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Association of follow-up infarct volume with functional outcome in acute ischemic stroke: a pooled analysis of seven randomized trials

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Key Points

Question: To what extent can the beneficial effect of endovascular therapy (EVT) on functional outcome be explained by treatment-associated reduction in follow-up infarct volume?

Findings: In this pooled data analysis including 1665 patients with an acute ischemic stroke presenting within 6 hours, a mere 12% of the beneficial effect of EVT on functional outcome is explained by a reduction in infarct volume.

Meaning: The infarct volume assessed on imaging post-treatment is currently not a valid proxy for estimating treatment effect in phase II studies.

ABSTRACT (344/350)

Importance: It is assumed that the positive treatment effect of endovascular therapy (EVT) is caused by the salvage and preservation of brain tissue. It remains unclear to what extent a reduction in follow-up infarct volume (FIV) explains the improved functional outcome after EVT in acute ischemic stroke patients.

Objective: To study whether FIV mediates the effect of EVT on functional outcome.

Design: Patient data of seven randomized trials were pooled. FIV was assessed on 24-hour or 1-week follow-up CT or MR after stroke onset. FIVs in patients allocated to EVT were compared to patients in the control group. Mediation analysis was performed to examine the causal chain in which EVT determines FIV and where FIV (the mediator) is presumed to determine the functional outcome.

Setting: Multicenter

Participants: 1690 of the 1764 patients had follow-up imaging acquired between 12 hours and 2 weeks after stroke onset. Twenty-five patients were additionally excluded, resulting in a total of 1665 included patients.

Main outcome and Measure: The primary outcome was the functional outcome as assessed on ordinal 90-day modified Rankin Scale (mRS).

Results: Median FIV of 1665 patients was 41mL (IQR 14-120), and median mRS was 3 (IQR 2-4). Patients allocated to EVT had significantly smaller FIVs compared to controls ($p<0.007$), with a median of 33mL (IQR 11-99) in the EVT group (n=821) and 51mL (IQR 18-134) in the control group (n=844). FIV was a strong predictor of functional outcome with an adjusted common odds ratio (acOR) of 0.46 (95%CI: 0.39–0.54, $p<0.001$). FIV partially mediated the relationship between treatment type and mRS, as EVT still had a substantial effect on functional outcome after adjustment for FIV (acOR of 2.22 (95%CI: 1.52–3.21, $p<0.001$). Merely 12% (95%CI: 1%-19%) of the beneficial effect of EVT on functional outcome is explained by a reduction in FIV.

Conclusions and Relevance: Reduction in FIV among patients with acute ischemic stroke treated with EVT only partially explains functional outcome, indicating that additional mechanisms underpin the benefits of EVT. FIV is not yet a valid proxy for estimating treatment effect in phase II studies.

INTRODUCTION

Endovascular therapy (EVT) substantially reduces disability in acute ischemic stroke patients with a large vessel occlusion in the anterior circulation.[1–7] It is assumed that this positive treatment effect is caused by the salvage and preservation of brain tissue. This idea is strengthened by many studies that have shown a strong association between the extent of ischemic tissue injury assessed at follow-up imaging and functional outcome.[8–11] With this in mind, the follow-up infarct volume (FIV) has been suggested as an early measure of treatment efficacy since this represents a potentially more objective estimate of the pathological response to treatment than traditional clinical outcomes. However, the validity of a potential surrogate outcome measure depends upon the demonstration that the effect of therapy on that surrogate accurately reflects and reliably predicts the effect on the clinical endpoint.[12] Formal testing through a causal mediation analysis is relevant to establishing the full potential of FIV as an early measure of treatment efficacy.

