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### Copula-Based Regression Models for Correlated Bivariate Binary Outcomes: Application to Ophthalmologic Data Structures

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**OBJECTIVES/SPECIFIC AIMS:** To account for association between the pair of binary outcomes, we adopt the Clayton and Frank copulas to indirectly specify their joint distributions. **METHODS/STUDY POPULATION:** We propose a regression model for the joint modelling of correlated bivariate outcomes using copulas. **RESULTS/ANTICIPATED RESULTS:** develop full maximum likelihood inference.

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### Defining the Extracellular Vesicle Content of Interstitial Fluid for Blood-Free Diagnostics; Extraction Methods and Initial Characterization

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**OBJECTIVES/SPECIFIC AIMS:** Recent advances in microneedle technology have enabled practical, in vivo dermal interstitial fluid (ISF) sampling. These minimally-invasive techniques allow for collection of ISF without damage to adjacent tissues and do not rely on blister formation. Initial reports of extracellular vesicle (EV) isolation from dermal ISF and paired blood samples suggest that EVs may be more abundant in ISF. Analysis of ISF-derived EVs may allow for more detailed study of intercellular communication at the tissue level, particularly in acute inflammatory conditions. The objective of this study is to describe the isolation and initial characterization of interstitial fluid-derived exosomes. **METHODS/STUDY POPULATION:** We apply electron microscopy, nanoparticle tracking analysis (NTA), immunochemical, and sequencing methods to describe and distinguish the EV content of interstitial fluid. We include apparently healthy adult human subjects with no active skin disease. We also study immunocompetent, CD-hairless rats to demonstrate the generalizability of the methods. **RESULTS/ANTICIPATED RESULTS:** We successfully isolated EVs from human and rat interstitial fluid using commercially available precipitation methods. The EVs were initially characterized using UV/Vis spectroscopy, electron microscopy, and NTA. While the study is ongoing, initial results suggest that the concentration and size distribution of EVs differs significantly between blood fractions and ISF. Further immunochemical and sequencing characterization is ongoing. **DISCUSSION/SIGNIFICANCE OF IMPACT:** We present here the initial characterization of EVs isolated from dermal interstitial fluid. This appears to be the first report of EV characterization using ISF collection methods that do not perturb adjacent tissues (such as with blister or microdialysis methods). The present study lays a foundation for further examination of ISF-derived EVs in acute inflammatory disease such as cellulitis or infectious neuritis. This may enable minimally invasive diagnostics and new research tools to understand intercellular communication in living organisms with increased spatial and temporal resolution.

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### Delayed Administration Of Angiotensin Receptor (AT2R) Agonist C21 Downregulates Diabetes Induced Pro-Inflammatory Microglia Activation To Improve Cognitive & Functional Recovery Post Stroke: Therapeutic Indications For The Treatment Of Vascular Cognitive

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**OBJECTIVES/SPECIFIC AIMS:** The study aim was to 1) elucidate mechanisms contributing to the evolution of PSCI using a clinically relevant model of diabetes, a major risk factor for stroke and cognitive impairment, and 2) develop angiotensin type 2 receptor (AT2R) agonism as a therapeutic target. **METHODS/STUDY POPULATION:** Diabetes was induced in male Wistar rats by a HFD & low dose streptozotocin combination. At 12-14 weeks of age a total of 69 control & diabetic rats were subjected to 1 hr middle cerebral artery occlusion (MCAO) or Sham surgery. 3 days post-MCAO, rats that met the pre-set inclusion criteria were administered C21 or saline in drinking water at a dose of 0.12 mg/kg/day Adhesive removal task (ART) & 2-trial Ymaze were utilized to test sensorimotor & cognitive function at baseline as well as 1, 2, 4 and 8 weeks post-stroke. At week 8 post-stroke cell suspensions from freshly harvested brains were analyzed by flow cytometry utilizing antibodies against cell surface markers for M1 (CD11b+/CD45 low/ CD86+/TNFa+), M2 (CD11b+/CD45 low/ CD206+/IL-10+), and residential microglia (CD11b+/CD45+/TMEM119+). **RESULTS/ANTICIPATED RESULTS:** Control rats progressively recovered from stroke-induced functional deficits by week 8, while diabetics still remained impaired (P< 0.05). 8 weeks post-MCAO only diabetic rats exhibited a decline in sensorimotor (P< 0.05) and cognitive function (P< 0.05) compared to Shams. Delayed administration of C21 on D2 post-stroke halted the decline and improved sensorimotor (P< 0.05) and cognitive function (P< 0.01). Flow cytometric analyses indicate that 8 post-stroke vehicle diabetics had an elevated M1/M2 ratio within the ipsilateral prefrontal cortex and hippocampus (P< 0.01, 0.01). They also had a larger percentage of non-residential microglia/macrophages, indicative of compromised blood brain barrier (BBB) integrity. Treatment with C21 significantly lowered the M1/M2 ratio (P< 0.05) and improved the BBB integrity. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Taken together this study suggests that the use of comorbid disease models such as diabetes, may allow for more translational evaluations of PSCI. Higher translational relevance may also lead to a higher number of successful clinical trials and more FDA approved stroke therapies. It also suggests that C21 may serve as a potential therapeutic to modulate the development of PSCI.

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### Development of a Contractile Fontan Circuit to Decrease Central Venous Pressures in Single Ventricle Patients

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**OBJECTIVES/SPECIFIC AIMS:** Children born with a single ventricle congenital heart defect requires three invasive open-heart surgeries in the first three years of life. The third operation, the