Impact of homocysteine levels on clinical outcome in patients with acute ischemic stroke receiving intravenous thrombolysis therapy

Lei Li1,*, Xiaoye Ma2,*, Li Zeng1, Sajan Pandey1, Ronghao Wan1, Rui Shen1 and Quanbin Zhang1

1 Department of Neurosurgery, Shanghai Tenth People’s Hospital, Tongji University School of Medicine, Shanghai, China
2 Department of Neurology, Shanghai Tenth People’s Hospital, Tongji University School of Medicine, Shanghai, China
* These authors contributed equally to this work.

ABSTRACT

Background: The purpose of this study was to retrospectively assess the potential correlation between clinical outcomes and homocysteine (Hcy) levels in acute ischemic stroke (AIS) patients after recombinant tissue plasminogen activator (rtPA) treatment.

Methods: AIS patients treated by rtPA were enrolled between September 2018 and March 2019 in the Stroke Center (Department of Neurology and Neurosurgery), Shanghai Tenth People’s Hospital, Tongji University School of Medicine. Demographics, baseline and clinical characteristics, and modified Rankin Scale (mRS) score after three months from the onset were retrospectively analyzed. Then we compared data about demographics, baseline and clinical characteristics between patients with favorable (mRS score 0–2) and unfavorable (mRS score 3–6) outcomes.

Results: Among 141 patients, 36 patients had poor outcome, for an incidence of 25.53%. Univariate analysis showed that higher Hcy levels (OR = 1.07, 95% CI [1.02–1.12]), older age (OR = 1.06, 95% CI [1.02–1.10]), longer door to needle time (DNT) (OR = 1.03, 95% CI [1.01–1.05]), higher D-Dimer levels (OR = 1.33, 95% CI [1.03–1.71]), and higher National Institutes of Health Stroke Scale (NIHSS) score before treatment (OR = 1.21, 95% CI [1.08–1.35]) were each associated with poor outcome. Also, without internal carotid artery plaque (OR = 0.30, 95% CI [0.10–0.92]) showed a protective effect on patients’ clinical outcome. Patients with higher levels of Hcy decline also showed an increased risk of poor outcome for AIS patients obtaining rtPA treatment (Non-adjusted: OR = 1.07, 95% CI [1.02–1.12]; Adjust model I adjusts for demographics (age, male): OR = 1.06, 95% CI [1.02–1.11]; Adjust model II adjusts for hospital care factors (onset to treatment, DNT): OR = 1.08, 95% CI [1.03–1.13]; Adjust model III adjusts for health and stroke factors (INR, D-Dimer, HGB, NIHSS score before treatment, smoking, drinking, hypertension, diabetes, coronary disease, hyperlipidemia, previous stroke, atrial fibrillation, hemorrhagic transformation, internal carotid artery plaque): OR = 1.06, 95% CI [1.02–1.11]). The results are very stable in all three models constructed.

Conclusion: The results of this study indicate that increased Hcy level independently predicts unfavorable outcome in AIS patients accepting thrombolytic therapy.

How to cite this article Li L, Ma X, Zeng L, Pandey S, Wan R, Shen R, Zhang Q. 2020. Impact of homocysteine levels on clinical outcome in patients with acute ischemic stroke receiving intravenous thrombolysis therapy. PeerJ 8:e9474 DOI 10.7717/peerj.9474
However, the contribution of Hcy to the outcome, although significant, is relatively small and perhaps not clinically significant when all the other confounders are considered.

Subjects Neurology, Surgery and Surgical Specialties
Keywords Acute ischemic stroke, Alteplase, Intravenous thrombolysis, Modified Rankin Scale, Homocysteine

INTRODUCTION
Acute ischemic stroke (AIS) is one of the principal causes of disability and death, which causes great economic and mental burden to patients’ families (Merlino et al., 2019; Rosenbaum Halevi et al., 2019). Intravenous thrombolytic therapy and recombinant tissue plasminogen activator (rtPA) have been broadly used in AIS patients within 4.5 h after symptom onset (Valdes Hernandez et al., 2014).

