MALDI IMS and Comparative Pathology: Defining Molecular Constituents of Staphylococcal Tissue Abscess Formation and Maturation

William J. Perry[1,2,3], Andy Weiss[3,4], Kelli L. Boyd[4], Nathan Heath Patterson[1,5], Jessica R. Sheldon[3,4], Jeffrey M. Spraggins[1,2,5], Eric P. Skaar[3,4], and Richard M. Caprioli[1,2,5,6,7]

1. Mass Spectrometry Research Center, Vanderbilt University, Nashville, TN, USA 2. Department of Chemistry, Vanderbilt University, Nashville, TN, USA 3. Vanderbilt Institute for Infection, Immunology, and Inflammation, Vanderbilt University, Nashville, TN, USA 4. Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN, USA 5. Department of Biochemistry, Vanderbilt University, Nashville, TN, USA 6. Department of Pharmacology, Vanderbilt University, Nashville, TN, USA 7. Department of Medicine, Vanderbilt University, Nashville, TN, USA

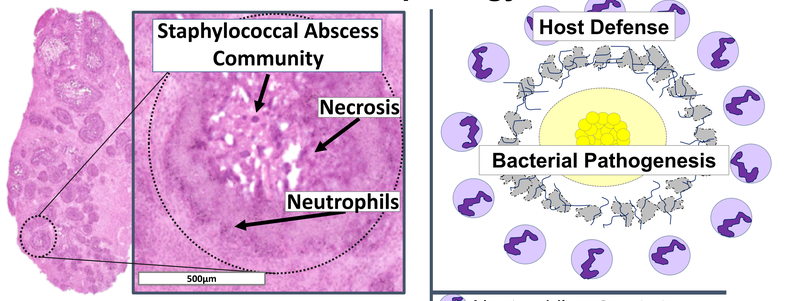
William J. Perry

VANDERBILT UNIVERSITY

INTRODUCTION

- Antibiotic resistant *S. aureus* causes ~20,000 yearly U.S. deaths[1,2].
- Infectious lesions (abscesses) can progress to life threatening conditions[1,2].
- Abscesses contain a central nidus (bacterial micro-colony) segregated by innate immune host structures and necrosis[3]. These structures form and grow as disease progresses.
- Spatial, molecular investigations allow for increased understanding of bacterial pathogenesis and host innate immune responses[4].

Mature Abscess Morphology

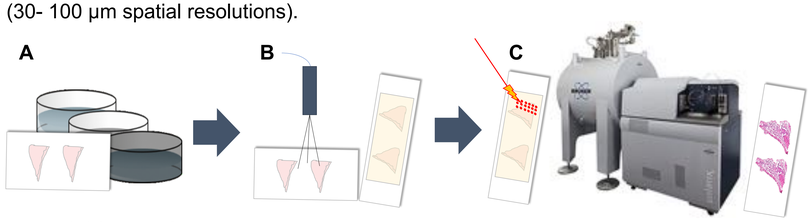


S. aureus Infected Mouse Kidney

METHODS

Infection Model and Tissue Preparation:
Female, six week old C57Bl/6 mice were infected with 1 x 10^7 CFUs of *S. aureus*. Kidney tissues were harvested 7 days post infection (DPI) and frozen on dry ice. Tissues were sectioned and thaw mounted on conductive glass slides.

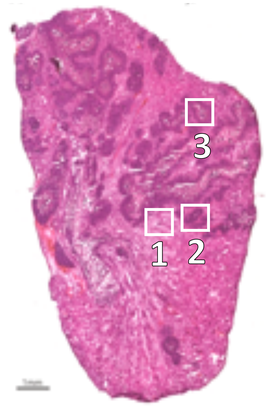
MALDI IMS:
Sections for lipid analyses were washed as reported previously[5]. (A) Sections for MALDI IMS analysis were homogeneously coated with matrix using a robotic aerosol sprayer (TM Sprayer, HTX Technologies) (metabolites: previously reported methods[6] and lipids: 1,5-diaminonapthalene (DAN)). (B) Data were acquired using a Bruker Solarix 9.4T or 15T FT-ICR MS (30- 100 μm spatial resolutions).

