Brain, Behavior, & Immunity - Health 20 (2022) 100422

Contents lists available at ScienceDirect

Brain, Behavior, & Immunity - Health

journal homepage: www.editorialmanager.com/bbih/default.aspx

Influence of BMI on adenosine deaminase and stroke outcomes in mechanical thrombectomy subjects

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ARTICLE INFO

Keywords:
Adenosine deaminase
Mechanical thrombectomy
Ischemic stroke
Obesity

ABSTRACT

Background: Emergent Large Vessel Occlusion (ELVO) strokes are ischemic vascular events for which novel biomarkers and therapies are needed. The purpose of this study is to investigate the role of Body Mass Index (BMI) on protein expression and signaling at the time of ELVO intervention. Additionally, we highlight the protein adenosine deaminase (ADA), which is a deaminating enzyme that degrades adenosine, which has been shown to be neuroprotective in ischemia. We investigate the relationship between ADA and BMI, stroke outcomes, and associated proteomic networks which might aid in personalizing prognosis and future treatment of ELVO stroke.
Methods: The Blood And Clot Thrombectomy And Collaboration (BACTRAC) study is a continually enrolling tissue bank (clinicaltrials.gov NCT03153683) and registry from stroke patients undergoing mechanical thrombectomy (MT). N = 61 human carotid plasma samples were analyzed for inflammatory and cardiometabolic protein expression by Olink Proteomics. Statistical analyses used t-tests, linear, logistic, and robust regressions, to assess the relationship between BMI, proteomic expression, and stroke-related outcomes.
Results: The 61 subjects studied were broken into three categories: normal weight (BMI 18.5–24.9) which contained 19 subjects, overweight (BMI 25–30) which contained 25 subjects, and obese (BMI $\geq$30) which contained 17 subjects. Normal BMI group was a significantly older population (mean 76 years) when compared to overweight (mean 66 years) and obese (mean 61 years) with significance of $p = 0.041$ and $p = 0.005$, respectively. When compared to normal weight and overweight categories, the obese category had significantly higher levels of adenosine deaminase (ADA) expression ($p = 0.01$ and $p = 0.039$, respectively). Elevated levels of ADA were found to have a significant positive correlation with both infarct volume and edema volume ($p = 0.013$ and $p = 0.041$, respectively), and were associated with a more severe stroke (NIHSS on discharge) and greater stroke related disability (mRS on discharge) with significance of $p = 0.053$ and $p = 0.032$, respectively.
Conclusions: When examined according to BMI, subjects undergoing MT for ELVO demonstrate significant differences in the expression of certain plasma proteins, including ADA. Levels of ADA were found to be significantly higher in the obese population when compared to normal or overweight groups. Increased levels of ADA in the obese group were predictive of increased infarct volume, edema volume, and worse NIHSS scores and mRS at discharge. These data provide novel biomarker candidates as well as treatment targets while increasing the personalization of stroke prognosis and treatment.

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https://doi.org/10.1016/j.bbih.2022.100422
Received 14 December 2021; Received in revised form 21 January 2022; Accepted 24 January 2022
Available online 26 January 2022

1. Introduction

The World Health Organization (WHO) reports obesity has tripled worldwide since 1975 and that in 2016, more than 1.9 billion adults were overweight and 650 million were obese (Organization, 2021). The WHO also reported in 2019, stroke was the second most common cause of death globally, following ischemic heart disease (Organization, 2021). The relationship between Body Mass Index (BMI), a metric representing the amount of body fat one has, and stroke has become an interesting topic over the last several years. Obesity has been shown to be an independent risk factor for stroke, and thus, physicians routinely encourage weight loss for primary prevention of vascular disease (Aparicio et al., 2017). However, many studies have reported better outcomes and lower mortality after stroke in the obese population, leading to a phenomenon dubbed the "obesity paradox." (Skolarus et al., 2014; Kawase et al., 2017; Park et al., 2019).

At the University of Kentucky Center for Advanced Translational Stroke Science, mechanical thrombectomy (MT) for emergent large vessel occlusions (ELVO) is utilized to treat and study stroke. Our institution developed the Blood And Clot Thrombectomy Registry And Collaboration (BACTRAC) protocol (clinicaltrials.gov NCT03153683). This protocol allows for both intracranial (i.e., distal to thrombus) and systemic (i.e., carotid) arterial blood samples to be obtained during MT. To date, our institution has published results from BACTRAC on arterial blood gasses (ABGs), electrolyte chemistry, genomics, neuroinflammatory cells, and proteomics (Maglinger et al., 2020; Martha et al., 2019; Martha SR et al., 2020; Shaw et al., 2021; Sands et al., 2021; Spears et al., 2021).

The objective of this study was to utilize systemic (carotid) arterial blood samples taken at time of MT to study how BMI affects proteomic expression and stroke severity. We first separated our thrombectomy cohort into BMI categories to investigate how our data compare to the contemporary literature and the obesity paradox. We utilized outcome measures including National Institute of Health Stroke Scale (NIHSS) score, modified Rankin scores (mRS), infarct volume and edema volume. We investigated how BMI relates to systemic expression of inflammatory and cardiometabolic proteins. We first identified proteins which were related to BMI, and then we investigated whether those proteins were important clinically and radiographically in order to identify predictive BMI-dependent biomarkers of stroke outcome. Adenosine deaminase (ADA) was chosen as the focus for this study for several reasons. First, ADA was found to have the strongest statistical correlations to BMI and the outcome measures of interest compared to all other proteins analyzed. Next, the main function of ADA is to break down adenosine, which has been reported to be neuroprotective in the literature. However, ADA itself is an under-reported protein in the stroke literature, so this study aims to provide novel data on ADA in the context of ischemic stroke. Other related proteins from our analyses will be subjects for future studies. These novel data provide candidates for predictive biomarkers and potential therapeutic targets for prognosis/treatment of a devastating disease.

2. Methods

2.1. Sample collection and analysis

The University of Kentucky Center for Advanced Translational Stroke Sciences previously established the Blood And Clot Thrombectomy Registry And Collaboration (BACTRAC). This registry is a continually enrolling tissue bank (clinicaltrials.gov NCT03153683) from ELVO stroke patients undergoing MT. This study is approved through the University of Kentucky Institutional Review Board (IRB). All subjects enrolled in this study consented through written documentation by either the subject or their legally authorized representative. Inclusion criteria included all ELVO thrombectomy candidates aged 18 or older and non-pregnant. Exclusion criteria included pregnancy, <18 years of age, imprisoned, or subjects unable to consent within the 72-h window outlined by the IRB. The BACTRAC tissue bank is a continuously enrolling bank; however, subjects included in this study were obtained between June 21, 2017, and March 1, 2021. Methods outlining tissue sample acquisition and tissue processing have been previously published (Maglinger et al., 2020; Fraser et al., 2018). Carotid arterial blood samples of N = 61 human subjects were analyzed for inflammatory and cardiometabolic protein expression by Olink Proteomics (Olink Proteomics, Boston, MA). We specifically selected Olink for our analyses for their ability to assess small-volume samples such as our intracranial samples (~1 ml). Proteomic results from Olink are reported in a Normalized Protein eXpression (NPX) value. NPX is a unit in log2 scale to minimize intra- and inter-assay variability allowing for identification of changes in protein levels across samples sets (https://www.olink.com/faq/what-is-npx/). Methods by which Olink Proteomics evaluates tissue samples is both well-established and published in over 780 articles to date (Folkersen et al., 2020; Larsson et al., 2015; Enroth et al., 2019).

