# Η «Τεχνολογία των Μεθόδων Αιμαφαίρεσης»

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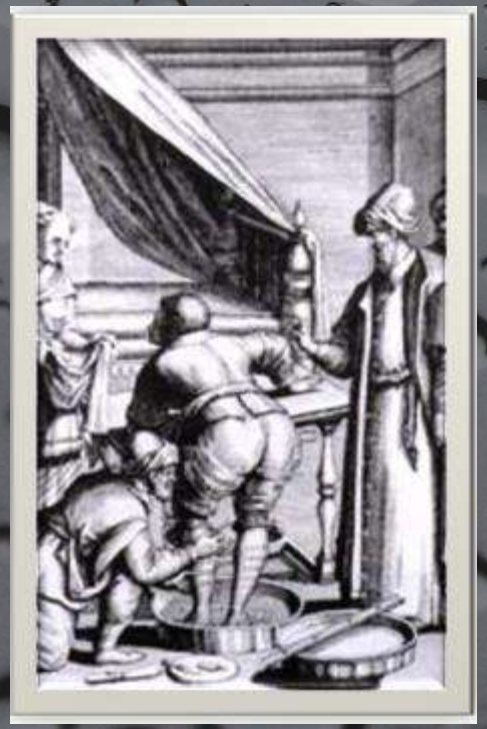
athens-NEPHROLOGY

# Apheresis History



# 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

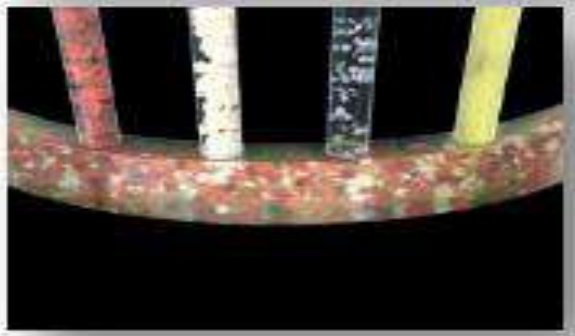
# Apheresis

- From the Greek - “to take away”
- Blood separation
  - Donor apheresis
  - Therapeutic apheresis

# Principles of Blood Separation

- Filtration

- Centrifugation

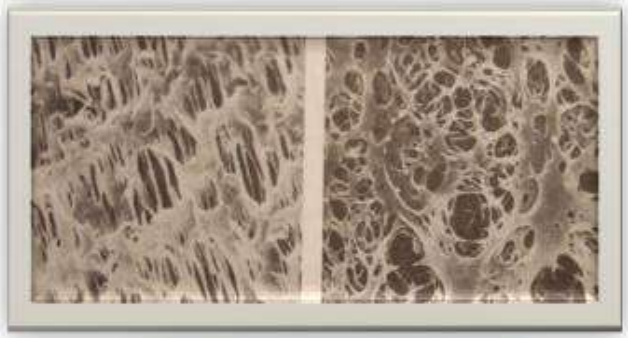


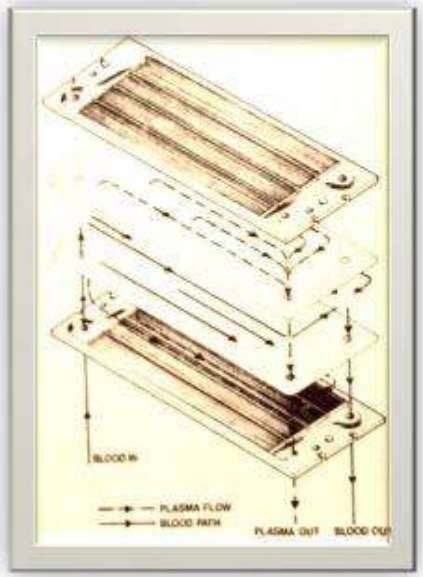
- Combined centrifugation and filtration

# Membrane Separation

- Blood is pumped through a membrane with pores allowing plasma to pass through whilst retaining blood cells.
- Available as a hollow fiber membrane (older devices used parallel-plate membranes)
- Pore diameter for plasma separation: 0.2 to 0.6µm.
- A number of parameters need to be closely controlled

# Membrane Blood Separation






## Detail of Membrane Separation

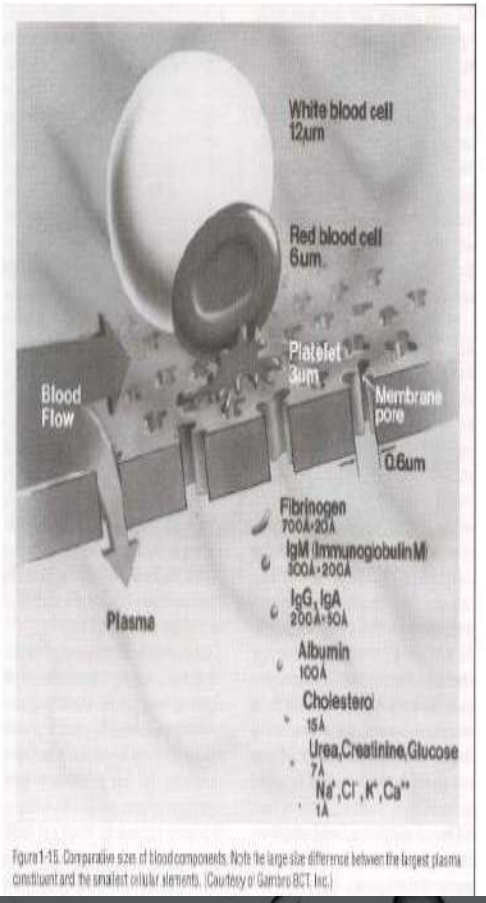
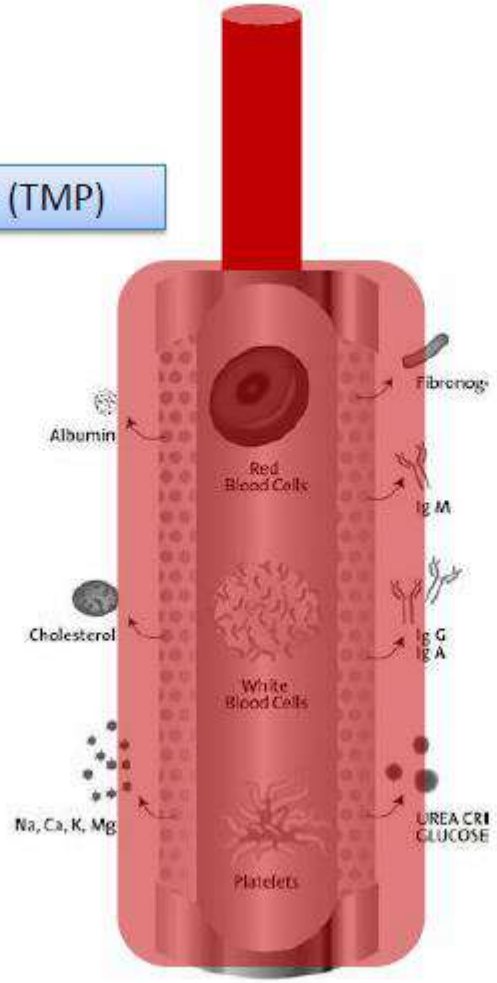
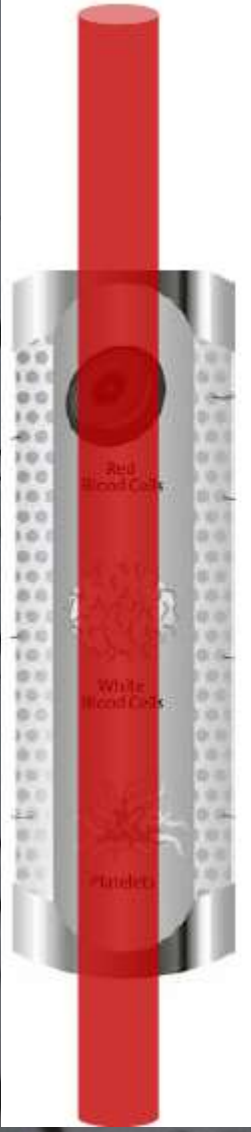



Figure 1-15. Comparative size of blood components. Note the large size difference between the largest plasma constituent and the smallest cellular elements. (Courtesy of Gambro BCT, Inc.)

Trans Membrane Pressure (TMP)

