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ABSTRACT

BACKGROUND AND PURPOSE: Perviousness is the differential attenuation on CT of an intracranial arterial occlusive thrombus before and after IV contrast administration. While perviousness/permeability has been shown to be related to various clinical outcomes and reflects histopathologic composition, it remains unclear whether perviousness is also associated with differences in proteomic composition.

MATERIALS AND METHODS: Retrieved clots from 59 patients were evaluated with quantitative mass spectrometry. Proteomic differences between high-perviousness (≥11 HU) and low-perviousness (<11 HU) clots were investigated. Perviousness as a continuous variable was also correlated with protein abundance. Last, an ex vivo lysis assay was performed to investigate the differential susceptibility to tPA, deoxyribonuclease, and ADAMTS13 thrombolysis as a function of perviousness.

RESULTS: In total, 2790 distinct proteins were identified. Thrombus perviousness was associated with distinct proteomic features, including depletion of the macrophage marker CD14 ($P = .039$, $z = 1.176$) and hemoglobin subunit ζ ($P = .046$, $z = 1.68$) in pervious clots. Additionally, proteins involved in platelet cytoskeleton remodeling (tropomyosin α-3-chain) and granule secretion/aggregation (synaptotagmin-like protein 4/FC region receptor II-a) were associated with increasing perviousness ($P < .006$), among numerous other proteins. Monocyte/macrophage-associated proteins (apoptosis-associated specklike protein containing a CARD/SAMHD1) were also depleted in pervious emboli ($P < .002$). Ex vivo lysis indicated that pervious clots were more susceptible to ADAMTS13-augmented tPA thrombolysis compared with impervious clots ($P < .05$), though without differences in deoxyribonuclease digestion.

CONCLUSIONS: Thrombus perviousness is associated with complex proteomic features, including differential abundance of platelet-related proteins in highly permeable clots with monocyte/macrophage depletion. This association may help to explain why highly pervious thrombi were also found more susceptible to ADAMTS13-augmented thrombolysis.

ABBREVIATIONS: CARD = Caspase Recruitment Domain; DNase = deoxyribonuclease; dNTPase = deoxynucleoside triphosphohydrolase; FcγRIIA = FC region receptor II-a; FC = fold change; log2FC = log 2 fold change; PYCARD/ASC = apoptosis-associated specklike protein containing a CARD; RBC = red blood cell; SYTL4 = synaptotagmin-like protein 4; TPM3 = tropomyosin α-3 chain; vWF = von Willebrand factor; WBC = white blood cell

NCCT and contrast-enhanced CTA are typically performed in suspected cases of acute ischemic stroke. Previous studies have demonstrated that characterization of ischemic stroke thrombus with CT can be used to delineate histopathologic composition and even stroke etiology.[1-4] One such imaging feature is thrombus perviousness, or permeability, which is defined as the degree of clot enhancement and the difference in attenuation between the NCCT and CTA phases, and it is thought to reflect the relative porosity of the thrombus.[2,5] Clot permeability has also been shown to be strongly associated with various clinical outcomes, including endovascular thrombectomy efficacy and susceptibility to IV thrombolysis.[6-8] While prior research has investigated how thrombus perviousness relates to histopathologic composition, it remains unclear whether this imaging feature is also linked to more distinguishable protein biology.[2-4,9] Likewise, susceptibility to different treatments or enzymatic lysis might be a function of granular protein content, not able to be delineated on rudimentary histopathology. In this study, we leveraged quantitative mass spectrometry to investigate whether the perviousness of ischemic stroke thrombus is associated with a distinct proteomic composition.

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MATERIALS AND METHODS

The authors certify that this article conforms to the International Committee of Medical Journal Editors recommendations for conduct, reporting, editing, and publication of scholarly work in medical journals and is hereby ethically sound. This study was approved by our institutional review board; documentation is available on request.

Sample Collection and Patient Characteristics

This study was approved by our institutional review board. Patients were consecutively enrolled during the study period and were included only after obtaining consent from either the patient when able or from next of kin/designated health care proxy. Whole ischemic stroke thromboembolic material was collected from 59 patients via mechanical thrombectomy at our single-center tertiary care institution. All thromboemboli were kept moist after being retrieved and were then moved into a Cryo Tube (Thermo Fisher Scientific) and immediately stored at −80°C until thawing for quantitative mass spectrometry analysis. Clinical data from all included patients were also collected and included sex, age, relevant medication, pertinent medical history, and clot location, among other clinical variables. A Fisher exact test was then used to evaluate any differences in patient clinical characteristics between patients with high- and low-thrombus permeability. Evaluation of clot permeability is further described below.

