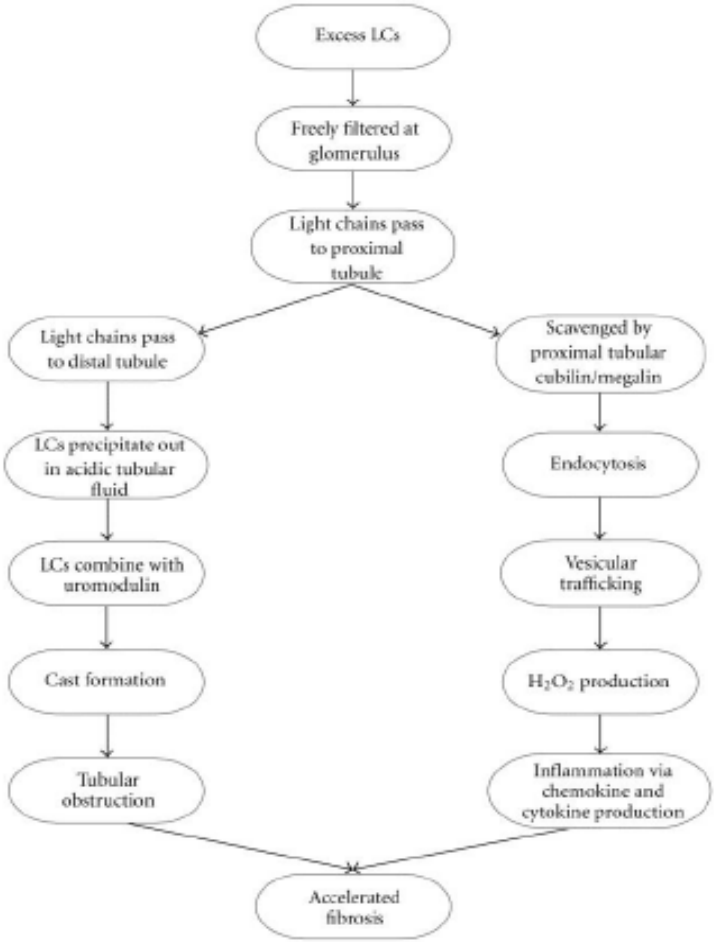


FIGURE 2: Renal handling of LCs.

Multiple Myeloma: Cast Nephropathy

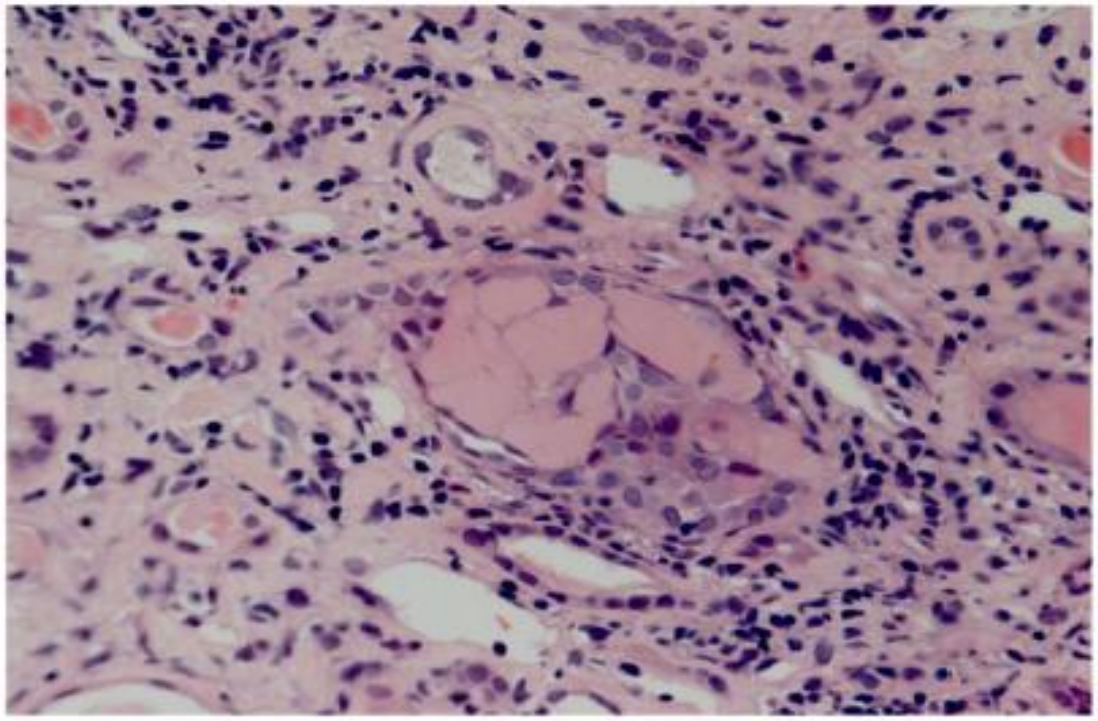


FIGURE 1: Renal biopsy showing cast nephropathy: distal tubular casts and interstitial inflammation and fibrosis.