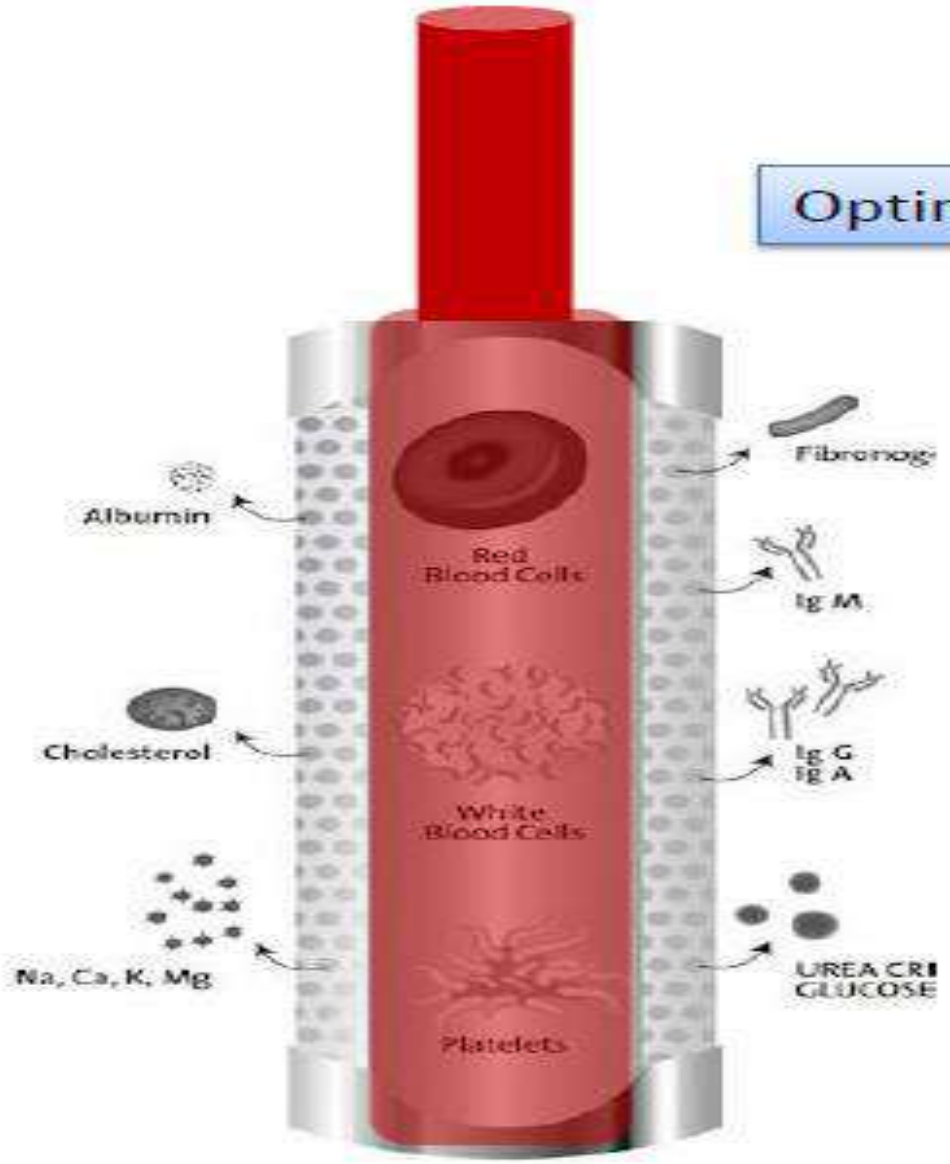



Too High = Hemolysis




TMP Too Low = No Separation

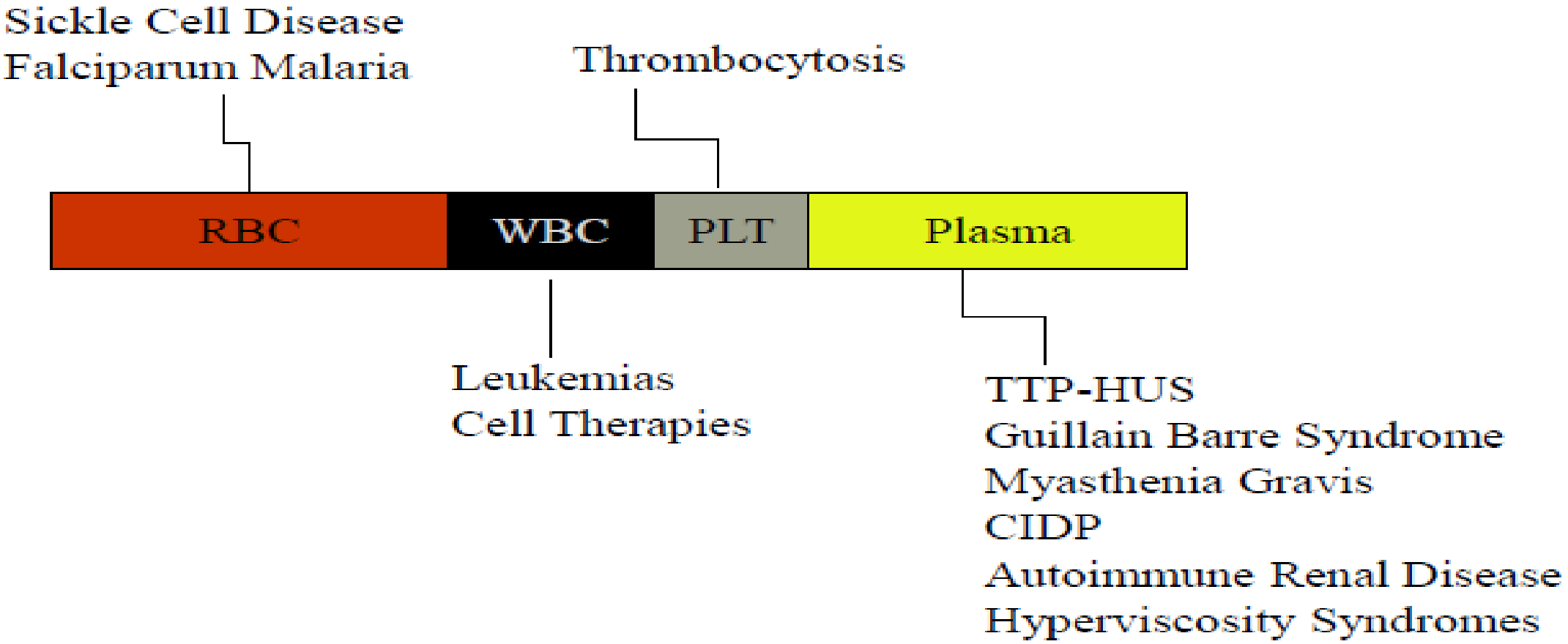
## Optimal TMP = Good Separation



# Centrifugation vs. Filtration Apheresis

| | Centrifugation Apheresis | Filtration Apheresis |
|---|---|---|
| Blood Flow | 10 – 100 ml/min | 150 ml/min |
| Efficiency of Plasma Removal | 60 – 65% | 30% |
| | | |
| | | |

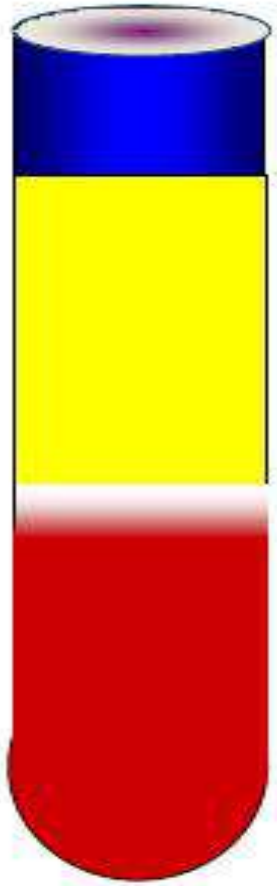
# Apheresis in Clinical Practice and Blood Banking

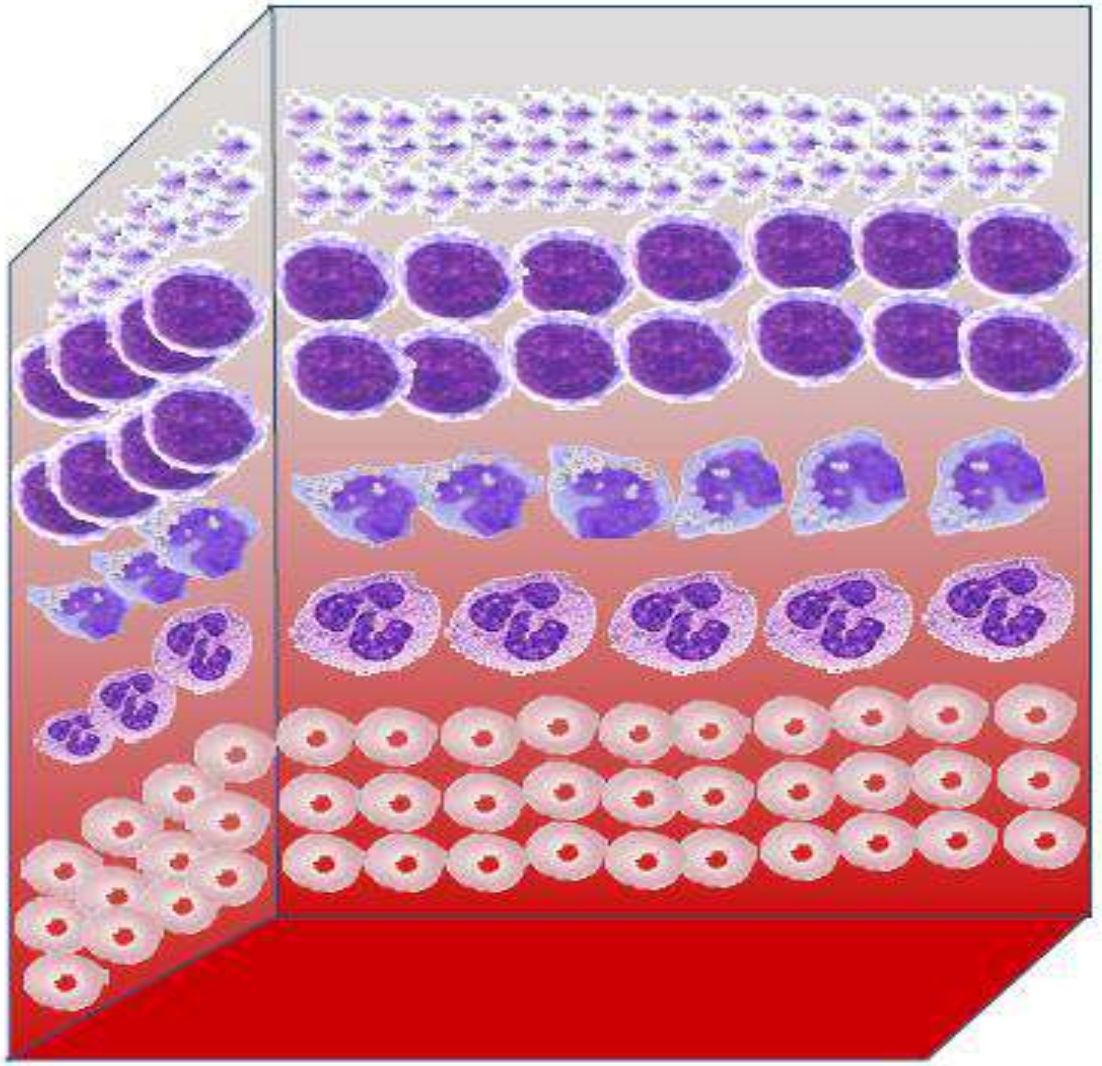


# Centrifugal Separation

- Based on the different **specific gravity** of the blood components.
- In some instruments, also based on the **cellular size** (Elutriation).
- All apheresis devices need to be able to control the **Separation Factor**.

