Apheresis Research: Challenges and Opportunities

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President, American Society for Apheresis

• Challenges
  – Funding
  – Relative rarity of many indications

• Opportunities (are many!)
  – Improved clinical data on indication and outcome
  – Mechanism of efficacy
  – Expanded indications
  – Novel approaches
  – Cellular therapy
Growth of Apheresis Research

- Summarize current state of the literature for a variety of disease states and reported clinical uses of apheresis based treatments
- Updated every 3 years
- Used GRADE methodology

Using the ASFA Guidelines to identify clinical knowledge gaps

- The Grade letter gives quality of current evidence
  - A: High quality evidence
    - RCTs without important limitations or overwhelming evidence from observational studies
  - B: Moderate quality evidence
    - RCTs with important limitations (inconsistent results, methodological flaws, indirect, or imprecise) or exceptionally strong evidence from observational studies
  - C: Low quality evidence
    - Observational studies or case series
- Indications/diseases with grade C are ripe for investigation
- Guidelines list numbers of subjects and types of studies which is also helpful
**Example:** TPE in ANCA-associated rapidly progressive glomerulonephritis

<table>
<thead>
<tr>
<th>Condition</th>
<th>ASFA Category</th>
<th>GRADE</th>
</tr>
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<tr>
<td>Dialysis dependent</td>
<td>I</td>
<td>1A</td>
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<tr>
<td>DAH</td>
<td>I</td>
<td>1C</td>
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<tr>
<td>Dialysis independent</td>
<td>III</td>
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</table>
State of the Science in Apheresis

- The National Heart Lung and Blood Institute (NHLBI) hosted a two-day state of the science symposium on therapeutic apheresis in November, 2012.
  - ASFA involved with many planners/speakers being ASFA members
- Discussed Current state of the science and knowledge gaps in apheresis medicine related to:
  - cardiovascular diseases
  - hematological and oncological diseases,
  - infectious diseases and sepsis
  - renal diseases
  - neurological diseases
- Proposed studies/trials to fill gaps

- **RED CELL EXCHANGE (RCE) FOR THE PREVENTION AND TREATMENT OF PAIN IN PATIENTS WITH SICKLE CELL DISEASE (SCD)**
  - Efficacy in pain crisis
  - Simple vs RBCx
  - Issues of iron and alloimmunization

- **USE OF TPE IN PATIENTS WITH ANTI-PF4/HEPARIN ANTIBODIES**
  - Treatment of acute HIT/T
  - Antibody reduction in sub-acute HIT

- **LEUKOCYTAPHERESIS vs. CYTOREDUCTIVE CHEMOTHERAPY IN THE URGENT MANAGEMENT OF ADULT HYPERLEUKOCYTIC ACUTE MYELOID LEUKEMIA**
  - Head to head comparison needed despite broad use of leukoreduction in these patients
IMMUNOLOGIC PREDICTORS OF LEFT VENTRICULAR FUNCTION FOLLOWING PROTEIN A IMMUNOADSORPTION FOR THE TREATMENT OF ACUTE MYOCARDITIS

- Focus is on antibody mediated cardiomyopathy
- What markers can be used to predict and/or monitor
  - Antibodies
  - Immune profiles
  - Other
- TPE also of interest

USING THERAPEUTIC LIPOPROTEIN-APHERESIS AS AN ADDITIONAL THERAPY IN DIABETIC PATIENTS WITH PERIPHERAL VASCULAR DISEASE

- Randomized trial for PVD specifically in diabetic patients
- Could also study PBD more broadly

Specific removal of pathogenic substances


The Top Clinical Trial Opportunities in Therapeutic Apheresis and Neurology

- Therapeutic Plasma Exchange in Neuromyelitis Optica
  - \(\alpha\)-aquaporin-4 in 85%
  - RTC needed
    - Acute disease
    - Maintenance TPE
- TPE Versus IVIG in Severe Acute Disseminated Encephalomyelitis
  - RTC, but rare
- TPE Versus IVIG in Anti-NMDA Encephalitis (paraneoplastic)
  - RTC, but rare
- Rare Neurologic Disease Registry and Biorepository
  - \(\alpha\)-muscle specific kinase associated Myasthenia Gravis
- Extracorporeal Photopheresis in Relapsing Remitting Multiple Sclerosis
  - Rational: T-cell involvement

ASFA “Consensus Conference in 2015
   - Full day conference with presentations, discussion
     and addressing of pre-written questions

Discussion on use of Red Blood Cell Exchange in SCD for a variety of indications

Identification of knowledge Gaps and areas in need of further research

Gaps in SCD knowledge relevant to apheresis

- Lack of high quality evidence for simple or exchange transfusion (RBCx) in priapism in SCD
  - ASFA Cat III, Grade 2C

- Lack of randomized, prospective trials (and data for adults in general) for the use of RBCx in Acute Chest Syndrome
  - ASFA Cat II, Grade 1C

- Randomized controlled trial of standard RBCx vs RBCx with isovolemic hemodilution
  - Clearly define relative risks and benefits head to head