Multiple Myeloma: Response Depends on Biopsy Diagnosis and SFLC Removal

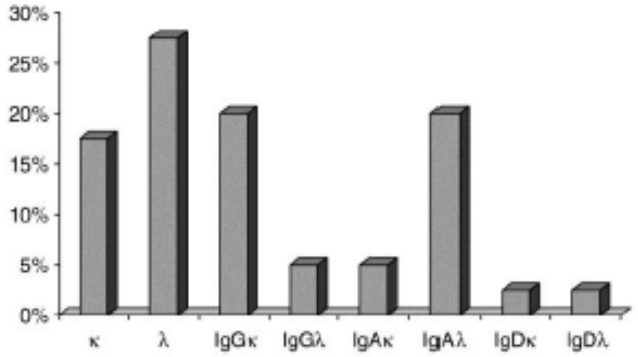
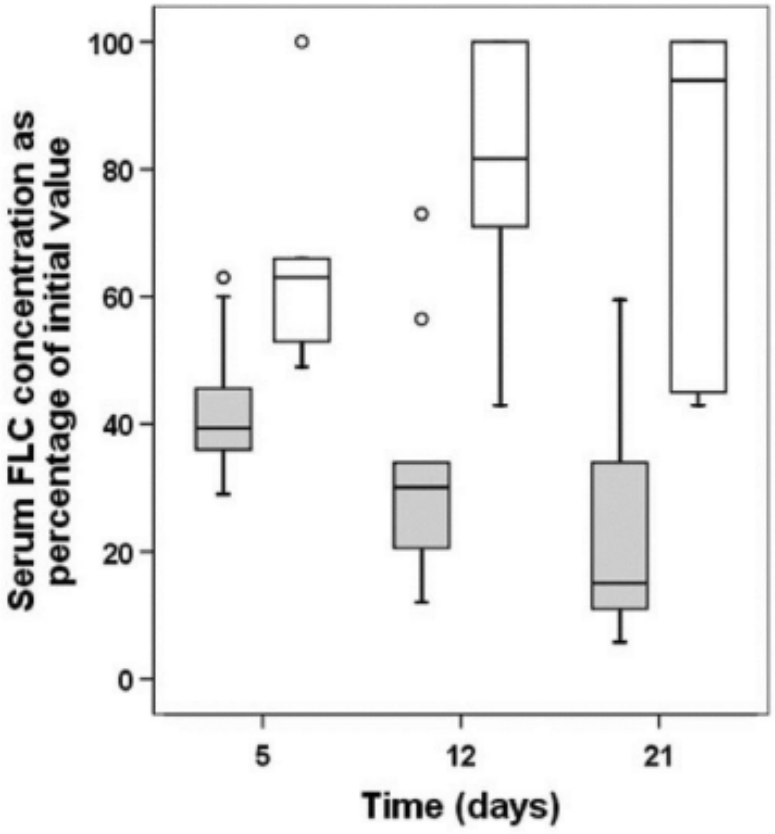


Figure 1 | The distribution of monoclonal proteins amongst patients with multiple myeloma and renal failure.

- Leung KI 2008; 73: 1282
- 40 patients with MM and renal failure
- TPE
- Positive biopsies with wide range of SFLC levels
- FLC reduction and renal recovery correlated only in those with cast nephropathy

Multiple Myeloma: Benefit of Concurrent Chemotherapy



- Hutchison CJASN 2009; 4: 745
- 19 patients, dialysis-dependent
- Open label, high cut-off hemodialysis technology
- Standard chemotherapy

CONCLUSIONS

- The clinical application of therapeutic plasma exchange to patients with kidney disease continues to evolve
- Likely to be a growing number of potential target molecules
- Need for more information about the relationship between target removal and clinical outcomes
- TPE likely to be coupled to other therapies
- Apheresis remains a safe but crude technology