Previous studies have shown that those who have hyperhomocysteinemia (Hhcy) have a higher risk of the development of AIS, and Hhcy is correlated with poor prognosis in AIS patients (Davis Armstrong et al., 2018; Zaric et al., 2019). Besides, the increased risk of cardiovascular disease could also be partially attributed to Hhcy (Borowczyk et al., 2019). Its possible underlying mechanism is that elevated serum homocysteine (Hcy) may lead to endothelial dysfunction (Esse et al., 2019; Wu et al., 2019; Yan et al., 2019), neurotoxicity (Moretti & Caruso, 2019) and up-regulation of thrombosis formation factors (Diao et al., 2019; Jin et al., 2018). However, the likelihood of applying Hcy to prognosticate AIS patients’ clinical outcomes after rtPA therapy hasn’t been thoroughly investigated. Therefore, we designed this retrospective study to assess the potential relationship between clinical outcomes and Hcy in patients with AIS after rtPA therapy.

MATERIALS AND METHODS
Patients
The study was designed as a retrospective study. Patients with AIS taking rtPA treatment were enrolled between September 2018 and March 2019 in a single Stroke Center (Department of Neurology and Neurosurgery, Shanghai Tenth People’s Hospital, Tongji University School of Medicine). The study population included 205 consecutive patients admitted with AIS within 4.5 h of their symptom onset.

Exclusion criteria
Evidence of severe infection, cardiopulmonary disease, cancer, hepatic or renal disease, or multiple organ dysfunction. Patients with mental disorders, severe cognitive dysfunction, a history of mental problems and abnormal behavior were also excluded.

Data and design
The institutional ethics committee of Shanghai Tenth People’s Hospital has approved this retrospective study. All investigations and methods were performed in accordance with Shanghai Tenth People’s Hospital’s guidelines and regulations. The consent form was not
required due to the retrospective nature and de-identified nature of this retrospective study. The standard cardiological and neurological examinations were performed immediately on the patients’ arrival in the emergency room. Before the initiation of therapy, the information about vital signs, blood chemistry and computed tomography scans was obtained. Information about previous and concomitant diseases were recorded. National Institutes of Health Stroke Scale (NIHSS) score was assessed (Brott et al., 1989). Adverse events of hemorrhage, including symptomatic intracerebral hemorrhage (sICH), were identified.

All patients underwent intravenous (i.v.) thrombolytic therapy using rtPA at a dose of 0.9 mg/kg. And those who were followed up for at least three months and had the available modified Rankin Scale (mRS) score (Van Swieten et al., 1988) at three months from symptom onset were involved in the final retrospective analysis. Then we compared data about demographics, baseline and clinical characteristics between patients with favorable (mRS score 0–2) and unfavorable (mRS score 3–6) outcomes.

**Statistical analysis**

Applying the Kolmogorov–Smirnov test to test if the metrological data followed the normal distribution or not. Mean ± standard deviation (SD) was used to express continuous variables that follow the normal distribution, and the median (quartiles) was used to indicate variables that do not follow a normal distribution. Frequencies or percentages were used to indicate categorical variables.

Chi-Square tests and Kruskal–Wallis H test or one-way ANOVA test were applied to detect any statistical difference between the means and proportions of the three groups.

Univariate analysis of baseline characteristics and clinical outcome were carried out. In this process, variables were added to the model with recoding into tertiles/binary or without recoding as needed.

Multiple logistic regression models were utilized to assess the association between serum Hcy levels and clinical outcome. Both non-adjusted and multivariate-adjusted models (variables adjusted for demographics (age, male)); hospital care factors (onset to treatment, door to needle time (DNT)); health and stroke factors (INR, D-Dimer, hemoglobin (HGB), NIHSS score before treatment, smoking, drinking, hypertension, diabetes, coronary disease, hyperlipidemia, previous stroke, atrial fibrillation, hemorrhagic transformation, internal carotid artery plaque) were applied. In this step, multivariate logistic regression models with the conditional forward selection method were used to reduce confounding factors’ contribution and evaluate the independent contribution of Hcy to clinical outcome after adjusting different factors. All analyses were performed with the SPSS 22.0. A two-sided significance level of 0.05 was applied to assess statistical significance.

**RESULTS**

**General patient characteristics**

Among 205 consecutive patients with AIS, 141 (68.78%) patients’ follow-up data could be analyzed. Among them, 36 patients had poor outcomes, for an incidence of 25.53%.
The data about baseline clinical findings, demographic characteristics and medical history records were summarized in Table 1.

