

# Intracranial carotid artery calcification and effect of endovascular stroke treatment: a MR CLEAN subgroup analysis

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*Stroke*. 2018 Dec;49(12):2961-2968.

## ABSTRACT

**Background and purpose** Previous studies suggest that intracranial carotid artery calcification (ICAC) volume might influence the clinical outcome of patients after endovascular treatment (EVT) for acute ischemic stroke (AIS). Importantly, ICAC can be subtyped into a medial or intimal pattern that may differentially influence the effect of EVT in patients with AIS.

**Methods** All 500 patients included in the Multicenter Randomized Clinical trial of Endovascular treatment for AIS in the Netherlands (MR CLEAN) were evaluated. Volume ( $\text{mm}^3$ ) and location pattern (tunica intima or tunica media) of ICAC could be determined on baseline non-contrast CT in 344 patients. Functional outcome at 90 days was assessed with the modified Rankin Scale. Next, we investigated the association of ICAC volume and pattern with functional outcome using adjusted ordinal logistic regression models. Effect modification by EVT was assessed with an interaction term between treatment allocation and ICAC aspect.

**Results** We found evidence for treatment effect modification by ICAC pattern (p-interaction 0.04). Patients with predominantly medial calcification had better functional outcome with EVT than without this treatment (acOR 2.32; 95%CI 1.23-4.39), but we observed no effect of EVT in patients with predominantly intimal calcifications (acOR 0.82; 95%CI 0.40-1.68). We did not find an association of ICAC volume with functional outcome (adjusted common odds ratio (acOR) per unit increase ICAC volume 1.01 (95%CI 0.89-1.13). Moreover, we found no evidence for effect modification by ICAC volume (p-interaction 0.61).

**Conclusions** The benefit of EVT in AIS patients with a medial calcification pattern is larger than the benefit in patients with an intimal calcification pattern.

## INTRODUCTION

In the clinical management of acute ischemic stroke, endovascular treatment (EVT) has recently been established as an effective treatment for patients with a large vessel occlusion<sup>1</sup>. Despite the large overall benefits, recent evidence shows that specific pre-procedural patient characteristics may substantially influence the prognosis and absolute treatment benefit of the patient after EVT in terms of functional outcome. In particular, a larger amount of highly prevalent intracranial carotid artery calcification (ICAC) may be an indicator of poor functional outcome in acute ischemic stroke patient treated by EVT<sup>2,3</sup>. One study reported an association between ICAC volume and poor recanalization status, which could not be explained by differences in procedural difficulties like accessibility of target occlusion, number of passes or periprocedural complications<sup>2</sup>.

Yet, given the retrospective design of previous studies, in combination with the older, less effective EVT techniques that were investigated, more evidence is required to establish whether ICAC influences functional outcome in acute ischemic stroke patients. Furthermore, due the lack of control (non-EVT) groups in previous studies modification of treatment effect by ICAC volume could not be assessed.

In addition to the volume of ICAC, two distinct morphological patterns of ICAC were recently highlighted that likely represent two different pathologic processes<sup>4,5</sup>. In brief, one of the patterns is characterized by calcification in the tunica intima (intimal calcification pattern), whilst in the other pattern calcification is primarily present in the tunica media (medial calcification pattern)<sup>6-8</sup>. A recently published study observed differences in cardiovascular risk factor profile between patients with intimal- or medial calcification patterns<sup>9</sup>. The two types of calcification may relate differently to functional outcome and EVT effect in these patients.

Against this background, we performed a post-hoc analysis of the Multicenter Randomized Clinical trial of Endovascular treatment of Acute ischemic stroke in the Netherlands (MR CLEAN), and investigated the effect of the volume and pattern of ICAC on functional outcome and on treatment effect<sup>10</sup>. This knowledge may directly contribute to our insight into factors influencing the success of EVT in acute ischemic stroke patients.

## MATERIAL AND METHODS

### Patients

Data originated from the MR CLEAN trial which investigated the effectiveness of EVT in acute ischemic stroke patients<sup>10</sup>. All patients had a radiographically confirmed proximal intracranial arterial occlusion and a minimal score of 2 on the National Institutes of

Health Stroke Scale (NIHSS) at baseline. Treatment had to be possible within 6 hours after symptom onset. Patients were randomized between EVT (intervention) or no EVT (control) along with usual medical care. Intravenous alteplase before randomization was allowed. Demographics, laboratory tests and medical cardiovascular history were collected at baseline as previously described<sup>11</sup>. Baseline imaging was performed with non-contrast computed tomography (NCCT) and CT angiography (CTA), evaluating Alberta Stroke Program Early Computed Tomography Score (ASPECTS), location of occlusion and collateral status<sup>12, 13</sup>. Written informed consent before randomization was provided by all patients or their legal representatives. The study protocol was approved by a central medical ethics committee and the research board of each participating center. Funders of the original study and this post-hoc study had no role in study design, data collection, data analysis, data interpretation or writing of the manuscript. All authors had full access to all the data in the study and approved the manuscript for publication.