Imaging Analysis of Thrombus Perviousness

All NCCT and CTA studies were obtained on either a 128-section (Ingenuity Core; Phillips Healthcare) or a 256-section (Brilliance iCT; Phillips Healthcare) helical CT scanner. Standard scan parameters for NCCT were the following: 120 kV(peak) tube voltage; 350-mA tube current with occasional fluctuation, no dose modulation; 2.5-mm section thickness. For CTA studies, parameters were the following: 120-kV(p) tube voltage and 415-mA tube current, without dose modulation. On the CTA study, the lowest section thickness (most often 0.8 mm) was used for analysis and data collection. All imaging data collection was completed blinded to clinical outcomes and proteomic composition. NCCT and CTA images were not coregistered but were, instead, read simultaneously on diagnostic monitors within the PACS system to best localize intraluminal thrombus. ROIs were placed to measure thrombus attenuation in Hounsfield units. Up to 3 ROIs were positioned for each clot on the basis of the size, location, and visualization on NCCT and CTA imaging. If >1 ROI was measured, the mean thrombus attenuation in Hounsfield units was calculated and used. Whenever possible, 3 ROIs were used because they have been shown to most accurately assess thrombus attenuation and limit observer variability.[10] This technique has been previously used to measure thrombus permeability/perviousness and has been well-described in prior studies.[2-4,9,10]

Absolute perviousness was calculated as the differences between Hounsfield units on NCCT (HU_{NCCT}) and CTA (HU_{CTA}) (or, perviousness = HU_{CTA} − HU_{NCCT}). The perviousness ratio was also calculated and was defined as HU_{CTA}/HU_{NCCT} to better account for the initial thrombus attenuation. For example, while 2 separate clots measuring 50 HU and 25 HU on initial NCCT could both enhance by 20 HU (HU_{CTA} = 70 and 45, respectively) and thus have the same perviousness, their perviousness ratios would differ (HU_{CTA}/HU_{NCCT} = 1.4 and 1.8, respectively). Thrombi were categorized as either "high perviousness" or "low perviousness" by the median value across all samples, which was calculated as ≥ 11 HU and is in line with prior investigations.[2,4] Similarly, the median value across all samples was also taken to categorize a high- or low-perviousness ratio, which was ≥1.2. An example of a thrombus permeability measurement and calculation can be seen in Fig 1.

Mass Spectrometry Proteomic and Bioinformatic Analyses

All retrieved thromboembolic specimens were gently washed with 1× phosphate buffered saline before being subsequently sonicated, solubilized, and centrifuged. Bicinchoninic acid (Thermo Scientific) was then used to determine the protein concentration within the supernatant with all samples and then was diluted to a final concentration of 1 mg/mL before being digested with trypsin and resuspended. Samples were then processed by our institutional core Mass Spectrometry Resource lab using an Orbitrap Fusion Lumos Tribrid quantitative mass spectrometer (Thermo Fischer Scientific). Resulting raw proteomic data were processed and analyzed using the library-free analysis mode in DIA-NN, Version 1.8.1 (https://github.com/vdemichev/DiaNN). Several bioanalytic methods were used to investigate how clot permeability relates to protein composition: 1) abundance analysis, to assess broad differences in the abundance of proteins between permeable and impermeable clots, and 2) linear analysis, to investigate associations between protein content and permeability as a spectrum/continuous variable.

Functional analysis was also completed to explain possible mechanistic differences based on thrombus perviousness using The Gene Ontology (GO; https://geneontology.org/) Resource enrichment analyses with g:Profiler (Version e109_eg56_p17_1d3191d; https://biit.cs.ut.ee/gprofiler/gost) to identify differentially over-represented GO terms in relation to clot permeability.[11] Wilcoxon rank-sum tests were used to compare the median abundance of individual proteins between clots with high perviousness compared with those with low perviousness. Throughout the investigation, significant differences (defined as a $P < .05$) are described by the fold change (FC) in abundance and are reported with the corresponding absolute z score. Furthermore, correlation between the continuous variable of the perviousness ratio as a function of specific protein abundance was investigated with Spearman rank correlation coefficients and described using the Spearman ρ test statistic.

Evaluation of Susceptibility to Ex Vivo Thrombolysis

To relate perviousness to clot lysis and susceptibly to various lytic enzymes, we completed an ex vivo lysis assay. There were sufficient samples for ex vivo thrombolysis in 26 of the total 59 samples. These samples were thawed from −80°C and portioned into 4 qualitatively representative segments. A small piece of each segment was collected and pooled for mass spectrometry to generate a proteome for the corresponding thrombus. The remainder of the 4 segments was subjected to 1 hour of in vitro lysis at 37°C in