Only one study by the Endovascular Revascularization With Solitaire Device Versus Best Medical Therapy in Anterior Circulation Stroke Within 8 Hours (REVASCAT) investigators[5] examined if the effect of EVT on functional outcome was mediated by FIV, and reported that FIV did not mediate the relation between treatment type and functional outcome in their study data.[13] However, no estimates were reported on the proportion of EVT effect that is explained by FIV. In an exploratory analysis of Multicenter Randomized Clinical Trial of Endovascular Treatment for Acute Ischemic Stroke in the Netherlands (MR CLEAN) data, only a small proportion of the treatment effect could be explained by FIV, but estimates were not precise.[14] Hence, it still remains unclear to what extent the beneficial effect of EVT on functional outcome is explained by treatment-associated reduction in FIV. We investigated the mediating effect of

FIV on the association between treatment and functional outcome by analyzing pooled individual patient data from seven randomized trials of thrombectomy for acute ischemic stroke.[1–7]

METHODS

Data in this study are from the pooled individual patient data of the Highly Effective Reperfusion Evaluated in Multiple Endovascular Stroke Trials (HERMES) collaboration. This collaboration was established by trial investigators of seven recent randomized controlled trials (Endovascular Treatment for Small Core and Anterior Circulation Proximal Occlusion with Emphasis on Minimizing CT to Recanalization Times [ESCAPE], Extending the Time for Thrombolysis in Emergency Neurological Deficits — Intra-Arterial [EXTEND-IA], Solitaire With the Intention For Thrombectomy as Primary Endovascular Treatment [SWIFT PRIME], REVASCAT, MR CLEAN, Pragmatic Ischemic Stroke Thrombectomy Evaluation [PISTE], and Trial and Cost Effectiveness Evaluation of Intra-arterial Thrombectomy in Acute Ischemic Stroke [THRACE]) that investigated the efficacy of EVT in patients with acute ischemic stroke due to large vessel occlusions. Design features and inclusion criteria have previously been described.[6,7,15]

All subjects enrolled in each trial, except for MR CLEAN, had 24-hour follow-up brain imaging with either non-contrast computed tomography (CT) or magnetic resonance (MR) imaging. The THRACE trial protocol additionally required follow-up imaging at day 7 or at hospital discharge. Participating centers in MR CLEAN were required to perform follow-up imaging at 5-7 days. In EXTEND-IA, ESCAPE, SWIFT PRIME, REVASCAT and PISTE, 5-day follow-up imaging occurred at the discretion of the intervention site. This study included all patients that had available follow-up imaging, acquired at least 12 hours after symptom onset with an upper limit of 2 weeks (336 hours).

Outcome measures

The primary outcome was the degree of disability as scored on the modified Rankin Scale (mRS) at 90 days, considered as an ordinal outcome.[16] Assessment of secondary imaging outcome measures was performed on follow-up CT or MR. When multiple follow-up image data were available, the latest scan within the 12 hours - 2 week time window was selected for assessment. In case both CT and MR images were acquired, MR was the modality of choice and in that, diffusion-weighted imaging (DWI) was the preferred sequence due to its sensitivity in the detection on early infarcts. Infarcts were identified as intra-axial hypodense (CT) or hyperdense (MR [DWI]) regions within the affected hemisphere. Areas with parenchymal hemorrhage (within or adjacent to the infarct), cerebral edema extending into the contralateral hemisphere, and those causing ventricular and sulcal effacement were included in the lesion. Infarcts in the ipsilateral hemisphere with characteristics of old infarct were categorized as preexistent and were not included in the FIV. In case of decompressive hemicraniectomy with no available pre-surgery scan, only the ischemic lesion within the theoretical boundaries of the skull was included. . In case of CT, validated software was initially used to segment infarcts and volumes were calculated based upon planimetry.[17] Infarct volumes on MR were calculated using planimetry, manually outlined by an experienced observer (A.M.M.B or I.G.H.J). All infarct boundaries (on both modalities) were checked by an expert neuroradiologist (W.vZ, L.F.B or C.B.M) and adjusted where necessary. A standardized window and level setting for CT was set in that to limit variation between observers; window width was 30 Hounsfield units (HU) and center level was 35 HU. Discrepancies were resolved by a consensus reading with the two neuroradiologists. FIVs were calculated in milliliters (mL). Infarct location was defined by laterality (left or right hemisphere) and involvement of the 10 distinct anatomical regions of the Alberta Stroke Program Early CT score (ASPECTS) template[18], assessed by one of the same

expert neuroradiologists (W.vZ, L.F.B or C.B.M). In case of MR, an ASPECTS region with an infarction encompassing more than 20% of that region was classified as an infarct positive region to minimize differences between MR and CT. Hemorrhagic transformations were scored according to the anatomical description of the Heidelberg Classification.[19] All readers were blinded to treatment assignment, trial, and clinical findings.