Demographic data are recorded for all subjects including age, sex, BMI, comorbid stroke risk factors, National Institute Health Stroke Scale/Score (NIHSS) on admission, NIHSS on discharge, modified Rankin Score (mRS) on admission and discharge, thrombolysis in cerebral infarction (TICI) score, computed tomography angiogram (CTA) collateral score, infarct time, infarct volume, edema volume, and discharge Montreal Cognitive Assessment (MoCA) scores. BMI is broken down into the following categories: BMI $\leq$18.5 kg/m^2 represents underweight, BMI 18.5–24.9 kg/m^2 represents normal weight, BMI 25–30 kg/m^2 represents overweight, and BMI $\geq$30 kg/m^2 represents obesity. For discharge MoCA scores, there were only n = 22 subjects who had MoCA scores: 6 in the normal BMI category, 7 in the overweight BMI category, and 9 in the obese BMI category. We chose to analyze CTA collateral score based on two categories: 0 or greater or equal to 1. This methodology was chosen as specific CTA scores have poor specificity and each score is not particularly useful in clinical decision making, however, the two categories may provide a useful snapshot of the collateral blood flow for each subject (Souza et al., 2012).

2.2. Statistical analysis

Prior to analysis, all continuous outcome variables were assessed for normality. Both infarct and edema volume were found to be positively skewed. They were therefore log-transformed to make normality a more tenable assumption. After log transformation, the normality of the transformed distribution was supported by both visual inspection and the Kolmogorov-Smirnov test. Differences in demographic and clinical characteristics between the three BMI categories were assessed using chi-square tests for categorical variables and analysis of variance (ANOVA) for continuous variables. The relationship between ADA expression and demographic and clinical characteristics was assessed with correlations for continuous variables and ANOVA for categorical variables. A multiple linear regression approach was used to predict infarct and edema volume from ADA expression and BMI category, while also controlling for age and sex. We chose to control for age and sex for multiple reasons. First, we found our BMI cohorts had significantly different ages and wanted to control for this heterogeneity. Secondly, we surveyed the literature and found studies reporting varying results on the influence of age and sex on BMI and stroke risks. For example, in a large meta-analysis, one study demonstrated no significant relationship between men and women regarding BMI as a risk factor for stroke (Strazzullo et al., 2010). However, in a population in China, one study reported both age- and sex-associated impacts of BMI on stroke risk (Gu et al., 2019). As our study focused on a specific population in Kentucky, we chose to control for both age and sex in our multiple linear regressions. Regression diagnostics determined the presence of several outliers for infarct and edema volume, as well as high leverage values on some of the predictors. To account for this, robust regression with MM estimation was used. In this method, rather than removing data points, observations with high

outlier and leverage values are given less weight in the analysis. The analysis predicting MoCA scores did not suffer from outliers or high leverage points and was analyzed using multiple linear regression. Ordinal logistic regression was used to assess the relationship between ADA, BMI, and the ordinal outcomes of mRS and NIHSS scores at discharge. For both the robust and ordinal regression analyses, and interaction between ADA and BMI category was introduced to the models to test if the relationship between ADA and outcome differed by BMI category. Non-significant interactions were removed from the final models. Network analysis was performed on STRING V11 (https://string-db.org/) using protein-protein interaction analysis data. All statistical analyses were performed using SAS v 9.4 (SAS Institute Inc, Cary, NC) or IBM SPSS Statistics. A p-value less than 0.05 was considered statistically significant.

3. Results

3.1. BMI demographics and outcome

Demographic data for N = 61 subjects are shown in Table 1. For this study, demographics were broken into three BMI categories: normal weight (BMI 18.5–24.9) which contained 19 subjects, overweight (BMI 25–30) which contained 25 subjects, and obese (BMI ≥30) which contained 17 subjects. The average age for normal weight subjects was 76 (SD 13), overweight subjects was 66 (SD 16), and for obese 61 (SD 14) demonstrating the normal BMI group was a significantly older population when compared to overweight and the obese group ($p = 0.041$ and $p = 0.005$, respectively). 15 of the 19 normal weight group, 11 of the 25 overweight group and 10 of the 17 obese weight group were female which was a non-significant difference across BMI categories. When comparing BMI groups, there were no significant differences in the presence of hypertension (HTN), diabetes type 2 (DMII), hypercholesterolemia (HLD), or whether the patient was a smoker.

Median and interquartile range (IQR) NIHSS score on admission for normal weight was 16 (13–22), for overweight was 19 (12–22), and for obese was 16 (10–19) with no statistical significance between groups. Median and IQR NIHSS score on discharge for normal weight was 8 (3–15), for overweight was 11 (2–18), and for obese was 8 (0.5–18.5) with no statistical significance between groups. NIHSS on discharge was further broken into three categories: minor stroke (1–4), moderate stroke (5–15), and severe stroke (≥16). For the normal BMI group, there were 7 minor strokes (36.8%), 8 moderate strokes (42.1%), and 4 severe strokes (21.1%). For the overweight BMI group, there were 12 minor strokes (48%), 7 moderate strokes (28%) and 6 severe strokes (24%). For the obese category there were 7 minor strokes (41.2%), 6 moderate strokes (35.3%), and 4 severe strokes (23.5%). Median and IQR mRS on discharge for the normal, overweight, and obese groups were 4 (2–5), 5 (4–5), and 4 (2–5), respectively. There were no significant differences in discharge mRS between BMI categories. Median and IQR infarct volumes for normal, overweight, and obese groups were 25,565 mm^3 (4621–73,180), 45,090 mm^3 (12,520–145,700), and 12,170 mm^3 (3796–106,900), respectively. There were no significant differences in infarct volumes between BMI categories. Median and IQR edema volumes for normal, overweight, and obese groups were 29,270 mm^3 (4621–97,210), 48,140 mm^3 (12,520–152,800), and 15,060 mm^3 (3447–106,900), respectively. There were no significant differences in edema volumes between BMI categories. There were no significant differences in discharge MoCA scores based on BMI category (n = 22). Additionally, when comparing BMI categories, there were no significant differences in CTA collateral score.

3.2. Proteins important in BMI and radiographic outcome

The top 10 proteins with the greatest differences in expression levels between BMI groups were adenosine deaminase (ADA), Beta-Ala-His dipeptidase (CNDP1), interleukin-7 receptor subunit alpha (IL7R), multiple epidermal growth factor-like domains protein (MEGF9), carbonic anhydrase 4 (CA4), transforming growth factor-beta-induced protein ig-h3 (TGFBI), carbonic anhydrase 1 (CA1), natural killer cell receptor 2B4 (CD244), latent-transforming growth factor beta-binding protein 2 (LTBP2), and cystatin D (CST5). For each of these proteins there was statistically significant difference ($p \leq 0.05$) in expression levels between BMI categories. Further, the 10 proteins with the strongest correlations between protein expression and infarct volume were ADA, monocyte chemotactic protein 1 (MCP1), eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1), nidogen-1 (NID1), CD40 L receptor (CD40), leukemia inhibitory factor receptor (LIFR), CUB domain-containing protein 1 (CDCP1), interleukin-18 receptor 1 (IL18R1), C-X-C motif chemokine 10 (CXCL10), signaling lymphocytic activation molecule (SLAMF1). The 8 proteins with the strongest correlations between protein expression and edema volume included ADA, CD40, LTBP2, CDCP1, 4E-BP1, CXCL10, MCP1, and LIFR. All correlations between protein expression and infarct volume or edema volume obtained a p-value of less than 0.05. As ADA expression was found to be significant between BMI categories as well as predictive of both infarct volume and edema, it was selected as a protein for further investigation.