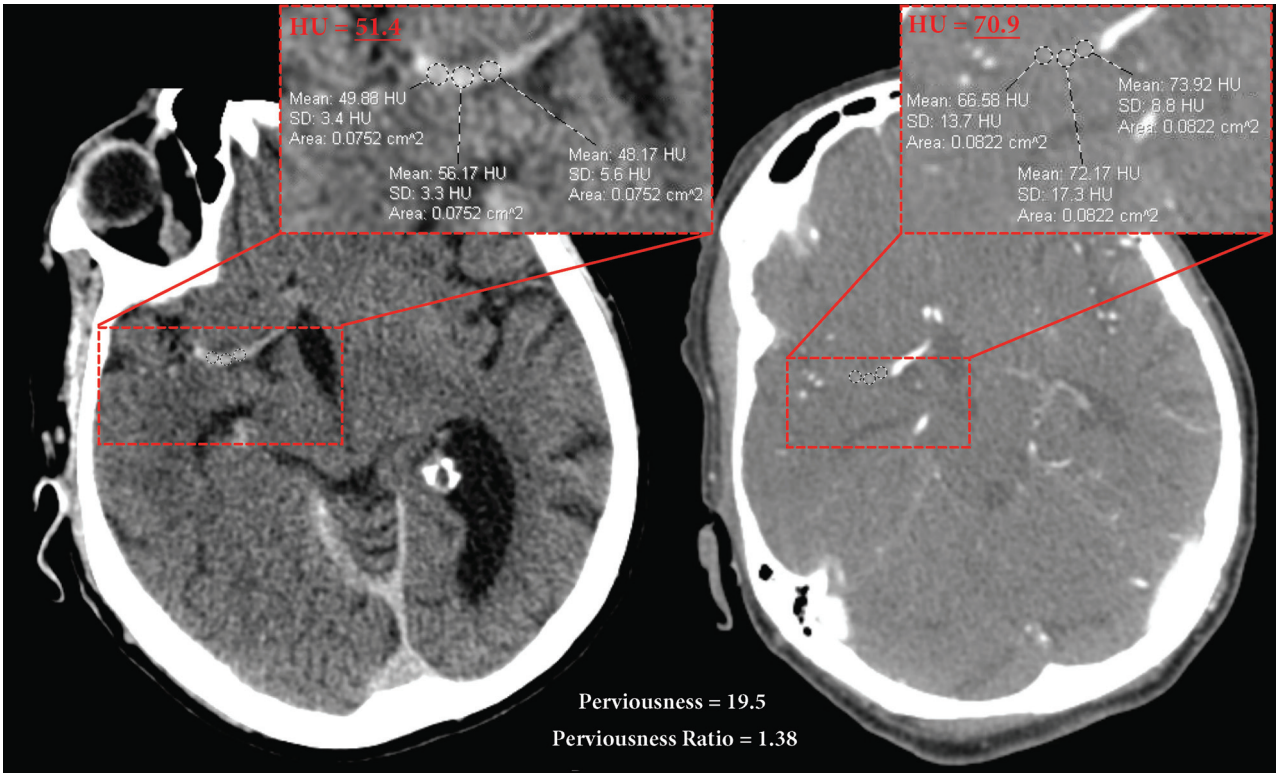

FIG 1. Sample calculation of thrombus perviousness/permeability on admission NCCT (*left*) and CTA (*right*). The image on the left demonstrates NCCT with 3 ROIs placed on the thrombus, with the thrombus attenuation in Hounsfield units calculated as the mean among the ROI values. The image on the right demonstrates a similar thrombus attenuation calculation on CTA. Perviousness was then calculated as the difference in Hounsfield units between CTA and NCCT. Likewise, the perviousness ratio was calculated as the ratio of Hounsfield units of the CTA over NCCT.

1 of 4 lytic solutions with the standard-of-care tPA at 5 μg/mL (T; MyBioSource, MBS142404), tPA + von Willebrand factor (vWF)-cleaving ADAMTS13 at 2.5 μg/mL (TA; MyBioSource, MBS636953), tPA along with DNA-cleaving DNase I at 100 U/mL (Td; MyBioSource, MBS142460), and all 3 enzymes (TDA) at the previously mentioned concentrations. Postlysis residual, characterized by the postlysis thrombus weight as a percentage of the prelysis weight, was compared across the 4 solutions. Spearman coefficients were used to describe the association between lysis and the abundance of all proteins identified by mass spectrometry.

RESULTS

Patient Characteristics

In total, 59 patients were included in the study with 30 (50.8%) retrieved clots being categorized as high perviousness (≥11 HU), and 29 (49.2%), as low perviousness (<11 HU). Overall, patients were an average age of 68.6 years, and 50.8% were men. There were no significant differences in medical history or overall clinical characteristics between the 2 groups (Online Supplemental Data). In total, a history of previously diagnosed atrial fibrillation was present in 30.5% of patients; prior stroke, in 11.9%; active cancer of any form, in 11.9%; hypertension, in 76.3%; and a history of hypercoagulability/clotting disorder, in 5.1% of patients. There was no significant difference in time from last known well to recanalization between clots with high perviousness (mean, 487 [SD, 384] minutes) and low perviousness (mean, 353 [SD, 286] minutes) (P = .14). Furthermore, 28.8% of patients were previously on aspirin, 10.2% were on warfarin, and 40.8% were on a statin, without differences between the 2 groups.