Statistical Analysis

Dichotomous variables were presented as proportions. Continuous variables were tested for normality using the Shapiro-Wilk test and reported as mean ± standard deviation (SD) when normally distributed, or as median and interquartile range (IQR) otherwise. Differences in FIVs between EVT and control group were tested for significance with the Wilcoxon Rank-sum test. The statistical approach in this study was based on a previous post-hoc analysis by the MR CLEAN investigators.[20]

Mediation of treatment effect by follow-up infarct volume

To assess the contribution of infarct size reduction on the positive effect of EVT on functional outcome, mediation analysis was performed using Baron and Kenny's template[21] with FIV as mediating variable. Figure 1 illustrates the causal model where the treatment type (EVT or control) determines the FIV after an acute ischemic stroke and where FIV (the mediator) is the determinant of functional outcome at 90 days. To perform mediation analysis, it is necessary to test three causal pathways: 1) association of treatment with 90-day mRS, 2) association of treatment with FIV, and 3) association of FIV with 90-day mRS, controlling for treatment type. If all three associations are confirmed, mediation (indirect effect) can be established in a fourth step through estimation of the direct effect (*c'*) (see Table 1). According to Baron and Kenny, the

mediating effect is 'full' when *c'* is zero, 'partial' when *c'* is greater than zero. Mediation is 'absent' when not all causal steps are satisfied.

All pathways were tested using univariable and multivariable regression analysis. FIV was log transformed (log+1) to best satisfy the linear model (distribution of residuals was normal and homoscedasticity of the data was preserved). The effect of treatment on FIV (pathway *a*) was tested using linear regression modeling and reported as adjusted and unadjusted βs with its 95% confidence interval (CI). All other pathways were tested using ordinal linear regression and reported as adjusted and unadjusted common odds ratios (acOR and cOR, respectively) with associated 95% CIs. Multivariable modeling included the variables infarct location, hemorrhage type, and the pre-specified prognostic variables age and score on the National Institute of Health Stroke Scale (NIHSS) at baseline. The proportion of the effect that is mediated through FIV was estimated by dividing the log odds ratios (OR) of the indirect effect (*ab*) by the log OR of the total effect (*c*).[22,23] Given the ordinal nature of the outcome measure, the method of VanderWeele and colleagues[23] was used to compute mediation effects based on OR, with 95% CIs derived from bootstrap methods. Missing variables were included after imputation of the relevant covariate with median values of the non-missing data. For all ORs and other parameter estimation, mixed-effects modeling with a random effect for trial were performed to account for between-trial variance.

Sensitivity analyses

Infarcts may still evolve within the first week after onset due to ongoing hypoperfusion or because vasogenic edema increases the lesion volume.[24,25] Accordingly, FIV assessment is dependent on the timing of image acquisition. A sensitivity analysis was conducted in subjects with FIVs assessed on imaging acquired after 48 hours of symptom onset. In addition, MR may

provide more accurate estimates of FIV than CT, since tissue contrast on MR is superior to CT. A second sensitivity analysis was performed with MR imaging only.

All statistical analyses were performed in SAS version 9.4 (SAS Institute, Cary, NC, USA) and R version 3.2 (R Foundation for Statistical Computing, Vienna, Austria). P-values were two-sided and $p<0.05$ indicated statistical significance in all analyses.

Role of the funding source

The funder of the study had no role in study design, data collection, analysis, or interpretation, writing of this article, or the decision to submit this study for publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

In HERMES, 1690 (95.8%) out of 1764 patients had follow-up imaging acquired between 12 hours and 2 weeks after stroke symptom onset with a median of 30 hours (IQR 24-137). Twenty-five patients were additionally excluded because of poor image quality or difficulties precluding accurate infarct determination, resulting in a total of 1665 patients for this analysis.