3.3. Signaling proteins related to BMI, infarct volume, and edema volume

As described above, the top 10 proteins with the greatest expression difference between BMI categories, as well as the top 10 proteins most predictive of infarct volume and top 8 proteins most predicative of edema

Table 1
Demographic and outcome data for thrombectomy subjects broken into respective BMI categories.

Demographic/comorbidity	Normal BMI n = 19	Overweight BMI n = 25	Obese BMI n = 17	p-value
Age	76 (13)	66 (16)	61 (14)	0.015
Female	15 (79%)	11 (44%)	10 (59%)	0.066
Hypertension	13 (68%)	17 (68%)	16 (94%)	0.108
Type II Diabetes	6 (32%)	9 (36%)	5 (29%)	0.897
Hyperlipidemia	3 (16%)	6 (24%)	5 (29%)	0.616
Previous stroke	5 (26.3%)	3 (12%)	2 (12%)	0.371
NIHSS admission median (IQR)	16 (13–22)	19 (12–22)	16 (10–19)	0.434
NIHSS discharge median (IQR)	8 (3–15)	11 (2–18)	8 (0.5–18.5)	0.859
Discharge mRS median (IQR)	4 (2–5)	5 (4–5)	4 (2–5)	0.786
[a]Mean MoCA score at discharge (SD)	22.7 (5.4)	23.9 (5)	19.3 (5)	
Infarct volume median (IQR)	25,565 mm^3 (4621–73,180)	45,090 mm^3 (12,520–145,700)	12,170 mm^3 (3796–106,900)	0.056
Edema volume median (IQR)	29,270 mm^3 (4621–97,210)	48,140 mm^3 (12,520–152,800)	15,060 mm^3 (3447–106,900)	0.091
CTA				
0	4 (21.1%)	6 (24%)	4 (26.5%)	0.938
	14 (73.7%)	16 (64%)	11 (64.7%)	
Unknown	1 (5.3%)	3 (12%)	2 (11.8%)	

[a] For MoCA scores, there were only 6 subjects in the normal BMI group, 7 subjects in the overweight BMI group, and 9 subjects in the obese BMI group.

volume were identified. Each set of proteins was used as the input for STRING analysis. STRING analyzes genome-wide proteomic connectivity integrations from existing literature to provide an output of physical and functional associations between proteins. STRING-generated outputs also include related network proteins associated with each biological function identified. Fig. 1A demonstrates a STRING network generated from proteins with greatest expression difference between BMI categories. Fig. 1B and C demonstrate a STRING network generated from proteins most predictive of infarct volume and edema volumes, respectively. Table 2A, 2B, and 2C illustrate the common network functions for each model along with additional corresponding proteins for each specific biological function. Network functions were ordered by smallest false discovery rate. Several related network proteins were consistent throughout many of the biological function outcomes, including interleukin 7 (IL-7), oncostatin M (OSM), and leukemia inhibitory factor receptor (LIFR).

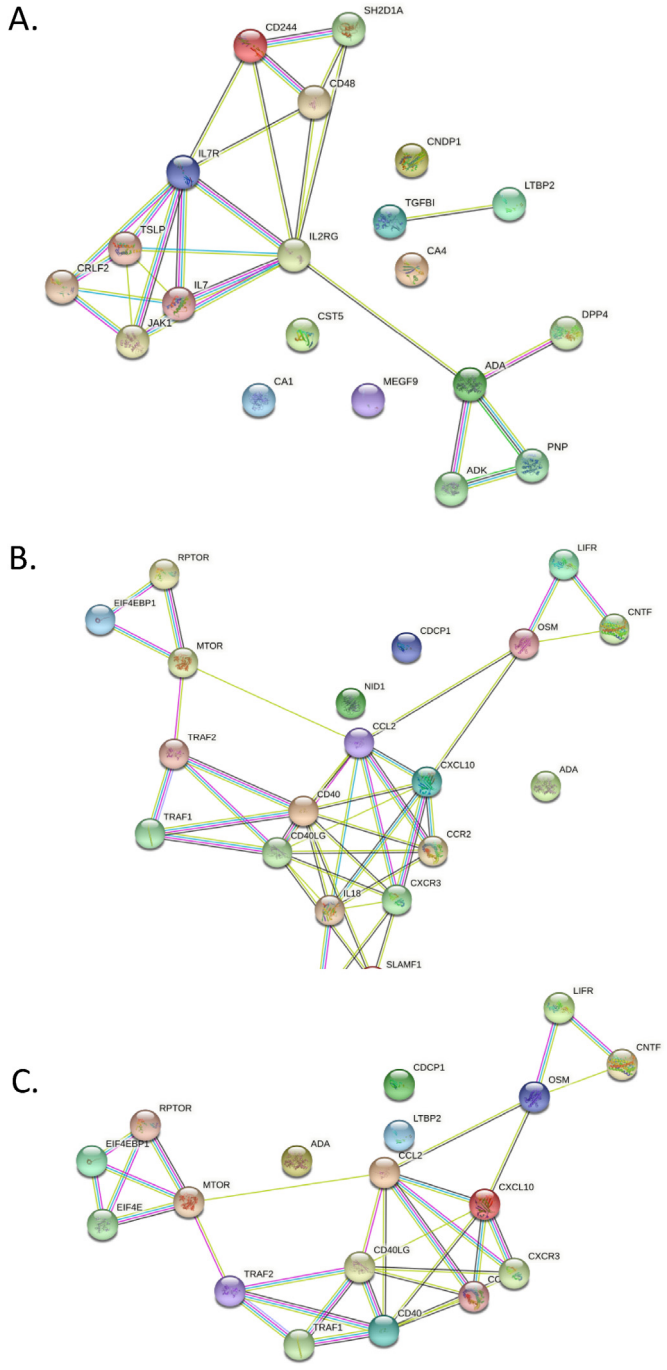

Fig. 1. STRING-generated proteomic network webs for proteins with significant expression differences across BMI categories (**A**), proteins most predictive of infarct volume (**B**), and proteins most predictive of edema volume (**C**).

3.4. ADA demographics and outcome

In our cohort of subjects, ADA was positively correlated with BMI, demonstrating higher BMI was associated with higher ADA levels ($r = 0.287$, $p = 0.025$). Specifically, when broken into BMI categories, expression of ADA was significantly higher in the obese weight category when compared to normal and overweight categories ($p = 0.01$ and $p = 0.04$, respectively). There was no significant difference in ADA expression between the normal and overweight groups. ADA expression was found to be negatively correlated with age, signifying lower levels of ADA in the older population ($r = -0.301$, $p = 0.019$). Expression of ADA was not significantly different between female and male subjects. There were no significant differences in ADA expression between subjects with and without HTN, DMII, HLD, or previous stroke. There was no significant difference in ADA expression between those who were current smokers versus those who had never smoked or who were previous smokers.