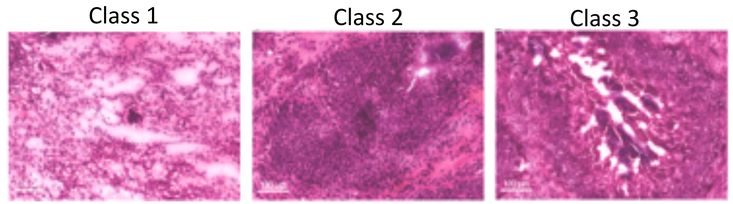


Post-acquisition analyses:
Post-MALDI IMS, tissue sections were washed of matrix and stained with hematoxylin and eosin (H&E) for histological analysis. (**C**) All IMS data were imported into SCiLS Lab software version 2017a for image generation and data analysis using receiver operating characteristic curve analysis.

William.J.Perry@Vanderbilt.edu

RESULTS: ABSCESSES ARE SEPARATED INTO 3 HISTOLOGICAL CLASSES



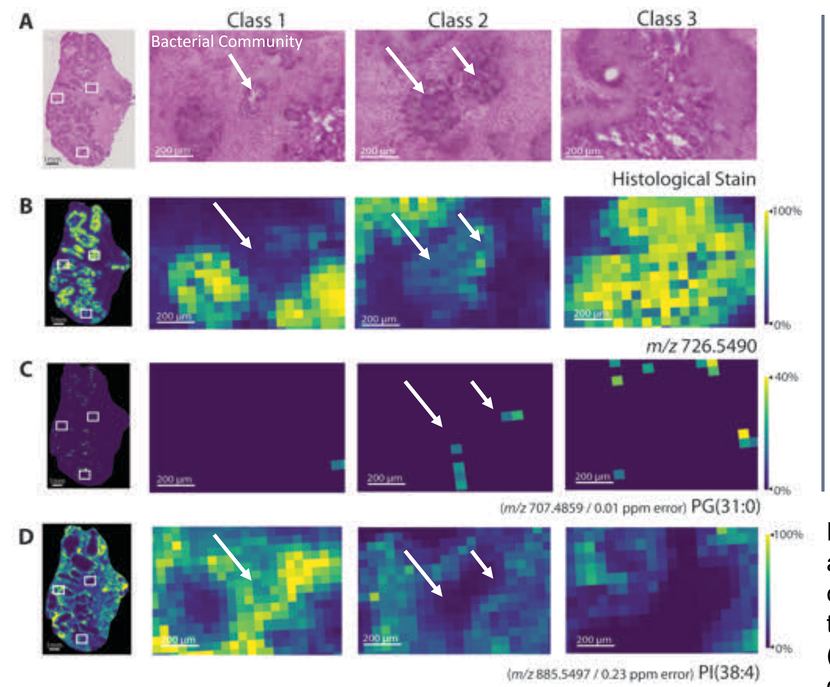


Abscesses and infectious lesions were segregated into 3 classes: abscess communities (class 1), abscess communities with immune cell recruitment (class 2), and abscess communities with immune cell recruitment and necrosis (class 3).

CONCLUSIONS AND FUTURE WORK:

- A staphylococci tissue abscess pathology classification system describing lesion morphology was developed and implemented to compare regions within downstream IMS data.
- Ions were isolated from IMS of lipids and metabolites with discriminative localizations across tissue abscesses.
- Future work includes identification of isolated ions from our approach.
- Other future work includes the use of micro computed tomography as an *in vivo* imaging approach to visualize abscess development and maturation.

RESULTS: DISCRIMINATIVE IONS ARE PRESENT ACROSS HISTOLOGICAL CLASSES



Histological stains show isolated classes of abscesses for IMS of lipids (**A**) and metabolites (**E**). Ions were isolated that increase in abundance from class 1 to class 3 (**B, C, F, & G**) or decrease (**D**). Specifically, we attribute the ion in **B** to be consistent with immune cell recruitment, and PI(38:4) (**D**) with healthy kidney tissue. All identifications are tentative and based on accurate mass measurements. Arrows point to bacterial foci.

REFERENCES: **1)** DeLeo, *et al., Lancet,* 2010. **2)** Klevens, *et al., JAMA,* 2007. **3)** Cheng, *et al., Trends Microbiol,* 2011. **4)** Cassat & Moore, *et. al., Sci Trans Med*, 2018. **5) 6)** Angel, *et al., Anal Chem,* 2012. **6)** Perry, *et al.*, PNAS, 2019.

ACKNOWLEDGEMENTS: This work was supported by the NIH/NIGMS (5P41 GM103391-08), NIH/NIAID (R01AI138581), & NIH/OD shared instrumentation grant (1S10 OD012359-01).