Baseline characteristics of both treatment groups are shown in Table 2. Eight-hundred-twenty-one subjects were allocated to the EVT arm and 844 to the control arm. Overall, median FIV was 41 mL (IQR: 14-120 mL) and 39% (651/1650) achieved functional independence (mRS 0-2) at 3 months. Patients allocated to EVT had significantly smaller FIVs compared to controls, with a median of 33 mL (IQR 11-99) in the EVT group and 51 mL (IQR 18-134) in the control group ($p<0.007$) (Table 3). Successful reperfusion (TICI 2b-3) was achieved in 76% (523/690) of the patients in the intervention arm with evaluable angiographic imaging. In the intervention group,

median FIV in the substantial reperfused patients was 28 mL versus 86 mL for those who were not (TICI 0-2a) ($p<0.001$).

Mediation analysis

EVT was independently associated with a better functional outcome (Step 1; acOR = 2.28 [95%CI: 1.55 – 3.36, $p<0.001$]), and with smaller FIV (log transformed) (Step 2; β = -0.13 [95%CI: -0.19 to -0.07, $p<0.001$], see Table 4). In adjusted analysis, FIV (log transformed) was a strong predictor of functional outcome (Step 3) with an acOR of 0.46 (95%CI: 0.39 – 0.54, $p<0.001$); EVT similarly predicted good function outcome (Step 4; OR of 2.22 (95%CI: 1.52 – 3.21, $p<0.001$) (Table 4 and Supplemental Table 1). Other independent predictors of functional outcome included age (acOR 0.62; $p<.001$), baseline NIHSS (acOR 0.82 per 5 points; $p=.001$), hemorrhagic infarct type 2 (acOR 0.73; $p=.043$), intraventricular hemorrhage (acOR 0.29; $p=0.002$), and involvement of the ASPECTS Internal Capsula (acOR 0.45; $p<0.001$) and M5 (acOR 0.77; $p=0.042$) regions.

In the mediation analysis steps one through three were satisfied. Step four established partial mediation of FIV on the association between treatment and 90-day mRS; after adjustment for the mediator FIV, EVT still had a substantial effect on functional outcome. For the adjusted model, 12% (95%CI: 1%-19%) of EVT effect on functional outcome was explained by the mediator FIV. This proportion was 18% (95%CI: 3%-34%) for the unadjusted model.

Figure 2 depicts the relation between FIV and estimated probability of functional independence for all patients, stratified by treatment type and adjusted for baseline characteristics. This illustrates that the difference in estimated probability between treatments was mainly present in patients with smaller FIVs, where the absolute benefit of EVT appears highest. Supplemental Figures 1 and 2 show this relation for subjects who achieved successful reperfusion (TICI score

2b to 3 in the EVT group), and who did not achieve any reperfusion (TICI score 0). These illustrate an average increased likelihood of good outcome when reperfusion therapy was successful, but lack statistical precision due to the dominant proportion of patients with successful reperfusion.

Sensitivity analyses

Results of the tested pathways in our sensitivity analyses are shown in Supplemental Table 2. The first sensitivity analysis, which only included follow-up imaging past 48 hours of symptom onset (n=688), showed no substantial differences from the main analysis. The proportions of explained mediated effect by FIV were 7% (95%CI: -5% to 22%) and 23% (95%CI: -2% to 55%) for the adjusted and unadjusted analysis, respectively. The second sensitivity analysis with MR imaging (n=279) showed proportions of 0% (95%CI: -25% to 24%, adjusted) and 10% (95%CI: -38% to 56%, unadjusted) for the explained mediated effect. However, treatment was not significantly associated with FIV (pathway *a*), meaning that mediation was absent in this analysis.

DISCUSSION

Our analysis of the pooled recent EVT trial data showed that FIV at subacute time points only partially mediates treatment effect on functional outcome at three months, despite being a strong outcome predictor. Merely one-eighth of the variance in functional outcome as captured by the mRS could be attributed to a difference in FIV, suggesting that there are multiple component causes of clinical outcome at 90 days.

Several studies have previously addressed the relation between FIV and functional outcome after an acute occlusion of the proximal anterior circulation.[9–11] In concordance with our results, all demonstrated that FIV is a strong predictor of functional outcome and found that subjects treated

with EVT had significantly smaller FIVs compared to controls. One possibility is that there were imprecise FIV measurements and varying infarct sizes due to differences in follow-up acquisition time. However, the fact that patients were randomized and that our sensitivity analysis in patients with late follow-up imaging did not alter our results stresses that these factors cannot clarify the small proportion of explained effect.