## Assessment of intracranial carotid artery calcification

### ICAC volume

All study participants underwent NCCT before randomization, on which ICAC volumes were quantified (mm<sup>3</sup>) in the symptomatic intracranial internal carotid arteries separately. ICAC was evaluated from the horizontal part of the petrous (horizontal) segment of the artery till its top (circle of Willis). All segmentations of ICAC volume were done manually by two experienced observers (KC; PC) with a custom-made, reliable and validated tool in ImageJ<sup>14</sup>. The number of pixels with a Hounsfield-unit (HU)  $\geq 130$  was multiplied by pixel-size and slice increment to obtain the volume of ICAC (mm<sup>3</sup>). Interobserver agreement has been published previously with an intraclass correlation coefficient of 0.99<sup>14</sup>.

### ICAC pattern

Pattern of ICAC was differentiated into intimal- and medial ICAC according to a recently developed and validated scoring method<sup>15</sup> (Figure 1). In short, this scoring method evaluates circularity, thickness and morphology of the calcification using a specific weighting to determine whether calcification is predominantly intimal (<7 points) or medial ( $\geq 7$  points). Two observers (KC; PC) independently graded all ICAC calcifications and were blinded to the symptomatic side during scoring. In case of disagreement, a consensus reading was performed between both observers.

### Outcome assessment

Functional outcome at 90 days after the intervention was assessed with the modified Rankin Scale (mRS) by an independent research nurse who was blinded for treatment allocation<sup>16</sup>. Recanalization status on follow-up CTA at 24 hours, evaluated by the modi-

fied Arterial Occlusive Lesion score (mAOL), was assessed by an independent core lab<sup>17</sup>. Follow up infarct volumes at 5 to 7 days follow up were semi-automatically segmented on NCCT scans with the use of validated software<sup>18</sup>. Safety end points were reported by local neurologists. Symptomatic intracranial hemorrhage was defined as neurologic deterioration (an increase of 4 or more points on the NIHSS score) and evidence of intracranial hemorrhage on imaging studies.

## Population for analyses

Patients with NCCT scans with a slice thickness over 3 mm, that could not be assessed reliably for ICAC, were excluded. Other reasons for excluding patients were movement artefacts, incomplete scans, inappropriate reconstruction, unavailable axial slices or unavailable NCCT scan.

## Statistical analysis

Baseline characteristics of included patients between both intervention and control group were compared by means of the Mann-Whitney U test for continuous variables due to non-normal distributions and  $\chi^2$  test was used for categorical variables. The correlation between ICAC volume and age was evaluated by Spearman's rank correlation coefficient. Cohen's kappa value ( $\kappa$ ) and proportion of agreement was calculated to define the level of interobserver agreement in grading ICAC pattern.

Regarding ICAC volume, data was handled in two approaches. First, for continuous analyses, ICAC volumes were natural log-transformed after adding 1.0 mm<sup>3</sup> to all volumes to deal with volumes of 0 mm<sup>3</sup> ( $\ln(\text{ICAC volume} + 1.0)$ ) due to the skewed distribution. Second, quartiles of ICAC volume of the total population were created. For illustration purposes we compared the outcomes between the lower three quartiles with the upper (fourth) quartile; defined as severe ICAC. We assessed the association of ICAC volume and pattern (intimal calcification versus medial calcification pattern) with functional outcome (mRS range 0-6) using ordinal logistic regression models (shift analysis). Relationships of ICAC volume or pattern with successful recanalization on follow-up CTA (mAOL) were assessed with adjusted ordinal logistic regression models. Linear regression was used to assess to association between ICAC volume and pattern with follow up infarct volume. Modification of treatment effect by ICAC volume and pattern was tested with a multiplicative interaction term. In the first model, adjustments for age and sex only were made. In a second model, additional adjustments were made for cardiovascular risk factors: smoking, diabetes mellitus, atrial fibrillation, myocardial infarction and history of hypertension according revised Framingham stroke risk profile<sup>19</sup>. In the third model, adjustments were also made for prestroke mRS, NIHSS at baseline, occlusion of the internal carotid artery terminus (ICA-T), collateral status on baseline CTA and time to randomization as proven predictors of outcome<sup>20</sup>. P values  $\leq 0.05$  were con-

sidered as statistically significant. Analyses were performed with R statistical software (version 3.4.2) using packages foreign, rms, MASS, irr and ggplot2.

## RESULTS

In total 128 patients were excluded because of a slice thickness over 3 mm on NCCT. Additional reasons for excluding patients were movement artefacts (n=20), incomplete scans (n=5), inappropriate reconstruction (n=1), unavailable axial slices (n=1) and unavailable NCCT scan (n=1). In total, 344 of the 500 patients (69%) in the MR CLEAN trial were included in this post hoc subgroup analysis (Suppl. Table I). Baseline characteristics of the study participants were equally distributed in the intervention and control group as shown in Table 1.