Journal of Clinical Apheresis 2016;
DOI: 10.1002/jca.21511
ASFA Research Committee

• Created in 2010 as a subcommittee of the applications committee
  – Address need for closing knowledge gaps in apheresis medicine
• Became a full committee in 2016
• Current chair – Ed Wong, MD

ASFA Research Committee Projects

• TTP/TMA (biorepository proposed)
  – Effect of taper type (gradual vs. complete stop) on relapse
  – Type of replacement fluid type on relapse
  – Effect of plasma volumes exchanged on relapse
• Heparin Induced Thrombocytopenia
  – Primary Objective
    • To create a comprehensive database of patient outcomes in HIT treated with TPE
  – Secondary objectives
    • To determine the efficacy of TPE for decreasing HIT-related complications
    • To determine the safety of TPE when used to treat HIT
    • To establish the optimal treatment parameters of HIT TPE treatment
  – Long-term goal
    • Multi-institutional randomized prospective trial of TPE for acute HIT
ASFA Research Committee Projects

- Sickle Cell Disease
- FSGS
- Neurological Disease (NMO, MUSK+MG)
  - Completion of entry of approximately 30 additional NMO patients so that approximately 50 patients TPE treatments and outcomes can be analyzed
  - MUSK+MG paper published
- Adverse Events of Therapeutic Apheresis
- Solid Organ Transplant
- Wilson’s Disease
- Extracorporeal Photopheresis
- Allied Health
  - Issues related to allied health practice and patient outcome

Report of the ASFA Apheresis Registry on Muscle Specific Kinase Antibody Positive Myasthenia Gravis

Chisa Yamada,1* Huy P. Pham,2 Yanyun Wu,2,3 Laura Cooling,3 Haewon C. Kim,4 Shanna Morgan,5 Joseph Schwartz,6 Jeffrey L. Winters,6 and Edward C.C. Wong1

- MuSK MG
  - Subset of MG
  - More severe and refractory
  - More rapid course
- Little data on use/effectiveness of TPE
- Multi-institutional registry created to prospectively collect data
  - MuSK MG exacerbation treated with TPE
  - Treatment Parameters
  - Outcome and safety data

MuSK MG registry

- 15 patients from 3 centers enrolled
- 2 – 10 TPE (1-1.5 PV) per patient QOD
  - Median 5 procedures
  - 92% with 5% Alb
- Objective symptoms resolved in 75%
- Subjective symptoms resolved in 94%
- 67% relapsed in a median of 7 weeks

LIPOPROTEIN APHERESIS IN PATIENTS WITH MAXIMALLY TOLERATED LIPID LOWERING THERAPY, LP(A) HYPERLIPIDEMIA AND PROGRESSIVE CARDIOVASCULAR DISEASE

• Prospective, observational, multicenter study
• 170 patients who began lipoprotein apheresis
  – Lp(a) hyperlipidemia
  – Progressive CVD
• Compared two years prior and first two years on lipoprotein apheresis
• Major Adverse Coronary Events (MACE)
  – CV death, non-fatal MI, CABG, PCI, stent


LIPOPROTEINS

| Table 2. Plasma Concentrations of Lipoproteins and Fibrinogen in 2 Years Before and in 2 Years During Steady State of Chronic LA |
|---|---|---|---|---|---|---|
|     | y-2 | y-1 | 1st LA | y-1, 6 mo | y-1, 12 mo | y-2, 6 mo | y-2, 12 mo |
| Lipa | 3.94±1.77 | 3.95±1.81 | 3.16±1.35 | 2.57±1.05 | 2.54±0.99 | 2.54±0.99 | 2.53±0.96 |
| LDL-C | 2.56±0.93 | 2.57±1.02 | 2.17±0.99 | 2.12±0.79 | 2.08±0.75 | 2.15±0.79 | 2.10±0.83 |
| Reduction, % | 59.2±14.1 | 66.6±11.5 | 68.5±8.4 | 68.8±3.8 | 69.0±3.8 |

MACE

- 78% decrease in MACE after initiation of lipoprotein apheresis

- ACVE: Adverse Cardiac and Vascular Events – MACE and Cerebrovascular events

Some thoughts on future focus

- Continue to collect efficacy data
  - More defined patient populations
  - More consistent treatment parameters
  - New indications

- Improved technologies
  - Focus on specific removal treatments

- Cellular collections
  - Improved blood product collections
  - Improved specificity of leucocyte collections
Some thoughts on future focus - 2

• Continue to work for funding
  – NIH/NHLBI
  – Corporate sources

• Improved interactions
  – Registries
  – Networks
  – Support

Questions?
Selective Removal of C-Reactive Protein (CRP): Clinical Advances

Steffen Mitzner
Rostock, Germany

CRP indicator and actor

- acute phase protein
- homopentamer - pentraxine - 115 kDa
- evolutionarily old
- well known diagnostic marker

- CRP agglutinates Bacteria
- CRP migrates into wounds and keeps them open

- CRP marks intact, adjacent cells for destruction and clearance
- CRP augments necrotic lesions and amplifies the scarring
Molecular Mechanism of cell damage by CRP