# Separation by Specific Gravity





**Platelets**
(1040)

**Lymphocytes**
(1050-1061)

**Monocytes**
(1065 - 1069)

**Granulocyte**
(1087 - 1092)

**RBC**

# Centrifugal Separation

## Discontinuous

- Blood is processed in batches of a size that can be tolerated by the subject.
- Once the separation of that blood is completed, the separation chamber must be emptied to repeat the process (cycle) again.
- Large extracorporeal volume (ECV).
- Pediatric tubing sets (with smaller ECV) must be used with small patients.

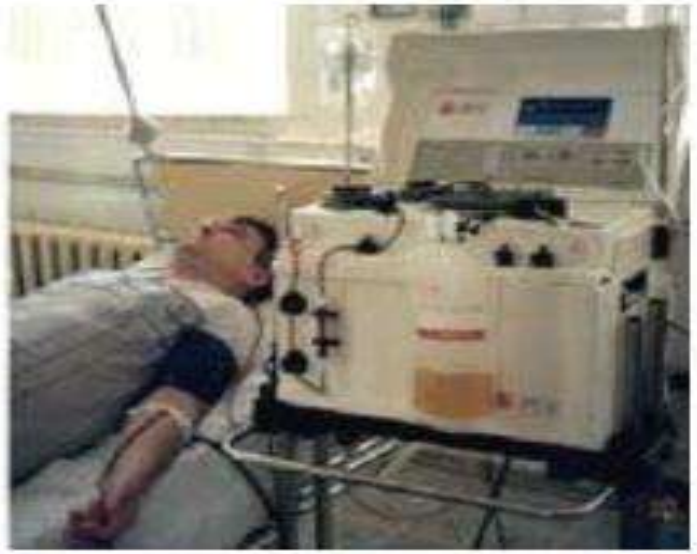
## Continuous

- Blood is processed and separated in a continuous way.
- Once the tubing set is primed, the separation chamber is not emptied till the end of the process.
- Medium – small ECV.
- No pediatric tubing sets are necessary; instead, a blood prime is performed with smaller patients.

# Centrifugal Apheresis Systems

- Intermittent / Discontinuous Flow
  - **Haemonetics:** PCS-2, MCS+ 8150 and 9000, Cymbal
  - Therakos UVAR-XTS
- Continuous Flow
  - **TerumoBCT**: COBE Spectra, Trima, Trima Accel, Spectra Optia
  - **Fenwal (Fresenius Kabi)**: Amicus, Alyx.
  - **Fresenius Kabi**: AS 104, Com.Tec

# Haemonetics Devices

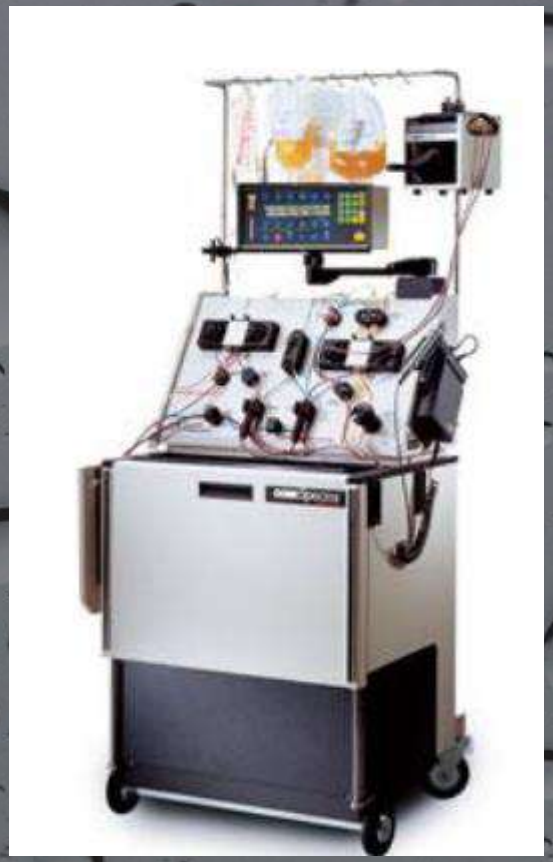


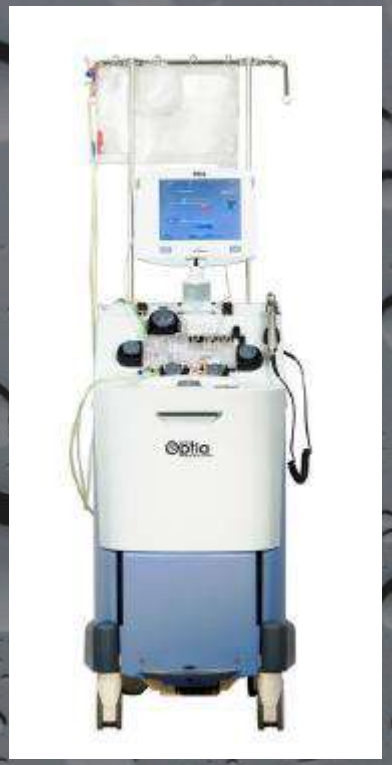
PCS 2

MCS+

Cymbal

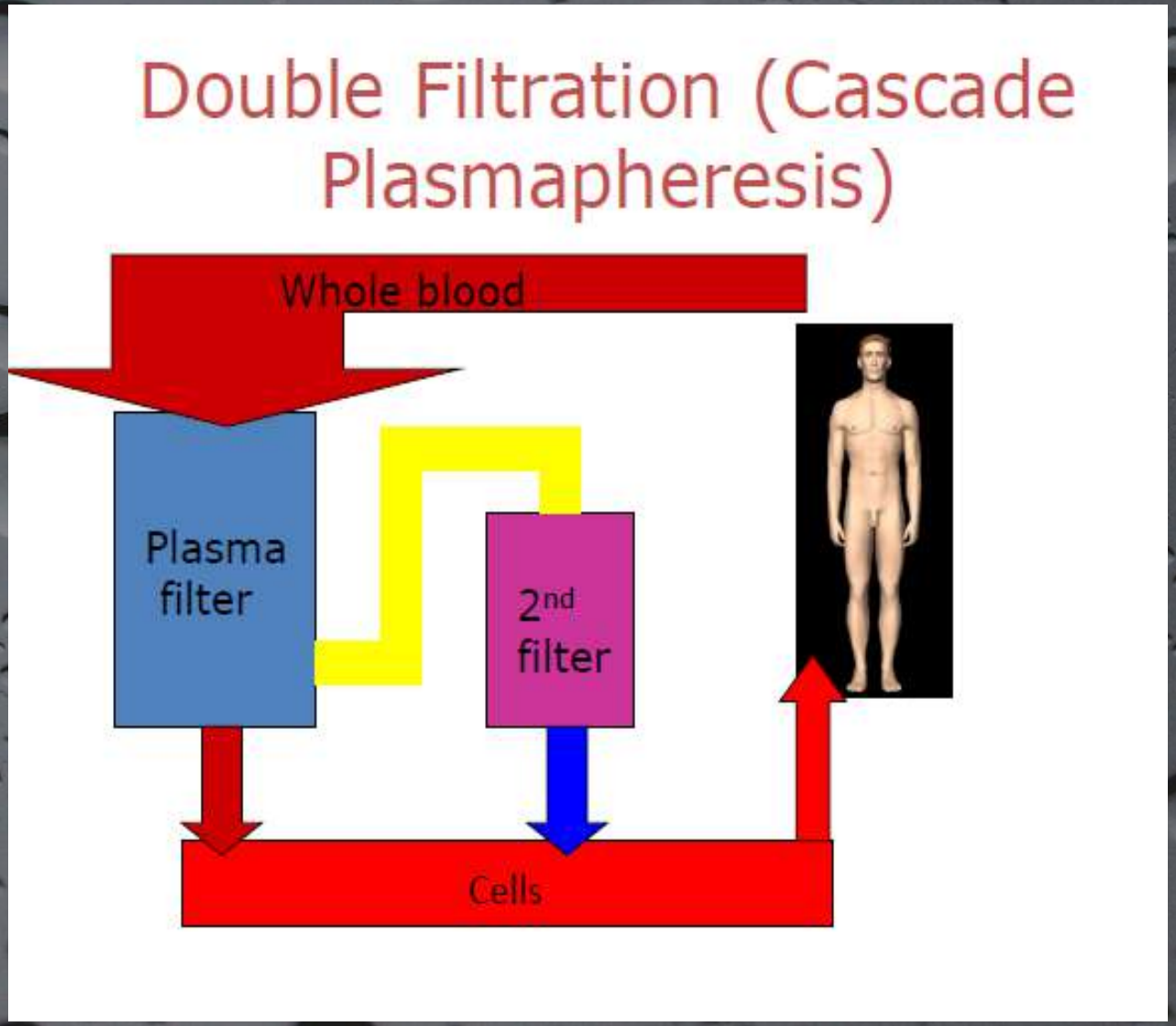
Courtesy of Haemonetics Corp.



