PROGRESS REPORT SUMMARY

A. SPECIFIC AIMS:

The specific aims are unchanged.

B. STUDIES AND RESULTS:

The spectrum of lesions in patients with diabetes who do not have classic or overt diabetic nephropathy is not established. Recently, there has been recognition that many patients with diabetes experience chronic kidney disease without significant albuminuria or evidence of other primary glomerulonephritis. Whether this injury is also attributable to diabetes or other underlying unrecognized injury processes is not established. The typical diabetic patient with nephropathy does not undergo renal biopsy. Current renal biopsies done in diabetic patients for clinical indications thus show a high incidence of disease superimposed on diabetes, such as IgA nephropathy, crescentic glomerulonephritis, proliferative glomerulonephritis, post-infectious glomerulonephritis, etc. Many show no other morphologic findings other than diabetic nephropathy, but diseases more advanced than predicted from the clinical data. Our goal is to use novel proteomic techniques to mine and phenotype diabetic nephropathy from existing archival renal biopsies, and prospectively to examine tissues obtained from nephrectomies in diabetic and non-diabetic patients with or without nephropathy, and in autopsies. These approaches will allow us to map the spectrum of lesions that may be attributable to diabetes or to other unrecognized abnormalities in diabetic patients. These pilot studies will importantly interact with a companion pilot grant proposing to establish a pre-consenting cohort for tissue collection that will be linked to a synthetic derivative electronic medical record (BioVU). Validation of a detailed phenotype of existing diabetic nephropathy will allow detailed prospective mining of such samples that we plan to collect, building on our expertise with BioVU. Our Aims for this project have been to test the feasibility of examining the presence of proteins, metabolites, and lipid derivatives in tissue, linked to the microenvironmental phenotype, by use of the novel and powerful technique of ***histology-directed MALDI imaging mass spectrometry (MALDI IMS)*** and fusion of these data with light and electron microscopic findings and to link novel identified compounds in the diabetic glomerulus to detailed phenotypic analysis by light microscopy with morphometry and electron microscopy. We are now testing feasibility of using a histology-directed mass spectroscopy profiling approach to link to the morphologic appearance in these biorepository specimens.

We have also developed novel strategies to allow MALDI IMS and microscopy to be fused into a single pseudo-modality. Specifically, we combine an imaging modality that has high spatial resolution and low chemical information (microscopy) with a modality that has relatively low spatial resolution but high chemical information (MALDI IMS) in order to produce a higher-resolution view of molecular expression in tissue. This concept of sharpening MALDI IMS images has been inspired by developments in the realm of remote sensing and satellite imaging. In the latter cases, the concept of pan-sharpening was introduced, in which a high resolution pan-chromatic (gray-level) image and a low-resolution multispectral (color) image can be merged into a single high resolution color image. Our goal is to achieve a similar result but at the micrometer-scale. However, given the difference in measurement principles and distortion sources, the algorithms and implementations from the satellite-imaging field cannot be transferred readily to MALDI IMS. Instead, we have developed a custom sharpening algorithm that has been optimized to work with mass spectrometry data as well as other relevant molecular imaging modalities. This allows us to take an ion image acquired at a set

spatial resolution and to obtain a higher-resolution estimate of that same ion image using the information gleaned from an accompanying tissue picture, be it a standard H&E stain or a specialized immunohistological stain.

In initial studies of human diabetic disease, we identified 32 diabetic nephropathy (DN) biopsies done for cause. Patients were selected with at least 3 years of follow-up. Biopsies were chosen with a range of severity of diabetic lesions, spanning class I to IV. Patients were further categorized as to progressors vs. nonprogressors, with 2 patients even showing improved eGFR over follow-up. MALDI IMS preliminary data showed feasibility of detecting differential glomerular expression in these categories, with statistically significantly different peaks in each grade of severity.

C. SIGNIFICANCE:

This pilot project was designed to develop and model new and powerful modalities that will allow us to utilize these kidney samples collected in the biorepository to link standard microscopy to proteomic analysis. Traditional methods of analysis of the proteome, lipidome or metabolome cannot provide insight into specific histologic alterations underlying diabetic nephropathy because they only provide data on total tissue homogenates. Methods such as isolation of glomeruli or laser capture microscopy may provide information about an individual glomerulus but do not allow coupling to histologic changes or identification of the cellular source of any identified proteins. Matrix assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS) is a technology that acquires molecular information from thin tissue sections in a spatially-defined manner. The spatial localization of hundreds of molecules can be detected from a single tissue section without the need for specific antibodies or *a priori* knowledge of what proteins are present. The resolution allows *in situ* scanning of an individual glomerulus and identification of proteomic changes at a cellular level. The coupling of detailed morphology by light microscopy, immunofluorescence and electron microscopy with analysis of metabolites, proteins and lipids can potentially yield powerful insights into local perturbations of structure and function, potentially pointing to novel pathways for intervention or understanding of mechanisms of disease.

In this pilot study, we are testing the application of this novel modality to the study of diabetic nephropathy and we will link mass spectroscopy imaging and state-of-the-art standard morphological abnormalities across a spectrum of diabetic lesions (from renal biopsies), comparing to tissues from patients without diabetes (donor biopsies), or patients with diabetes without overt diabetic nephropathy (nephrectomy remnant tissue or autopsy).

D. PLANS:

Our overarching goal for the pilot phase of this resource expansion is to enable the capability to extract focused data and histological images and request accompanying biospecimens for further study. Ultimately, human tissue banking could enable a broad range of research questions including those related to environmental exposures, disease progression, reproductive outcomes, and cancer. Specifically, linking tissue specimens to DNA samples and clinical information will be important in the genetic and biochemical characterization of diabetic nephropathy risk and disease progression. We have submitted an R24 proposal to build on these pilot studies, and plan, if funded, to expand to collect several hundred specimens a year.

E. PUBLICATIONS (directly and partially supported by this grant):
None

F. PROJECT-GENERATED RESOURCES:
N/A