**Table 1.** Baseline characteristics of analyzed patients

	Control group (n=188)	Intervention group (n=156)	p
<b>Age (median [IQR])</b>	66 [56-76]	66 [57-76]	0.97
<b>Sex (male) (%)</b>	108 (57.4)	96 (61.5)	0.51
<b>NIHSS at baseline (median [IQR])</b>	18 [14-22]	18 [14-21]	0.29
<b>Previous stroke (%)</b>	19 (10.1)	22 (14.1)	0.33
<b>Atrial fibrillation (%)</b>	48 (25.5)	44 (28.2)	0.66
<b>Diabetes mellitus (%)</b>	22 (11.7)	26 (16.7)	0.24
<b>Smoking (%)</b>	54 (28.7)	47 (30.1)	0.87
<b>Myocardial infarction (%)</b>	32 (17.0)	23 (14.7)	0.58
<b>Hypertension (%)</b>	89 (47.3)	60 (38.5)	0.12
<b>Pre-stroke mRS ≤2 (%)</b>	171 (91.0)	141 (90.4)	1.00
<b>Systolic blood pressure at baseline (mmHg) (median [IQR])</b>	142 [130-160]	143 [128-159]	0.51
<b>Treatment with IV alteplase (%)</b>	170 (90.4)	134 (85.9)	0.26
<b>ASPECTS ≥ 8 at baseline (%) *</b>	153 (82.3)	116 (74.8)	0.12
<b>Location of intracranial occlusion on baseline CTA (%) §</b>			0.92
ICA	3 (1.6)	1 (0.6)	
ICA-T	48 (25.5)	42 (26.9)	
M1	116 (61.7)	97 (62.2)	
M2	19 (10.1)	15 (9.6)	
A1 or A2	2 (1.1)	1 (0.6)	
<b>Collateral status on baseline CTA (%) †</b>			0.71
Absent collaterals	11 (6.0)	8 (5.2)	
Poor collaterals	47 (25.5)	47 (30.3)	
Moderate collaterals	77 (41.8)	57 (36.8)	
Good collaterals	49 (26.6)	43 (27.7)	

**Table 1.** Baseline characteristics of analyzed patients (*continued*)

	Control group (n=188)	Intervention group (n=156)	p
<b>Extracranial ICA <math>\geq</math> 50% stenosis (%)</b>	19(10.1)	212 (13.5)	0.43
<b>Extracranial ICA occlusion (%)</b>	14 (7.4)	11 (7.1)	1.00
<b>No ICAC at symptomatic side of stroke (%)</b>	14 (7.4)	11 (7.1)	1.00
<b>ICAC volume at symptomatic side of stroke (median [IQR])</b>	41 [1-120]	34 [2-114]	0.81
<b>ICAC pattern at symptomatic side of stroke (%)</b>			0.66
No calcification	40 (21.3)	34 (21.8)	
Intimal calcification	61 (32.4)	57 (36.5)	
Medial calcification	87 (46.3)	65 (41.7)	
<b>Time from stroke onset to randomization (minutes) (median [IQR]) ‡</b>	188 [144-260]	191 [147-240]	0.57

\* Alberta Stroke Program Early Computed Tomography Score (ASPECTS) was missing for three patients.

† Collaterals were graded on baseline CTA on a four grade scale: absent collaterals (0% filling of occluded territory), poor collaterals (0 – 49% filling of occluded territory), moderate collaterals (50 – 99% filling of occluded territory) and good collaterals (100% filling of occluded territory)

‡ Data was missing for two patients.

## ICAC volume

ICAC in the symptomatic intracranial carotid artery of ischemic stroke (symptomatic ICAC) was present in 270 (78%) patients: 122/156 (78%) patients in the intervention group and 148/188 (79%) patients in the control group. Median ICAC volume in the symptomatic carotid artery was 69.8 mm<sup>3</sup> (interquartile range (IQR): 19.2–171.1 mm<sup>3</sup>). A moderate correlation ( $\rho$  0.6;  $p < 0.001$ ) between symptomatic ICAC volume and age was observed. There was no statistically significant difference in median volume of symptomatic ICAC between both treatment allocations (resp. 65.5 mm<sup>3</sup> vs. 81.9 mm<sup>3</sup>;  $p$  0.81).

