

LOYOLA UNIVERSITY DEPARTMENT OF PATHOLOGY AND LABORATORY MEDICIN

DIFFERENTIAL HEPARIN NEUTRALIZATION STUDIES USING  
PROTAMINE SULFATE AND A NOVEL SYNTHETIC PEPTIDE

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# INTRODUCTION

- Heparin is a variable length glycosaminoglycan that binds to antithrombin III to increase its activity
  - Antithrombin III primarily possesses anti-IIa and anti-Xa activity
  - Clinically, this manifests as higher aPTT, ACT, and smaller amplitude values in thromboelastography
  - Bleeding after heparin can be problematic
    - Protamine sulfate is a naturally derived protein indicated for the reversal of various types of heparin
    - In the case of heparin rebound, protamine is also indicated for continued reversal

# PROTAMINE SULFATE ADVERSE REACTIONS

- Literature has reported severe anaphylactic reactions in some patients\*
  - Reactions:
    - Bronchospasms
    - Severe hypotension
    - Bradycardia
    - Pulmonary vasoconstriction
  - Incidence frequency ranges from 0.06% to 10.6% according to one review of literature\*\*

\*C. Boer, M. Meester, D. Veerhoek, A.B.A. Vonk, Anticoagulant and side effects of protamine in cardiac surgery: a narrative review, British Journal of Anaesthesia, volume 120, Issue 5, 2018, Pages 927-934, ISSN 0007-0912, <https://doi.org/10.1016/j.bja.2018.01.023>.

\*\*Nybo M, Madsen JS. Serious anaphylactic reactions due to protamine sulfate: a systematic literature review. Basic Clin Pharmacol Toxicol. 2008 Aug;103(2):192-6. doi: 10.1111/j.1742-7843.2008.00274.x. PMID: 18816305.

# REPORTS OF ADVERSE REACTIONS TO PROTAMINE SULFATE

- 55-year-old woman with diabetes mellitus, a history of hypertension, hyperlipidemia, chronic anemia, and hypothyroidism presented to the hospital with chest pains \*
  - On the fourth day of stay, she received a cardiac catheterization
  - She was administered 20 mg of protamine over 5 minutes to reverse the 5000 U of heparin used, which resulted in severe hypotension and bronchospasms
  - She thankfully had a full recovery

# REPORTS CONTINUED

- 65-year-old man with history of type II diabetes mellitus and neuropathy, chronic obstructive pulmonary disease, and benign prostatic hypertrophy presents with foot ulcers\*
  - Previous administration of protamine sulfate had not caused any adverse reactions
  - Bilateral carotid bruits were noted with otherwise normal expiration, heart sounds, and abdomen
  - Total occlusion of the left superficial femoral artery was discovered
    - Femoral popliteal bypass graft was performed with 10,000 U of heparin
    - Administration of 50 mg protamine 45 minutes later over 5 minutes resulted in wheezing and cyanosis
    - Systolic pressure fell to 50 mmHg with an HR of 35 beats per minute
    - He was successfully resuscitated and treated, making a full recovery

**HOW DO WE SOLVE THE PROBLEM?**

# MECHANISM OF PROTAMINE AND HEPA-REMOVE

- Heparin has a high number of negatively charged sulfate moieties
  - Protamine is a positively charged glycoprotein capable of exploiting this characteristic of heparin by forming a stable complex with it
  - The sequestering of heparin with protamine sulfate prevents binding of heparin to antithrombin III which inhibits coagulation factors
- HEPA-Remove also binds heparin to prevent activation of antithrombin III and subsequent deactivation of coagulation factors to restore normal blood clotting