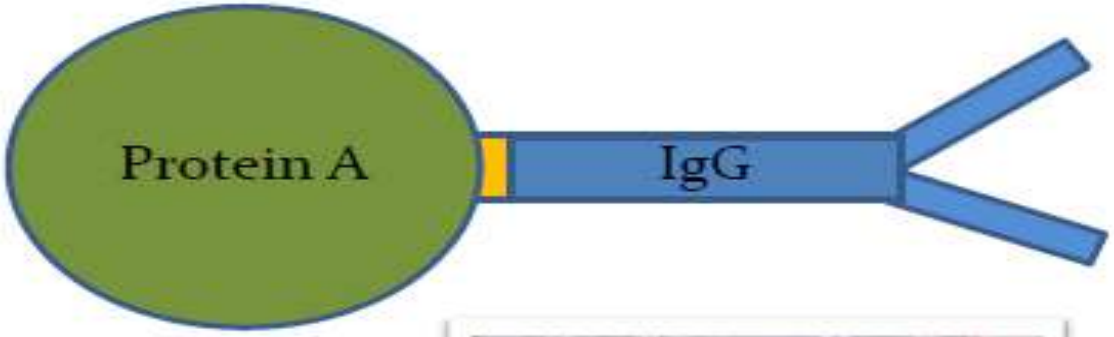

Optia

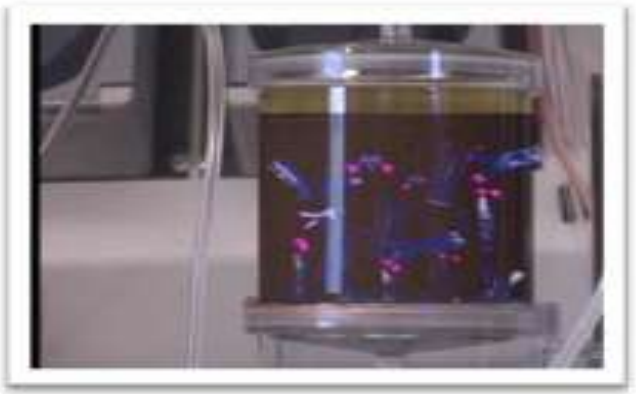
# Double Filtration (Cascade Plasmapheresis)

- First filter
  - Separates plasma from whole blood
- Second filter
  - Removes a specific plasma component



# Specific Removal of Substances from Plasma
## *Immunoadsorption*




- **Liposorber LA-15** column
- Plasma Separator **MA-01**
- *Plasma is removed with a hollow fiber membrane device (MA-01).*
- *Plasma is subsequently filtered with the LA-15 column that keeps the LDL cholesterol by selective adsorption, then returned to the patient.*
- *Most HDL cholesterol is returned to the patient in the filtered plasma.*

# H.E.L.P. System

- *"Heparin-induced Extracorporeal LDL Precipitation"*
- *Plasma is removed by membrane filtration.*
- *Heparin in an acid Acetate solution (pH 4.85) is added, causing a selective precipitation of LDL cholesterol.*
- *The LDL precipitate is removed by filtration and finally, plasma is returned to the patient after being ultra-filtered and dialyzed.*

# The Therakos® Photopheresis Process

## Photopheresis Process

### WBC Collection

1. Whole blood drawn and separated via centrifugation.
2. Plasma and RBCs immediately returned to patient.
3. Leukocyte (WBC) enriched Buffy Coat isolated and collected.
4. Methoxsalen added to Buffy Coat and exposed to UVA light.
5. Photoactivated Buffy Coat returned to patient.

### Re-Infusion

Therakos® Photopheresis delivers photopheresis therapy in single, integrated device

# 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)

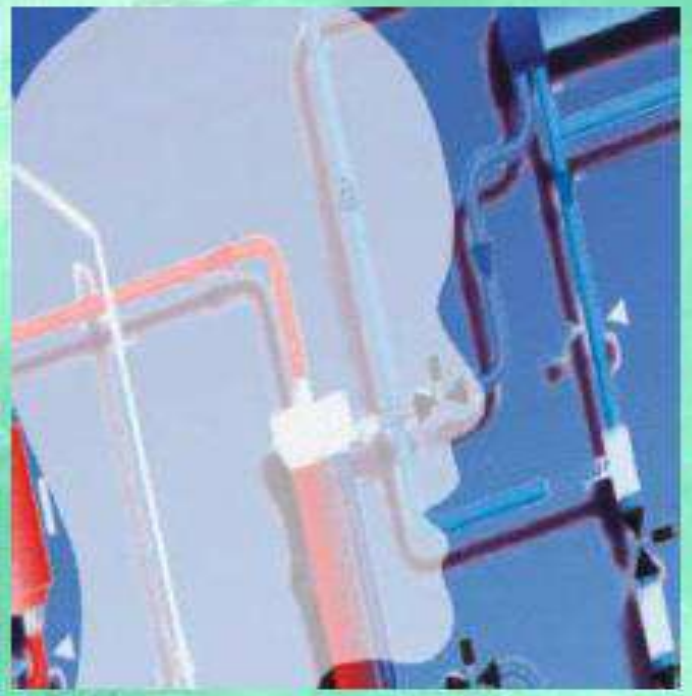
Only ~1/3 of the therapies currently marketed (~13) required and received regulatory approval. In contrast, an estimated 90% of the therapies in development are "products" requiring pre-market approval.

* 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.



# 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

# 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).

# 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 |

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*]

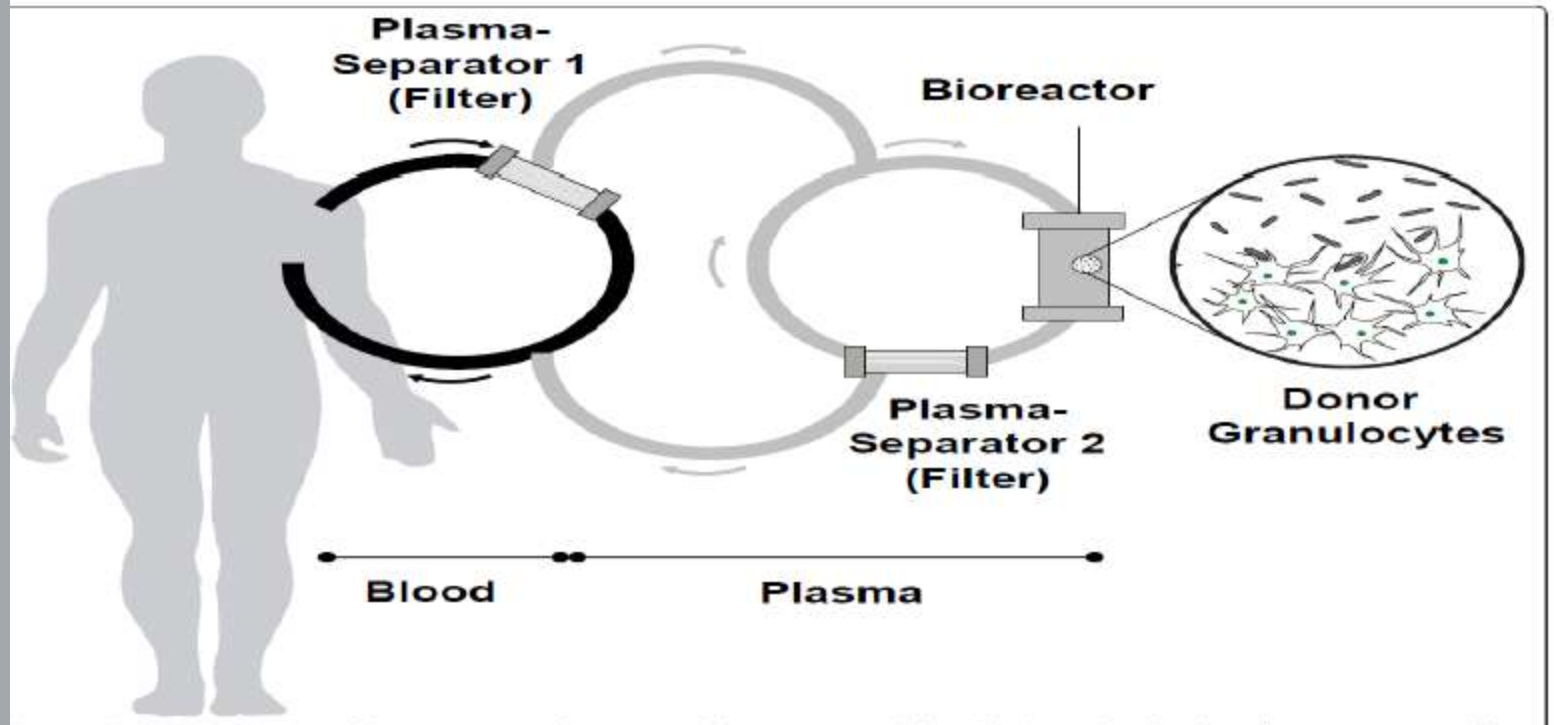
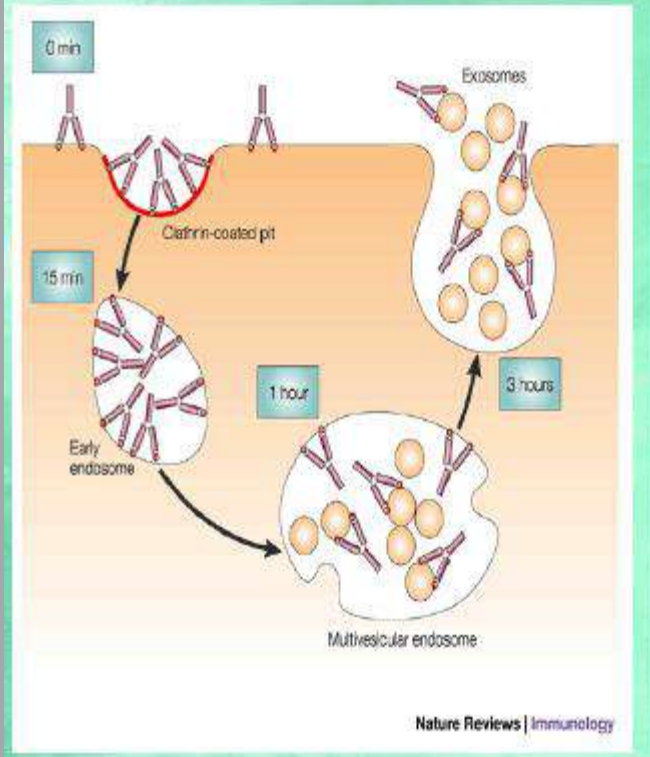



**Figure 2 Schematic drawing of the extracorporeal treatment**. Plasma is separated from blood, transferred to the cell-compartment, and then returned to the patient.