Overall, larger symptomatic ICAC volumes were not significantly associated with poorer functional outcome (adjusted common odds ratio (acOR) per unit increase in log-transformed ICAC volume 1.01; 95% confidence interval (95%CI) 0.89-1.13) in our first model. After additional adjustments, symptomatic ICAC volume was still not significantly associated with functional outcome (acOR 0.99; 95%CI 0.83-1.03). Treatment effects were similar in patients with severe ICAC volume ( $>119.2$  mm<sup>3</sup>) and patients with non-severe ICAC (Table 2) and no effect modification was observed ( $p$ -interaction 0.61). Furthermore, effect of treatment on final recanalization status and final infarct volume was comparable in patients with severe ICAC volume and patients with non-severe ICAC volume without a significant effect modification ( $p$ -interaction 0.66 and 0.77, respectively).

**Table 2.** Association of treatment allocation with functional outcome\*, recanalization on CTA and follow-up infarct volume on CT according to severity of calcification volume of the intracranial carotid artery at the symptomatic side of ischemic stroke.

	Functional outcome*	Recanalization†	Follow-up infarct volume‡
	acOR (95% CI)	acOR (95% CI)	β (95% CI)
<b>Intervention versus control group</b>			
<b>Non-severe ICAC (n=258)</b>			
Model 1	1.64 (1.05-2.55)	5.53 (2.99-10.28)	-0.19 (-0.51-0.14)
Model 2	1.71 (1.09-2.69)	5.86 (3.11-11.01)	-0.21 (-0.53-0.12)
Model 3	1.69 (1.06-2.70)	7.91 (3.94-15.90)	-0.17 (-0.47-0.13)
<b>Severe ICAC (n=86)</b>			
Model 1	1.99 (0.91-4.35)	8.83 (2.75-28.39)	-0.63 (-1.31-0.06)
Model 2	1.67 (0.75-3.74)	9.12 (2.54-32.76)	-0.55 (-1.24-0.14)
Model 3	1.91 (0.80-4.57)	6.11(1.54-24.27)	-0.61 (-1.28-0.06)

\* Effect parameters is the adjusted common odds ratio (acOR) for a shift in the direction of a better outcome on the modified Rankin Scale (mRS) in favor of the intervention.

Model 1: adjusted for age and sex.

Model 2: model 1 and plus adjustments for smoking, diabetes mellitus, atrial fibrillation, myocardial infarction and history of hypertension.

Model 3: model 2 plus adjustments for prestroke mRS, NIHSS at baseline, occlusion of the internal carotid artery terminus, collateral status at baseline CTA and time to randomization.

† Data on recanalization on follow-up CTA was missing for 64 (24%) patients of which 28 patients died.

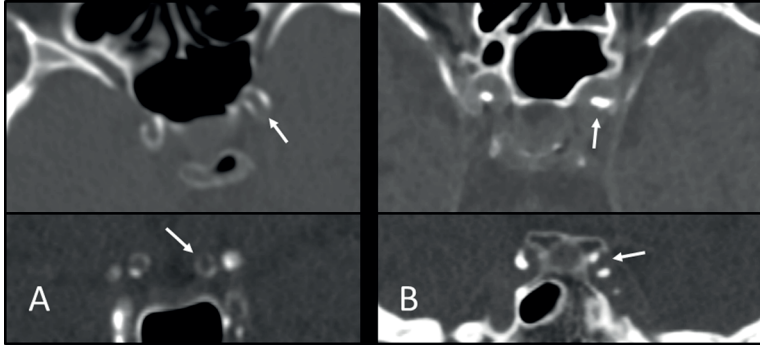
‡ Data on follow-up infarct volume on NCCT 5-7 days was missing for 26 patients (8%) of which 8 patients died before assessment.

## ICAC pattern

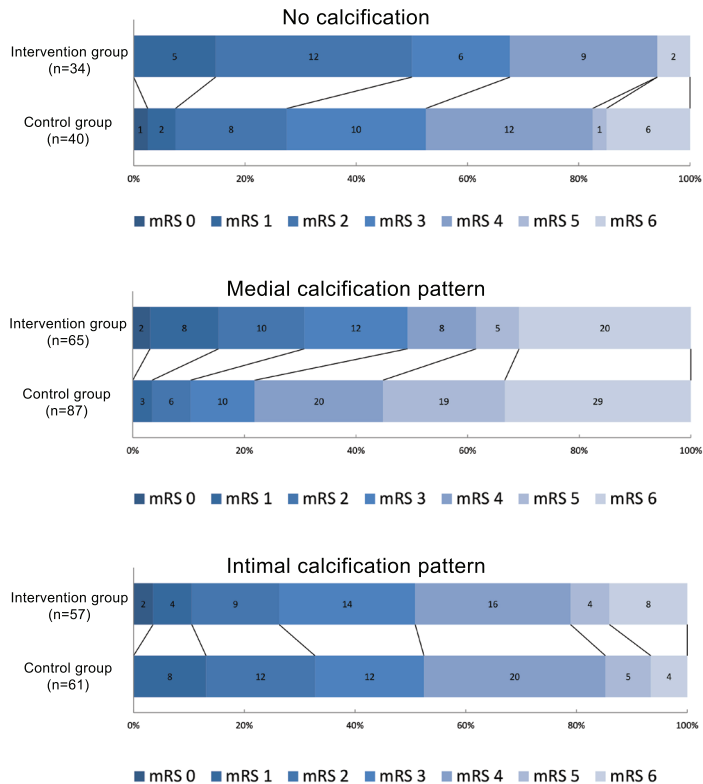
In the 270 patients with ICAC, we found 118 intimal calcification patterns and 152 medial calcification patterns in the symptomatic intracranial carotid artery (Figure 1). A good interobserver agreement was found in grading ICAC pattern (total agreement 93.9%;  $\kappa$  0.88). No difference in distribution of ICAC pattern was observed between the intervention and control group ( $p$  0.43). Patients with a medial ICAC pattern were in general older, more often female and had more often a history of diabetes mellitus, myocardial infarction, hypertension and poorer collateral status on baseline CTA (Suppl. Table II).