**Univariate logistic regression analysis**

Table 2 presents the association between outcomes at 90 days and each baseline characteristic. Consistent with the available literature, higher Hcy levels (OR = 1.07, 95% CI [1.02–1.12]), older age (OR = 1.06, 95% CI [1.02–1.10]), longer DNT (OR = 1.03, 95% CI [1.01–1.05]), higher D-Dimer levels (OR = 1.33, 95% CI [1.03–1.71]), and higher NIHSS score before treatment (OR = 1.21, 95% CI [1.08–1.35]) were each associated with poor outcome. Without internal carotid artery plaque (OR = 0.30, 95% CI [0.0.10–0.92]) showed a protective effect on patients’ clinical outcome. The OR for poor outcomes was 0.36 (95% CI [0.14–0.95]), 0.24 (95% CI [0.08–0.67]), 0.24 (95% CI [0.08–0.67]), 0.09 (95% CI [0.03–0.34]) in individuals with Hcy levels, age, D-Dimer levels, NIHSS score before treatment in the bottom tertile compared with those in the top tertile (Table 2).
| Exposure                              | Statistics          | Clinical outcome at 3 months | P value |
|--------------------------------------|---------------------|-----------------------------|---------|
| Hcy, μmol/L                          | 12.20 (9.00–16.45) | 1.07 (1.02, 1.12)           | 0.006   |
| Hcy tertile                          |                     |                             |         |
| Bottom tertile                       | 47 (33.33%)         | 0.36 (0.14, 0.95)           | 0.039   |
| Middle tertile                       | 47 (33.33%)         | 0.54 (0.22, 1.33)           | 0.18    |
| Top tertile                          | 47 (33.33%)         | reference                   |         |
| Gender                               |                     |                             |         |
| Male                                 | 93 (65.96%)         | 1.04 (0.47, 2.33)           |         |
| Female                               | 48 (34.02%)         | reference                   | 0.917   |
| Age, year                            | 66.00 (60.00–73.50) | 1.06 (1.02, 1.10)           | 0.006   |
| Age tertile                          |                     |                             |         |
| Bottom tertile                       | 47 (33.33%)         | 0.24 (0.08, 0.67)           | 0.006   |
| Middle tertile                       | 47 (33.33%)         | 0.55 (0.23, 1.33)           | 0.187   |
| Top tertile                          | 47 (33.33%)         | reference                   |         |
| Onset to treatment, min             | 139.00 (100.00–182.5) | 1.00 (1.00, 1.01)           | 0.405   |
| Onset to treatment tertile           |                     |                             |         |
| Bottom tertile                       | 47 (33.33%)         | 0.56 (0.21, 1.46)           | 0.233   |
| Middle tertile                       | 47 (33.33%)         | 0.90 (0.37, 2.20)           | 0.820   |
| Top tertile                          | 47 (33.33%)         | reference                   |         |
| DNT, min                             | 45.00 (37.50–55.00) | 1.03 (1.01, 1.05)           | 0.013   |
| DNT tertile                          |                     |                             |         |
| Bottom tertile                       | 47 (33.33%)         | 0.52 (0.21, 1.32)           | 0.170   |
| Middle tertile                       | 47 (33.33%)         | 0.52 (0.21, 1.32)           | 0.170   |
| Top tertile                          | 47 (33.33%)         | reference                   |         |
| INR                                  | 0.97 (0.93–1.02)    | 11.61 (0.32, 416.15)        | 0.179   |
| INR tertile                          |                     |                             |         |
| Bottom tertile                       | 47 (33.33%)         | 0.71 (0.27, 1.82)           | 0.473   |
| Middle tertile                       | 47 (33.33%)         | 1.00 (0.41, 2.47)           | 1.000   |
| Top tertile                          | 47 (33.33%)         | reference                   | 0.433   |
| D-Dimer, μg/mL                       | 0.54 (0.31–1.21)    | 1.33 (1.03, 1.71)           | 0.027   |
| D-Dimer tertile                      |                     |                             |         |
| Bottom tertile                       | 48 (33.57%)         | 0.24 (0.08, 0.67)           | 0.006   |
| Middle tertile                       | 47 (32.87%)         | 0.55 (0.23, 1.33)           | 0.187   |
| Top tertile                          | 48 (33.57%)         | reference                   |         |
| Smoking                              |                     |                             |         |
| Yes                                  | 55 (39.01%)         | reference                   |         |
| No                                   | 86 (60.99%)         | 1.64 (0.73, 3.68)           | 0.231   |
| Drinking                             |                     |                             |         |
| Yes                                  | 28 (19.86%)         | reference                   |         |
| No                                   | 113 (80.14%)        | 1.33 (0.49, 3.58)           | 0.579   |
| Hypertension                         |                     |                             |         |
| Yes                                  | 109 (77.30%)        | reference                   |         |
| No                                   | 32 (22.70%)         | 0.47 (0.17, 1.32)           | 0.150   |