Mechanisms other than FIV are at play that affects this causal pathway, such as the eloquence of certain brain areas. A previous study demonstrated large differences between brain regions in functional outcome when affected by a stroke, even when corrected for infarct volume.[26] Patients with small infarcts in eloquent regions are likely to have poor outcome despite a small total infarct volume. We used laterality and involvement of follow-up ASPECTS regions as a measure of infarct location in our analysis, and found that involvement of the internal capsule and M5 region was inversely associated with functional outcome. Considering the fact that these areas are linked to the motor cortex and corticospinal tract underlines the importance of brain eloquence. This association is additionally likely because the modified Rankin Scale is heavily weighted towards motor functions, particularly walking. Unfortunately, we do not have more detailed measurement of infarct location to better discriminate the total effect of brain eloquence.

Interestingly, we found that patients in the EVT arm had significantly better functional outcome than controls, even after controlling for FIV. This finding remained consistent in our sensitivity analyses with comparable effect sizes. The difference between treatment arms was mainly driven by subjects with smaller infarcts, where the impact of treatment was more pronounced. This effect was even stronger in patients who had successful reperfusion. Several hypotheses could possibly explain this phenomenon. First, studies have reported significant infarct growth between 24 hours and 1 week follow-up imaging[24] (whether or not driven by edema), but little is known

about the course of infarction after the follow-up imaging period of 1 week. It could be that patients randomized to the control arm continue to have hypoperfusion and consequently, true infarct growth even a week after ictus. Second, the binary definition of infarcted versus non-infarcted may be an over-simplification. There may be variation in the severity of injury within tissue defined as infarcted and potentially this may be less marked in EVT patients. Moreover, tissue outside the defined infarct may have undetected injury (e.g. selective neuronal loss) and this may be less severe in EVT patients.[27] Future studies are encouraged to use more sophisticated imaging approaches to increase insight in the pathophysiological process. Follow-up imaging near the 90-day mRS evaluation time point or magnetization transfer ratio imaging to assess axonal damage might help addressing this issue. Third, one could speculate about the possibility that the apparent benefits of EVT outside of FIV restriction are not from treatment alone, but that these may also result from differences in after care. Unfortunately, evidence is scarce to support these theories. [28]

Our study has limitations. First, because FIV measurements might be less accurate on CT and because treatment may have other pathophysiological effect that one cannot see on CT, we performed a sensitivity analysis with MR imaging only. In this analysis, effect sizes did not differ significantly from the main analysis. However, mediation could not be established due to absence of an association between treatment and FIV. This can possibly be explained by the small number of patients in this sub-analysis. In addition, MR is also not immune to FIV measurement error. However, it is noteworthy that a previous study investigating the association between FIV and functional outcome showed similar strengths of correlations for both MR and CT with mRS, as well as early and late imaging. [ref JNIS] Second, the fact that the last scan of each patient was selected for FIV assessment could have led to a bias, as patients with

complications and deterioration would have had more late imaging. Third, to obtain trustworthy results from mediation analysis, unmeasured confounding must not exist between parameters in the examined causal model. This is a strong assumption, especially when we consider that the independent contributions of the many interconnected biological processes to the final clinical outcome are not fully understood and are likely to vary among individuals. However, we can expect that this unmeasured confounding effect is minimal as patients across all trials were randomized and all observers were blinded to information outside of relevant imaging material. Fourth, the proportions of explained mediated effect could never reach the theoretical value of 0%. This is because our model would no longer suffice in such a situation, as the mediator FIV would not remain significant. The same applies for the hypothetical value of 100%, as all variables (even when that biological pathway is completely non-existent) exert some form of influence, albeit being noise. Further work needs to be done to understand the mediation pathway and the limitations of mediation analysis.

In conclusion, our results show that while FIV is a strong predictor of functional outcome, successful treatment with EVT resulting in smaller FIV is only for a modest one-eighth explained by a reduction in infarct volume. FIV as it is currently measured is not yet a valid proxy for estimating treatment effect in phase II studies of acute ischemic stroke.

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REFERENCES

1 Berkhemer O, Fransen P, Beumer D, *et al.* A Randomized Trial of Intraarterial Treatment for Acute Ischemic Stroke. *New Engl J Med* 2015; **372**: 11–20.

