# Analysis of retrieved stroke thrombi from mechanical thrombectomy using X-ray fluorescence imaging and Fourier-transform infrared spectroscopy.

Vedashree R. Meher[1], Nicole J. Sylvain[2], M. Jake Pushie[2], Huishu R. Hou[2], Roland N. Auer[3], Lissa Peeling[2] and Michael E. Kelly[2]

[1]Department of Health Sciences, College of Medicine, University of Saskatchewan; [2]Department of Surgery, College of Medicine, University of Saskatchewan, [3]Department of Pathology and Laboratory Medicine, College of Medicine, University of Saskatchewan

## Background

With recent advances in endovascular therapy (EVT), a technique whereby the thrombus is mechanically retrieved [1] has demonstrated significant improvement in the health outcomes of patients with large vessel occlusions [2]. However, interventionists occasionally have to perform multiple passes to extract the thrombus using multiple deployment approaches and sometimes are still unable to completely recanalize. We **hypothesize** that this is due to two main reasons: 1. endothelialization, and 2. trapped biological elements within the core of the thrombus that result in further compaction. We **aim** to characterize the distribution of elements and biological macromolecules that contribute in densifying clots, to understand whether EVT outcomes are influenced by thrombus composition.

**Objectives:**

1. To characterize elemental composition using synchrotron-based imaging, X-ray fluorescence imaging (XFI).
2. To identify amide bands, protein, glutamate and fibrinogen distribution in thrombi using Fourier transform infrared spectroscopy (FTIR).
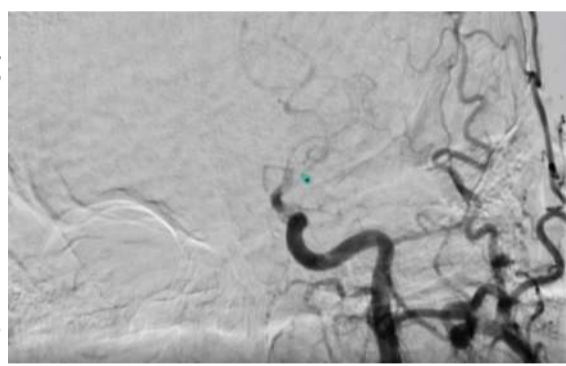
## Methods



**Figure 1.** Demonstrates the steps for sample preparation from the time the clot is freshly retrieved by EVT (A) until it is flash frozen in isopentane within 60 mins of recanalization (C). White asterisk indicates stent retrieval of clot during EVT.

**Study population:** Since September 2017, we have enrolled 19 patients with AIS who received EVT at Royal University Hospital. In all patients, symptomatic large vessel occlusions, and infarction were confirmed by computed tomography angiograms (CTA) and CT scans. Clinical, and radiological data including surgical approaches, attempts, and devices were prospectively collected.