On CTA, the thrombi were located within the ICA in 3.4% of cases and the ICA with extension into the M1 division of the MCA in 13.6% of cases. In 47.5% of the occlusions, the thrombus was located only in the M1 division of the MCA, 20.3% of occlusions were in the M1 with extension into the M2 division, and 10.2% of cases were isolated M2 occlusions. Last, 5.1% of clots were in the basilar artery. Additionally, in 86% (25/29) of low-pervious clots and in 93% (28/30) of high-pervious clots, the location was congruent on CTA and neuroangiography, without significant difference between the groups (P = .38). We also observed no differences in first-pass effect, 90-day functional outcomes (mRS 0–2), or 90-day mortality between the 2 groups. Full details regarding baseline patient characteristics within the study can be seen in the Online Supplemental Data.

Thrombus Perviousness is Associated with Distinguishable Proteomic Features

From the 59 mechanical thrombectomy–retrieved thromboembolic samples, a total of 2790 distinct proteins were identified by mass spectrometry, of which 147 were significantly enriched or depleted on the basis of thrombus permeability (Fig 2). Two proteins were found to be significantly more abundant with a >0.05 log2FC: immunoglobulin heavy variable 1 (P = .04, z = 1.7) and BCL2 interacting protein (P = .16, z = 2.15) (Fig 2). Several other

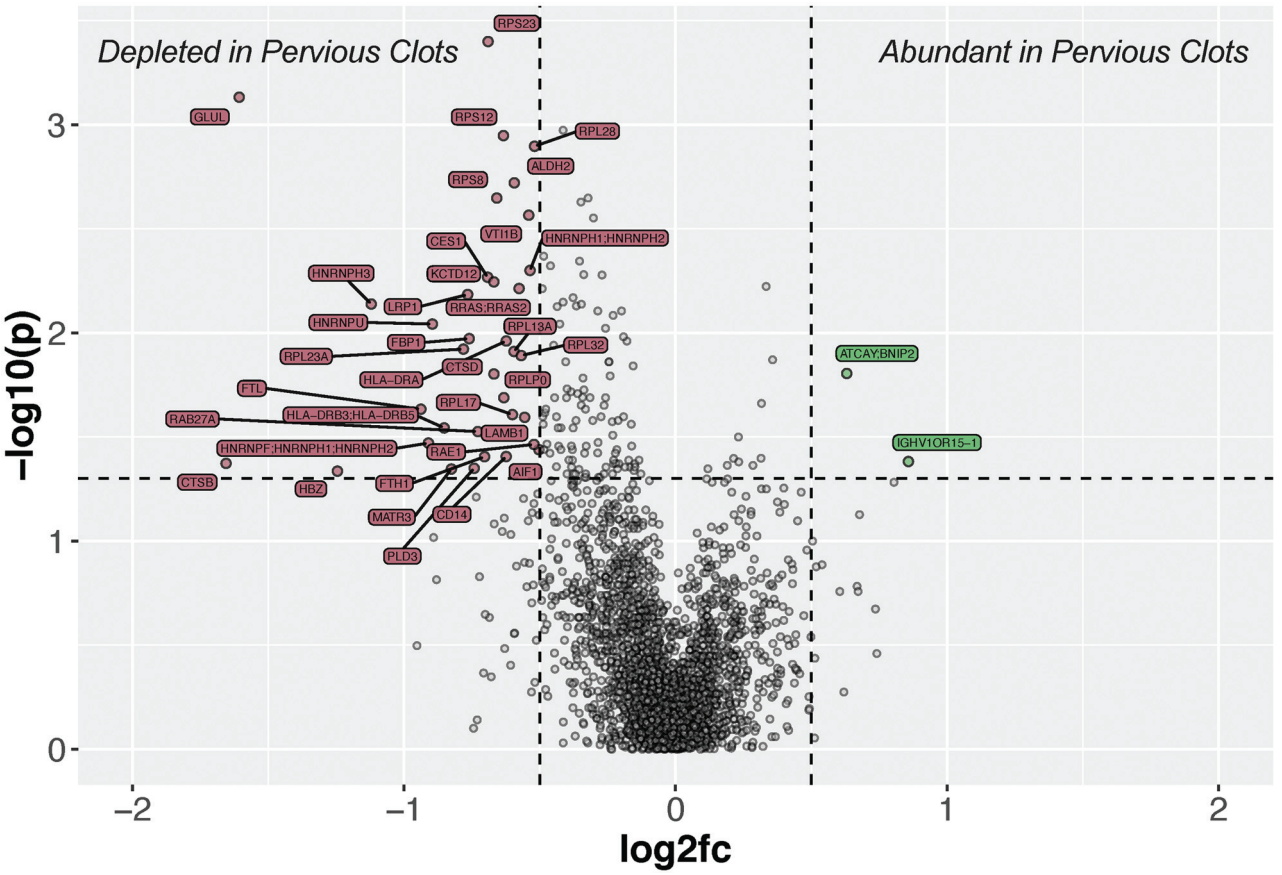

FIG 2. Volcano plot depicting the proteomic landscape of pervious acute ischemic stroke clots. The *left side* of the graph depicts proteins that are significantly depleted (red), while the *right side* depicts those that are differentially abundant (green).

proteins ($n = 12$) were significantly more abundant in highly pervious clots though with a log2FC of <0.05, notable among which were tropomyosin α-3 chain ($P = .0059$, $z = 2.51$), farnesyl pyrophosphate synthase ($P = .013$, $z = 2.21$), and *GTPase NRAS* ($P = .022$, $z = 2.02$).