(according to Volanakis 2001; Sheriff, Kalden, and Herrmann, unpublished)

CRP and membrane phospholipids

phosphatidylcholine  lysophosphatidylcholine
CRP and membrane phospholipids

low CRP amounts
no effect on cell vitality

Necrosis induction (e.g. AMI)

damage to the cell

sPLA2 IIa
Necrosis induction (e.g. AMI)

Opsonisation by CRP
CRP mediates complement binding

CRP induction

C1q complement
C2
C3
C4

CRP

liver

CRP

IL6

MØ

macrophage
CRP in the necrotic area

High CRP amounts vital cells die at the center of inflammation

CRP enlarges AMI + stroke area in rat, rabbit, pig

C-reactive protein and complement are important mediators of tissue damage in acute myocardial infarction.
Griselli M, Herbert J, Hutchinson WL, Taylor KM, Sohail M, Krausz T, Pepys MB.

C-reactive-protein-associated increase in myocardial infarct size after ischemia/reperfusion.
Barrett TD, Hennan JK, Marks RM, Lucchesi BR.

Human C-reactive protein increases cerebral infarct size after middle cerebral artery occlusion in adult rats.
Gill R, Kemp JA, Sabin C, Pepys MB.

Targeting C-reactive protein for the treatment of cardiovascular disease.
Pepys MB et al.

Inhibiting C-reactive protein for the treatment of cardiovascular disease: promising evidence from rodent models.
Szalai AJ et al.

Selective Apheresis of C-Reactive Protein: A New Therapeutic Option in Myocardial Infarction?
CRP enlarges AMI in rats

C-reactive Protein and Complement Are Important Mediators of Tissue Damage in Acute Myocardial Infarction

By M. Griselli, J. Herbert, W.L. Hutchinson, K.M. Taylor, M. Sohail, T. Krausz, and M.B. Peps

From the *Immunological Medicine Unit, Division of Medicine, †Cardiothoracic Unit, Department of Surgery, and ‡Department of Histopathology, Imperial College School of Medicine, Hammersmith Hospital, London W12 0NN, United Kingdom

Summary

Myocardial infarction in humans provokes an acute phase response, and C-reactive protein (CRP), the classical acute phase plasma protein, is deposited together with complement within the infarct. The peak plasma CRP value is strongly associated with postinfarct morbidity and mortality. Human CRP binds to damaged cells and activates complement, but rat CRP does not activate complement. Here we show that injection of human CRP into rats after ligation of the coronary artery reproducibly enhanced infarct size by ~40%. In vivo complement depletion, produced by cobra venom factor, completely abrogated this effect. Complement depletion also markedly reduced infarct size, even when initiated up to 2 h after coronary ligation. These observations demonstrate that human CRP and complement activation are major mediators of ischemic myocardial injury and identify them as therapeutic targets in coronary heart disease.

Key words: heart • ischemia • necrosis • inflammation • acute phase response

J. Exp. Med Volume 190, Number 12, December 20, 1999 1733–1739

*Animal model (rabbit) - AMI

50% increase of area
125% increase in volume

Plasma CRP after s.c. injection of croton oil

*Barrett et al, J Pharm Exptl Ther 2002, 303, p 1007
CRP increases stroke volume in rats

Figure 1. Human C-reactive protein increases cerebral infarct volume after middle cerebral artery occlusion. Each point represents the infarct volume in an individual rat measured within the hemisphere as a whole, the cortex, or caudate nucleus alone. Rats received a dose of 20 mg/kg of either human C-reactive protein or human serum albumin (in the control group) immediately after middle cerebral artery occlusion and then again at 24 and 48 hours. Infarct size was assessed at 72 hours after occlusion. Horizontal lines indicate the median values for each group. *P values are from one-tailed Mann-Whitney tests.

CRP is also an actor in AMI

Myocardial infarction
Vascular obliteration => Anoxia => Inflammation

The CRP amount is responsible for the magnitude of the lesion after MI or stroke.*

Blockade of CRP after AMI?

Targeting C-reactive protein for the treatment of cardiovascular disease
Pepys, M. et al.

NATURE CHEMICAL BIOLOGY
NEWS AND VIEWS
Chemical knockout of C-reactive protein in cardiovascular disease
Heinecke, JW (2006) 6

(2006) 355

LIMITING MYOCARDIAL DAMAGE DURING ACUTE MYOCARDIAL INFARCTION BY INHIBITING C-REACTIVE PROTEIN
Richard N. Kris, M.D., and Sahuvaral Jath, M.D., Ph.D.

The Inflammatory Hypothesis: Any Progress in Risk Stratification and Therapeutic Targets?
Blankenberg & Yusuf (2006) 114

Selective Apheresis of C-Reactive Protein: A New Therapeutic Option in Myocardial Infarction?