# PURPOSE AND HYPOTHESIS

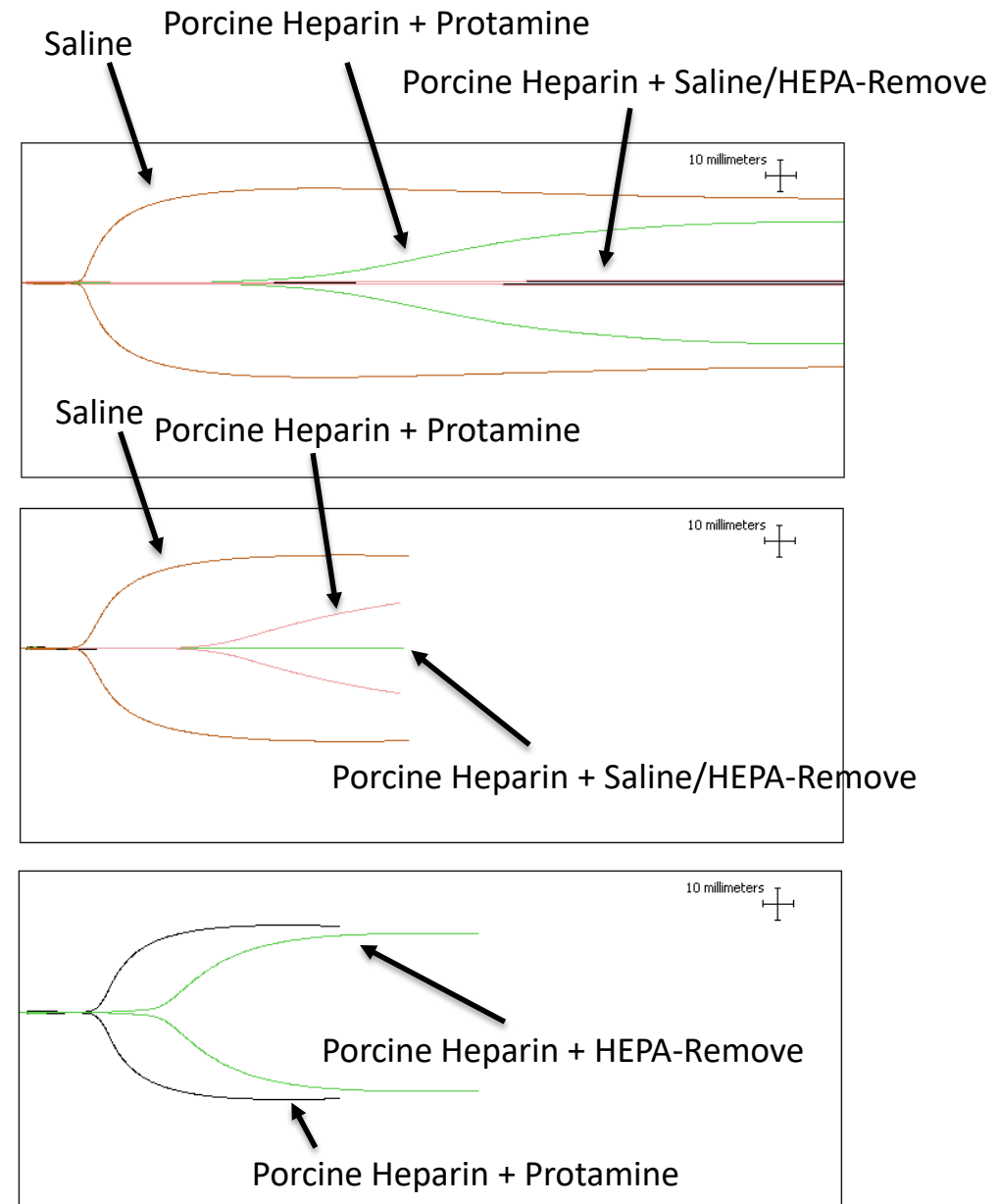
- Purpose
  - Comparison of reversal potential of HEPA-Remove, a novel heparin antagonist, to protamine sulfate in conventional assays
  - HEPA-Remove was originally developed as a laboratory reagent for heparinized blood and plasma samples, however it may have the potential to be developed in the clinical arena
  - More options for heparin reversal are needed
- Hypothesis
  - HEPA-Remove would have approximately comparable reversing potential as protamine sulfate