In patients with ICAC, a medial calcification pattern was not associated with a shift to a poorer functional outcome (acOR 0.62; 95% CI 0.38 – 1.04). A significant EVT treatment effect was observed in patients with medial calcification pattern (acOR 2.32; 95% CI 1.23 – 4.39). This in contrast to patients with intimal calcification pattern, in whom we observed no treatment effect (acOR 0.82; 95% CI 0.40 – 1.68) (Table 3 and Figure 2). Consequently, a significant effect modification by ICAC pattern was noted ( $p$ -interaction 0.04) (Table 4). In EVT-treated patients, we observed a lower impact of reperfusion on functional outcome in patients with intimal ICAC pattern compared to patients with medial ICAC pattern (Suppl. Table III).





**Figure 1.** Patterns of medial and intimal intracranial carotid artery calcification on no-contrast CT. Medial calcification pattern is identified as a thin, continuous, and almost circular calcification patterns in axial viewing plane (A; upper) and coronal viewing plane (A; lower). Intimal calcification pattern is identified as a thick, irregular, and noncircular calcification patterns in axial viewing plane (B; upper) and coronal viewing plane (B; lower).



**Figure 2.** Distribution of modified Rankin Scale (mRS) scores at 90 days in patients with no calcification, medial, or intimal calcification pattern of the intracranial carotid artery at symptomatic side of ischemic stroke. A significant difference in the distribution of scores between both groups was observed in patients with medial calcification pattern but not in patients with intimal calcification pattern and no calcification. Numbers in bars are absolute numbers.

**Table 3.** Association of treatment allocation with functional outcome\*, follow-up infarct volume on CTA and recanalization according to calcification pattern.

	Functional outcome*	Recanalization†	Follow-up infarct volume‡
	acOR (95% CI)	acOR (95% CI)	β (95% CI)
<b>Intervention versus control group</b>			
<b>Total sample (n=344)</b>			
Model 1	1.70 (1.16-2.50)	5.90 (3.44-10.12)	-0.29 (-0.58-0.01)
Model 2	1.74 (1.18-2.56)	6.19 (3.56-10.77)	-0.31 (-0.61- -0.02)
Model 3	1.70 (1.13-2.52)	7.47 (4.15-13.45)	-0.27 (-0.58- -0.01)
<b>No calcification (n=74)</b>			
Model 1	2.22 (0.96-5.14)	4.42 (1.52-12.03)	-0.46 (-1.06-0.13)
Model 2	2.27 (0.95-5.42)	4.63 (1.59-13.49)	-0.51 (-1.14-0.12)
Model 3	2.43 (0.99-6.05)	5.86 (1.81-18.93)	-0.45 (-0.96-0.06)
<b>Medial calcification pattern (n=152)</b>			
Model 1	2.63 (1.43-4.83)	11.11 (4.11-30.06)	-0.46 (-0.90- -0.02)
Model 2	2.55 (1.38-4.72)	12.10 (4.28-34.15)	-0.41(-0.83-0.01)
Model 3	2.32 (1.23-4.39)	11.26 (3.86-32.90)	-0.28 (-0.69-0.13)
<b>Intimal calcification pattern (n=118)</b>			
Model 1	0.81 (0.42-1.54)	4.32 (1.76-10.60)	0.08 (-0.44-0.61)
Model 2	0.76 (0.39-1.50)	3.91 (1.51-10.11)	0.13 (-0.41-0.68)
Model 3	0.82 (0.40-1.68)	7.69 (2.41-24.59)	0.00 (-0.49-0.49)

\* Effect parameters is the adjusted common odds ratio (acOR) for a shift in the direction of a better outcome on the modified Rankin Scale (mRS) in favor of the intervention.

Model 1: adjusted for age and sex.

Model 2: model 1 and plus adjustments for smoking, diabetes mellitus, atrial fibrillation, myocardial infarction and history of hypertension.