Ahmed Sheriff,1,*, Ralf Schindler,1,*, Birgit Vogt,1, Hassan Abdel-Aty,2, Juliane K. Unger,3, Christopher Bock,1, Frank Gebauer,1, Anna Slagman,4, Timo Jerichow,4, Dörte Mans,4, Gülcen Yapici,1, Gunnar Janelt,1, Malte Schröder,4, Rudolf Kunze,5, and Martin Möckel4

1Department of Nephrology and Internal Intensive Care Medicine, Charité – Universitätsmedizin Berlin, Berlin, Germany
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5Office Campus Max Delbrück Centrum, Berlin, Germany
Amount of CRP correlates with MACE

Myocardial infarction (MI) develops through anoxia (vascular obliteration), whereby inflammation in the myocardium evokes.

The CRP kinetic correlates with the clinical course.*

(*Dimitrijevic 2006)

Scheme of the CRP adsorber

Matrix and ligand are sterilisable
Acute Myocardial Infarction CAMI1: Endpoints

80 patients including controls - small exploratory clinical trial

Primary endpoint:
• Infarct size determined by MRI of the heart 2-5 days as well as 12 weeks (± 2 weeks) after the infarction

Secondary endpoints:
• Incidence of expected and unexpected adverse effects of CRP apheresis. For the assessment, a continuous monitoring of the vital parameters of blood pressure and heart rate, the general condition of the subjects as well as a venous blood gas analysis during the apheresis are performed.
• LVEF (left ventricular ejection fraction), determined by MRI of the heart 2-5 days and 12 weeks (± 2 weeks) after the infarction.
• Major Adverse Cardiac Events (MACE) 6 and 12 months after the infarction (phone interview). MACEs are defined as: death for any reason, not fatal infarction or stroke, unstable angina pectoris, congestive heart failure requiring inpatient treatment, and coronary arterial revascularization (percutaneous coronary angioplasty or coronary artery bypass). The time to the first event is used as the end point.

Acute Myocardial Infarction CAMI1: Inclusion criteria

80 patients including controls - small exploratory clinical trial

Inclusion criteria:
• STEMI is defined according to the guidelines of the European Society of Cardiology (ESC) for the therapy of acute myocardial infarction in patients with ST elevation
• TIMI grade III after PCI (stent implantation)
• Killip Class ≤ II
• Start of symptoms up to coronary recanalization 2 - 12 h
• Enlightened clarification and the existence of a written declaration of consent
• Business performance
Acute Myocardial Infarction CAMI1: Exclusion criteria

80 patients including controls - small exploratory clinical trial

Exclusion criteria:
• Age <18 ≥ 80 years
• Earlier myocardial infarction
• Acute infectious disease (body temperature (auricular, sublingual)> 38.0 ° C)
• Systolic blood pressure <100 mmHg
• Known hypersensitivity to therapeutic apheresis
• Cardiac shock
• Renal insufficiency requiring dialysis
• Preceding coronary artery bypass surgery
• Contraindication for MRI (eg non-MRI-compatible implants, claustrophobia, unstable circulatory situation)
• Malignant or chronic inflammatory disease
• Pregnancy or lactation
• Foreseeably limited possibility of follow-up (e.g., patient living abroad)
• Participation in other interventional examinations

Acute Myocardial Infarction CAMI1: Treatment scheme 1

Treatment scheme for 40 patients with PentraSorb® CRP

Treatment scheme:
• The primary treatment of acute myocardial infarction is in accordance with guidelines for percutaneous coronary intervention (PCI) (inter alia ESC guideline "Myocardial revascularization", 2014 [19]).
• The CRP apheresis is performed as an additional therapeutic measure within the scope of the purpose of the adsorber "PentraSorb® CRP" (Pentracor GmbH, 16761 Hennigsdorf) in two treatment groups.
• 40 patients received 2 treatments at intervals of approx. 24 hours (from the start of the previous treatment). The first treatment begins 10-24 hours after the onset of the symptoms.
### Acute Myocardial Infarction CAMI1: Treatment scheme 2

**Treatment scheme for 40 patients with PentraSorb® CRP**

**Treatment scheme:**
- If the CRP concentration increases to over 30 mg/l approximately 6 hours after the end of the second treatment, a third treatment is performed.
- For each treatment, up to 6000 ml are processed, preferably in 6 cycles (change of loading and regeneration) per 1000 ml. The duration of a treatment is up to approx. 4 hours.

### Acute Myocardial Infarction CAMI1: Clinical study centres

- **Germany**
  - **Non University**
    - Flensburg (initiated in September 2015)
    - Kempten (initiated in May 2016)
  - **University**
    - Berlin Charité (~ May 2017)
    - Dresden (initiated in February 2017)
    - Rostock (initiated in ~ February 2017)
    - Klinikum Neukölln Berlin (initiated in January 2017)

**approved by BfArM**

**current status:**
21 patients recruited
Wider inclusion criteria then in CAMI1

1. Turkey
2. Estonia (initiated in January 2017)
3. Hungary (~ March 2017)

CRP apheresis performed with very high CRP concentration

Reduction of 66% of the original level
CRP apheresis performed with 5 different patients