The interaction between ADA expression and BMI category was not statistically significant for any of the outcomes and was therefore dropped from all final models. The robust linear regressions found that higher ADA levels were associated with both higher infarct volume and higher edema volume ($p = 0.021$ and $p = 0.036$, respectively). Ordinal logistic regression found that higher levels of ADA were associated with increased odds of greater stroke related disability based on mRS at discharge ($p = 0.032$). Similarly, ordinal logistic regression demonstrated higher levels of ADA were associated with a near-significant increase of odds of a more severe stroke based on NIHSS at discharge ($p = 0.053$). Further, robust linear regressions were also run while controlling for age and sex. Neither age nor sex were associated with infarct volume. Overweight individuals had significantly higher infarct volumes than obese individuals ($p = 0.017$), but no other inter-BMI comparisons were significant for infarct volume (Fig. 2A). Fig. 2B illustrates the significant positive correlation between ADA expression and infarct volume, with higher levels of ADA associated with larger infarct volumes ($p = 0.008$). Similarly, overweight individuals had significantly higher edema volumes than obese individuals ($p = 0.012$), with no additional inter-BMI comparisons being significant. Higher levels of ADA were also associated with larger edema volumes ($p = 0.003$). An ordinal logistic regression was used to predict discharge NIHSS categories from BMI group and systemic ADA expression, while also controlling for age, sex, and NIHSS admission scores. BMI was not significantly related to stroke severity as measured by the NIHSS score. Systemic ADA expression was related to stroke severity ($p = 0.011$). A doubling of the relative concentration of ADA was associated with increased odds (nearly 2.5 times) of being in a more severe NIHSS category. Additionally, both age ($p = 0.009$) and NIHSS admission scores ($p = 0.008$) were positively associated with stroke severity, demonstrating older individuals and individuals with larger NIHSS admission scores were more likely to be in a more severe stroke category. When ADA was assessed in relation to MoCA scores at discharge, there were no significant correlations.

4. Discussion

Many studies have reported on the relationship of obesity to stroke, both as a risk factor as well as a potential positive factor regarding recovery (Aparicio et al., 2017; Skolarus et al., 2014; Kawase et al., 2017; Park et al., 2019; Strazzullo et al., 2010; Gu et al., 2019; Li et al., 2019). Our current study of ELVO subjects treated with mechanical thrombectomy demonstrates the obese group experience ELVO on average 15 years earlier than normal weight patients. Comorbid conditions such as hypertension, hypercholesterolemia, diabetes, and stroke severity were not significantly different among these groups. When examining the

Table 2
STRING-generated biological processes as well as matching network proteins for proteins with significant expression differences across BMI categories (**A**), proteins most predictive of infarct volume (**B**), and proteins most predictive of edema volume (**C**).

Term ID	Biological Process	Strength	False Discovery Rate	Matching Proteins in Network
A. Proteins with significant expression differences across BMI categories				
GO:0038111	interleukin-7-mediated signaling pathway	2.29	1.66e-08	IL7, IL7R, TSLP, JAK1, IL2RG, CRLF2
GO:0002696	positive regulation of leukocyte activation	1.31	0.00012	IL7, IL7R, TSLP, DPP4, PNP, ADA, CRLF2
GO:0002682	regulation of immune system process	0.85	0.00016	IL7, IL7R, TSLP, JAK1, DPP4, PNP, CD244, SH2D1A, ADA, CRLF2, CD48
GO:0002684	positive regulation of immune system process	0.97	0.0004	IL7, IL7R, TSLP, DPP4, PNP, CD244, SH2D1A, ADA, CRLF2
GO:0022409	positive regulation of cell-cell adhesion	1.33	0.00056	IL7, IL7R, JAK1, DPP4, PNP, ADA
GO:0043101	purine-containing compound salvage	2.29	0.0013	ADK, PNP, ADA
GO:0045582	positive regulation of T cell differentiation	1.64	0.0029	IL7, IL7R, PNP, ADA
GO:0050870	positive regulation of T cell activation	1.37	0.0029	IL7, IL7R, DPP4, PNP, ADA
GO:0019221	cytokine-mediated signaling pathway	1	0.0038	IL7, IL7R, TSLP, JAK1, IL2RG, CRLF2, CA1
GO:0030155	regulation of cell adhesion	0.98	0.0041	IL7, IL7R, JAK1, DPP4, PNP, ADA, TGFBI
B. Proteins most predictive of infarct volume				
GO:0019221	cytokine-mediated signaling pathway	1.27	1.27E-10	OSM, CCL2, TRAF2, LIFR, IL18, CCR2, CXCL10, CNTF, CD40LG, CD40, CXCR3, TRAF1, IL18R1
GO:0008284	positive regulation of cell population proliferation	1.14	2.94E-09	OSM, LIFR, IL18, CCR2, CXCL10, SLAMF1, RPTOR, MTOR, CNTF, CD40LG, CD40, ADA, CXCR3
GO:0048584	positive regulation of response to stimulus	0.84	1.38E-08	OSM, CCL2, TRAF2, IL18, CCR2, CXCL10, SLAMF1, RPTOR, MTOR, CNTF, CD40LG, CD40, ADA
GO:0071310	cellular response to organic substance	0.82	1.95E-08	OSM, CCL2, TRAF2, LIFR, IL18, CCR2, CXCL10, RPTOR, EIF4EBP1, MTOR, CNTF, CD40LG, CD40
GO:0010033	response to organic substance	0.74	2.82E-08	OSM, CCL2, TRAF2, LIFR, IL18, CCR2, CXCL10, RPTOR, EIF4EBP1, MTOR, CNTF, CD40LG, CD40
GO:0009967	positive regulation of signal transduction	0.92	4.72E-08	OSM, CCL2, TRAF2, IL18, CCR2, SLAMF1, RPTOR, MTOR, CNTF, CD40LG, CD40, ADA, TRAF1
GO:0042127	regulation of cell population proliferation	0.92	4.72E-08	OSM, CCL2, LIFR, IL18, CCR2, CXCL10, SLAMF1, RPTOR, MTOR, CNTF, CD40LG, CD40, ADA
GO:1902533	positive regulation of intracellular signal transduction	1.05	8.10E-08	OSM, CCL2, TRAF2, IL18, SLAMF1, RPTOR, MTOR, CD40LG, CD40, ADA, TRAF1, IL18R1
GO:0007166	cell surface receptor signaling pathway	0.8	1.60E-07	OSM, CCL2, TRAF2, LIFR, NID1, IL18, CCR2, CXCL10, EIF4EBP1, CNTF, CD40LG, CD40, CXCR3,
GO:0048522	positive regulation of cellular process	0.52	7.71E-07	OSM, CCL2, TRAF2, LIFR, NID1, IL18, CCR2, CXCL10, SLAMF1, RPTOR, EIF4EBP1, MTOR, CNTF
C. Proteins most predictive of edema volume				
GO:0010033	response to organic substance	0.79	4.28E-09	OSM, CCL2, TRAF2, LTBP2, LIFR, CCR2, CXCL10, RPTOR, EIF4EBP1, MTOR, CNTF, CD40LG, CD40, ADA, CXCR3
GO:0071310	cellular response to organic substance	0.87	4.28E-09	OSM, CCL2, TRAF2, LTBP2, LIFR, CCR2, CXCL10, RPTOR, EIF4EBP1, MTOR, CNTF, CD40LG, CD40, CXCR3
GO:0019221	cytokine-mediated signaling pathway	1.25	1.40E-08	OSM, CCL2, TRAF2, LIFR, CCR2, CXCL10, CNTF, CD40LG, CD40, CXCR3, TRAF1
GO:0008284	positive regulation of cell population proliferation	1.11	2.14E-07	OSM, LIFR, CCR2, CXCL10, RPTOR, MTOR, CNTF, CD40LG, CD40, ADA, CXCR3
GO:0042127	regulation of cell population proliferation	0.9	2.83E-06	OSM, CCL2, LIFR, CCR2, CXCL10, RPTOR, MTOR, CNTF, CD40LG, CD40, ADA, CXCR3
GO:0048584	positive regulation of response to stimulus	0.8	5.63E-06	OSM, CCL2, TRAF2, CCR2, CXCL10, RPTOR, MTOR, CNTF, CD40LG, CD40, ADA, CXCR3, TRAF1
GO:0007166	cell surface receptor signaling pathway	0.78	7.39E-06	OSM, CCL2, TRAF2, LTBP2, LIFR, CCR2, CXCL10, EIF4EBP1, CNTF, CD40LG, CD40, CXCR3, TRAF1
GO:0009967	positive regulation of signal transduction	0.86	4.21E-05	OSM, CCL2, TRAF2, CCR2, RPTOR, MTOR, CNTF, CD40LG, CD40, ADA, TRAF1
GO:0072678	T cell migration	2.13	4.21E-05	CCL2, CCR2, CXCL10, CXCR3
GO:0001934	positive regulation of protein phosphorylation	0.98	0.0001	OSM, CCL2, TRAF2, RPTOR, MTOR, CNTF, CD40LG, CD40, TRAF1