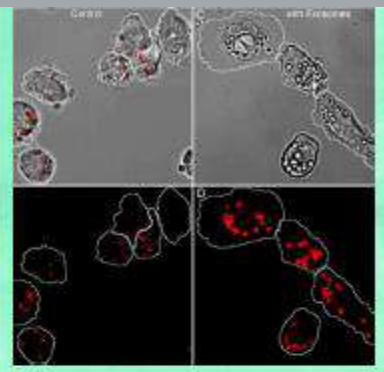


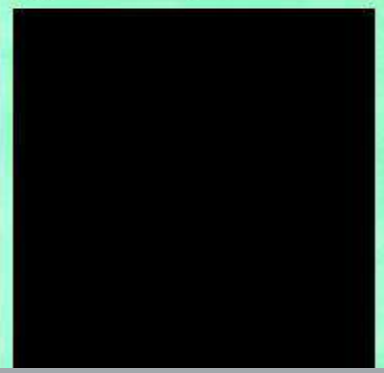

# Exosomes

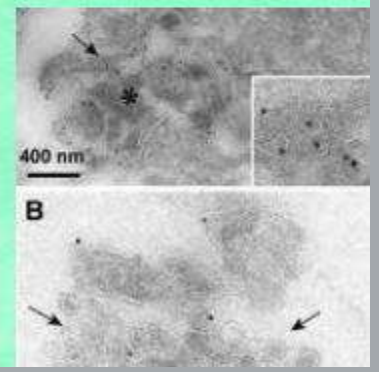
- A specific subset of membrane-bounded vesicles formed intracellularly within vesicular endosomes.
- Released into the extracellular environment by many cells from different tissues and organs.
- Exist in a wide range of biological fluids, including blood and urine.
- Between 30 and 100 nm in diameter.

# 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.

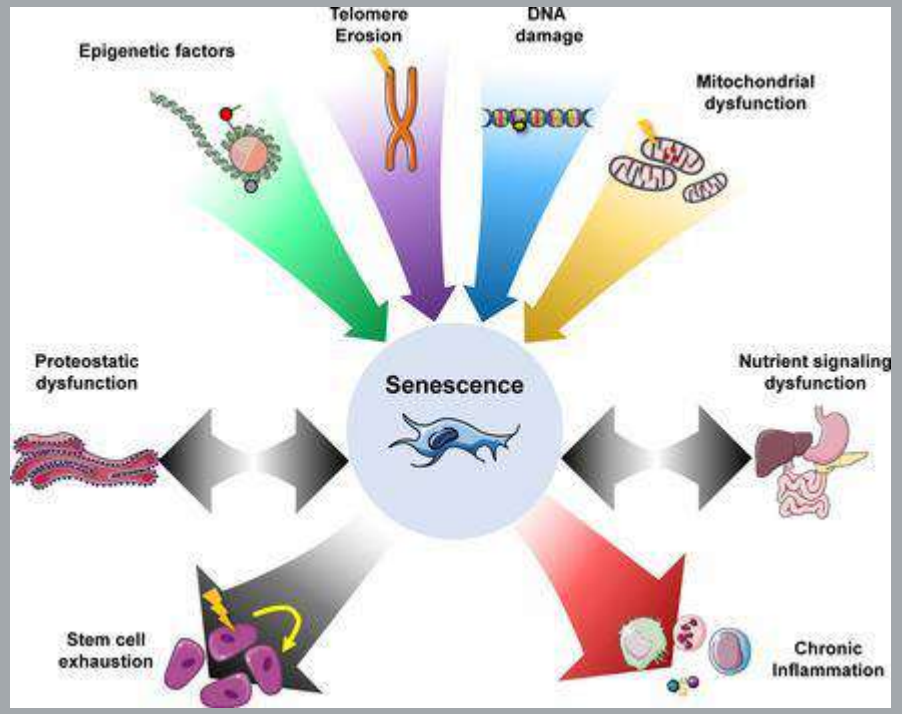
**In studies on aging, the primary objective is to improve the quality of life, not necessarily with the goal of extending life for a much longer period, but by combating disease.**

01 Reversing genome damage caused by aging

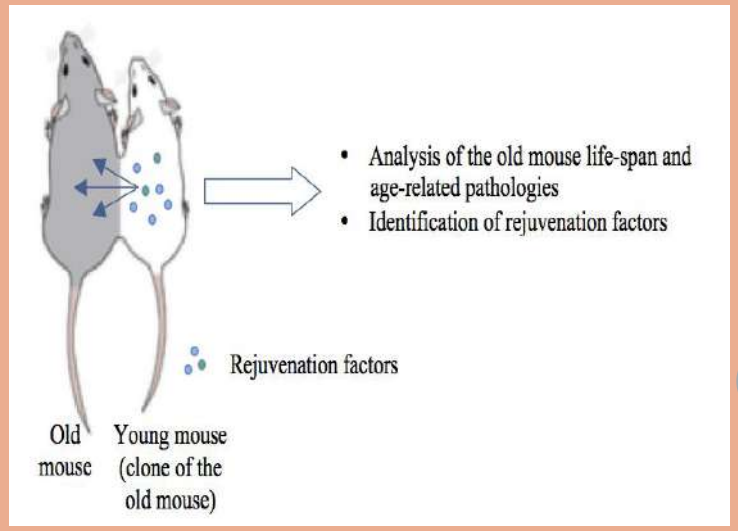
02 Diet, and environmental factors

03 researching new tissue and blood

04 Reprogramming senescent cells


Telomere Erosion
DNA damage
Epigenetic factors
Mitochondrial dysfunction
Proteostatic dysfunction
Senescence
Nutrient signaling dysfunction
Stem cell exhaustion
Chronic Inflammation


ELSEVIER
Biochemical and Biophysical Research Communications
journal homepage: www.elsevier.com/locate/ybbrc
The NADase CD38 is induced by factors secreted from senescent cells providing a potential link between senescence and age-related cellular NAD+ decline
Claudia Chini, Kelly A. Hogan, Gina M. Warner, Mariana G. Tarragó, Thaís R. Peclat, Tamar Tchkonia, James L. Kirkland, Eduardo Chini
ARTICLE INFO
ABSTRACT
1. Introduction




The general hypothesis is that young molecules help repair damage and detoxify old mice. Using parabiosis with blood exchange between old and young mice that new brain cells form in the hippocampus of young mice with young blood, but when young mice received old blood, brain cell formation slows demonstrating that old blood in mice contains substances that can cause health decline

**WHILE MORE RESULTS MAY BE FORTHCOMING FROM THESE TRIALS IN ALZHEIMER'S DISEASE PATIENTS, A MAJOR QUESTION STILL EXISTS: IS IT MORE IMPORTANT TO REMOVE THE "BAD" MOLECULES OR REPLACE WITH THE "GOOD"? ARE CRYOGLOBULINS ONE OF THE "BAD" MOLECULES?**