Behandlung = Treatment, P02 = Patient 2, Zyklus = cycle, CRP vor Apherese = CRP before apheresis, Nach Apherese = after apheresis

Cardiac MRI Analysis

CAMI1 Study
Cardiac MRI during acute phase of MI and at follow-up after 3 months

Dr. Tomas Lapinskas
Dr. Sebastian Kelle
German Heart Center Berlin (DHZB)

Blinded analysis of CMRI by the CORE-LAB of the DHZB
quantitative measurements of local myocardial function

4-chamber view
longitudinal strain

Yellow line indicates the segment with the infarction

mid short axis view
rotation of LV

Green line indicates the segment with the infarction

Reduction of the Infarct Area

Initial-CMRI
Infarktareal

3 Monate-CMRI
Infarktareal

LGE
- Late Gadolinium Enhancement
Change of LVEF after CRP apheresis

Blinded evaluation by a CoreLab of the DHZB
Changes in infarction after CRP apheresis

Blinded evaluation by a CoreLab of the DHZB

Therapeutic options for CRP apheresis

- Acute Myocardial Infarction (no treatment alternatives)
- Stroke (no treatment alternatives)
- Sero-negative Rheumatic Arthritis (no treatment alternatives)
- Crohn’s disease (no treatment alternatives)
- Pancreatitis (no treatment alternatives)
- CRP apheresis after Coronary Bypass (no treatment alternatives)
In Crohn's disease, increased serum CRP levels can be detected already hours after the onset of symptoms. To date, it has been assumed that CRP is formed in the liver and thus systematically responds to the inflammation. For Crohn's disease there is another source, the mesenteric adipose tissue. This has a particular phenotype in the disease, the adipose tissue not only increases but also grows around the inflamed intestine. It is currently unclear whether this rather maintains the disease or those morphological changes could even play a protective function. Also for the Crohn's disease has been shown by work in animal models that complement activation contributes to the disease process. Thus, it can be assumed that, in addition to its diagnostic and prognostic use as a marker of inflammatory reactions, CRP may also be involved in the pathogenesis of Crohn's disease.
CRP apheresis after coronary bypass

CRP apheresis after coronary bypass: Rational

- A goal in patients after coronary bypass surgery is not only the rapid restoration of coronary perfusion, but also the rapid and uncomplicated healing of mainly the coronary and also the thoracic wound surfaces.
- This therapy strategy leads to a reduction in the size of the scars and thus to the improvement of the "outcome".
- Extracorporeal circulation (ECC) in cardiac surgery may also produce hemodynamic, humoral, and inflammatory symptoms.
- The development of a systemic inflammatory response syndrome (SIRS) caused by the contact of the blood components with the external surfaces of the extracorporeal circulation often leads to complications which, despite intensive medical care, are complex and lengthy to treat.

- The object of the study is to reduce the plasma concentration of C-reactive protein (CRP) by CRP apheresis in patients after guidelines-compliant elective, isolated, primary coronary bypass surgery.
- The aim of the study is to analyze the effect of CRP reduction on myocardial tissue damage caused by surgical intervention.
- A possible protective effect of CRP apheresis with regard to the development of such damage should be determined by functional parameters.
Acute Pancreatitis

Acute Pancreatitis: Rational

- Acute pancreatitis is defined as an acute, inflammatory process of pancreatic tissue.
- Very often adjacent pancreatic tissue and surrounding organs are also affected.
- A severe acute pancreatitis can be associated with necrosis, abscesses or pseudocysts, sometimes even organ failure.
- In patients with acute pancreatitis, a cascade of immune mediators is activated very fast by an enzymatically-mediated self-digestion of the pancreas.
- This leads to a primary abacterial inflammatory reaction.
- The pathophysiology of the acute pancreatitis is still not completely understood.
- Severe complications with septic complications, a systemic inflammatory response syndrome (SIRS) or multiorgan failure can occur.
- The aim of the study is to reduce the plasma concentration of C-reactive protein (CRP) by CRP apheresis in patients with acute pancreatitis.
- Another purpose is to analyze the effect of a CRP reduction on the pancreatic tissue damage.
- A possible protective effect of CRP apheresis with regard to such damage should be determined by functional parameters, scores, magnetic resonance tomography (MRI), nuclear magnetic resonance imaging (NMR) or computer tomography (CT).
Stroke

Stroke: Rational

• Damage to the cell membranes occurs after a stroke in the infarct zone (so-called area at risk). Within a few hours after the onset of the symptoms, increased serum CRP levels can be detected.

• Several studies have described a relationship between high CRP values after acute stroke and adverse effects in the course of the disease.

• Muir et al. have shown that the CRP level, measured within 72 h after a stroke, predicts mortality over an observation period of up to 4 years.

• According to Winbek et al. the CRP levels 24 and 48 h after the onset of the symptoms have an effect on the prognosis but not on their concentration during the admission.