proteomic profile associated with obesity, ADA expression was found to be positively correlated with BMI, more importantly ADA was only significantly higher in the obese category when compared to normal and overweight categories. Finally, higher ADA levels were indicative of a poor prognosis as evident by its relationship to infarct volume, edema volume, mRS at discharge, and NIHSS at discharge.

Although the literature remains inconsistent, the “obesity paradox” highlights obesity as a risk factor for stroke; however, those with stroke who have higher BMI typically have better outcomes. For example, a study discussed BMI to be an important risk factor for both ischemic stroke and hemorrhagic stroke, and suggested BMI may influence both types through blood pressure, blood glucose, and cholesterol (Song et al., 2004). Another study reported the incidences of lacunar, non-lacunar, and cardioembolic stroke were all significantly positively associated with the degree of obesity (Yatsuya et al., 2010). A study from Japan reported the cumulative average BMI demonstrated a positive linear effect on lacunar, large vessel occlusive, and cardioembolic strokes in both sexes, with an exception of cardiometabolic stroke in women (Li et al., 2019). Additionally, in 2017, a group conducted a systematic review of 25 studies encompassing 299,750 patients looking at associations of body weight and clinical outcome after stroke (Oesch et al., 2017). This review reported the association between obesity and favorable outcomes after stroke was strong and consistent among many studies, and the associations mostly remained statistically significant after adjusting for confounders, however, no data were reported for patients undergoing mechanical thrombectomy. When considering our data in the context of the literature, it is important to highlight that in our cohort, the obese population was significantly younger than the normal weight group. From these findings, it is intuitive that younger patients will recover better than older ones. Our findings may also align closer to those focusing only on the most severe type of ischemic stroke, ELVO, suggesting that patient demographics and type of stroke also play a role in

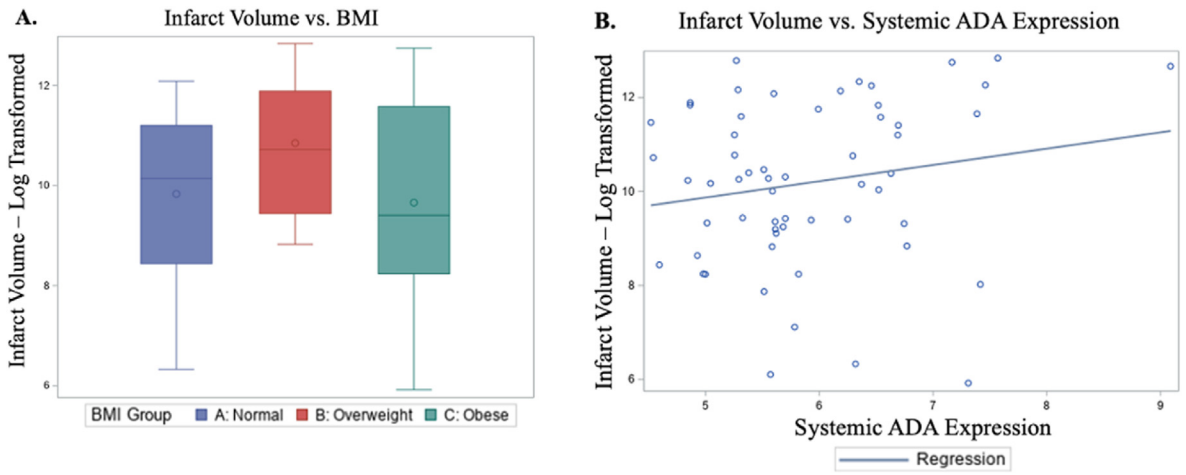

Fig. 2. Overweight BMI category had significantly higher infarct volumes than Obese BMI group with p = 0.017 (**A**). Significant positive correlation between systemic ADA expression and infarct volume with p = 0.008 (**B**).

understanding this "obesity paradox." Additionally, for our analyses, we investigated BMI categorically for clinical relevance, however, analyzing BMI as a continuous variable may be useful for subset analyses in the future.

Although our study found no significant sex differences, a group from Italy investigated sex differences of metabolically unhealthy overweight patients by assessing National Institutes of Health Stroke Scale score (NIHSS) at admission and modified Rankin scale (mRS) at discharge. This study reported a significantly increasing trend in NIHSS and mRS along with increase in BMI category in women but a significantly decreasing trend in NIHSS and non-significant trend in mRS with increasing BMI category for men (Viticchi et al., 2020). While the 'obesity paradox' appears to be an observed phenomenon, studies have also demonstrated that elevated BMI is associated with lower likelihood of being discharged directly home from the hospital and a longer stay on an inpatient stroke service after the ischemic event (Razinia et al., 2007). More recently, studies have investigated the role BMI may play on interventional outcomes of ischemic stroke. For example, a study investigating clinical outcome after intravenous thrombolysis reported BMI category did not significantly influence clinical outcome after intervention (Chatzikonstantinou et al., 2016). Other studies, more closely related to this one, have reported on stroke outcomes after mechanical thrombectomy based on BMI. Two of these studies, including a post hoc analysis of the MR CLEAN trial, reported no significant interaction between BMI and endovascular treatment effect (Bouslama et al., 2020; Pirson et al., 2019). A final study found BMI did not affect procedural success, however, they report both high and low BMI were associated with worse functional independence based on mRS(Chen et al., 2020).