2 Goyal M, Demchuk AM, Menon BK, *et al.* Randomized assessment of rapid endovascular treatment of ischemic stroke. *N Engl J Med* 2015; **372**: 1019–30.

3 Saver JL, Goyal M, Bonafe A, *et al.* Stent-Retriever Thrombectomy after Intravenous t-PA vs. t-PA Alone in Stroke. *N Engl J Med* 2015; **372**: 2285–95.

4 Campbell BC V, Mitchell PJ, Kleinig TJ, *et al.* Endovascular therapy for ischemic stroke with perfusion-imaging selection. *N Engl J Med* 2015; **372**: 1009–18.

5 Jovin TG, Chamorro A, Cobo E, *et al.* Thrombectomy within 8 Hours after Symptom Onset in Ischemic Stroke. *N Engl J Med* 2015; **372**: 1–11.

6 Muir KW, Ford GA, Messow C-M, *et al.* Endovascular therapy for acute ischemic stroke: the Pragmatic Ischemic Stroke Thrombectomy Evaluation (PISTE) randomized, controlled trial. *J Neurol Neurosurg Psychiatry* 2017; **88**: 38–44.

7 Bracard S, Ducrocq X, Mas JL, *et al.* Mechanical thrombectomy after intravenous alteplase versus alteplase alone after stroke (THRACE): a randomized controlled trial. *Lancet Neurol* 2016; **15**: 1138–47.

8 Yoo AJ, Chaudhry ZA, Nogueira RG, *et al.* Infarct Volume Is a Pivotal Biomarker After Intra-Arterial. 2012; **I**: 1323–30.

9 Zaidi SF, Aghaebrahim A, Urra X, *et al.* Final infarct volume is a stronger predictor of outcome than recanalization in patients with proximal middle cerebral artery occlusion

treated with endovascular therapy. *Stroke* 2012; **43**: 3238–44.

10 Albers GW, Goyal M, Jahan R, *et al.* Relationships Between Imaging Assessments and Outcomes in Solitaire With the Intention for Thrombectomy as Primary Endovascular Treatment for Acute Ischemic Stroke. *Stroke* 2015; **46**: 2786–94.

11 Al-Ajlan FS, Goyal M, Demchuk AM, *et al.* Intra-Arterial Therapy and Post-Treatment Infarct Volumes: Insights From the ESCAPE Randomized Controlled Trial. *Stroke* 2016; **47**: 777–81.

12 Prentice RL. Surrogate endpoints in clinical trials: definition and operational criteria. *Stat Med* 1989; **8**: 431–40.

13 Al-Ajlan FS, Al Sultan AS, Minhas P, *et al.* Posttreatment Infarct Volumes when Compared with 24-Hour and 90-Day Clinical Outcomes: Insights from the REVASCAT Randomized Controlled Trial. *Am J Neuroradiol* 2017; published online Nov 23. DOI:10.3174/ajnr.A5463.

14 Lingsma HF, Companje K, Boers AMM, *et al.* Is infarct volume on CT a useful proxy for functional outcome? Results from the MR CLEAN trial, 3rd Europe. 2017.

15 Goyal M, Menon BK, van Zwam WH, *et al.* Endovascular thrombectomy after large-vessel ischemic stroke: a meta-analysis of individual patient data from five randomized trials. *Lancet* 2016; **387**: 1723–31.

16 Saver JL. Novel End Point Analytic Techniques and Interpreting Shifts Across the Entire Range of Outcome Scales in Acute Stroke Trials. *Stroke* 2007; **38**: 3055–62.

17 Boers AM, Marquering HA, Jochem JJ, *et al.* Automated Cerebral Infarct Volume

Measurement in Follow-up Noncontrast CT Scans of Patients with Acute Ischemic Stroke. *Am J Neuroradiol* 2013; **34**: 1522–7.

18 Pexman JH, Barber PA, Hill MD, *et al.* Use of the Alberta Stroke Program Early CT Score (ASPECTS) for assessing CT scans in patients with acute stroke. *Ajnr Am J Neuroradiol* 2001; **22**: 1534–42.