Many more proteins were found to be significantly depleted in pervious clots relative to impervious clots: most substantially glutamine synthetase ($P = .0007$, $z = 3.2$), several ribosomal 40S/60S proteins (S2/S12/L28/L27, all $P < .003$, $z < -2.8$), and aldehyde dehydrogenase 2 ($P = .002$, $z = -2.90$). Other notable proteins included the following: monocyte/macrophage differentiation antigen CD14 ($P = .039$, $z = -1.176$), NF-kB-kinase subunit β ($P = .007$, $z = -2.44$), and receptor of activated protein C kinase 1 ($P = .009$, $z = -2.36$). A summary of those proteins with the most significant FCs is seen in the Table. For a complete list of all statistically significant differentially abundant proteins as a function of thrombus permeability refer to the Online Supplemental Data.

GO analysis using g:Profiler (Version e109_eg56_p17_1d3191d) of 35 depleted proteins with both $P < .05$ and a logFC of −0.05 in pervious clots revealed that the most significantly represented molecular functions involved ribosomal constituents or RNA binding proteins ($P < .0001$). Similarly, the most significantly depleted biologic processes included those involved in translation ($P < .0001$), with cytosolic ribosomes being the most significantly represented cellular compartment among the proteins. Furthermore, pervious clots were depleted in intracellular ferritin complex binding and iron sequestration (all $P < .05$).

Differential protein abundance was also evaluated as a function of the thrombus perviousness ratio as a surrogate for clot permeability. Among 2790 proteins, 254 were significantly enriched or depleted at a threshold of $P < .05$, and 69, at a threshold of $P < .01$. Notable among the latter group, low-affinity immunoglobulin γ FC region receptor II-a (*FCGR2A*/FcγRIIA) was significantly associated with increasing perviousness ($P = .0091$, $R = 0.34$) (Fig 3*A*). An increasing thrombus perviousness/perviousness ratio was also associated with increased abundance of synaptotagmin-like protein 4 (SYTL4) ($P = .0057$, $R = 0.36$) (Fig 3*B*) and tropomyosin α-3 chain (TPM3) ($P = .00089$, $R = 0.42$) (Fig 3*C*). Conversely, there was a significant negative association between perviousness and hemoglobin subunit ζ ($P = .0046$, $R = -0.37$) (Fig 3*D*), apoptosis-associated specklike protein containing a CARD (PYCARD/ASC) ($P = .0019$, $R = -0.4$) (Fig 3*E*), and deoxynucleoside triphosphohydrolase (dNTPase) SAMHD1 ($P = .0019$, $R = -0.4$) (Fig 3*F*). Additional numerous proteins were either positively or negatively associated with clot perviousness and are listed in the Online Supplemental Data.

Thrombus Perviousness is Associated with Differential Susceptibility to ADAMTS13 Ex Vivo Thrombolysis

To investigate whether clot permeability is related to susceptibility to thrombolysis by different enzymatic degradations, we conducted

Proteins with the most significant abundance and depletion in highly pervious clots

Protein Name	log2fc	*P* Value
40S Ribosomal protein S23	–0.690129341	.00039
Glutamine synthetase	–1.606714657	.00073
40S Ribosomal protein S2	–0.413995286	.001
40S Ribosomal protein S12	–0.633724478	.001
60S Ribosomal protein L28	–0.519178688	.001
Aldehyde dehydrogenase, mitochondrial	–0.593729139	.0019
60S Ribosomal protein L27	–0.321892776	.0022
40S Ribosomal protein S8	–0.658129234	.0022
Electron transfer flavoprotein-ubiquinone oxidoreductase, mitochondrial	–0.348210926	.0023
Vesicle transport through interaction with t-SNAREs homolog 1B	–0.540383664	.0027
Immunoglobulin heavy variable 1/OR15-1 (nonfunctional) (fragment)	0.85769177	.04
BCL2/adenovirus E1B 19 kDa protein-interacting protein 2.0.311	0.630567877	.015
Farnesyl pyrophosphate synthase	0.357444057	.013
Tropomyosin α-3 chain.0.2616	0.333697844	.005
GTPase NRAS.0.1703	0.316709736	.02
Cullin-4A..0.636	0.314913909	.04
V-type proton ATPase subunit G 1	0.285104241	.04
Protein phosphatase 1 regulatory subunit 7	0.232882868	.04
Dedicator of cytokinesis protein 10	0.231574532	.03