(Continued)
Subgroup analysis

We further explored the role of other covariables on the association between Hcy levels and outcome. The impact of Hcy levels on outcome exhibited a significant difference in DNT subgroups ($P = 0.037$), whereas no difference in the following subgroups: age, gender, onset to treatment, INR, D-Dimer, smoking, drinking, hypertension, diabetes, coronary disease, hyperlipidemia, previous stroke, atrial fibrillation, hemorrhagic transformation, internal carotid artery plaque, HGB and NIHSS score before treatment (all $P$ value for interaction is more than 0.05) (Table 3).
Table 3 Subgroup analysis of Hcy levels with clinical outcome according to covariates by logistic regression.

| Subgroup                        | OR (95% CI)     | P value | P value for interaction |
|---------------------------------|-----------------|---------|-------------------------|
| Age tertile                     |                 |         |                         |
| Bottom tertile                  | 1.09 [1.00–1.19]| 0.055   |                         |
| Middle tertile                  | 1.05 [0.98–1.13]| 0.186   |                         |
| Top tertile                     | 1.06 [0.97–1.15]| 0.239   |                         |
| Gender                          |                 |         |                         |
| Male                            | 1.06 [1.01–1.12]| 0.024   |                         |
| Female                          | 1.12 [1.00–1.25]| 0.047   |                         |
| Onset to treatment tertile      |                 |         |                         |
| Bottom tertile                  | 1.07 [0.98–1.17]| 0.120   |                         |
| Middle tertile                  | 1.01 [0.93–1.10]| 0.812   |                         |
| Top tertile                     | 1.13 [1.03–1.23]| 0.010   |                         |
| DNT tertile                     |                 |         |                         |
| Bottom tertile                  | 1.15 [1.04–1.28]| 0.008   |                         |
| Middle tertile                  | 1.08 [1.00–1.18]| 0.064   |                         |
| Top tertile                     | 1.00 [0.92–1.09]| 0.923   |                         |
| INR Tertile                     |                 |         |                         |
| Bottom tertile                  | 1.09 [0.99–1.21]| 0.070   |                         |
| Middle tertile                  | 1.07 [1.00–1.14]| 0.052   |                         |
| Top tertile                     | 1.04 [0.94–1.15]| 0.466   |                         |
| D-Dimer tertile                 |                 |         |                         |
| Bottom tertile                  | 1.18 [1.02–1.37]| 0.030   |                         |
| Middle tertile                  | 1.05 [0.98–1.12]| 0.204   |                         |
| Top tertile                     | 1.03 [0.94–1.13]| 0.585   |                         |
| Smoking                         |                 |         |                         |
| Yes                             | 1.09 [1.00–1.18]| 0.048   |                         |
| No                              | 1.07 [1.00–1.13]| 0.035   |                         |
| Drinking                        |                 |         |                         |
| Yes                             | 1.08 [0.96–1.23]| 0.196   |                         |
| No                              | 1.07 [1.01–1.12]| 0.013   |                         |
| Hypertension                    |                 |         |                         |
| Yes                             | 1.05 [0.99–1.10]| 0.101   |                         |
| No                              | 1.16 [0.97–1.38]| 0.105   |                         |
| Diabetes                        |                 |         |                         |
| Yes                             | 1.13 [1.04–1.23]| 0.004   |                         |
| No                              | 1.03 [0.98–1.09]| 0.212   |                         |
| Coronary disease                |                 |         |                         |
| Yes                             | 1.08 [0.81–1.44]| 0.595   |                         |
| No                              | 1.07 [1.02–1.12]| 0.007   |                         |
| Hyperlipidemia                  |                 |         |                         |
| Yes                             | 1.07 [0.96–1.20]| 0.218   |                         |
| No                              | 1.06 [1.01–1.12]| 0.020   |                         |