**Sample Processing:** Flash frozen samples were cryosectioned at 7μm for histology and for FTIR (collected on CaF$_2$ discs), 30 μm for XFI (collected on metal-free thermanox coverslips).
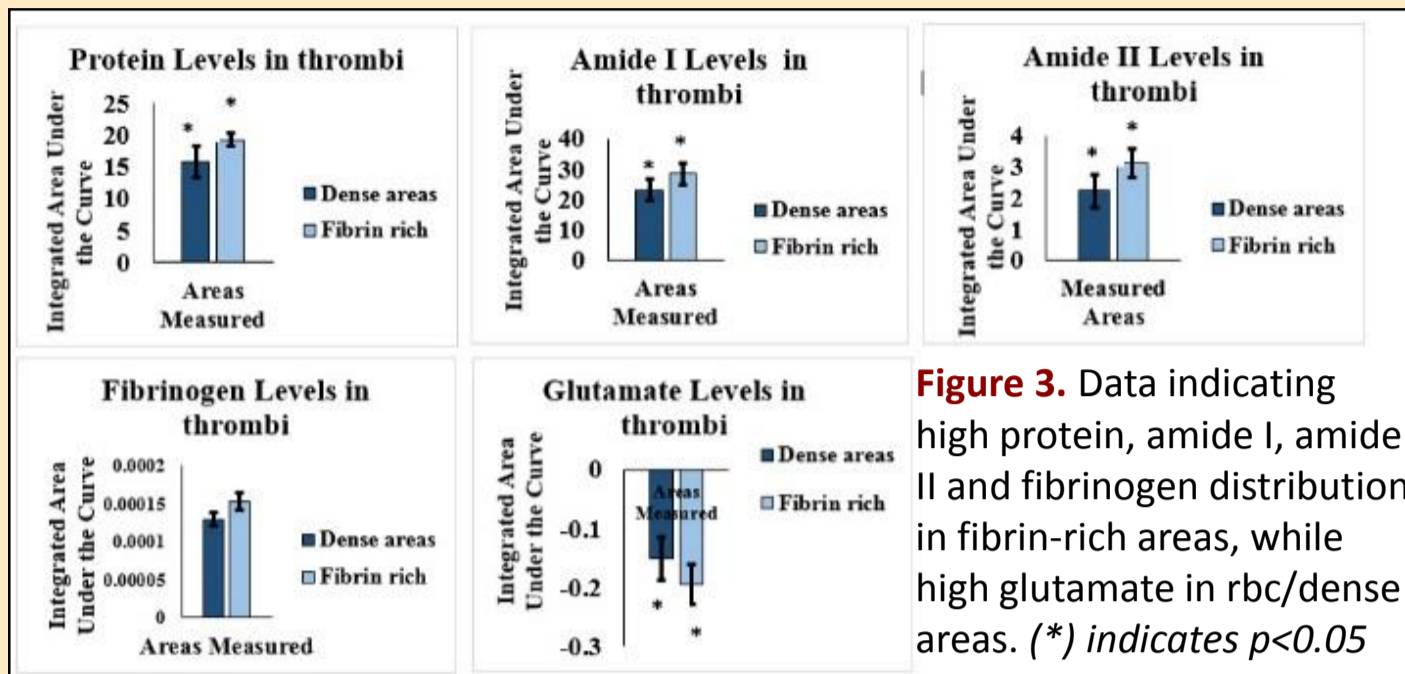
## FTIR Results
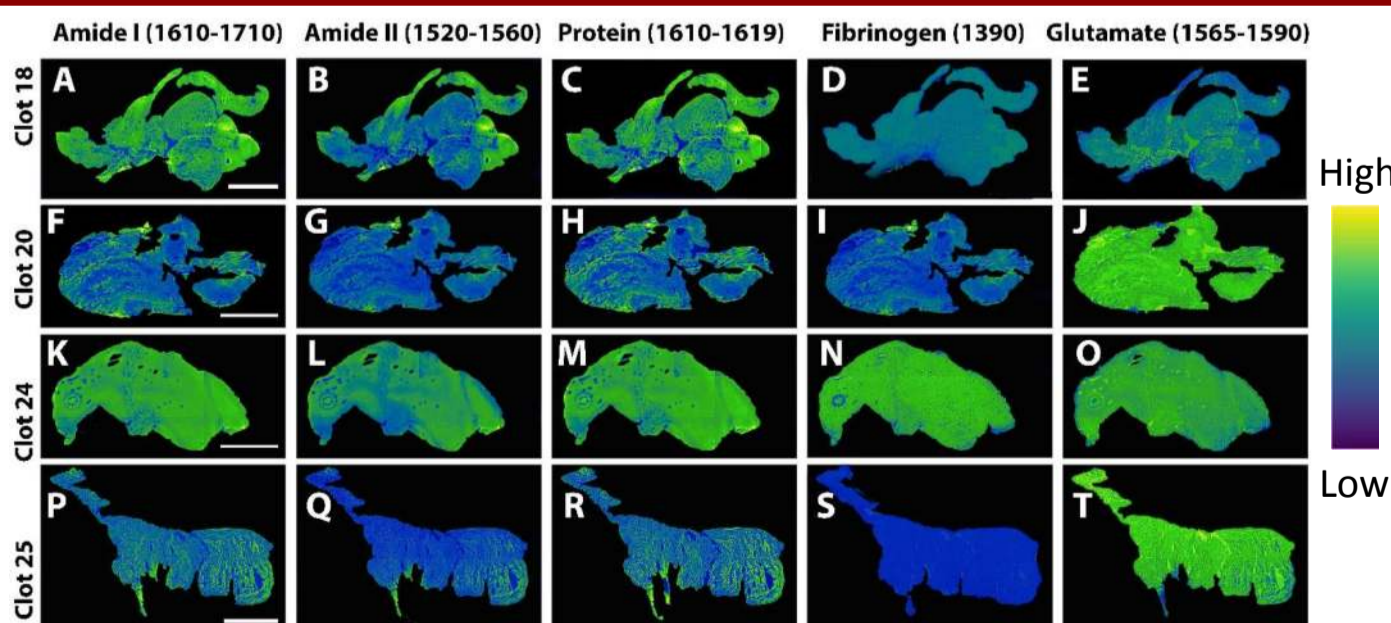