an ex vivo thrombolysis assay on 26 of the same mechanical thrombectomy–retrieved thromboemboli. We found no difference in thrombolysis between high- and low-pervious clots when defined as being greater or less than 11 HU (Fig 4*A*). Given this null result, we subsequently evenly distributed the 26 clots on the basis of perviousness, which stratified them by ≥18 HU, and found that clots with high perviousness ($n = 13$) were more susceptible to thrombolysis with tPA-combined ADAMTS13 than clots with low perviousness ($n = 13$) (mean, 66.1% [SD, 22.3%] versus 91.1% [SD, 28.6%], respectively; $P = .034$) (Fig 4*B*). However, there was no difference in ex vivo thrombolysis with tPA alone, combining tPA with DNase, or when combining tPA with ADAMTS13 and DNase (Fig 4*B*). Likewise, clots with a high perviousness ratio defined as ≥1.2 ($n = 15$) were more susceptible to thrombolysis with tPA combined with ADAMTS13 than thrombi with low perviousness ($n = 11$) (mean, 68.1% [SD, 21.8%] versus 92.9% [SD, 30.5%], respectively; $P = .047$) (Fig 4*C*). However, there was no difference in thrombolysis with the other enzymatic combinations. Most important, there was no significant difference in prethrombectomy IV tPA use between the clots with high (≥18 HU) and low perviousness ($P = .19$), or based on a high- and low-perviousness ratio ($P = .07$).

DISCUSSION

We present the first study to investigate whether ischemic stroke thrombus perviousness is associated with a distinguishable proteomic signature. Leveraging quantitative mass spectrometry, we found that retrieved thromboemboli have distinct proteomic features when categorized as high (≥11 HU) or low (<11 HU) perviousness. We also found direct associations between numerous proteins and increasing perviousness, providing insights into cerebral thrombus biology not previously gleaned from prior histopathologic studies. Last, we related clot permeability to an ex vivo lysis assay, revealing that highly pervious clots may be particularly vulnerable to ADAMTS13-augmented tPA thrombolysis. Moreover, this study further demonstrates the feasibility of relating clot imaging characteristics to proteomic composition and ex vivo lysis, a novel technique for thrombus characterization of ischemic stroke.

Thrombus permeability/perviousness has been shown to be associated with clinical outcomes in acute ischemic stroke. For example, post hoc analysis of 165 patients from the COMPASS Trial: A Direct Aspiration First Pass Technique[7] found that clot perviousness was related to first-pass success with frontline aspiration thrombectomy, whereas stent retriever success was not dependent on thrombus permeability. Likewise, data from the The Multicenter Randomized Clinical Trial of Endovascular Treatment for Acute Ischemic Stroke in the Netherlands (MR CLEAN) trial[6] also found that thrombus perviousness was strongly associated with higher recanalization rates, smaller final infarct volume, and improved functional outcomes. Permeable thrombi have also been shown to be more likely to result in recanalization and favorable functional outcomes following IV tPA administration.[6,12] However, likely due to suspected underpowering, we did not detect a difference in clinical outcomes based on clot perviousness within our small cohort of 59 specimens.

A handful of studies have investigated how clot permeability is associated with histopathologic composition; however, data are conflicted, and no studies have examined how it relates to proteomic composition. Benson et al[2] studied retrieved thrombi from 57 patients and found that pervious clots were more red blood cell (RBC)-rich, whereas impervious clots had a higher proportion of fibrin and white blood cells (WBCs). By contrast, both Patel et al[3] and Berndt et al[9] reported that pervious clots had a lower proportion of RBCs and a higher fibrin/platelet content. Additionally, a previous study investigated 24 retrieved ischemic stroke clots and explored how the hyperdense cerebral artery sign was related to proteomic composition; however, thrombus permeability was not evaluated as an imaging variable.[13]

Our proteomic data suggest depletion of RBCs and iron processing with an abundance of platelet proteins in pervious thrombi. We found that RBC hemoglobin ζ was significantly depleted in pervious clots (Online Supplemental Data) and that decreasing