(Continued)
Multivariate logistic regression analysis

Patients with higher levels of Hcy decline also show an elevated risk of poor outcome for AIS patients obtaining rtPA treatment (Non-adjusted: OR = 1.07, 95% CI [1.02–1.12]; Adjust model I adjusts for demographics (age, male): OR = 1.06, 95% CI [1.02–1.11]; Adjust model II adjusts for hospital care factors (onset to treatment, DNT): OR = 1.08, 95% CI [1.03–1.13]); Adjust model III adjusts for health and stroke factors (INR, D-Dimer, HGB, NIHSS score before treatment, smoking, drinking, hypertension, diabetes, coronary disease, hyperlipidemia, previous stroke, atrial fibrillation, hemorrhagic transformation, internal carotid artery plaque): OR = 1.06, 95% CI [1.02–1.11]. The results are very stable in all three models constructed (Table 4).

DISCUSSION

Currently, intravenous thrombolysis (IVT) with alteplase is the standard treatment approved for AIS patients within 4.5 h of the onset of their symptoms (Valdes Hernandez et al., 2014). In this study, we found that high level of Hcy, as a predictor for poor prognosis at three months in AIS patients receiving rtPA therapy, has important clinical implications. We also demonstrated that the risk of poor prognosis in AIS patients taking
IVT increased by approximately 10% for every 1 μmol/L increase in serum Hcy concentration.

In the past decade, an enormous amount of epidemiological evidence supports the correlation between high Hcy levels and increased risk of the development of AIS (Cheng et al., 2018; Lu et al., 2018b), and some researchers have discovered that increased serum Hcy levels are related to increased hematoma volume (Hacke et al., 2008; Zhou et al., 2015). Previous studies also demonstrated that increased Hcy levels are correlated with functional disability in the acute phase of stroke (Mizrahi et al., 2005; Song et al., 2009). A prospective multicenter research conducted by Kwon et al. unveiled that the risk of early neurological deterioration increased along with the increase of Hcy levels in patients with ischemic stroke (Kwon et al., 2014). However, whether high Hcy levels can be regarded as an independent risk factor for unfavorable clinical outcome in ischemic stroke patients accepting IVT has not been well addressed. Though a small number of studies have already been published, the prognostic value of Hcy levels in patients with AIS after IVT remains controversial. Some investigations revealed that Hhcy was correlated with unfavorable outcomes in patients with ischemic stroke (Ling et al., 2018; Luo et al., 2019; Yao et al., 2016), whereas some other studies indicated that there was no significant correlation between Hhcy and ischemic stroke patients’ clinical outcome (Ribo et al., 2004a, 2004b). To further confirm the association between Hcy levels and clinical outcome of AIS patients treated with thrombolytic therapy, we conducted this retrospective study. Consistent with the previous retrospective studies conducted by Ling et al. (2018), Luo et al. (2019) and Yao et al. (2016), we found that there exists a correlation between Hcy levels and poor prognosis after acute thrombolytic therapy in AIS patients in this retrospective study. And we indicated that higher Hcy levels have a negative impact on prognosis. The result of our study implements new proof that Hhcy has a negative impact on ischemic stroke patients’ clinical outcome.

The underlying mechanism may be due to impaired vascular wall integrity and disturbance of cerebrovascular permeability resulting from increased levels of Hcy, which may lead to endothelial dysfunction, damage to elastic structures and damage to the basal layer of cerebral arterioles and microvessels (Fan et al., 2017; Mach et al., 1997). Some studies have consistently shown that hyperhomocysteinemia is an independent risk factor for atherosclerosis’ development, suggesting that raised plasma levels of Hcy are relevant to endothelial dysfunction (Borowczyk et al., 2019; Lu et al., 2018a; Wang et al., 2017). Besides, high Hcy levels also increase low-density lipoproteins oxidation, and the

| Exposure | Non-adjusted OR (95% CI) | P value | Adjust Model I OR (95% CI) | P value | Adjust Model II OR (95% CI) | P value | Adjust Model II OR (95% CI) | P value |
|----------|------------------------|---------|---------------------------|---------|-----------------------------|---------|---------------------------|---------|
| Hcy      | 1.07 [1.02–1.12]       | 0.006   | 1.06 [1.02–1.11]          | 0.006   | 1.08 [1.03–1.13]            | 0.003   | 1.06 [1.02–1.11]          | 0.009   |

Notes:
The non-adjusted model adjusts for none.
Adjust model I adjust for: demographics (age, male).
Adjust model II adjust for: hospital care factors (onset to treatment, DNT).
Adjust model III adjust for: health and stroke factors (INR, D-Dimer, HGB, NIHSS score before treatment, smoking, drinking, hypertension, diabetes, coronary disease, hyperlipidemia, previous stroke, atrial fibrillation, hemorrhagic transformation, internal carotid artery plaque).
dominant mechanism by which Hcy adversely affects vascular endothelial function involves oxidative stress and bioactive nitric oxide consumption (Miyazaki et al., 2014; Seo et al., 2010).