**Figure 2.** FTIR distribution map to simultaneously visualize biological molecules using agilent FTIR microspectrometer and Orange software. Wavelength is indicated in brackets (cm$^{-1}$). Scale bar= 1mm (A-O), 2mm (P-T).
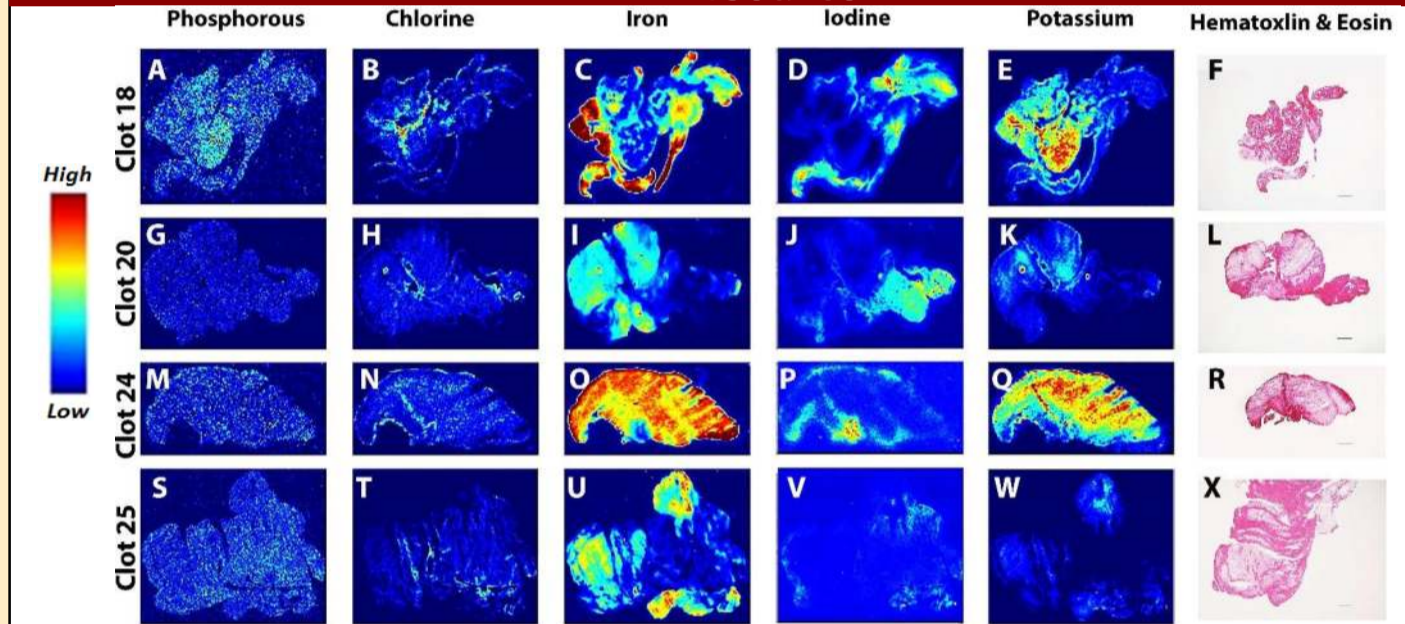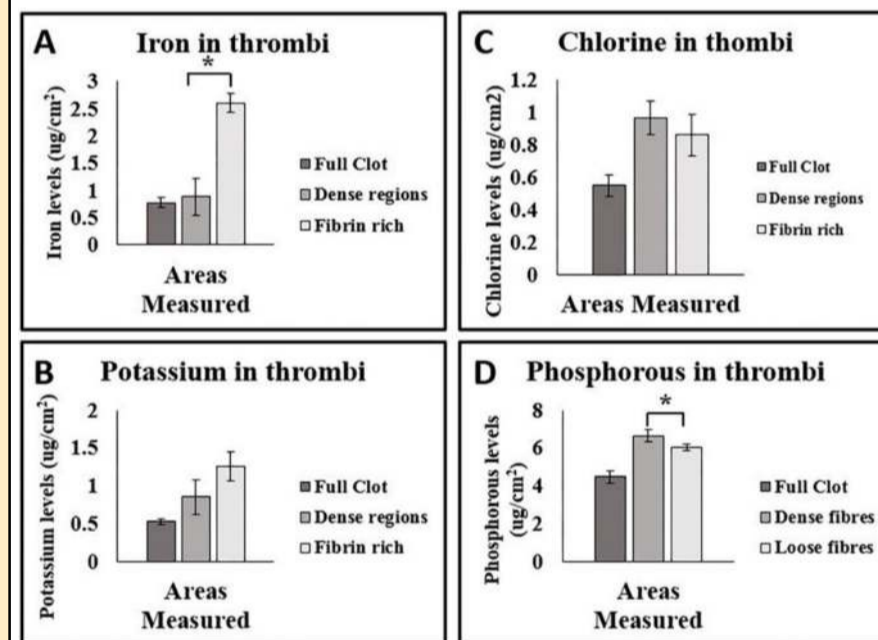