19 von Kummer R, Broderick JP, Campbell BCV, *et al.* The Heidelberg Bleeding Classification. *Stroke* 2015; **46**: 2981–6.

20 Compagne KC., Boers AMM, Marquering H., *et al.* Effect of endovascular treatment on infarct volume and functional outcome in acute ischemic stroke: a causal mediation analysis. 2017.

21 Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. *J Pers Soc Psychol* 1986; **51**: 1173–82.

22 Freedman LS, Graubard BI, Schatzkin A. Statistical validation of intermediate endpoints for chronic diseases. *Stat Med* 1992; **11**: 167–78.

23 VanderWeele TJ, Vansteelandt S. Odds Ratios for Mediation Analysis for a Dichotomous Outcome. *Am J Epidemiol* 2010; **172**: 1339–48.

24 Bucker A, Boers AM, Bot JCJ, *et al.* Associations of Ischemic Lesion Volume With Functional Outcome in Patients With Acute Ischemic Stroke. *Stroke* 2017; **48**: 1233–40.

25 Krongold M, Almekhlafi MA, Demchuk AM, Coutts SB, Frayne R, Eilaghi A. Final infarct volume estimation on 1-week follow-up MR imaging is feasible and is dependent

on recanalization status. *NeuroImage Clin* 2015; **7**: 1–6.

26 Ernst M, Forkert ND, Brehmer L, *et al.* Prediction of Infarction and Reperfusion in Stroke by Flow- and Volume-Weighted Collateral Signal in MR Angiography. *Am J Neuroradiol* 2015; **36**.

27 Baron J-C, Yamauchi H, Fujioka M, Endres M. Selective neuronal loss in ischemic stroke and cerebrovascular disease. *J Cereb Blood Flow Metab* 2014; **34**: 2–18.

28 Pendlebury ST, Lee MA, Blamire AM, Styles P, Matthews PM. Correlating magnetic resonance imaging markers of axonal injury and demyelination in motor impairment secondary to stroke and multiple sclerosis. *Magn Reson Imaging* 2000; **18**: 369–78.

TABLES

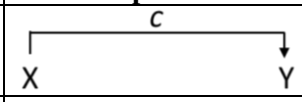
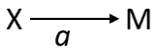
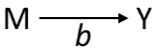
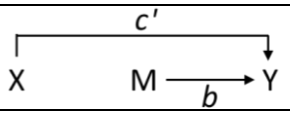
Table 1. Four-step approach for testing mediation		
Step	**Analysis**	**Visual representation**
Step 1	Regression analysis with X predicting Y to test for path c, $Y = B_0 + B_1X + e$	
Step 2	Regression analysis with X predicting M to test for path a, $M = B_0 + B_1X + e$	
Step 3	Regression analysis with M predicting Y to test the significance of path b, $Y = B_0 + B_1M + e$	
Step 4	Multiple regression analysis with X and M predicting Y, $Y = B_0 + B_1X + B_2M + e$	

Table 2 - Baseline characteristics			
Characteristic	**EVT (n=821)**	**Control (n=844)**	**p-value**
Age (years), median [IQR]	68 [57-76]	68 [58-76]	0.79
Female sex, % (n/N)	47.3% (388/821)	46.6% (393/844)	0.81
Left hemisphere infarct, % (n/N)	47.1% (381/809)	48.7% (409/840)	0.52
Onset to follow-up imaging acquisition in hours, median [IQR]	29 [24-138]	31 [24-141]	0.61
Modality, % (n/N)			0.36
CT	82.2% (675/821)	83.9% (708/844)	
MR	17.8% (146/821)	16.1% (136/844)	
NIHSS at baseline, median [IQR]	17 [14-20]	17 [13-21]	0.93
Alteplase (tPA) delivered, % (n/N)	88.2% (724/821)	91.5% (772/844)	0.028
Diabetes mellitus, % (n/N)	14.5% (119/819)	17.9% (151/842)	0.063
Hypertension, % (n/N)	53.6% (439/819)	59.0% (497/843)	0.030
Tobacco use, % (n/N)	37.7% (280/742)	36.8% (286/777)	0.71
Onset to alteplase (tPA) (min), median [IQR]	115 [85-155]	119 [85-161]	0.075
Onset to randomization (min), median [IQR]	181 [141-240]	184 [140-248]	0.80
Onset to reperfusion (min), median [IQR]	291 [230-355]	NA	NA
ASPECTS at baseline, median [IQR]	8 [7-9]	8 [7-9]	0.20
Occlusion location, % (n/N)			0.91
Not available	5.7% (47/821)	5.5% (46/844)	
ICA	24.5% (201/821)	26.2% (221/844)	
M1	61.8% (507/821)	61.3% (517/844)	
M2	7.9% (65/821)	7.0% (59/844)	
Other	0.1% (1/821)	0.1% (1/844)	
Collateral score, % (n/N)			0.75
0	1.0% (6/606)	1.1% (7/627)	
1	14.9% (90/606)	17.1% (107/627)	
2	43.7% (265/606)	42.3% (265/627)	
3	40.4% (245/606)	39.6% (248/627)	
NIHSS, National Institutes of Health and Stroke Scale score; ASPECTS, Alberta Stroke Program Early CT; ICA, internal carotid artery			