# RESULTS

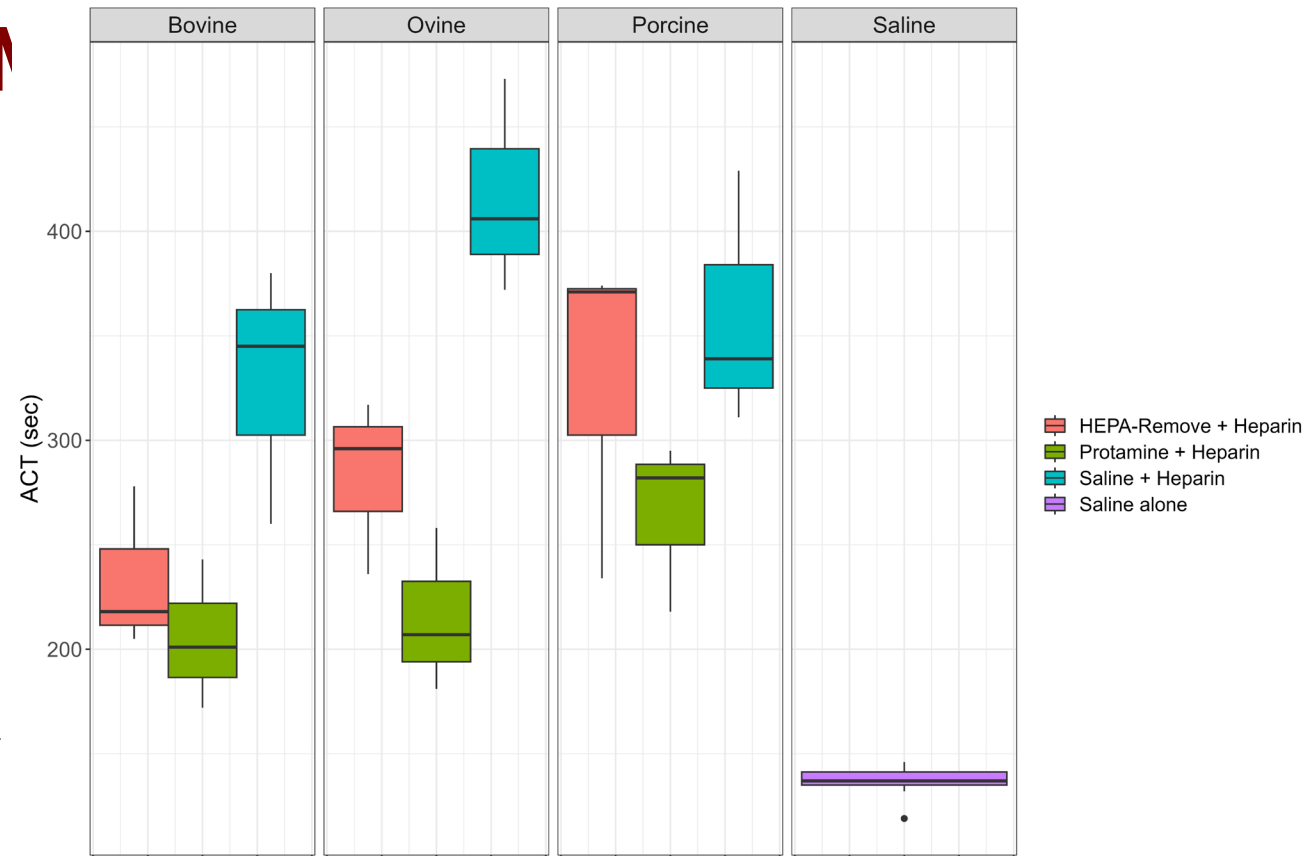
# THROMBOELASTOGRAPHY

- Fresh blood was drawn with informed consent and placed in citrate tubes
- Blood was transferred from citrate tubes and partitioned into 4 TEG cups and treated with saline or porcine heparin with a reversal all at 1  $\mu\text{g}/\text{mL}$  and calcium chloride to initiate clotting
- HEPA-Remove surprisingly did not show any reversal at this concentration
- Protamine showed weak reversal
- Run at higher concentrations (bottom, 10  $\mu\text{g}/\text{mL}$ ) both protamine and HEPA-Remove show almost complete reversal