Model 3: model 2 plus adjustments for prestroke mRs, NIHSS at baseline, occlusion of the internal carotid artery terminus, collateral status at baseline CTA and time to randomization.

† Data on recanalization on follow-up CTA was missing for 64 (24%) patients of which 28 patients died.

‡ Data on follow-up infarct volume on NCCT 5-7 days was missing for 26 patients (8%) of which 8 patients died before assessment.

Recanalization grades on follow-up CTA were significantly higher in the intervention group in patients with medial- and intimal calcification pattern (resp. acOR 11.26 (95%CI 3.86-32.90) and acOR 7.69 (95%CI 2.41-24.59)) with no observed significant treatment modification by ICAC pattern (p-interaction 0.28).

In the control group, median follow up infarct volumes were significantly larger in patients with medial calcification pattern compared to intimal calcification pattern (resp. median volume 99.46 mL vs 69.52mL; p 0.01). However, in the intervention group infarct volumes did not differ between both calcification patterns (resp. median volume 51.93 mL vs 55.12 mL; p 0.40). Compared to patients with intimal calcification pattern,

**Table 4.** P-interaction values between treatment groups (intervention versus control) and intracranial carotid artery calcification volume or pattern.

	ICAC volume			ICAC pattern		
	Functional outcome	Recanalization	Follow-up infarct volume	Functional outcome	Recanalization	Follow-up infarct volume
Model 1	0.428	0.385	0.593	0.009	0.109	0.115
Model 2	0.561	0.391	0.684	0.021	0.126	0.177
Model 3	0.607	0.657	0.770	0.036	0.276	0.511

Model 1: adjusted for age and sex.

Model 2: model 1 and plus adjustments for smoking, diabetes mellitus, atrial fibrillation, myocardial infarction and history of hypertension.

Model 3: model 2 plus adjustments for prestroke mRs, NIHSS at baseline, occlusion of the internal carotid artery terminus, collateral status at baseline CTA and time to randomization.

patients with medial calcification pattern showed a larger effect on follow infarct volume. However, no significant effect modification was observed (p-interaction 0.51).

There was no difference in occurrence of serious adverse events or symptomatic intracerebral hemorrhage between patients with medial calcification pattern in the treatment and control groups (Suppl. Table IV). In patients with intimal calcification pattern, serious adverse events occurred more often in the intervention group. However, the absolute difference of 18% between was statistically nonsignificant (p 0.07).

## DISCUSSION

We did not find an association of ICAC volume with functional outcome after acute ischemic stroke due to large vessel occlusion nor modification of EVT treatment effect by ICAC volume. We found a trend towards a worse outcome in patients with medial ICAC pattern. Notwithstanding, patients with a medial ICAC pattern benefited from EVT in contrast to patients with intimal ICAC pattern.

In our analysis, volume of ICAC was not associated with functional outcome as opposed to a recent observational study<sup>2</sup>. This discrepancy may be explained by different study design but selection of patients may have contributed to the findings. In that study, EVT was not yet standard care. Patients were only eligible for EVT if intravenous thrombolysis was contra-indicated and specific clinical characteristics (NIHSS  $\geq 6$  and ASPECTS  $> 6$ ) were present. Due to the pragmatic design of the randomized MR CLEAN trial, the patients included in our study reflect the population encountered in clinical practice. Another added value of our study compared to previous studies was the randomized

controlled design which allowed us to investigate treatment effect modification by ICAC volume and pattern.

Two other studies that investigated effect of ICAC on revascularization and functional outcome, assessed ICAC qualitatively using different evaluating approaches which did not differentiate between volume or pattern. One study included patients with a middle cerebral artery occlusion who received EVT and/or intravenous thrombolysis and found a significant association between high calcification burden and poor functional outcome (defined as mRS 5 or 6) <sup>3</sup>. However, the analysis was not stratified by type of treatment. Another study included EVT patients by perfusion imaging selection and found no association between total Carotid Siphon Calcium score and successful reperfusion (TICI  $\geq 2b$ ) or good functional outcome (mRS  $\leq 2$ ) <sup>21</sup>. All three previous studies used a dichotomized mRS as primary outcome, and thus reported only the proportion of patients with a good functional outcome. Evaluating the entire mRS range with ordinal analysis, allows patients with suboptimal, but clinically important improvements to be captured and might outperform dichotomized outcomes used in other studies <sup>22</sup>.

