The Blood and Clot Thrombectomy Registry and Collaboration (BACTRAC) Protocol: Evaluation of Optimal RNA Extraction and Proteomic Analyses in Stroke

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Abstracts will be considered for both poster and platform presentations

Stroke/Neurovascular

BACKGROUND: Mechanical thrombectomy offers a unique opportunity to examine neurophysiological changes occurring during stroke. We developed a tissue banking protocol to capture intracranial thrombi as well as the blood immediately proximal and distal. These tissues provide a rare resource to correlate changes occurring at the time of stroke to patient outcomes. The purpose of this study is to develop a tissue banking protocol in order to provide high-quality yields of RNA for gene expression analysis and protein for proteomics.

METHODS: We instituted an IRB-approved protocol for tissue processing during mechanical thrombectomy (www.clinicaltrials.gov NCT03153683). The protocol is a joint clinical/basic science effort among multiple laboratories and the NeuroInterventional Radiology service line. We developed a step-by-step process for specimen retrieval, initial processing, and storage, which begins in a designated workspace constructed for the study in the angiography suite. Further processing and analysis is performed in our basic science laboratories, and includes extraction of RNA from thrombi with distal and proximal blood samples to assess RNA integrity number (RIN) values. To demonstrate viability of the collected plasma, proteomics of distal and proximal blood was evaluated through an outside vendor (Olink Proteomics).

RESULTS: We have enrolled 34 subjects (age = 66 ± 13.3, 14 males) in the BACTRAC registry thusfar. In our preliminary dataset, the process yielded adequate RNA for gene expression analysis and protein for proteomics. RNA integrity analysis reveal mean RIN values (± SD) for distal arterial blood (n = 8) were 8.89 ± 0.41 (range from 8.30 to 9.60) and mean RIN values (± SD) for proximal arterial blood (n = 8) were 6.98 ± 0.29 (range from 6.60 to 7.50), indicating moderate to high quality RNA with minimal degradation. Mean RIN values for the thrombi (n = 7) were undetectable. The first subject samples of distal and proximal blood were analyzed for proteomics. Duplicates of these samples passed quality control, and were run on all 11 of their proteomic panels (92 proteins/panel) for a total of 1012 proteins. 100% of proteins were detected on cardiovascular 3 and neurology panels. The organ damage panel detected the fewest, with 61% of this panel. The cardiometabolic and cardiovascular 3 panels each showed the most proteins with the greatest differences in expression.

CONCLUSION: We have developed a novel tissue banking protocol utilizing mechanical thrombectomy to capture thrombus along with arterial blood distal and proximal to it. The protocol provides high-quality specimens facilitating analysis of the initial molecular response to ischemic stroke in the human condition for the first time. In addition, preliminary integrity analyses demonstrated high-quality yields for RNA and protein in distal and proximal blood samples.