Hemoglobin Species in Plasma of Acquired Thrombotic Thrombocytopenic Purpura Patients: A Novel Therapeutic Target?

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Disclosures

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Background

• Thrombotic thrombocytopenic purpura (TTP)
  » Thrombocytopenia
  » Microangiopathic hemolytic anemia
  » Presumptive diagnosis is made if no other clear etiology explains these findings

• Incidence = ~5 per 1,000,000 per year in U.S.

• Without treatment, >90% patients die
Pathophysiology

• Autoantibodies directed against enzyme ADAMTS13 (vWF-cleaving protease)

• Depressed enzyme function decreases conversion of ULvWF into smaller vWF multimers

• Disrupts normal endothelial-VWF-platelet homeostasis
  » Hyperactive interactions between ULvWF and platelets

• Results in platelet-vWF thrombi within microvasculature

• “Innocent bystander” RBCs undergo mechanical hemolysis
Why Therapeutic Plasma Exchange (TPE) Works?

• **First-line therapy** consists of daily TPE until treatment response achieved

• **By removing patient plasma**
  » ADAMTS13 autoantibodies

• **By giving donor plasma**
  » Provide functional protease

• Decreases mortality to <10%

Kiss JE. *Int J Hematol* 2010
The Problem: Plasma Hemoglobin (Hgb)

Reduced-state Hgb
Oxidized-state Hgb

Plasma Hgb

• Elevated plasma hgb results in
  » Nitric oxide (NO) consumption/dysregulation
  » Vasoconstriction and endothelial dysfunction
  » ↓ cleavage of ULVWF by ADAMTS13
    • Interference at binding site on A2 domain
  » ↑ platelet activation and adhesion to ULVWF
    • ADP-hgb complexes
    • ↓ NO-mediated platelet inhibition

Plasma Hgb

• Haptoglobin reserves in an average-sized patient
  » Capable of binding ~4500 mg plasma hgb (20 mL RBCs)
  » Half-life 3.5-5 days

• Average hemoglobin for TTP patient = 8.2 g/dL
  » Approximately 5 g/dL acute decrease compared to baseline
  » Hemolyzing 4-5 units of packed RBC mass (~1500 mL)

Rother RP, Bell P, Hillmen P, Gladwin MT. JAMA 2005
Raval JS, Harm SK, Rollins-Raval MA, Kiss JE. J Clin Apher 2014
Raval JS, Wearden PD, Orr RA, Kiss JE. J Clin Apher 2012
Schaer DJ, Buehler PW, Alayash Al, Belcher JD, Vercellotti GM, Blood 2013
Plasma Hgb

- 0.00625 mM (10 mg/dL) plasma hgb inhibits normal aortic ring dilatation

- Concentrations measured in sickle cell disease patients
  - Up to 0.021 mM (33 mg/dL) at steady state
  - 0.026 mM (41 mg/dL) during crises

- 0.09-0.150 mM (150-250 mg/dL) results in kidney failure, hypertensive crises, and end-organ injury with plasma hgb
Plasma Hgb

- Hemolysis in these patients have been associated with
  - Pulmonary hypertension
  - ↓ oxygen saturations
  - ↑ cardiac dysfunction
  - Abdominal pain
  - Hemoglobinuria, proteinuria
  - ↑ mortality

Walk-PHASST Investigators. Haematologica 2013
Schaer DJ, Buehler PW, Alayash AI, Belcher JD, Vercellotti GM, Blood 2013
Plasma Hgb

- No data has been published in TTP patients

- Sickle cell disease patients ≠ TTP patients
  - ≠ Malaria patients
  - ≠ PNH patients
  - ≠ AIHA patients
The Problem

• “Innocent bystander” hemolysis is known to occur in TTP

• Details about the species and impact of plasma hgb in TTP patients are unknown

• Plasma hgb-mediated vasoconstriction could further impair blood flow in TTP
  » Via NO sequestration resulting in unopposed vasoconstriction
Objectives of Our Study

• Primary: Characterize the degree and speciation of plasma hgb in TTP patients

• Secondary: Correlate plasma hgb findings with impact on microvascular blood flow
Methods

• **TTP clinical database**
  » Demographics
  » TPE parameters
  » Laboratory data
  » Clinical responses
  » Outcomes

• **TTP biosample repository**
  » First 50 mL of waste plasma during performance of daily TPE collected
  » Aliquotted and frozen ≤4 hours at -80°C
Methods