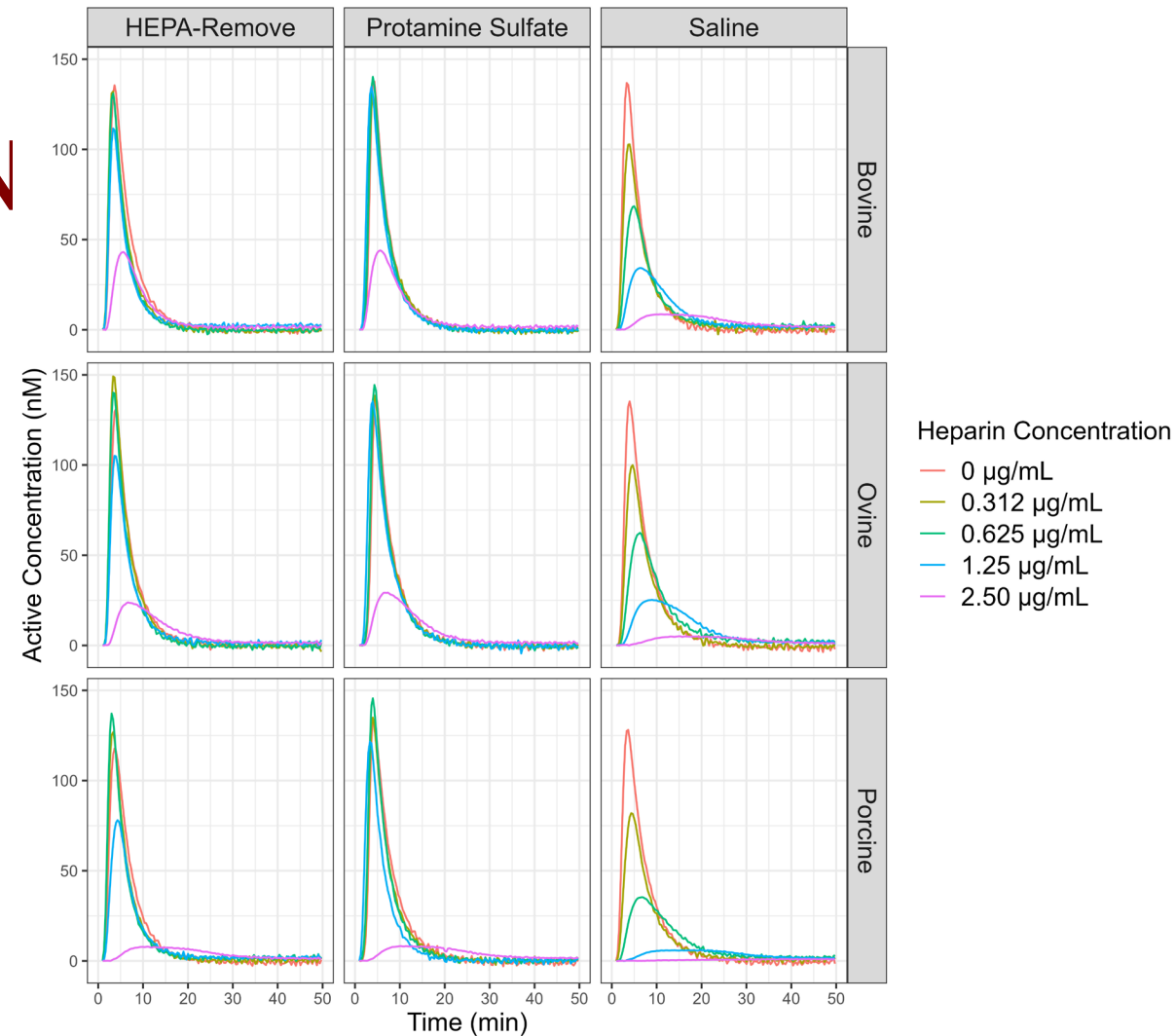
# ACTIVATED CLOTTING TIME

- Activated clotting time revealed a significant decrease in clotting time for bovine and ovine heparins (heparin and reversal at 10 $\mu$ g/mL)
- Interestingly, there was a very wide distribution of times for porcine heparin
  - This is likely due to a low number of participants for each reversal and varying sensitivities to heparin
- Bovine heparin showed very similar clotting times for both reversals most likely due to its low anticoagulant potency