JAAM

ANTI-AGING MEDICINE

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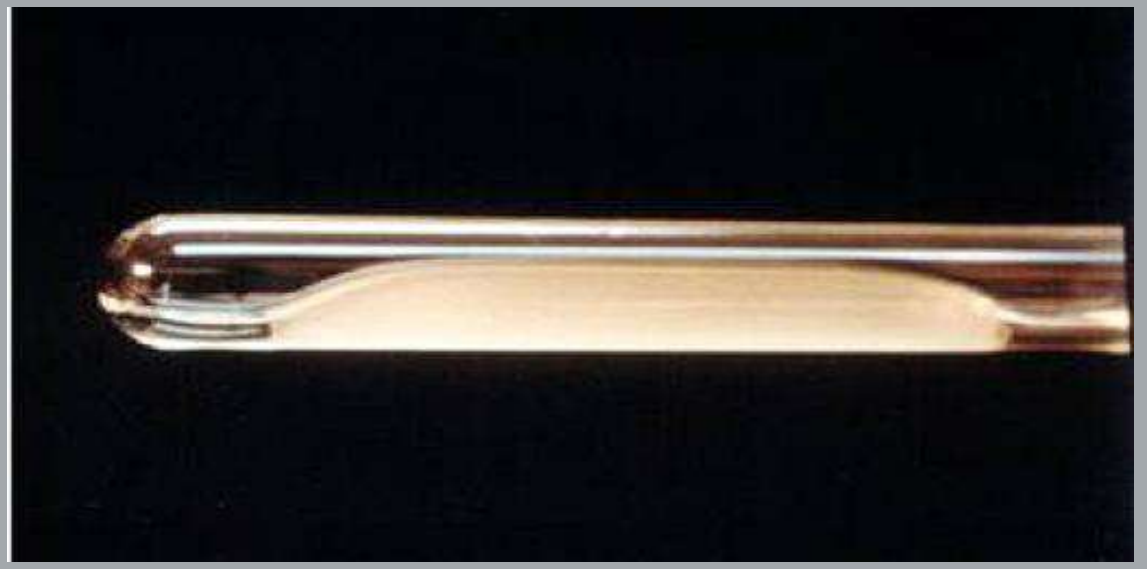
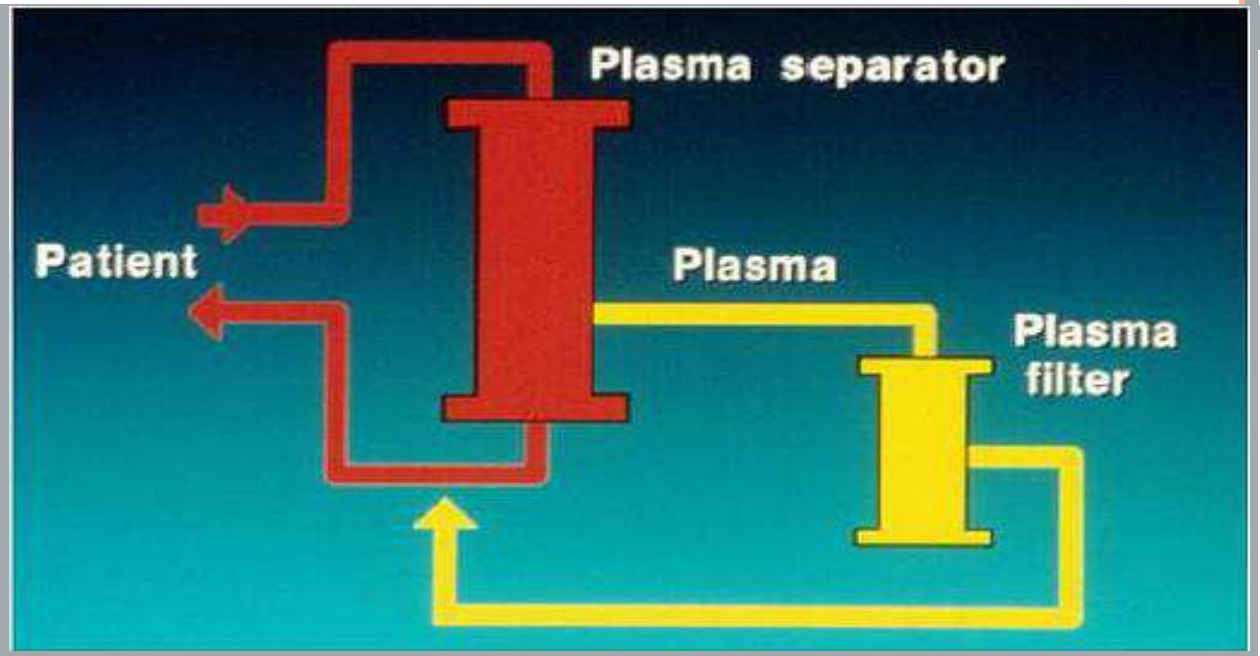
*Review Article*

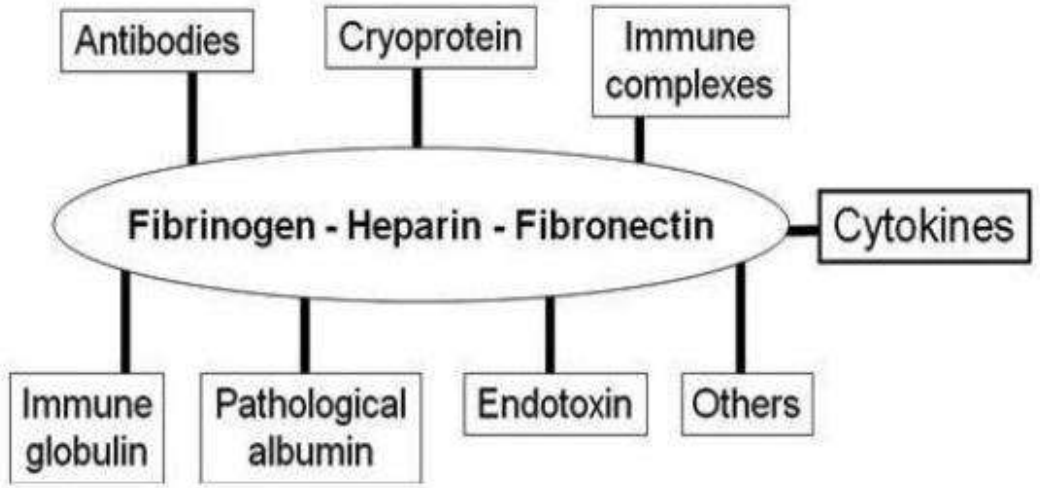
# Can an Apheresis Therapy become an Effective Method for Anti-Aging Medicine?

Hiroshi Miyamoto, Yukihiko Nosé

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





# CAN THERAPEUTIC APHERESIS SUPPORT OUR BIOLOGY TO IMPROVE LONGEVITY OR DECREASE MORBIDITY AND MORTALITY?

**100** diseases of varying types affecting the major organ systems are associated with abnormal or high concentrations of macromolecular proteins and other chemistries in plasma that would lend themselves to therapeutic apheresis.

Many diseases state (metabolic and immunologic) exhibit abnormalities of higher molecular weight solutes or protein-bound solutes.
The identification of the most appropriate and cost-effective separation/removal means is important.
It is also critically important that the effectiveness and long term safety of apheresis be tested in randomized clinical trials.

***Plasma membrane filtration technologies***

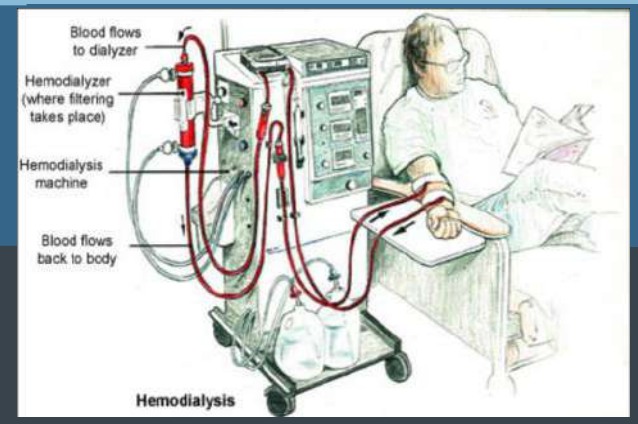
Cryofiltration

Thermofiltration

Cryoprecipitable proteins are suppressive to the immunological system such as inhibiting the blastogenesis of normal mononuclear cells and inhibiting neutrophil phagocytosis in a concentration-dependent manner

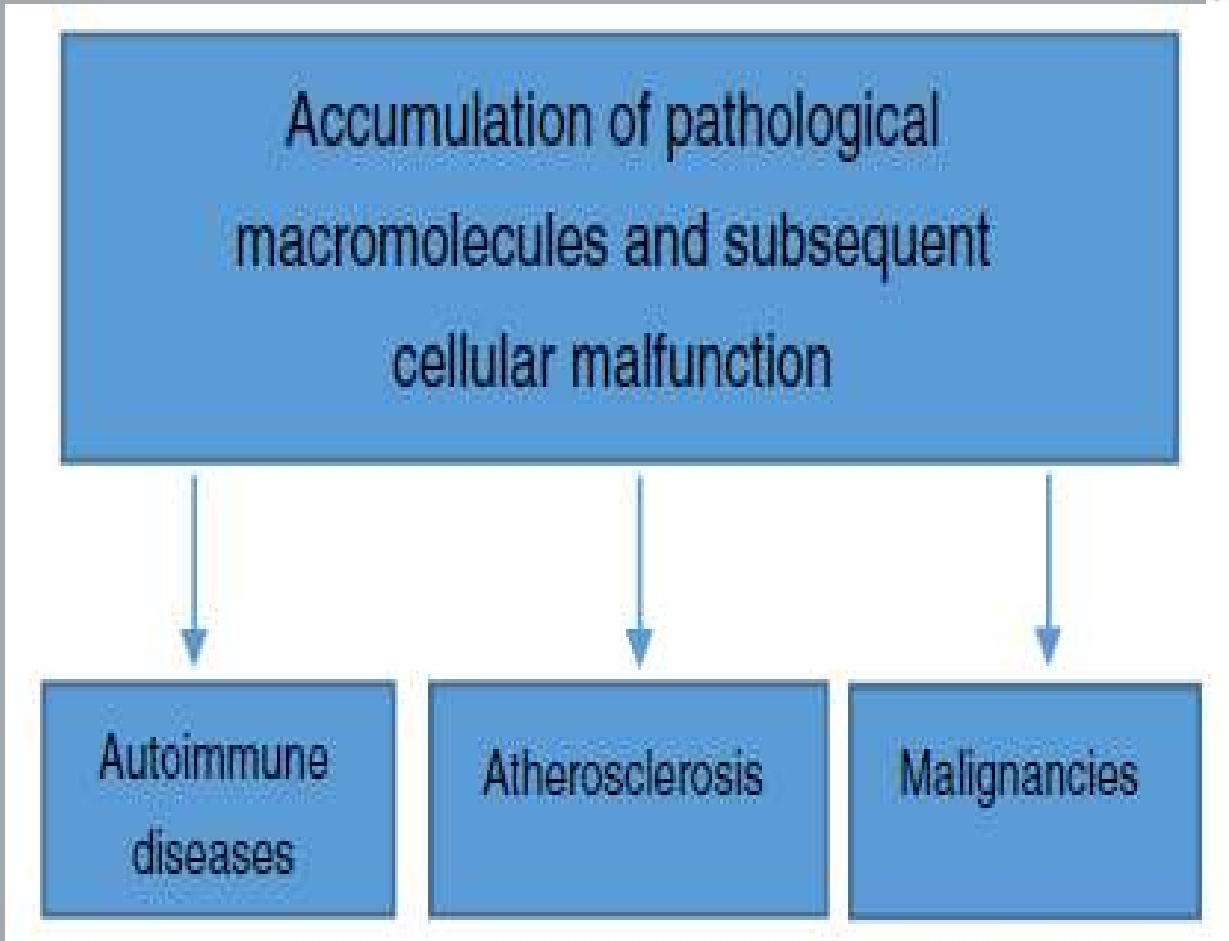
Cryoprecipitable proteins had a suppressive effect on normal lymphocyte proliferation

Patient plasmas with cryoglobulinemia were inhibitory to normal granulocyte chemotaxis .