**Figure 3.** Data indicating high protein, amide I, amide II and fibrinogen distribution in fibrin-rich areas, while high glutamate in rbc/dense areas. *(*) indicates p<0.05*

## XFI Results



**Figure 4.** XFI analysis representing elemental distribution. Maps have pseudo-colours to show high concentration areas in red and low concentration areas in blue Scale bar = 1mm (A-R), 2mm (S-X).



**Figure 5.** Quantitative measurement of elemental distribution indicating high concentration of Fe and K in fibrin rich areas (A-B), and high concentration of Cl and P in rbc rich dense regions (C-D). Analysis was done using Sams' microanalysis kit (SMAK) software. (*) indicates *p<0.05*

**Findings:** H&E stained adjacent sections showed high fibrin rich areas in clot 18-24 and high rbc rich regions in clot 25 (Fig 4). XFI distribution map showed high potassium and iron in fibrinous regions while phosphorus and chlorine were higher in dense rbc regions, as confirmed by quantitative analysis (Fig 5). Iodine rich areas probably indicate distal ends of thrombi first exposed to Isovue-300 contrast agent during CTA scans.

## Conclusion

There are many factors that influence clinical outcomes and EVT success. These factors include infarct size, recanalization rate, clot size, clot length, size and location of vessel blockage, collateral flow status, and clot characteristics [3]. It is essential to identify effective approach and device combinations that work quickly to retrieve clots in a time-efficient manner, and minimize post-stroke damage. Defining clot composition using advanced-synchrotron based imaging, will take us one step closer to this goal.

## Acknowledgements

## References

1. Castano C, Remollo S, Garcia MR, Hidalgo C, Hernandez-Perez M and Ciorba M. Mechanical thrombectomy with 'ADAPT' technique by transcervical access in acute ischemic stroke. Neuroradiol J. *2015* 28(6): 617–622
2. Yoshimura S, Sakai N, Okada Y, Kitagawa K, Kimura K, Tanahashi N, Hyogo T, Yamagami H, and Egashira Y. Efficacy of Endovascular Treatment for Acute Cerebral Large-Vessel Occlusion: Analysis of Nationwide Prospective Registry. Journal of Stroke and Cerebrovascular Diseases *2014* 23(5): 1183-1190
3. Alves HC, et al. Associations Between Collateral Status and Thrombus Characteristics and Their Impact in Anterior Circulation Stroke. Stroke. *2018*