• IRB-approved retrospective pilot, exploratory study

• Samples from 25 randomly selected de novo TTP patients with severe deficiency of ADAMTS13 activity (<10%) were chosen for analysis
  » “Initial sample” = Immediately prior to initial TPE treatment
  » “Final sample” = From the final day of daily TPE after achieving treatment response (PLT >150,000/μL x 2 days)
Methods: Primary Objective

• Paired samples (for each patient) were interrogated by
  » Absorption spectroscopy (for hgb detection except met-hgb)
  » Electronic paramagnetic resonance (EPR) spectroscopy (for met-hgb detection)
Absorption Spectroscopy

Hgb A Basis Spectra

Absorbance (mM⁻¹ cm⁻¹)

Wavelength (nm)

oxyHbA
deoxyHbA
metHbA
HbNO
metHbNO
EPR Spectroscopy
Methods: Secondary Objective

• To ascertain endothelial impact of hgb species, we selected cardiac troponins as a surrogate marker of microvascular blood flow
  » Measured on every patient prior to TPE
  » Aortic and coronary vasculature known to be sensitive to plasma hgb
  » Increased troponins proportional to impaired microvascular blood flow secondary to:
    • Physical occlusion: Platelet-vWF microthrombi
    • Narrowing: Vasoconstriction

## Results

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean Value or %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>42.9 years</td>
</tr>
<tr>
<td>Female</td>
<td>60% (15/25)</td>
</tr>
<tr>
<td>Black</td>
<td>80% (20/25)</td>
</tr>
<tr>
<td>Mortality</td>
<td>8% (2/25)</td>
</tr>
</tbody>
</table>
**Results**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean Value Prior to TPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Total plasma hgb]</td>
<td>0.041 mM = 64.9 mg/dl</td>
</tr>
</tbody>
</table>

*Oxy-hgb and met-hgb predominant bioactive hgb species identified.*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean Value Prior to TPE</th>
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</thead>
<tbody>
<tr>
<td>Survivor [Total plasma hgb]</td>
<td>0.035 mM = 54.8 mg/dl</td>
</tr>
<tr>
<td>Non-survivor [Total plasma hgb]</td>
<td>0.119 mM = 190.1 mg/dl</td>
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*All plasma hgb species normalized in the 23 patients that survived to achieve treatment response.*
Cardiac Troponin (ng/ml) vs. [Total Plasma Hemoglobin] (mM Heme)

- $R^2 = 0.92$
- Spearman $r = 0.99$
- $P < 0.0001$
$R^2 = 0.90$
Spearman $r = 0.92$
$P < 0.0001$
$R^2 = 0.08$
Spearman $r = -0.08$
P = 0.72
Deleterious Effects of Plasma Hgb

- Oxy-Hgb consumes NO in 1:1 molar ratio
  - Heme concentrations as low as 0.006 mM have been found to impair NO-dependent vasodilation *in vivo* in SCD patients
  - Our TTP patients had [plasma oxy-hgb] from 0.007-0.078 mM (11-125 mg/dL)

- Our patients also had markedly high levels of met-hgb
  - Ranging from 0.0005-0.142 mM (0.8-226 mg/dL)
  - Indicates deficiency in normal reductive capacity
  - Associated with anemic stress and hypoxic injury
    - Damage can still be caused via NO-independent mechanisms

Hare GM, Mu A, Romaschin A, Tsui AK, Shehata N, Beattie WS, Mazer CD. Can J Anaesth 2012
Conclusions

- **Plasma hgb markedly elevated in TTP patients**
  - At concentrations higher than in SCD patients during crisis

- **Variability of hgb species in TTP patients**
  - Either oxy-hgb, met-hgb, or a combination

- **[Total plasma hgb] significantly greater in non-survivors vs. survivors**
Conclusions

• Association of progressively worsening microvascular blood flow with increasing [plasma oxy-hgb]

• Patients had high amounts of met-hgb
  » Deficiency of reductive capacity
  » Associated with increased risk of hypoxic injury, possibly via impacting NO-independent mechanisms
Conclusions

• RBC may not be such an “innocent bystander”
  » “Unwilling accomplice”?

• In TTP patients with marked hemolysis, vascular dysfunction, and/or severe disease activity
  » More aggressive TPE
  » Agents targeting plasma hgb
  » Agents increasing [NO]

• Failed clinical trials of NO support (arginine, inhaled NO) and haptoglobin administration in other hemolytic states
  » These may be beneficial in TTP patients
• Many investigations are still required

• Prospective, multi-institutional collaborations are necessary

• ASFA TTP/TMA Subcommittee is one place to begin to answer these questions