Therapeutic Apheresis and Dialysis

*Therapeutic Apheresis and Dialysis* 2018; 22(4):312–316
doi: 10.1111/1744-9987.12706


Editorial

# Aging, Disease, and Therapeutic Apheresis

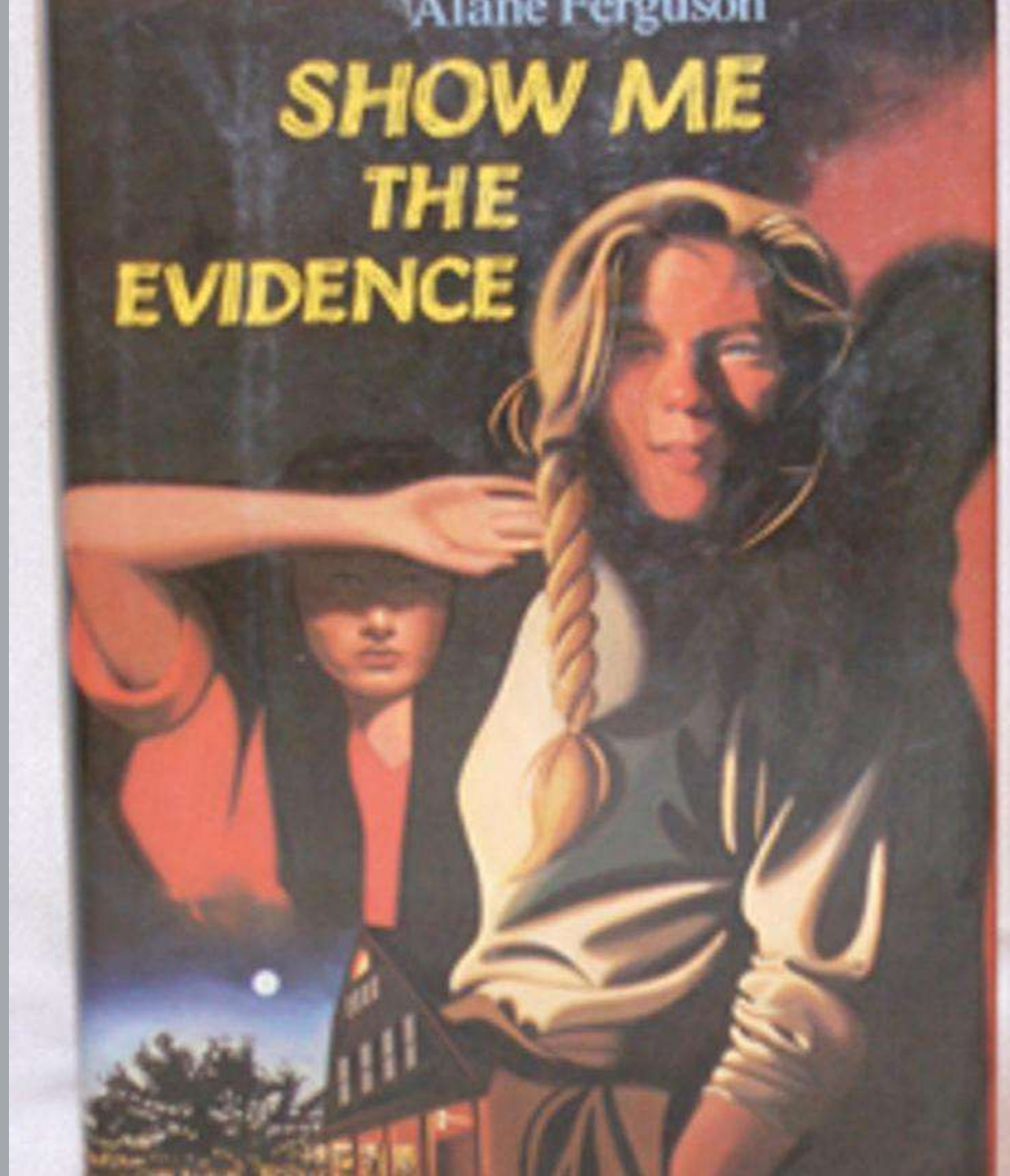
## X-Effect Hypothesis

ing of the "biological smoke", those abnormally high concentration and toxic macromolecules, can activate the biological system to return to normalcy and allow pharmaceutical agents to work more effectively. The normal detoxification processes are lacking in disease states and in aging. "Factors"

# Conclusion

Generally, aging process should be a physiological phenomenon. However, if its speed is too fast or too strong, it might produce a disease. If this hypothesis would be true, "apheresis therapy", which could remove pathological molecules accumulated excessively inside of the living old body and could return them to the normal level, should be an effective interventional method as an Anti-Aging medical therapy. For such purpose, the cryofiltration or cryoaggregate filtration should be an ideal therapeutic artificial organ called "Juzo" or to prevent aging processes of patients. We should proceed to investigate and expand the possibility of "apheresis therapy" as an active Anti-Aging medical therapy in the future.

Various types of apheresis procedures have been performed on a clinical basis for many years, but the number of patients and types of diseases treated have risen significantly in the last 5 years. This increase is partially due to increased understanding of the disease and partially due to engineering advances in equipment technologies. By almost any standard, treatment by apheresis is still in relatively early stages of development—there are no ideal protocols based on a thorough understanding of reasons for its efficacy. Nevertheless, there is an increasing flow of clinical data, sometimes describing dramatic patient improvement, supporting the view that apheresis is a rapidly emerging technology with significant promise (117). Such evidence of treatment effec-



1981

L. F. Rothschild, Unterberg, Towbin, "Therapeutic Apheresis," unpublished, New York, Sept. 11, 1981.

*Effectiveness is the health benefit as measured under average conditions of use. *Efficacy* is the health benefit as measured under controlled conditions such as those in a randomized clinical trial (104).

**Outcomes**

What is being measured?

**Patients Selection**

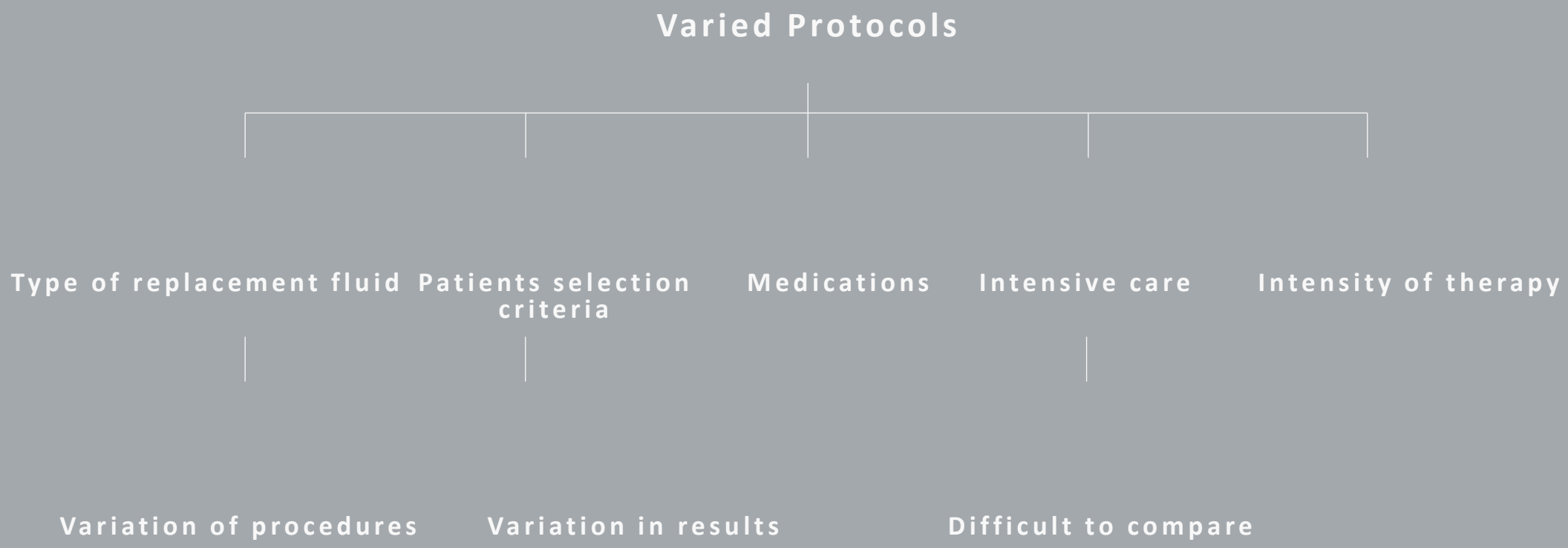
Which subject population are involved in the research?

**Research Desigh**

What is being compared?