Table 3. Outcomes per treatment allocation group			
Characteristic	EVT (n=821)	Control (n=844)	p-value
Follow-up infarct volume (mL), median [IQR]	33 [11-99]	51 [18-134]	0.007
mRS at 90 days, median [IQR]	3 [1-4]	4 [2-5]	<0.001
Hemorrhage [a], % (n/N)			
Hemorrhagic infarct type 1 (HI-1)	14.1% (116/821)	13.4% (113/844)	0.67
Hemorrhagic infarct type 2 (HI-2)	12.3% (101/821)	11.1% (94/844)	0.49
Parenchymal hematoma type 1 (PH-1)	8.5% (70/821)	6.5% (55/844)	0.14
Parenchymal hematoma type 2 (PH-2)	8.3% (68/821)	5.9% (50/844)	0.069
Remote parenchymal hematoma (rPH)	1.6% (13/821)	0.9% (8/844)	0.28
Intraventricular hemorrhage (IVH)	2.4% (20/821)	2.7% (23/844)	0.76
Subarachnoid hemorrhage (SAH)	2.8% (23/821)	1.7% (14/844)	0.14
Subdural hemorrhage (SDH)	0.0% (0/821)	0.4% (3/844)	0.25
Reperfusion, % (n/N)			
TICI 2b-3	75.7% (518/684)		NA

EVT = endovascular therapy; TICI = thrombolysis in cerebral ischemia;
[a] Hemorrhages scored according to the anatomical description of the Heidelberg Classification
TICI 2b-3 indicates successful reperfusion

Table 4. Mediating effect of follow-up infarct volume on the association between treatment and ordinal 90-day modified Rankin Scale, FIV transformed by ln(FIV+1)

Pathway	Unadjusted				Adjusted			
	Effect measure	Value	95% CI	p-value	Effect measure	Value	95% CI	p-value
a	β	-0.28	-0.41 -- -0.14	<0.001	β	-0.13	-0.19 -- -0.07	<0.001
b	cOR	0.45	0.42 – 0.48	<0.001	acOR	0.46	0.39 – 0.54	<0.001
c	cOR	2.17	1.38 – 3.41	<0.001	acOR	2.28	1.55 - 3.36	<0.001
c’	cOR	1.87	1.25 – 2.81	0.002	acOR	2.08	1.44 – 3.00	<0.001

Path a represents the regression coefficient of the association between treatment (control or endovascular therapy) and FIV;
b between FIV and 90-day mRS; c between treatment and 90-day mRS; and c’ between treatment and 90-day mRS, controlling for FIV. Multivariable regression analysis included FIV, location, hemorrhage type, age, and National Institutes of Health and Stroke Scale score.
FIV = follow-up infarct volume; mRS = modified Rankin Scale; cOR = common odds ratio; CI = confidence interval

FIGURE LEGENDS

Figure 1: A pictorial representation of the causal pathway in acute ischemic stroke patients. Total effect (*c*) = direct effect (*c'*) + indirect effect (*ab*).

Figure 2: Relation between FIV and estimated probability of functional independence (point estimates ± 95% CI), stratified by treatment and adjusted for baseline characteristics