In a recently published study the risk factors of intimal- and medial calcification patterns in patients with suspected ischemic stroke were investigated. Similar results to our observations were described with regard to clinical characteristics across the different ICAC patterns. Patients with medial calcification pattern were significantly older and less often male, suffered more often from diabetes and smoked less often <sup>9</sup>. The poor functional outcome in patients with medial calcification pattern could be explained by arterial stiffening, characterized by an increasing pulse pressure, which causes impaired regulation of distal blood flow <sup>23</sup>. This may lead to an impaired distal microvascular cerebral perfusion and thereby failure to improve microvascular function (Windkessel effect) <sup>24</sup>. Earlier studies investigated the pattern of calcifications in relation to clinical outcome in other cardiovascular diseases. They identified medial calcification as a risk factor for foot amputation in patients with diabetes compared to intimal calcifications, and it also appeared to be a strong prognostic marker for mortality in dialysis patients with end-stage renal disease <sup>25-27</sup>.

We found a significant treatment effect in favor of EVT in patients with medial calcification pattern but not in patients with an intimal calcification pattern. One could hypothesize that patients with medial calcifications which is accompanied by arterial stiffening have already a compromised microcirculation and have developed already a micro-collateral pathways. Another reason for our findings could be that the thrombus is different in patients with intimal and medial calcifications and that EVT is less effective in removing the thrombus in toto in patients with intimal calcification pattern. Furthermore, although infarct volumes in EVT-treated patients did not differ between the two ICAC patterns, the beneficial effect of EVT regarding prevention of infarct volume might be reduced in patients with intimal calcification pattern. Intimal calcifications are as-

sociated with local atherosclerotic plaques which might lead to plaque disruption and microemboli during stent retrieval. Besides, EVT might cause damage to the vascular endothelium as has been showed in different studies<sup>28</sup>. It is known that atherosclerosis is also related to endothelial damage<sup>29-31</sup>. It might be possible that endothelium in patients with intimal ICAC pattern is more prone to damage which may results in secondary injury of brain tissue.

A recent post-mortem histopathologic correlation study showed that the pattern of ICAC can be reliably assessed on NCCT and the developed scoring method was used in our study<sup>15</sup>. Since NCCT is daily practice in acute ischemic stroke patients, determining the pattern of ICAC could be an interesting prognostic marker for selection of patients for endovascular treatment. Our experience is that this scoring method can be easily applied in clinical practice, as it can be executed quickly, and has a good interobserver agreement.

There are several limitations to our study. Firstly, the quality of NCCT scans varied between patients due to different scanning protocols used in the participating centers of the MR CLEAN trial. Exclusion of patients in our analysis was slice thickness >3 mm which could have led to under- or overestimation of ICAC volume and misclassification of ICAC pattern. Therefore, our post-hoc study included a limited number of patients, which contributed to the fairly wide confidence intervals. Consequently, the results of the current study are rather hypothesis-generating than definitive results that merit a change in imaging-based selection of acute ischemic stroke patients for EVT. Future studies dedicated to this topic must be performed to investigate whether these results hold. Secondly, significant observed baseline characteristics were observed between both ICAC patterns which may be important confounders. Although, we adjusted for these confounding variables using covariable-adjusted regression analyses. However, residual confound might still be present. An important note with regard to these baseline differences is that these differences may also partly reflect the presence of the dominant ICAC patterns in these persons as recently published<sup>9</sup>. Regarding the observed differences in treatment effect, confounding will be marginal due to randomization between EVT and non-EVT. Thirdly, our study used the recently published scoring method of Kockelkoren et al<sup>15</sup>. Another possible scoring method is the modified Woodcock scale which visually characterizes ICAC from 0 (absent) to 3 (thick, continuous calcification)<sup>32,33</sup> combining volume and pattern in one score which is not desirable for our study. The score by Kockelkoren et al. is developed to specifically determine the pattern of ICAC and it is histopathologically validated. Finally, we could not assess impaired cerebral microperfusion between both ICAC patterns in our study. In the MR CLEAN trial, CT or MRI perfusion scans were not performed by protocol. For future research, it would also be interesting to investigate the association between both degree of white matter hyperintensities and both ICAC patterns.

Further studies on patients from larger cohorts and randomized controlled trials are necessary to confirm our findings, but also to comprehend the underlying pathophysiological mechanism that determines the relation between ICAC pattern, treatment effect and functional outcome.

The benefit of EVT in AIS patients with a medial calcification pattern is larger than the benefit in patients with an intimal calcification pattern.

