profiles observed in patients before DAA therapy showed a strong macrophage-mediated inflammatory response against HCV infection in the liver that shifted significantly to a tissue remodeling microenvironment after treatment.

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Breast Cancer Biopsy Triage with Ex-Vivo Microscopy for Downstream Analysis*

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OBJECTIVES/GOALS: In this study, the ability of a pathologist to detect malignancy on digital pseudo-H&E slides obtained via structured illumination microscopy (SIM) imaging of fresh diagnostic breast biopsies was assessed. The speed of imaging and processing was also assessed for potential clinical implementation. METHODS/STUDY POPULATION: This study was conducted in accordance with an Ochsner Medical Center of New Orleans IRB. 200 patients undergoing either stereotactic or ultrasound-guided diagnostic breast biopsies were consented and an additional core from the suspicious lesion was collected for research use. Research biopsies were transported to the lab and stained with DRAQ5 and Eosin-Y and imaged with SIM before being submitted for histology processing. Imaging and digital processing times were recorded. The resulting SIM images and histology slides were given to a pathologist for blind review to assess accuracy. RESULTS/ANTICIPATED RESULTS: The ex-vivo structured illumination microscopy images and subsequent histology slides from 79 research cores have been assessed to date. Some samples were excluded from the total data set and not included in the final assessment due to technical failures of the imaging protocol. Of the current set, the pathologist has a specificity of 88% and a sensitivity of 65%, as well as an NPV of 88% and a PPV of 65%. Staining time for each biopsy was completed within 3 and a half minutes and imaging at 20x magnification took between 4 and 12 minutes, depending on size and implementation of autofocus to the imaging system. Image processing took approximately 5 minutes per biopsy and is a direct function of biopsy size. DISCUSSION/SIGNIFICANCE: Decreased time between cancer suspicion and treatment will improve the prognosis of breast cancer patients. SIM imaging of fresh breast biopsies could ultimately allow primary and secondary histology to be performed simultaneously and minimize histopathology time, thus allowing clinicians and patients to implement treatment course more quickly.

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Characterization of Glycosylation Patterns of Single IgA Molecules Using Single-Molecule Fluorescence Microscopy

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OBJECTIVES/GOALS: IgA1 nephropathy, which can lead to kidney failure, is caused by complexes formed between aberrant galactose-deficient IgA1 and antibodies directed against it. Our goals are to characterize shifting glycosylation patterns at the level of single IgA1 molecules and to apply this to patient samples for early detection and understanding of the disease. METHODS/

STUDY POPULATION: To characterize glycosylation patterns on single IgA1 molecules, labelled IgA1 in low concentration was physisorbed to borosilicate glass in a fluidic cell and labelled Jacalin was flowed in to bind with Glycans on IgA1. The samples were observed with a Nikon TiE epi-fluorescence microscope. FRET images were created by exciting the Jacalin dye with a blue laser and recording the red emission of the IgA1 dye with an EMCCD camera. FRET emission intensities of individual IgA1 molecules over time were analyzed to determine how frequent and how long Jacalin binds to each of them. The rate of binding is roughly inversely proportional to the amount of abnormal glycans on a given IgA1 molecule. After the method is perfected, we intend to compare the glycosylation patterns of healthy and diseased patient samples. RESULTS/ANTICIPATED RESULTS: Addition of the competitive binder Galactose to the solution led to an increase in the off times of Jacalin and IgA1 and a decrease in the on times in a concentration-dependent manner, yielding an increase in the dissociation constant. Dissociation constants of individual molecules within a single experiment vary by 3 orders of magnitude, which cannot be attributed to stochastic fluctuations but rather reflects differences in the adsorption geometry. Nevertheless, the unaffected dissociation constant can be identified. We expect that when this method is applied to samples from healthy and IgA1 Nephropathy patients, specific IgA1 molecules from patients will have higher dissociation constants for Jacalin compared to those from healthy patients. DISCUSSION/SIGNIFICANCE: The binding rates of Jacalin to single IgA1 vary by 3 orders of magnitude. The observed heterogeneity shows the Jacalin probe can differentiate between different IgA1 populations. An understanding of which IgA1 molecules in patient samples are problematic and what their distribution of Glycans is can lead to discovering biomarkers and treatments.

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Connecting Electronic Medical Record Sub-phenotypes of Obstructive Sleep Apnea to Adverse Outcome Risks

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OBJECTIVES/GOALS: Prior work has established subtypes of OSA and linked them to risks of future adverse events but rarely with the longitudinality and richness of data available in the EMR. Our goal is to leverage EMR data identify clinically meaningful sub-phenotypes of OSA and better study how they affect risks of adverse outcomes. METHODS/STUDY POPULATION: Vanderbilt's EMR database has over 61,000 adult patients with a literature-validated EMR definition of OSA with a median EMR follow-up period of 4 years after OSA diagnosis. Of these patients, 12,516 have fully recorded sleep study data in addition to EMR variables such as age at study and most recent BMI. We applied several clustering methods including to identify natural sub-phenotypes of OSA and assessed cluster quality. We also applied techniques which allow a single patient to belong to multiple clusters in various degrees. After selecting final clusters, we plan to analyze the associations between OSA sub-phenotypes and risks using statistical tools like logistic regression and Cox proportional hazards regression, with and without adjusting for factors such as age, gender, and certain medications. RESULTS/ANTICIPATED RESULTS: Preliminary clustering with primarily sleep study data has shown overlap with literature-described patient clusters, including a severe, high non-REM stage 1 sleep, high BMI cluster and a high