INTRODUCTION
Since 2015, mechanical thrombectomy is the standard treatment for emergent large vessel occlusion stroke. Using standard techniques during mechanical thrombectomy, the Blood and Clot Thrombectomy Registry and Collaboration (BACTRAC) protocol (clinicaltrials.gov NCT03153683) isolates intracranial blood within the artery immediately downstream from the thrombus, systemic arterial blood proximal to the thrombus, and the thrombus itself. BACTRAC is the first protocol utilizing the thrombectomy technique to collect local whole blood samples during brain infarction. We aimed to augment the current collection protocol to reproducibly obtain and study local leukocyte populations during human stroke.

METHODS
We started with the established BACTRAC protocol (PMID: 30064997) and modified the tissue collection protocol to isolate lymphocytes for flow cytometry and to bank the systemic compared to intracranial blood of stroke patients compared to controls. Myeloid cells including monocytes, granulocytes, endothelial cells, and progenitors, respectively. Cells were washed twice in FACS buffer and fixed in 1% paraformaldehyde plus 0.1% EDTA on ice for 30 minutes and analyzed on a FACS Symphony the same day. All gating and event analyses were performed in FlowJo V10 (TreeStar) and all statistical analyses were performed in GraphPad Prism 7 (GraphPad Software).

RESULTS
The average lymphocyte isolation yield in the intracranial blood samples of stroke patients was 2.26 × 10^6 cells/ml (SEM 4 × 10^5 cells/ml). The average viability of intracranial samples was 94.41% (SEM 2.53%). The average lymphocyte isolation from the systemic arterial blood was 4.04 × 10^6 cells/ml (SEM 5.1 × 10^5 cells/ml). The average viability of intracranial blood was 94.41% (SEM 2.53%). The average lymphocyte isolation from the systemic arterial blood was 4.04 × 10^6 cells/ml (SEM 5.1 × 10^5 cells/ml). The average viability of intracranial blood was 94.41% (SEM 2.53%). The average lymphocyte isolation from the systemic arterial blood was 4.04 × 10^6 cells/ml (SEM 5.1 × 10^5 cells/ml). The average viability of intracranial blood was 94.41% (SEM 2.53%). The average lymphocyte isolation from the systemic arterial blood was 4.04 × 10^6 cells/ml (SEM 5.1 × 10^5 cells/ml). The average viability of intracranial blood was 94.41% (SEM 2.53%). The average lymphocyte isolation from the systemic arterial blood was 4.04 × 10^6 cells/ml (SEM 5.1 × 10^5 cells/ml). The average viability of intracranial blood was 94.41% (SEM 2.53%). The average lymphocyte isolation from the systemic arterial blood was 4.04 × 10^6 cells/ml (SEM 5.1 × 10^5 cells/ml). The average viability of intracranial blood was 94.41% (SEM 2.53%). The average lymphocyte isolation from the systemic arterial blood was 4.04 × 10^6 cells/ml (SEM 5.1 × 10^5 cells/ml). The average viability of intracranial blood was 94.41% (SEM 2.53%). The average lymphocyte isolation from the systemic arterial blood was 4.04 × 10^6 cells/ml (SEM 5.1 × 10^5 cells/ml). The average viability of intracranial blood was 94.41% (SEM 2.53%). The average lymphocyte isolation from the systemic arterial blood was 4.04 × 10^6 cells/ml (SEM 5.1 × 10^5 cells/ml). The average viability of intracranial blood was 94.41% (SEM 2.53%). The average lymphocyte isolation from the systemic arterial blood was 4.04 × 10^6 cells/ml (SEM 5.1 × 10^5 cells/ml). The average viability of intracranial blood was 94.41% (SEM 2.53%). The average lymphocyte isolation from the systemic arterial blood was 4.04 × 10^6 cells/ml (SEM 5.1 × 10^5 cells/ml). The average viability of intracranial blood was 94.41% (SEM 2.53%). The average lymphocyte isolation from the systemic arterial blood was 4.04 × 10^6 cells/ml (SEM 5.1 × 10^5 cells/ml). The average viability of intracranial blood was 94.41% (SEM 2.53%). The average lymphocyte isolation from the systemic arterial blood was 4.04 × 10^6 cells/ml (SEM 5.1 × 10^5 cells/ml). The average viability of intracranial blood was 94.41% (SEM 2.53%). The average lymphocyte isolation from the systemic arterial blood was 4.04 × 10^6 cells/ml (SEM 5.1 × 10^5 cells/ml). The average viability of intracranial blood was 94.41% (SEM 2.53%). The average lymphocyte isolation from the systemic arterial blood was 4.04 × 10^6 cells/ml (SEM 5.1 × 10^5 cells/ml). The average viability of intracranial blood was 94.41% (SEM 2.53%).

CONCLUSIONS
This modification to the existing BACTRAC protocol provides the opportunity, for the first time, to study changes in local leukocyte populations in the arteries undergoing ischemic stroke in the human condition. Efficient processing of lymphocytes with subsequent flow cytometry analyses will provide insight into the neuroinflammatory microenvironment of the occlusion during stroke. Future studies will be aimed at investigating changes in specific leukocyte populations and how they might relate to patient demographics, patient co-morbidities, infarct volume, and functional recovery. These data will help accelerate translational stroke research to elucidate novel approaches for drug discovery and prognosis.

DISCLOSURES
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