Our research also has some restrictions. First, this study is a single-center retrospective study and the sample size is limited. Second, we did not include patients who did not receive thrombolytic therapy, thus may resulting in a selective bias. Third, the Hcy level could be affected by various factors, such as genetic factors and drugs. But we didn’t evaluate the reason for Hhcy in this study cohort. Also, almost all ORs for Hcy are close to 1 in our results. The contribution of Hcy to the outcome, although significant, is relatively small and perhaps not clinically significant when considering all other confounders. Hence, the results should be explained with caution, and the results should be further confirmed in a multicenter prospective study with a larger cohort to clearly establish the correlation between Hhcy and unfavorable outcome in ischemic stroke patients accepting IVT.

CONCLUSIONS
In conclusion, the results of this study indicate that increased Hcy level independently predicts unfavorable outcome in AIS patients accepting thrombolytic therapy. However, the contribution of Hcy to the outcome, although significant, is relatively small when all the other confounders are considered. To better guide clinical practice, the further multicenter prospective study still needs to be done to clearly clarify the correlation between Hcy level and clinical outcome of AIS patients treating with intravenous thrombolysis.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding
This work was supported by the Shanghai Shenkang Hospital Development Center (No. SHDC12017X17). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures
The following grant information was disclosed by the authors:
Shanghai Shenkang Hospital Development Center: SHDC12017X17.

Competing Interests
The authors declare that they have no competing interests.

Author Contributions
- Lei Li performed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Xiaoye Ma performed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Li Zeng analyzed the data, prepared figures and/or tables, and approved the final draft.
Human Ethics
The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

The study was approved by the Institutional Ethics Committee of Shanghai Tenth People’s Hospital.

Data Availability
The following information was supplied regarding data availability:

The raw data are available in Table S1.

Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.9474#supplemental-information.

REFERENCES
Borowczyk K, Piechocka J, Glowacki R, Dhar I, Midtun O, Tell GS, Ueland PM, Nygard O, Jakubowski H. 2019. Urinary excretion of homocysteine thiolactone and the risk of acute myocardial infarction in coronary artery disease patients: the WENBIT trial. Journal of Internal Medicine 285(2):232–244 DOI 10.1111/joim.12834.

Brott T, Adams HP Jr, Olinger CP, Marler JR, Barsan WG, Biller J, Spilker J, Holleran R, Eberle R, Hertzberg VJS. 1989. Measurements of acute cerebral infarction: a clinical examination scale. Stroke 20(7):864–870 DOI 10.1161/01.STR.20.7.864.

Cheng L-S, Tu W-J, Shen Y, Zhang L-J, Ji K. 2018. Combination of high-sensitivity C-reactive protein and homocysteine predicts the post-stroke depression in patients with ischemic stroke. Molecular Neurobiology 55(4):2952–2958 DOI 10.1007/s12035-017-0549-8.

Davis Armstrong NM, Chen W-M, Brewer MS, Williams SR, Sale MM, Worrall BB, Keene KL. 2018. Epigenome-wide analyses identify two novel associations with recurrent stroke in the vitamin intervention for stroke prevention clinical trial. Frontiers in Genetics 9:358 DOI 10.3389/fgene.2018.00358.

Diao L, Bai L, Jiang X, Li J, Zhang Q. 2019. Long-chain noncoding RNA GAS5 mediates oxidative stress in cardiac microvascular endothelial cells injury. Journal of Cellular Physiology 234(10):17649–17662 DOI 10.1002/jcp.28388.

Esse R, Barroso M, De Almeida IT, Castro R. 2019. The contribution of homocysteine metabolism disruption to endothelial dysfunction: state-of-the-art. International Journal of Molecular Sciences 20(4):867 DOI 10.3390/ijms20040867.