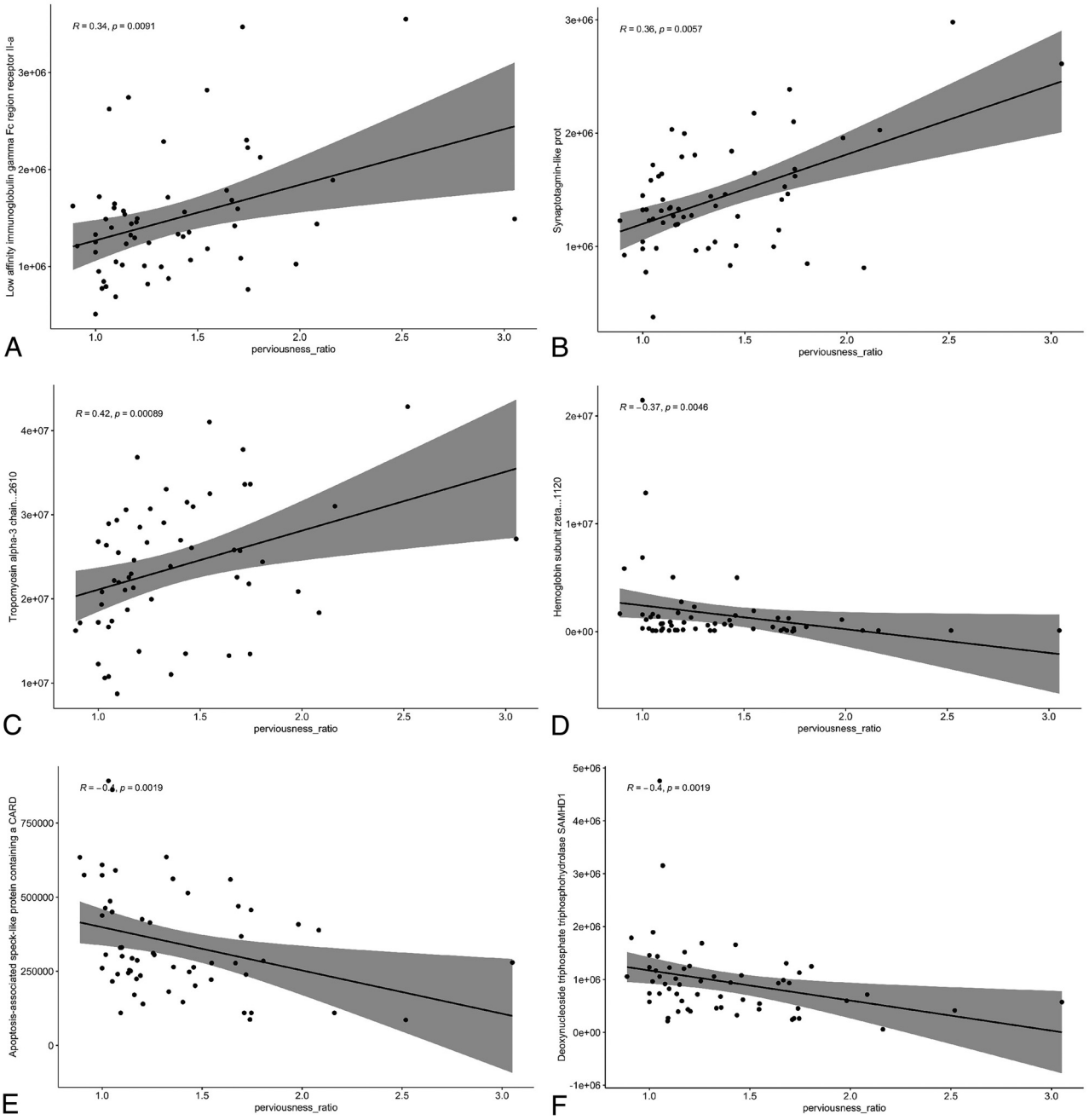

FIG 3. Association between specific protein content and the thrombus perviousness ratio. The relation between increasing perviousness ratio and low-affinity *FCGR2A*/FcγRIIA (*A*), SYTL4 (*B*), TPM3 (*C*), hemoglobin subunit ζ (*D*), PYCARD/ASC (*E*), and dNTPase SAMHD1 (*F*).

perviousness was significantly correlated with decreasing hemoglobin ζ content ($P = .0046$, Fig 3*D*), though the remaining hemoglobin subunits were not significantly different on the basis of clot permeability. GO data found that pervious clots were significantly depleted in ferritin-processing and iron-binding, suggesting depletion of iron-rich RBCs. On the other hand, several platelet activation–related proteins were directly related to thrombus permeability on CT. TPM3 was associated with increasing perviousness and has been suggested to play a role in platelet cytoskeleton remodeling ($P = .00089$, Fig 3*C*).[14] Likewise, SYTL4 was also significantly associated with increased thrombus perviousness ($P = .0057$, Fig 3*B*). SYTL4 is expressed in human platelets and has been shown to regulate attenuated granule secretion.[15,16] Moreover, human platelets express FcγRIIA, an immune complex receptor that fully activates platelets for aggregation/secretion and plays a complex role in immunothrombosis and was found to be significantly correlated with clot perviousness ($P = .0091$, Fig 3*A*).[17,18]

Our observed differences in several monocyte-associated proteins may suggest specific roles of immune-mediated thrombosis in relation to clot permeability. The macrophage-specific marker CD14 was significantly less abundant in pervious clots ($P = .039$, $z = 1.176$). Similarly, PYCARD/ASC, which is a component of the macrophage/monocyte inflammasome, was negatively associated