• In another study, patients who died during the study period had a significantly higher CRP level in the intake than the survivors. Also, the CRP values correlated with the modified Rankin scale after 3 months.
Autoimmune Autonomic Disorders

Immune-Mediated Postural Orthostatic Tachycardia Syndrome (iPOTS)
Acute Autonomic Dysfunction

- Toxic (Chemotherapy, heavy metals, hexamides, endotoxins, e.g. renal failure)
- Autoimmune
  - Guillain-Barre Syndrome
  - Parainfectious (e.g. HIV, Lyme)
  - Post-infectious
  - Post-vaccination
  - Paraneoplastic
  - Other AIDs (e.g. Sjogren’s Syndrome)
- Metabolic
  - Acute Intermittent Porphyria
  - Fulminant Diabetes
  - Rapid treatment of diabetes ("Insulin Neuritis", TIND)
  - Acute renal failure
- Electrical burn

Chronic Dysautonomia

- Acute causes
- Hereditary (e.g. HSAN)
- Neurodegenerative
  - CNS = Pre-Ganglionic
    - MSA
    - LBD
    - PD
  - Post-ganglionic = PAF
Autoimmune Autonomic Ganglionopathy (AAG)

- Widespread autonomic dysfunction
- Only 50% of severe cases have ganglionic AChR antibodies (G-AChR)
  - Nicotinic alpha 3
- Cholinergic dysfunction ~ underactivity
- Antibody level \( \propto \) severity

Orthostatic Intolerance

- Symptoms when upright relieved by lying down.
- Immediate or delayed.
- Immediate symptoms – typical pre-syncope
- Delayed symptoms – can be vague and weird
Delayed (Pernicious) Symptoms

• Fatigue
• Headache
• “Brain Fog”
• Nausea
• Chest pain
• Abdominal pain
• Discoloration of distal lower limbs
• Abnormal sweating
• Odd seizure-like events
• Vertigo
• Palpitations

Neurogenic Orthostatic Intolerance

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<th></th>
<th>BP</th>
<th>HR</th>
<th>CBF</th>
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<tr>
<td>Neurogenic Orthostatic Hypotension</td>
<td>↓</td>
<td>=</td>
<td>↓</td>
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<tr>
<td>POTS</td>
<td>=</td>
<td>↑↑ (&gt; 30)</td>
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<tr>
<td>Orthostatic Cerebral Hypoperfusion Syndrome</td>
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POTS Sub-Types

- Neuropathic – low noradrenaline levels, sudomotor dysfunction
- Hyperadrenergic – high noradrenaline levels
- Autoimmune – acetylcholine ganglionic receptor (nicotinic or muscarinic) or adreno-receptor antibodies.
A. Mutually Distinct Subtypes (Assumed)

- Hyperadrenergic
- Neuropathic
- Autoimmune

B. Overlapping Subtypes in POTS (Reality)

- Hyperadrenergic
- Neuropathic
- Autoimmune
Autoimmune POTS

- Autoantibody to α1AR with partial antagonist effect → compensatory sympathetic α1AR tachycardia
- Autoantibody to β1 AR stimulates β1 ± β2 AR activation → tachycardia.

Li et al. 2014
Case Study: TD, M, 47 yo, Physician

- 2011 orthostatic presyncope with tachycardia, immediate-onset, and exercise intolerance = POTS
- 2015 delayed orthostatic symptoms with sustained standing
- Corticosteroids, IVIg ~ good responses but inconsistent

TD Comorbidity

- Joint hypermobility syndrome (JHS)
- Mast cell activation disorder (MCAD)
- Sicca syndrome (Sjogren’s Syndrome)
TD: Investigations

- QSweat abnormal (sudomotor)
- Skin biopsy – small fiber polyneuropathy
- Alpha 1 adrenergic and angiotensin 2 receptor autoantibodies

TD’s serum partially inhibits alpha 1 AR activity.

Partially blocks effect of phenylephrine stimulation.
TD’s serum suppresses angiotensin 1 receptor activity.

TD’s serum suppresses angiotensin 1 receptor activity, even with max. stimulation by AT2.

Note 10-fold difference in scale with addition of Ang II

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TD

- July 2016 ~ bedbound, instantaneous presyncope and HR surges 40-50 BPM attempting to sit up
- In-patient plasmapheresis, 10 exchanges over 3 weeks → incremental improvement
- At discharge, could sit all day, walk short distances
- Droxidopa added, with benefit
TD

• Post-discharge, no TPE for 1 week → acute relapse
• Re-admitted, TPE x 3 → better
• Outpatient TPE 3x /week
• Tapering TPE failed, rituximab started 4 x 4 regimen
• Now, sit upright all day, all ADLs, walk 6000 steps, returning to work part-time

POTS Associations

• Other autonomic system disorders: GI dysmotility, thermal regulation, bladder
• Ehlers-Danlos Syndrome, hypermobility type (hEDS)
• Mast cell activation disorder (MCAD)
• Chronic Fatigue Syndrome (CFS)
What is a Mast Cell?