Fan CD, Sun JY, Fu XT, Hou YJ, Li Y, Yang MF, Fu XY, Sun BL. 2017. Astaxanthin attenuates homocysteine-induced cardiotoxicity in vitro and in vivo by inhibiting mitochondrial
dysfunction and oxidative damage. *Frontiers in Physiology* 8:1041
DOI 10.3389/fphys.2017.01041.

Hacke W, Kaste M, Bluhmki E, Brozman M, Davalos A, Guidetti D, Larrue V, Lees KR, Medeghri Z, Machnig T, Schneider D, Von Kummer R, Wahlgren N, Toni D. 2008. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *New England Journal of Medicine* 359(13):1317–1329 DOI 10.1056/NEJMoa0804656.

Jin P, Bian Y, Wang K, Cong G, Yan R, Sha Y, Ma X, Zhou J, Yuan Z, Jia S. 2018. Homocysteine accelerates atherosclerosis via inhibiting LXRα-mediated ABCA1/ABCG1–dependent cholesterol efflux from macrophages. *Life Sciences* 214:41–50 DOI 10.1016/j.lfs.2018.10.060.

Kwon HM, Lee YS, Bae HJ, Kang DW. 2014. Homocysteine as a predictor of early neurological deterioration in acute ischemic stroke. *Stroke* 45(3):871–873 DOI 10.1161/STROKEAHA.113.004099.

Ling C, Hong Z, Yu W, Yu L, Xin S. 2018. The serum homocysteine level in patients with acute ischemic stroke (AIS) after thrombolysis and its relationship with clinical outcomes. *Revista da Associação Médica Brasileira* 64(5):438–442 DOI 10.1590/1806-9282.64.05.438.

Lu S, Deng J, Liu H, Liu B, Yang J, Miao Y, Li J, Wang N, Jiang C, Xu Q, Wang X, Feng J. 2018a. PKM2-dependent metabolic reprogramming in CD4+ T cells is crucial for hyperhomocysteinemia-accelerated atherosclerosis. *Journal of Molecular Medicine* 96(6):585–600 DOI 10.1007/s00109-018-1645-6.

Lu SS, Xie J, Su CQ, Ge S, Shi HB, Hong XN. 2018b. Plasma homocysteine levels and intracranial plaque characteristics: association and clinical relevance in ischemic stroke. *BMC Neurology* 18(1):200 DOI 10.1186/s12883-018-1203-4.

Luo Y, Jin H, Guo ZN, Zhang P, Zhang LY, Chen J, Yu Y, Wang Y, Liu J, He QY, Sun X, Yang Y. 2019. Effect of hyperhomocysteinemia on clinical outcome and hemorrhagic transformation after thrombolysis in ischemic stroke patients. *Frontiers in Neurology* 10:592 DOI 10.3389/fneur.2019.00592.

Mach F, Schonbeck U, Bonnefoy JY, Pober JS, Libby P. 1997. Activation of monocyte/macrophage functions related to acute atheroma complication by ligation of CD40: induction of collagenase, stromelysin, and tissue factor. *Circulation* 96(2):396–399 DOI 10.1161/01.CIR.96.2.396.

Merlino G, Corazza E, Lorenzut S, Gigli G, Cargnelutti D, Valente M. 2019. Efficacy and safety of intravenous thrombolysis in patients with acute ischemic stroke and pre-existing disability. *Journal of Clinical Medicine* 8(3):400 DOI 10.3390/jcm8030400.

Miyazaki A, Sagae N, Usami Y, Sato M, Kameda T, Yoshimoto A, Ishimine N, Matsuda K, Sugano M, Haru M, Honda T, Tozuka M. 2014. N-homocysteinylation of apolipoprotein A-I impairs the protein’s antioxidant ability but not its cholesterol efflux capacity. *Biological Chemistry* 395(6):641–648 DOI 10.1515/hsz-2013-0262.

Mizrahi EH, Fleissig Y, Arad M, Adunsky A. 2005. Plasma homocysteine level and functional outcome of patients with ischemic stroke. *Archives of Physical Medicine and Rehabilitation* 86(1):60–63 DOI 10.1016/j.apmr.2004.01.031.

Moretti R, Caruso P. 2019. The controversial role of homocysteine in neurology: from labs to clinical practice. *International Journal of Molecular Sciences* 20(1):231 DOI 10.3390/ijms20010231.