**Treatment Desigh**

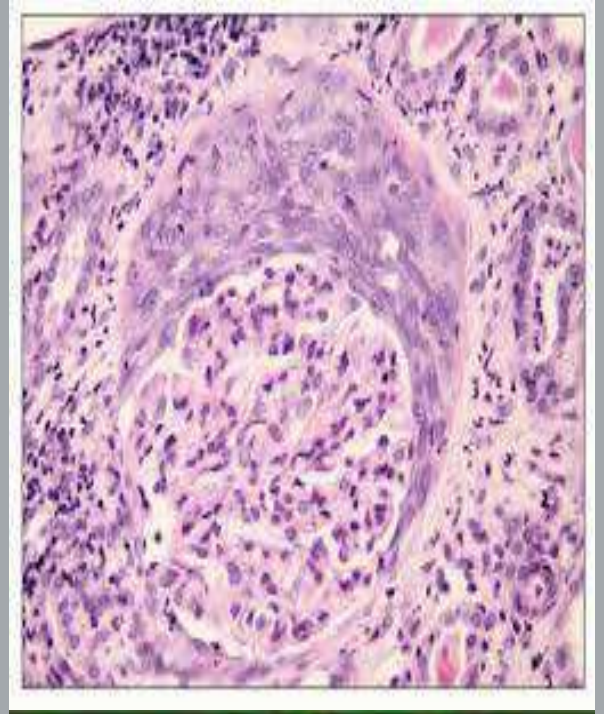
What is being tested?

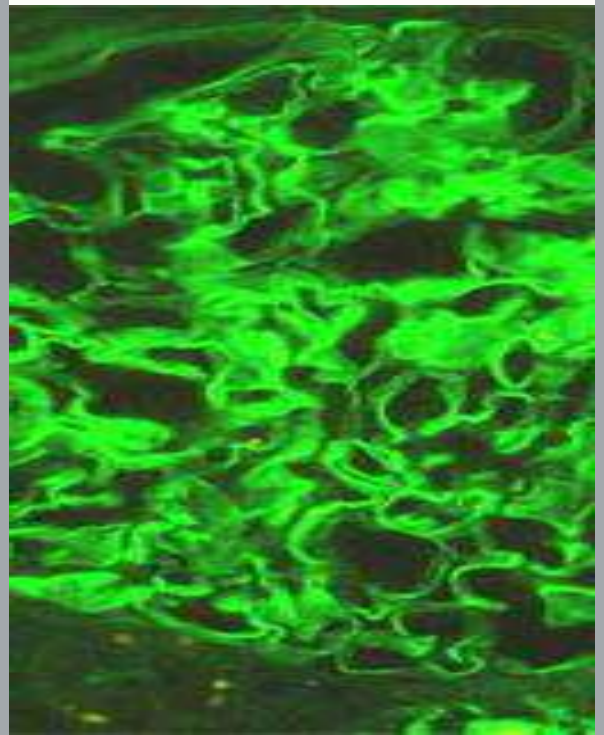

Varied Protocols
Type of replacement fluid
Patients selection criteria
Medications
Intensive care
Intensity of therapy
Variation of procedures
Variation in results
Difficult to compare

Even if standardized protocols could be developed, however, it maybe difficult or undesirable to administer them.

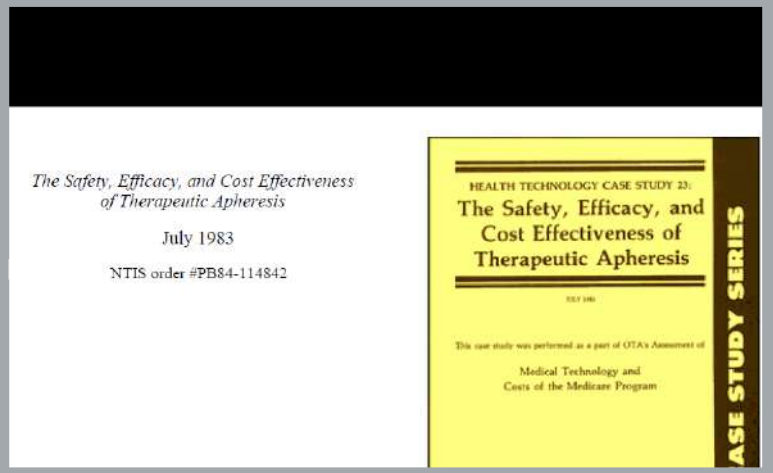
This is particularly problematic
if, for research purposes, assignment to
one group or another is required.

Another obvious ethical concern is whether treatment can be denied patients in near-fatal, disease states in which apheresis has served as the treatment of last resort.





A last treatment design problem has to do with possible placebo effects of the therapy itself.



# Patient Selection Criteria

Perhaps the most severe sampling problem in apheresis studies stems from the use of the therapy as a last resort, i.e., for the “worst cases.”

**Eligibility for treatment**

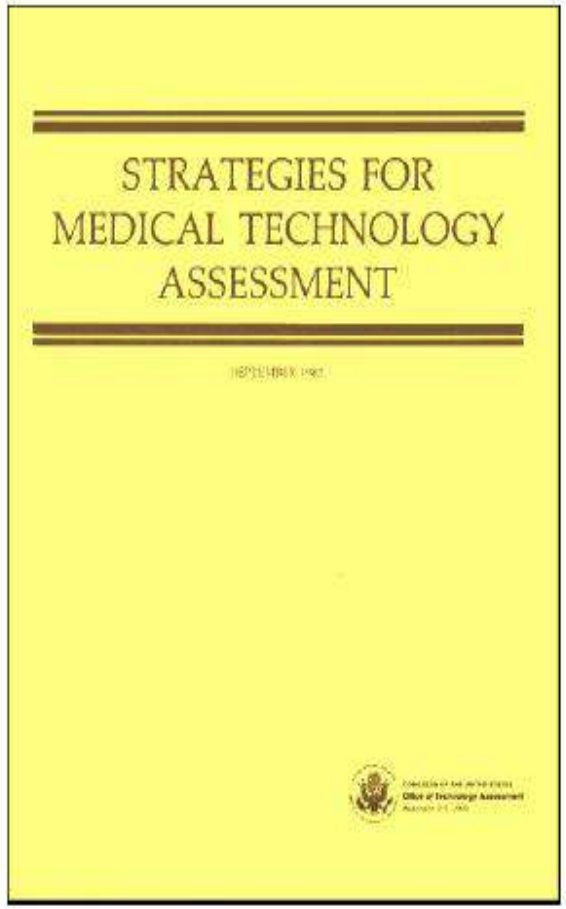
**Selection for participation in research**

**Availability for follow-up research**

*Strategies for Medical Technology Assessment*

September 1982

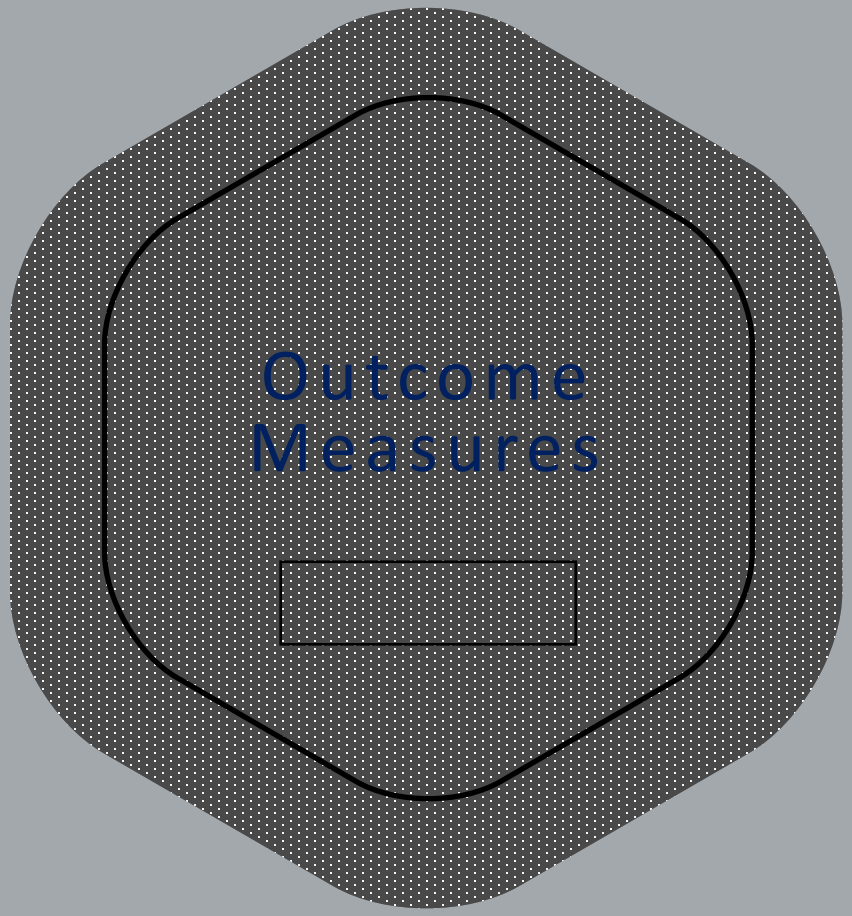
NTIS order #PB83-113274




Statistical regression arises when patients are chosen because of their extreme value on a laboratory test or other measure relevant to treatments.

Investigators have found that subjects with high pretreatment measures tend to have lower scores after the treatment-when, in fact, no change has taken place.

This is the statistical regression effect and it can deceive clinicians into believing that apheresis has been effective when it really has not.




A recurring critical issue in any attempt to analyze the effectiveness of a medical technology is the selection **of appropriate endpoints for evaluating the success or failure of the intervention.**

**The way in which outcomes of apheresis therapies are measured significantly affects interpretation of apheresis therapy research.**

Interpretation of clinical improvement for many diseases treated by apheresis is further confounded by the variability produced by a basic “remitting exacerbating” nature of the illness.

- Rheumatoid arthritis
- SLE
- myasthenia gravis
- Guillain-Barré syndrome

Finally, outcome measures probably suffer from the lack of systematic documentation of adverse effects.
As a new technology is developed, used, and reported, researchers and practitioners may also champion the technology for a variety of personal and professional reasons .

Apheresis therapy reporting may have been biased by the tendency to report the more successful uses of the new therapy.

# 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