In our cohort of ELVO stroke subjects treated with mechanical thrombectomy, we began by investigating the influence of BMI on factors important in stroke such as demographics and comorbidities. As mentioned above, we found that BMI category was significantly related to age, as our normal BMI group was an older population when compared to the overweight and the obese groups. It is known that obesity is an independent risk factor for stroke (Aparicio et al., 2017). Similarly, due to the cumulative effects of aging on the cardiovascular system as well as the additive effects of comorbidities, it is known that age typically increases stroke risk (Meschia et al., 2014). To our knowledge, there is not a clearly reported consensus on age-related BMI influences on ischemic stroke risk/outcomes. One study from China including subjects <65 years of age, reported their age- and sex-associated impacts of BMI on stroke risk including being overweight increased the risk of ischemic stroke in men whereas being obese increased the risk of ischemic strokes in women (Gu et al., 2019). One study from Japan which investigated BMI and risks of ischemic stroke reported a higher cumulative average BMI was associated with younger aged men but older aged women (Li et al., 2019). Other studies have reported a positive association between elevated BMI and stroke among subjects aged 65 or greater as well as higher BMI as a stroke risk factor for subjects aged 70 and older (Meschia et al., 2014; Dey et al., 2002). As a part of the aging process, body fat is often redistributed, most commonly to the abdominal cavity. Due to this redistribution, some suggest body circumference measurements may be more important measurements than BMI (Dey et al., 2002). Our study adds to the literature age-related BMI influences on a specific stroke subtype treated with MT. Unlike age, when our BMI categories were compared based on comorbidities, we found no significant differences in the presence of hypertension, diabetes type 2, hypercholesterolemia, or whether the patient was a smoker. Additionally, across BMI categories, we found no significant differences in NIHSS admission scores, NIHSS discharge scores, mRS discharge scores, infarct volume, or edema volume. As mentioned above, some prior studies have found increased BMI to be related to increased NIHSS and mRS, unlike in our study where we found no statistical correlation (Viticchi et al., 2020).

We identified several proteins with significant differences across BMI categories and adenosine deaminase was the most important protein associated with both BMI and outcome measurements following stroke. ADA is an important deaminating enzyme in purine metabolism that acts by catalyzing the transformation of adenosine to inosine. One well known and critical role of ADA is for T-cell maturation and survival. Individuals born with ADA deficiency develop a severe immunodeficiency classified as a subtype of severe combined immunodeficiency (SCID) (Giblett et al., 1972). As a response to cerebral ischemia and hypoxia, increased ATP breakdown leads to an increase in accumulation of adenosine (Chen et al., 1999). Several studies have aimed to elucidate biochemical modalities that may act to increase extracellular levels of adenosine. Findings from these studies include formation of intracellular adenosine with transmembrane export into the extracellular space, necrotic or apoptotic cell damage leading to leakage of high levels of intracellular ATP, storage vesicles containing hormones, transport vesicles and a subset of lysosomes (Burnstock, 2006; Elliott et al., 2009; MacDonald et al., 2006; Zhang et al., 2007, 2009; Lazarowski et al., 2003). Several early studies reported that adenosine was a neuroprotective agent in cerebral ischemia and that inhibiting adenosine phosphorylation, degradation or by using adenosine analogs, ischemia-induced neuronal injury may be attenuated (Hagberg et al., 1987; Matsumoto et al., 1992; Rudolphi et al., 1992; Deckert and Gleiter, 1994; Pignataro et al., 2007). As more information on adenosine receptors emerged, studies began focusing on modulation of specific adenosine receptor subtypes such as the A_1 and A_{2A} (Johansson et al., 2001). For example, one study that utilized an A_{2A} knock out mouse

model demonstrated that inactivation of the A_{2A} receptor had neuroprotective effects including smaller infarct volume and reduced neurologic deficits after transient middle cerebral artery occlusion followed by reperfusion (Chen et al., 1999). Due to properties such as vasodilation, reduction of excitatory transmitter release, and inhibition of platelets, adenosine has been thought to attenuate neural degeneration during cerebral injury such as ischemia (Phillis, 1989). Lastly, an adenosine receptor knockout mouse model demonstrated the A2B adenosine receptor may modulate inflammation, specifically through leukocyte adhesion to vasculature (Yang et al., 2006).

Although many studies have looked at the role of adenosine or the adenosine receptors in relation to ischemic events, fewer have investigated the role of ADA in this context. One study investigated ADA in ischemia reperfusion injury in patients with myocardial infarction. They found increased ADA activity in erythrocytes after reperfusion and suggest these elevated levels contribute to the depletion of adenosine, which increases the production free radicals as adenosine typically activates antioxidants (Kaul et al., 2006). Another study suggests ADA has a neuroprotective role during ischemic conditions in the striatum (Tamura et al., 2016). Taken together, these studies suggest adenosine, adenosine receptors, and ADA play a complex role in cerebral ischemia. Adenosine may act as an anti-inflammatory mediator during hypoxia (Sitkovsky et al., 2004; Ohta and Sitkovsky, 2001). However, some studies have demonstrated that chronically elevated adenosine levels due to ADA deficiency, may facilitate chronic inflammation in disease states such as lung disease (Chunn et al., 2005; Sun et al., 2005). To further investigate the balance of adenosine and ADA in inflammation, one study set out to uncover mechanisms of extracellular adenosine degradation during hypoxia (Eltzschig et al., 2006). This study revealed a hypoxia-induced parallel induction of ADA and CD26 at both the mRNA and protein level and suggest that induced ADA and CD26 form a membrane bound complex which can facilitate adenosine catabolism, decreasing neuroprotection.

There are a few articles that investigate a possible relationship between ADA or adenosine levels and BMI. Our study found ADA expression negatively correlates with age, but positively with BMI. One study which included 100 Indian subjects evaluated levels of ADA in fasting blood samples and reported a positive correlation between ADA and BMI (Jadhav and Jain, 2012). They propose that anti-inflammatory adenosine is upregulated in response to inflammation produced by adipose tissue, which in turn upregulates ADA levels. Additionally, it has been reported that ADA activity is increased in type 2 diabetes patients which is a major co-morbidity (Lee et al., 2011). In our study, we found no significant differences in ADA levels in our diabetes subjects. A separate study reported increased serum ADA levels in nonobese type 2 diabetes subjects when compared to healthy controls (Khemka et al., 2013). They report no correlation between ADA and BMI in their cohort.

As the literature on ADA expression in cerebrovascular ischemia in humans is relatively sparse, we set out to investigate the relationships between ADA and patient comorbidities, patient outcome, and related proteomic networks. As mentioned above, we found significant relationships between ADA and BMI as well as ADA and age. However, we found no sex differences, and no differences in those with or without hypertension, hyperlipidemia, diabetes type 2, previous stroke, or smoking status. To further establish the relationship between ADA and radiographic outcome and stroke severity, we ran additional analyses which controlled for potential confounding factors such as age and sex. Importantly, even when controlling for age and sex, we found that ADA expression was still positively correlated with BMI category and that ADA expression was also predictive of both infarct volume and edema volume. Additionally, in our cohort, after controlling for age, sex, and NIHSS on admission, increased ADA expression was related to worse stroke severity based on NIHSS at discharge.