Ribo M, Montaner J, Molina CA, Arenillas JF, Santamarina E, Alvarez-Sabín J. 2004a. Admission fibrinolytic profile predicts clot lysis resistance in stroke patients treated with tissue plasminogen activator. *Thrombosis and Haemostasis* 91(6):1146–1151 DOI 10.1160/TH04-02-0097.
Ribo M, Montaner J, Molina CA, Arenillas JF, Santamarina E, Quintana M, Alvarez-Sabin J. 2004b. Admission fibrinolytic profile is associated with symptomatic hemorrhagic transformation in stroke patients treated with tissue plasminogen activator. *Stroke* 35(9):2123–2127 DOI 10.1161/01.STR.0000137608.73660.4c.

Rosenbaum Halevi D, Bursaw AW, Karamchandani RR, Alderman SE, Breier JI, Vahidy FS, Aden JK, Cai C, Zhang X, Savitz SI. 2019. Cognitive deficits in acute mild ischemic stroke and TIA and effects of rt-PA. *Annals of Clinical and Translational Neurology* 6(3):466–474 DOI 10.1002/acn3.719.

Seo H, Oh H, Park H, Park M, Jang Y, Lee M. 2010. Contribution of dietary intakes of antioxidants to homocysteine-induced low density lipoprotein (LDL) oxidation in atherosclerotic patients. *Yonsei Medical Journal* 51(4):526–533 DOI 10.3349/ymj.2010.51.4.526.

Song IU, Kim JS, Ryu SY, Lee SB, Lee SJ, Jeong DS, Kim YI, Lee KS. 2009. Are plasma homocysteine levels related to neurological severity and functional outcome after ischemic stroke in the Korean population? *Journal of the Neurological Sciences* 278(1–2):60–63 DOI 10.1016/j.jns.2008.11.011.

Valdes Hernandez MC, Piper RJ, Bastin ME, Royle NA, Maniega SM, Aribisala BS, Murray C, Deary IJ, Wardlaw JM. 2014. Morphologic, distributional, volumetric, and intensity characterization of periventricular hyperintensities. *AJNR American Journal of Neuroradiology* 35(1):55–62 DOI 10.3174/ajnr.A3612.

Van Swieten JC, Koudstaal PJ, Vischot MC, Schouten HJ, Van Gijn J. 1988. Interobserver agreement for the assessment of handicap in stroke patients. *Stroke* 19(5):604–607 DOI 10.1161/01.STR.19.5.604.

Wang R, Wang Y, Mu N, Lou X, Li W, Chen Y, Fan D, Tan H. 2017. Activation of NLRP3 inflammasomes contributes to hyperhomocysteinemia-aggravated inflammation and atherosclerosis in apoE-deficient mice. *Laboratory Investigation* 97(8):922–934 DOI 10.1038/labinvest.2017.30.

Wu X, Zhang L, Miao Y, Yang J, Wang X, Wang CC, Feng J, Wang L. 2019. Homocysteine causes vascular endothelial dysfunction by disrupting endoplasmic reticulum redox homeostasis. *Redox Biology* 20:46–59 DOI 10.1016/j.redox.2018.09.021.

Yan P, Sun C, Ma J, Jin Z, Guo R, Yang B. 2019. MicroRNA-128 confers protection against cardiac microvascular endothelial cell injury in coronary heart disease via negative regulation of IRS1. *Journal of Cellular Physiology* 234(8):13452–13463 DOI 10.1002/jcp.28025.

Yao ES, Tang Y, Xie MJ, Wang MH, Wang H, Luo X. 2016. Elevated homocysteine level related to poor outcome after thrombolysis in acute ischemic stroke. *Medical Science Monitor* 22:3268–3273 DOI 10.12659/MSM.900010.

Zaric BI, Obradovic M, Bajic V, Haidara MA, Jovanovic M, Isenovic ER. 2019. Homocysteine and hyperhomocysteinaemia. *Current Medicinal Chemistry* 26(16):2948–2961 DOI 10.2174/0929867325666180313105949.

Zhou F, Chen B, Chen C, Huang J, Chen S, Guo F, Hu Z. 2015. Elevated homocysteine levels contribute to larger hematoma volume in patients with intracerebral hemorrhage. *Journal of Stroke and Cerebrovascular Diseases* 24(4):784–788 DOI 10.1016/j.jstrokecerebrovasdis.2014.11.005.