- Bag of Granules
- Located in connective tissue
  - close to blood vessels
- Histamine released
  - Increase blood flow
  - Increase vascular permeability
  - Binds to H1, H2 receptors
Mast Cell Activation Syndrome

I. Systemic
   - Anaphylaxis
   - Faintness
   - Fatigue

II. Dermatologic
   - Flushing
   - Rashes
   - Itching
   - Hives

III. Cardiovascular
   - Blood pressure changes and shock
   - Chest pain
   - Rapid heart rate

IV. Pulmonary
   - Wheezing
Mast Cells and Autoimmunity

- Mast cells involved in AIDs (MS, RA, Bullous pemphigoid).

DAME Syndrome (95% Female)

- Dysautonomia, POTS included
- Autoimmune disease activity
- Mast cell activation disorder
- Ehlers-Danlos Syndrome, hypermobility type

- Plus universal CFS, Cognitive complaints (Brain Fog).
**DAME and PEx**

- Dysautonomia ~ Cardiovascular instability
- Autoimmune ~ PEx indicated as Rx, procoagulant
- MCAD ~ strongly prone to allergy-like reactions (FFP, lines, medications)
- EDS ~ local anesthetic resistance, fragile connective tissue

**Tides-Boats**

- AID aggravated by immune stimulation = acute infections, vaccinations
- AID reduced by decreased immune stimulation
Reducible Immune Stimulation

- External = mold, rodents, other allergic stimuli
- Internal
  - Latent viruses = herpes group (HSV, VZV, EBV): Trial of antiviral (valacyclovir)
  - Leaky gut syndrome: probiotics, prebiotics, dietary changes, supplements

Immune Therapy

- Block Immune-Mediated Inflammation
  - Steroids
  - IVIg
- Remove antibodies, inflammatory mediators = TPE
- Reduce antibody production = non-steroidal immunosuppressants (e.g. mycophenolate, tacrolimus, rituximab, cyclophosphamide)
Conclusions

- Antibody-mediated autonomic dysfunction exists, often mistaken as psychogenic
- Might present as part of known disorder
- Look for other immune system overactivity (e.g. MCAD)
- Removal of pathological immune mediators by TPE can be effective

Conclusions

- Cardiovascular instability and Mast cell overactivity challenging for TPE.
- Objective outcome measures are desirable to validate improvement from treatment, e.g. orthostatic HR.
Metabolomics and Exosome Biology

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Departments of Medicine, Pediatrics, and Pathology
The Mitochondrial and Metabolic Disease Center
UCSD School of Medicine
February 24, 2017

A Sample of Blood is Like a Sample of Water from a River or Ocean Ecosystem
2 Types of Metabolomics

**Untargeted**
Accurate Mass TOF
LC-qTOF

- 10,000 “features” (anonymous ions)
- Typically, about 30-100 can be unambiguously identified.
- Strong for new molecule discovery

**Targeted**
Triple Quads
LC-MS/MS

- 500 unique chemical entities are typically identified.
- Strong for rapid pathway analysis and quantitation

UCSD Metabolomics

- 20-100 µl samples of urine and blood
- 10-50 mg of Liquid Nitrogen powdered tissue
- Sample Extraction (to remove proteins, DNA, and RNA)
- Polar + Nonpolar Metabolites
- Biochemical Pathway Visualization

**Internal Standards**

- UHPLC
- HILIC-NH₂

**Comprehensive Metabolite Quantitation**
(Scheduled MRM in both positive and negative mode)

**Biochemical Pathway Visualization**

- Volcano Plots
- Quantitation of About 700 Metabolites, 300 Drugs, Toxins, and Xenobiotics
Metabolomics Platforms in 2017
Many being explored. No clear “winner” yet. Like NextGen DNA sequencing in 2007 before Illumina became the market leader.

Metabotypes
Can be organized
In Pathways
Metabotypes can be used for chronic disease diagnosis

NextGen Metabolomics can be used to translate data into actionable clinical knowledge to personalize treatment for both monogenic and complex diseases.

Diagnostics II—
Area Under the Receiver Operator Characteristic (AUROC) Curves = 85-98%

AUC 0.88 “Excellent”
AUC 0.86 “Excellent”
AUC 0.91 “Outstanding”
AUC 0.91 “Outstanding”
AUC 0.98 “Outstanding”
AUC 0.86 “Excellent”
“The brain controls metabolism.”
(Through the autonomic nervous and endocrine systems.)

--All brain disorders produce a signature of abnormalities that can be detected in the blood and other biofluids.

**Metabolic “Reflexes” are Patterned Responses to Environmental Triggers**

- The Cell Danger Response (CDR) is Metabolic
- Metabolism regulates gene expression

See Naviaux. Metabolic Features of the Cell Danger Response. PMID: 23981537
Metabolomics Identified Biological Similarities Between Chronic Fatigue Syndrome And Dauer in C. elegans, and the Cell Danger Response and Metabolic Syndrome.