After determining the importance of ADA regarding BMI and stroke outcomes, we utilized STRING to investigate ADA-related protein-protein signaling networks to elucidate biological functions and additional proteins relevant at the time of infarct. Common biological functions among the models were: 'cytokine-mediated signaling pathway,' 'regulation of cell population proliferation,' 'response to organic substance,' cell surface receptor signaling,' and 'positive regulation of response to stimulus.' These biological functions represent a unique snapshot of the biochemical processes occurring at time of stroke intervention that relate to stroke severity, such as infarct and edema volume. STRING-generated output also includes network proteins that match the predictive model entered. Common proteins among the models included interleukin 7, interleukin 7 receptor, oncostatin M, and leukemia inhibitory factor receptor. Interestingly, these proteins have been reported in the literature in relation to cerebral ischemia. As part of our ongoing BACTRAC study, our group has reported that using machine learning analysis, the gene expression of IL-7 is associated with infarct and edema volume (Martha SR et al., 2020). Both IL7 and IL7R have been reported on in the context to ischemic stroke and prior studies have found plasma levels of both IL7 and IL7R were significantly lower in stroke cohorts as compared to controls (Oberheiden et al., 2012; Li et al., 2020). Oncostatin M (OSM) is a cytokine belonging to the IL-6 family that is typically released by monocytes, macrophages, dendritic cells, and T lymphocytes under inflammatory conditions (Hermanns, 2015). Human OSM can bind either a type I receptor complex, which consists of gp130 and LIFR, or a type II receptor complex consisting of gp130 and OSMRβ (Hermanns, 2015). A separate study reported that stroke reduced OSMRβ, and a diminished OSMRβ level was associated with exacerbated cerebral damage, whereas, OSMRβ overexpression protected the brain against ischemia reperfusion injury (Guo et al., 2015). This study also found that giving human OSM to rodents, improved ischemia-reperfusion-induced brain injury and that OSM may be a future neuroprotective therapy. As OSM can bind LIFR, it is not surprising that STRING generated output also generated LIFR as an associated network protein. Previous studies have demonstrated that LIF treatment after stroke promotes neuroprotection and recovery (Davis et al., 2018). These network-related proteins help bring together pieces of a complex system of biochemical signaling occurring during cerebral ischemia. These proteins serve as candidates for future studies regarding their relationship with BMI, stroke severity, and stroke outcome.

An important limitation of our study was the sample size of $n = 61$ subjects. Although preliminary, data reported here will be validated through increasing enrollment in our BACTRAC study. Additionally, there are potential confounding variables in our data which may limit conclusions such as the spectrum of stroke severity, differing infarct times, and comorbid conditions. A great deal of effort was taken to control for as many potential confounders as possible throughout our analyses. When studying proteomic expression, one must also recognize that there may be differing levels of baseline proteomic expression across subjects as well as differing levels of proteases which may influence expression. Further, this study focused solely on the expression level of ADA, which is an enzyme, and the expression level and enzymatic activity might not be perfectly correlated in the context of cerebral ischemia. There were other proteins in addition to ADA that were found to be significantly changed between BMI groups that warrant further investigation in future studies. When we employed STRING analysis to determine the protein networks significantly changed in our obese population as well as predictive of infarct volume and edema volume, ADA was not connected to any other network proteins. This may be due to multiple factors including an insufficient amount of literature on ADA in ischemia to make these connections, but another possible explanation is the expression and activity level of ADA might not perfectly align under these pathologic circumstances. To address this potential limitation in the future, studies can be directed at enzymatic activity in addition to protein expression. Finally, our institution treats a specific patient demographic in Central Kentucky that is primarily Caucasian with comorbid conditions ubiquitous to the area, however, this population is generally an underserved population for which targeted research is needed to improve quality of care. As our enrollment grows and our tissue bank expands, we will be able to combat study limitations and

produce analyses with larger power and generalizability. Development of multi-institutional and multi-national collaborations for this registry is fully underway.

In conclusion, this study utilizes a thrombectomy registry and tissue bank to study proteomic expression at time of mechanical thrombectomy for ELVO stroke. We found subjects with higher BMI had a significantly earlier age of onset of stroke as well as higher ADA levels which were predictive of higher infarct volume, edema volume, and worse mRS and NIHSS score on discharge. We highlight adenosine deaminase as a protein important in ischemic stroke and underreported in the stroke literature. ADA and related proteins elucidated by STRING, may serve as novel and personalized prognostic biomarkers as well as potential therapeutic targets in the future.

Ethics

All subjects enrolled in this study were consented and approved by the IRB.

Verification

This work has never been previously published nor is it being considered for publication elsewhere.

Funding

Funding from UK Center for Clinical and Translational Science (CCTS).

Declaration of competing interest

Authors KRP, AMS, and JFF are co-founders/equity holders in Cerelux, LLC.