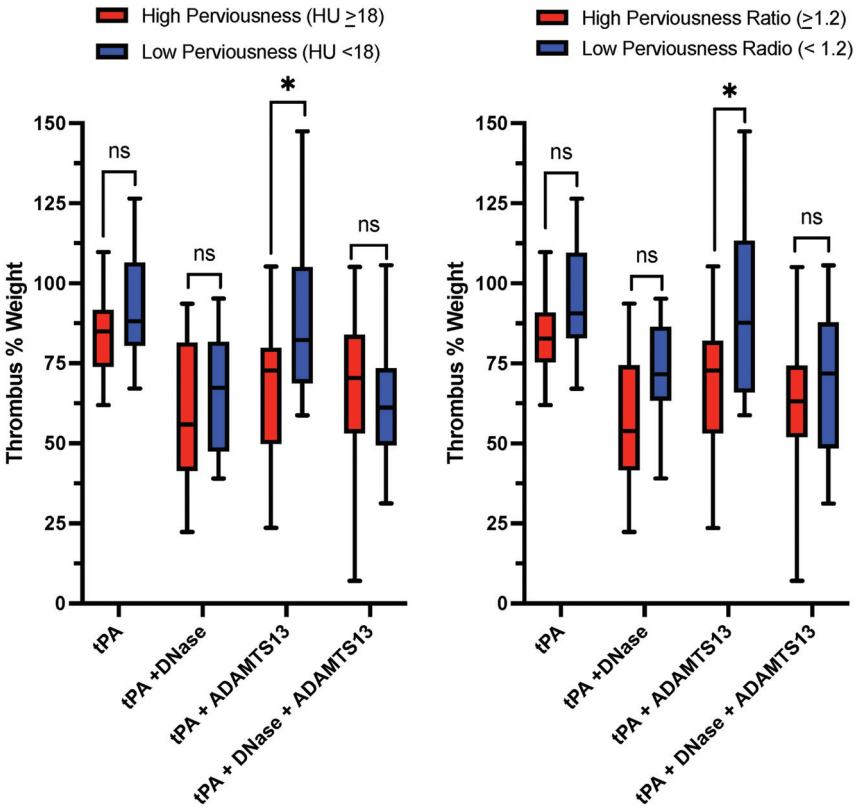

FIG 4. Thrombus perviousness is associated with differential susceptibility to ADAMTS13-augmented thrombolysis. Ns indicates not significant. *$P < .05$.

with increasing thrombus permeability ($P = .0019$, Fig 3*E*).[19] Likewise, dNTPase of SAMHD1, which is expressed in monocytes/macrophages, was negatively associated with increasing permeability ($P = .0019$, Fig 3*F*).[20] These findings are in line with prior observations by Benson et al,[2] who reported that impervious clots were more likely to be WBC-rich relative to permeable thrombi ($P = .01$). Because we did not observe differences in other granulocyte abundances, this prior finding may be reflective of monocyte content. However, Patel et al[3] found no significant difference in WBC content based on clot permeability in 40 received embolectomy samples. Evolving research is indicating a complex role of immune cells in acute ischemic stroke thrombogenesis, with significant heterogeneity within the thrombus immune landscape.[21]

Enhanced susceptibility to ADAMTS13 of pervious clots was the only difference on ex vivo lysis, validating the importance of a preponderance of platelet-associated proteins in pervious emboli on proteomic analysis. ADAMTS13 is an enzyme that cleaves vWF and thus inhibits vWF-mediated platelet aggregation and fibrinogenesis. A prior study found a positive association between fibrin content and vWF concentration in ischemic stroke clots ($P = .0085$) and that vWF-rich/tPA-resistant clots were dose-dependently dissolved by ADAMTS13 administration.[22] Together, our findings provide further evidence that pervious clots are fibrin/platelet rich, which might be leveraged to enhance stroke thrombolysis with ADAMTS13. However, we detected a difference in ADAMTS13 lysis only when clots were stratified by 18 HU (the median perviousness value of those clots that were lysed), suggesting that only very highly permeable clots might be vulnerable to this lysis strategy.

This study has several limitations that need to be considered. First, as with all studies of retrieved stroke thromboemboli, this investigation reflects only clots that were retrievable, with nonretrievable/failed thrombectomy samples being inherently excluded, and may have separate proteomic features. Furthermore, the ex vivo lysis assay was completed on only 44% (26/59) of the thrombus samples, making this analysis relatively less robust compared with the other proteomic analyses reported here. Another consideration is that prethrombectomy IV tPA may have unknowingly influenced some of the results. Although it has been previously shown that IV tPA does not alter histopathologic composition[23] and we found no significant difference in tPA use between groups in both the proteomic and lysis analyses, this influence should still be considered. Additionally, given that stroke imaging proteomics is essentially uncharted territory, we used a less stringent methodology for protein identification without a strict false discovery rate criteria. Consequently, it is necessary to bolster and expand our work with larger sample sizes that allow more stringent statistical analysis.

CONCLUSIONS

CT perviousness of acute ischemic stroke thrombus is associated with proteomic differences related to platelet and immune protein content, among other differences. Given that pervious thrombi exhibit enhanced lysis with ADAMTS13, this feature may open the door for better chemical lysis pathways in which tPA may be less effective.

Disclosure forms provided by the authors are available with the full text and PDF of this article at www.ajnr.org.

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