<table>
<thead>
<tr>
<th>Plasma Metabolites</th>
<th>Chronic Fatigue Syndrome</th>
<th>Dauer</th>
<th>Cell Danger Response(?)</th>
<th>Metabolic Syndrome</th>
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</thead>
<tbody>
<tr>
<td>Sphingolipids</td>
<td>Decreased (M+F)</td>
<td>Decreased(62)</td>
<td>Increased(63)</td>
<td>Increased(64)</td>
</tr>
<tr>
<td>Glycosphingolipids</td>
<td>Decreased (M+F)</td>
<td>Decreased(62)</td>
<td>Increased(63)</td>
<td>Increased(65)</td>
</tr>
<tr>
<td>Phospholipids (most species)</td>
<td>Decreased (M+F)</td>
<td>Decreased(66)</td>
<td>Increased(67)</td>
<td>Increased(68)</td>
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<tr>
<td>PC(18:1/22:6)—Oleoyl/DHA phospholipids</td>
<td>Increased (M+F)</td>
<td>No data</td>
<td>Decreased(67)</td>
<td>Decreased(13)</td>
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<tr>
<td>Cholesterol, Sterol Synthesis</td>
<td>Decreased (M+F)</td>
<td>Decreased(69)</td>
<td>Increased(70)</td>
<td>Increased(71)</td>
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<tr>
<td>Purines</td>
<td>Decreased (M+F)</td>
<td>Decreased(72)</td>
<td>Increased(73)</td>
<td>Increased(74)</td>
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<tr>
<td>Uric Acid</td>
<td>Decreased (M)</td>
<td>N/A</td>
<td>Increased(75)</td>
<td>Increased(76)</td>
</tr>
<tr>
<td>Pyrroline-5-Carboxylate/Arginine</td>
<td>Increased (M+F)</td>
<td>No data</td>
<td>Decreased(77)</td>
<td>No data</td>
</tr>
<tr>
<td>FAD/Riboflavin</td>
<td>Decreased (M+F)</td>
<td>Decreased(72)</td>
<td>Increased(20)</td>
<td>No data</td>
</tr>
</tbody>
</table>

*M+F = males and females. M = males only. F = females only

N/A: The end products of purine metabolism in worms are glyoxylate and ammonia, not uric acid.

Exosome Biomarkers of Disease

<table>
<thead>
<tr>
<th>Urine Exosomes</th>
</tr>
</thead>
</table>

**Males**

- 1.4 x 10^{13} Exosomes/day
- 1 x 10^{10} Exosomes/ml

**Females**

- 2-30 x 10^{13} Exosomes/day
- 1-20 x 10^{10} Exosomes/ml

**Tamm-Horsfall Protein**: 50-100 mg/dL
**Albumin**: 2-100 mg/day
**Tumor Susceptibility Gene 101**
**Podocalyxin**
**Neuron Specific Enolase**
**Annexin V**
**Aquaporin 2**
**Angiotension Converting Enzyme**
>1000 other Proteins
Ultrastructure of Urine Exosomes

Female (25 yo)
Buoyant Density Analysis
Male (52 yo)
Low Density Particles
BD= 1.23 g/ml
Female (PM 49 yo)

Urine Exosomes are Membrane-Bound Nanoparticles 14-200 nm in Diameter
2D Gels Reveal Over >1600 Proteins in Urine Exosomes

Exosomal DNA Stained with SybrGreen
Urinary Exosomes Contain mtDNA

**Southern Analysis**

Exosomal DNA purified from 50 ml of Urine and probed for human mtDNA

<table>
<thead>
<tr>
<th>Lane</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 ng Platelet mtDNA</td>
</tr>
<tr>
<td>2</td>
<td>Uncut Exosome DNA</td>
</tr>
<tr>
<td>3</td>
<td>BamHI-cut Exosome DNA</td>
</tr>
</tbody>
</table>

Urine Exosomes
EM 64,000x
1.23 g/ml band

Visceral Adipose Produces 5-40 Fold More Exosomes than Superficial Adipose in Culture

In collaboration with Bob Henry, Ted Ciaraldi, and Susan Philips
The Lipidome of Urine Exosome is Enriched in Sphingomyelins

Mass spectrum of exosome membrane lipids

Exosome Membrane Lipids Stained with BODIPY Lipid Probe

In collaboration with Lee Hagey

Urine Exosomes are Metabolically Active---Consume Oxygen and Produce Peroxide

CO₂ concentration (µmol/ml)
Exosomes produce NO in the presence of L-arginine

This is followed by CPTIO conversion to CPTI by NO trapping as detected by EPR spectroscopy.

This is the chemistry and spectroscopy involved in the reaction.

Urine Exosomes of Patients with Diabetes Contain Mitochondrial Protein Damage Associated Molecular Patterns (DAMPs)

In collaboration with Laura Dugan and Sameh Ali

In collaboration with Kumar Sharma and Satish Rao
Summary

• Metabolomics is a powerful new tool for systems biology, disease diagnosis, and for monitoring treatment
• Chemical signatures have been found for many chronic diseases using Targeted NextGen Metabolomics
• Pathway analysis is more robust than single “biomarker” studies
• Exosomes contain over 1000 proteins, RNA, DNA, mtDNA, and miRNA
• Exosomes are enriched in sphingolipids and mitochondrial proteins in diabetes and other conditions
• Exosomes change according to cellular stress and disease