References

Aparicio, H.J., et al., 2017. Overweight, obesity, and survival after stroke in the Framingham heart study. J. Am. Heart Assoc. 6 (6).
Bouslama, M., et al., 2020. Body mass index and clinical outcomes in large vessel occlusion acute ischemic stroke after endovascular therapy. Interv. Neurol. 8 (2–6), 144–151.
Burnstock, G., 2006. Historical review: ATP as a neurotransmitter. Trends Pharmacol. Sci. 27 (3), 166–176.
Chatzikonstantinou, A., Ebert, A.D., Wolf, M.E., 2016. The impact of body mass index on the thrombolytic treatment of acute ischemic stroke. Cerebrovasc. Dis. 42 (3–4), 240–246.
Chen, J.F., et al., 1999. A(2A) adenosine receptor deficiency attenuates brain injury induced by transient focal ischemia in mice. J. Neurosci. 19 (21), 9192–9200.
Chen, S.H., et al., 2020. Effect of body mass index on outcomes of mechanical thrombectomy in acute ischemic stroke. World Neurosurg. 143, e503–e515.
Chunn, J.L., et al., 2005. Adenosine-dependent pulmonary fibrosis in adenosine deaminase-deficient mice. J. Immunol. 175 (3), 1937–1946.
Davis, S.M., et al., 2018. Leukemia inhibitory factor modulates the peripheral immune response in a rat model of emergent large vessel occlusion. J. Neuroinflammation 15 (1), 288.
Deckert, J., Gleiter, C.H., 1994. Adenosine–an endogenous neuroprotective metabolite and neuromodulator. J. Neural. Transm. Suppl. 43, 23–31.
Dey, D.K., et al., 2002. Waist circumference, body mass index, and risk for stroke in older people: a 15 year longitudinal population study of 70- year-olds. J. Am. Geriatr. Soc. 50 (9), 1510–1518.
Elliott, M.R., et al., 2009. Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. Nature 461 (7261), 282–286.
Eltzschig, H.K., et al., 2006. Endothelial catabolism of extracellular adenosine during hypoxia: the role of surface adenosine deaminase and CD26. Blood 108 (5), 1602–1610.
Enroth, S., et al., 2019. High throughput proteomics identifies a high-accuracy 11 plasma protein biomarker signature for ovarian cancer. Commun. Biol. 2, 221.
Folkersen, L., et al., 2020. Genomic and drug target evaluation of 90 cardiovascular proteins in 30,931 individuals. Nat. Metab. 2 (10), 1135–1148.
Fraser, J.F., et al., 2018. The Blood and Clot Thrombectomy Registry and Collaboration (BACTRAC) protocol: novel method for evaluating human stroke. J. Neurointerventional Surg.
Giblett, E.R., et al., 1972. Adenosine-deaminase deficiency in two patients with severely impaired cellular immunity. Lancet 2 (7786), 1067–1069.
Gu, H., et al., 2019. Age- and sex-associated impacts of body mass index on stroke type risk: a 27-year prospective cohort study in a low-income population in China. Front. Neurol. 10, 456.
Guo, S., et al., 2015. Oncostatin M confers neuroprotection against ischemic stroke. J. Neurosci. 35 (34), 12047–12062.
Hagberg, H., et al., 1987. Extracellular adenosine, inosine, hypoxanthine, and xanthine in relation to tissue nucleotides and purines in rat striatum during transient ischemia. J. Neurochem. 49 (1), 227–231.
Hermanns, H.M., 2015. Oncostatin M and interleukin-31: cytokines, receptors, signal transduction and physiology. Cytokine Growth Factor Rev. 26 (5), 545–558.
Jadhav, A.A., Jain, A., 2012. Elevated adenosine deaminase activity in overweight and obese Indian subjects. Arch. Physiol. Biochem. 118 (1), 1–5.
Johansson, B., et al., 2001. Hyperalgesia, anxiety, and decreased hypoxic neuroprotection in mice lacking the adenosine A1 receptor. Proc. Natl. Acad. Sci. U. S. A. 98 (16), 9407–9412.
Kaul, A., Chandra, M., Misra, M.K., 2006. Adenosine deaminase in ischemia reperfusion injury in patients with myocardial infarction. J. Enzym. Inhib. Med. Chem. 21 (5), 543–546.
Kawase, S., et al., 2017. Association between body mass index and outcome in Japanese ischemic stroke patients. Geriatr. Gerontol. Int. 17 (3), 369–374.
Khemka, V.K., et al., 2013. Raised serum adenosine deaminase level in nonobese type 2 diabetes mellitus. Sci. World J., 404320
Larsson, A., et al., 2015. The body mass index (BMI) is significantly correlated with levels of cytokines and chemokines in cerebrospinal fluid. Cytokine 76 (2), 514–518.
Lazarowski, E.R., Boucher, R.C., Harden, T.K., 2003. Mechanisms of release of nucleotides and integration of their action as P2X- and P2Y-receptor activating molecules. Mol. Pharmacol. 64 (4), 785–795.
Lee, J.G., et al., 2011. Changes in adenosine deaminase activity in patients with type 2 diabetes mellitus and effect of DPP-4 inhibitor treatment on ADA activity. Diabetes Metab. J 35 (2), 149–158.
Li, Y., et al., 2019. Body mass index and risks of incident ischemic stroke subtypes: The Japan Public Health Center-based prospective (JPHC) study. J. Epidemiol. 29 (9), 325–333.
Li, Z., et al., 2020. Identifying the pattern of immune related cells and genes in the peripheral blood of ischemic stroke. J. Transl. Med. 18 (1), 296.
MacDonald, P.E., et al., 2006. Release of small transmitters through kiss-and-run fusion pores in rat pancreatic beta cells. Cell Metabol. 4 (4), 283–290.
Maglinger, B., et al., 2020. Proteomic changes in intracranial blood during human ischemic stroke. J. Neurointerventional Surg.
Martha, S.R., Fraser, J.F., Pennypacker, K.R., 2019. Acid-base and electrolyte changes drive early pathology in ischemic stroke. NeuroMolecular Med.
Martha SR, C.Q., Fraser, J.F., Gong, L., Collier, L.A., Davis, S.M., Lukins, D., Alhajeri, A., Grupke, S., Pennypacker, K.R., 2020. Expression of cytokines and chemokines as predictors of stroke outcomes in acute ischemic stroke. Front. Neurol.
Matsumoto, K., et al., 1992. Flow thresholds for extracellular purine catabolite elevation in cat focal ischemia. Brain Res. 579 (2), 309–314.
Meschia, J.F., et al., 2014. Guidelines for the primary prevention of stroke: a statement for healthcare professionals from the American Heart Association/American Stroke Association. Stroke 45 (12), 3754–3832.
Oberheiden, T., et al., 2012. Platelet and monocyte activation in acute ischemic stroke–is there a correlation with stroke etiology? Clin. Appl. Thromb. Hemost. 18 (1), 87–91.
Oesch, L., et al., 2017. Obesity paradox in stroke - Myth or reality? A systematic review. PLoS One 12 (3), e0171334.
Ohta, A., Sitkovsky, M., 2001. Role of G-protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. Nature 414 (6866), 916–920.
Organization, W.H., 2021 June 9. Obesity and overweight [online]. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight.
Park, H., et al., 2019. Body mass index and prognosis in ischemic stroke patients with type 2 diabetes mellitus. Front. Neurol. 10, 563.
Phillis, J.W., 1989. Adenosine in the control of the cerebral circulation. Cerebrovasc. Brain Metab. Rev. 1 (1), 26–54.
Pignataro, G., Simon, R.P., Boison, D., 2007. Transgenic overexpression of adenosine kinase aggravates cell death in ischemia. J. Cerebr. Blood Flow Metabol. 27 (1), 1–5.
Pirson, F.A.V., et al., 2019. The effect of body mass index on outcome after endovascular treatment in acute ischemic stroke patients: a post hoc analysis of the MR CLEAN trial. Cerebrovasc. Dis. 48 (3–6), 200–206.
Razinia, T., et al., 2007. Body mass index and hospital discharge outcomes after ischemic stroke. Arch. Neurol. 64 (3), 388–391.
Rudolphi, K.A., et al., 1992. Neuroprotective role of adenosine in cerebral ischaemia. Trends Pharmacol. Sci. 13 (12), 439–445.
Sands, M., et al., 2021. Antimicrobial protein REG3A and signaling networks are predictive of stroke outcomes. J. Neurochem.
Shaw, B.C., et al., 2021. Isolation and identification of leukocyte populations in intracranial blood collected during mechanical thrombectomy. J. Cerebr. Blood Flow Metabol., 271678X211028496
Sitkovsky, M.V., et al., 2004. Physiological control of immune response and inflammatory tissue damage by hypoxia-inducible factors and adenosine A2A receptors. Annu. Rev. Immunol. 22, 657–682.
Skolarus, L.E., et al., 2014. Association of body mass index and mortality after acute ischemic stroke. Circ. Cardiovasc. Qual. Outcomes 7 (1), 64–69.
Song, Y.M., et al., 2004. Body mass index and ischemic and hemorrhagic stroke: a prospective study in Korean men. Stroke 35 (4), 831–836.
Souza, L.C., et al., 2012. Malignant CTA collateral profile is highly specific for large admission DWI infarct core and poor outcome in acute stroke. AJNR Am. J. Neuroradiol. 33 (7), 1331–1336.

Spears, R.C., et al., 2021. Alterations in Local Peri-Infarct Blood Gases in Stroke Patients Undergoing Thrombectomy. World Neurosurg.
Strazzullo, P., et al., 2010. Excess body weight and incidence of stroke: meta-analysis of prospective studies with 2 million participants. Stroke 41 (5), e418–e426.
Sun, C.X., et al., 2005. A protective role for the A1 adenosine receptor in adenosine-dependent pulmonary injury. J. Clin. Invest. 115 (1), 35–43.
Tamura, R., et al., 2016. Neuroprotective effects of adenosine deaminase in the striatum. J. Cerebr. Blood Flow Metabol. 36 (4), 709–720.
Viticchi, G., et al., 2020. Sex influence in ischemic stroke severity and outcome among metabolically unhealthy overweight patients. J. Neurol. Sci. 416, 116955.
Yang, D., et al., 2006. The A2B adenosine receptor protects against inflammation and excessive vascular adhesion. J. Clin. Invest. 116 (7), 1913–1923.
Yatsuya, H., et al., 2010. Associations of obesity measures with subtypes of ischemic stroke in the ARIC Study. J. Epidemiol. 20 (5), 347–354.
Zhang, Z., et al., 2007. Regulated ATP release from astrocytes through lysosome exocytosis. Nat. Cell Biol. 9 (8), 945–953.
Zhang, Q., Li, Y., Tsien, R.W., 2009. The dynamic control of kiss-and-run and vesicular reuse probed with single nanoparticles. Science 323 (5920), 1448–1453.