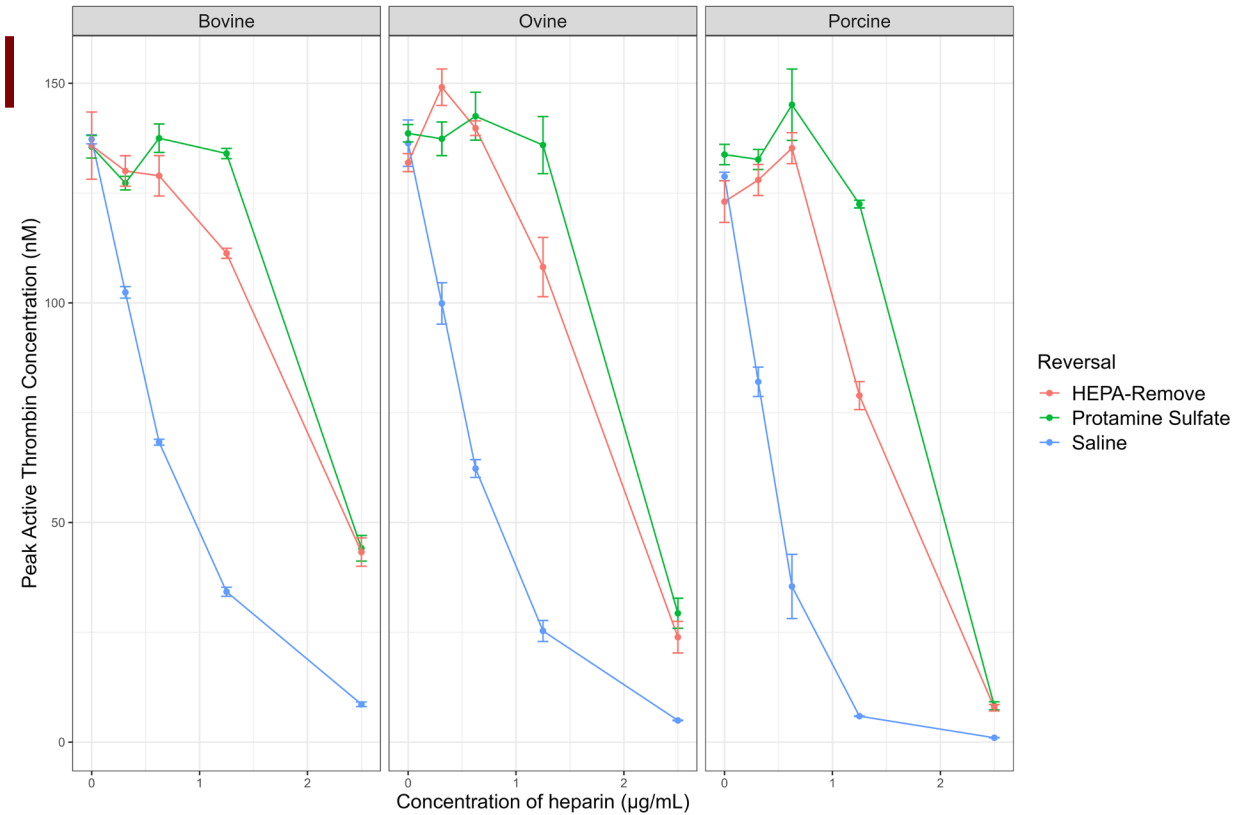
# THROMBIN GENERATION

- Citrated platelet-poor plasma was retrieved from Loyola University Medical Center blood bank
  - Samples were treated with 2.50 mcg/mL of reversal or saline and measured for thrombin generation over a period of 90 minutes in triplicate



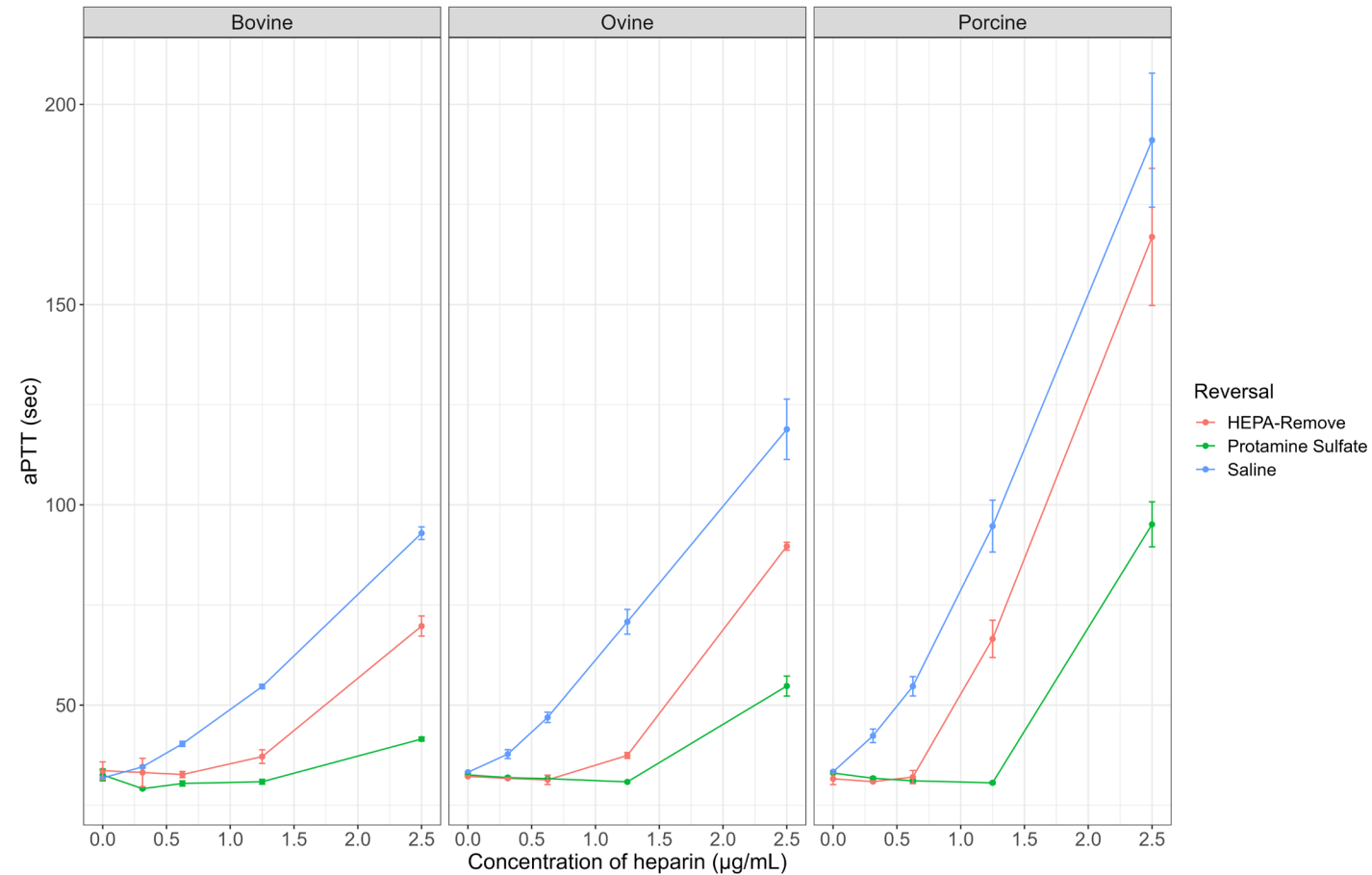
# PEAK ACTIVE THROMBIN

- Among the most insightful parameters is peak active thrombin concentration
  - Both HEPA-Remove and protamine displayed strong reversal



# APTT

- Pooled blood blank plasma was treated with varying concentrations of heparin and 2.50  $\mu\text{g}/\text{mL}$  of reversal
- HEPA-Remove and protamine both produced significant decreases in aPTT



# CONCLUSION

- HEPARemove shows lower potency than protamine sulfate
  - This was notable in all assays
  - HEPA-Remove's ability to inhibit factor XII may be the reason for this among other possible unknown mechanisms
- HEPA-Remove may prove to be a viable alternative when adjusted for potency

# ACKNOWLEDGEMENTS

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# FINANCIAL RELATIONSHIPS

The authors declare no financial relationships or conflicts of interest.

**THANK YOU!**



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