## REFERENCES

1. Goyal M, Menon BK, van Zwam WH, Dippel DW, Mitchell PJ, Demchuk AM, et al. Endovascular thrombectomy after large-vessel ischaemic stroke: A meta-analysis of individual patient data from five randomised trials. *Lancet*. 2016;387:1723-1731
2. Hernandez-Perez M, Bos D, Dorado L, Pellikaan K, Vernooij MW, Lopez-Cancio E, et al. Intracranial carotid artery calcification relates to recanalization and clinical outcome after mechanical thrombectomy. *Stroke*. 2017;48:342-347
3. Lee SJ, Hong JM, Lee M, Huh K, Choi JW, Lee JS. Cerebral arterial calcification is an imaging prognostic marker for revascularization treatment of acute middle cerebral arterial occlusion. *J Stroke*. 2015;17:67-75
4. Kovacic JC, Moreno P, Nabel EG, Hachinski V, Fuster V. Cellular senescence, vascular disease, and aging: Part 2 of a 2-part review: Clinical vascular disease in the elderly. *Circulation*. 2011;123:1900-1910
5. Persy V, D'Haese P. Vascular calcification and bone disease: The calcification paradox. *Trends Mol Med*. 2009;15:405-416
6. Thompson B, Towler DA. Arterial calcification and bone physiology: Role of the bone-vascular axis. *Nat Rev Endocrinol*. 2012;8:529-543
7. Vos A, Van Hecke W, Spliet WG, Goldschmeding R, Isgum I, Kockelkoren R, et al. Predominance of nonatherosclerotic internal elastic lamina calcification in the intracranial internal carotid artery. *Stroke*. 2016;47:221-223
8. Lanzer P, Boehm M, Sorribas V, Thiriet M, Janzen J, Zeller T, et al. Medial vascular calcification revisited: Review and perspectives. *Eur Heart J*. 2014;35:1515-1525
9. Vos A, Kockelkoren R, de Vis JB, van der Schouw YT, van der Schaaf IC, Velthuis BK, et al. Risk factors for atherosclerotic and medial arterial calcification of the intracranial internal carotid artery. *Atherosclerosis*. 2018;276:44-49
10. Berkhemer OA, Fransen PS, Beumer D, van den Berg LA, Lingsma HF, Yoo AJ, et al. A randomized trial of intraarterial treatment for acute ischemic stroke. *N Engl J Med*. 2015;372:11-20
11. Fransen PS, Beumer D, Berkhemer OA, van den Berg LA, Lingsma H, van der Lugt A, et al. Mr clean, a multicenter randomized clinical trial of endovascular treatment for acute ischemic stroke in the netherlands: Study protocol for a randomized controlled trial. *Trials*. 2014;15:343
12. Barber PA, Demchuk AM, Zhang J, Buchan AM. Validity and reliability of a quantitative computed tomography score in predicting outcome of hyperacute stroke before thrombolytic therapy. Aspects study group. Alberta stroke programme early ct score. *Lancet*. 2000;355:1670-1674
13. Jansen IGH, Berkhemer OA, Yoo AJ, Vos JA, Lycklama ANGJ, Sprengers MES, et al. Comparison of cta- and dsa-based collateral flow assessment in patients with anterior circulation stroke. *AJNR Am J Neuroradiol*. 2016;37:2037-2042
14. Bos D, Ikram MA, Elias-Smale SE, Krestin GP, Hofman A, Witteman JC, et al. Calcification in major vessel beds relates to vascular brain disease. *Arterioscler Thromb Vasc Biol*. 2011;31:2331-2337
15. Kockelkoren R, Vos A, Van Hecke W, Vink A, Bleys RL, Verdoorn D, et al. Computed tomographic distinction of intimal and medial calcification in the intracranial internal carotid artery. *PLoS One*. 2017;12:e0168360
16. van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJ, van Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. *Stroke*. 1988;19:604-607

17. Khatri P, Neff J, Broderick JP, Khoury JC, Carrozzella J, Tomsick T, et al. Revascularization end points in stroke interventional trials: Recanalization versus reperfusion in ims-i. *Stroke*. 2005;36:2400-2403
18. Boers AM, Marquering HA, Jochem JJ, Besselink NJ, Berkhemer OA, van der Lugt A, et al. Automated cerebral infarct volume measurement in follow-up noncontrast ct scans of patients with acute ischemic stroke. *AJNR. American journal of neuroradiology*. 2013;34:1522-1527
19. Dufouil C, Beiser A, McLure LA, Wolf PA, Tzourio C, Howard VJ, et al. Revised framingham stroke risk profile to reflect temporal trends. *Circulation*. 2017;135:1145-1159
20. Venema E, Mulder M, Roozenbeek B, Broderick JP, Yeatts SD, Khatri P, et al. Selection of patients for intra-arterial treatment for acute ischaemic stroke: Development and validation of a clinical decision tool in two randomised trials. *BMJ*. 2017;357:j1710
21. Haussen DC, Gaynor BG, Johnson JN, Peterson EC, Elhammady MS, Aziz-Sultan MA, et al. Carotid siphon calcification impact on revascularization and outcome in stroke intervention. *Clin Neurol Neurosurg*. 2014;120:73-77
22. Saver JL, Gornbein J. Treatment effects for which shift or binary analyses are advantageous in acute stroke trials. *Neurology*. 2009;72:1310-1315
23. Mitchell GF. Effects of central arterial aging on the structure and function of the peripheral vasculature: Implications for end-organ damage. *J Appl Physiol (1985)*. 2008;105:1652-1660
24. O'Rourke MF, Hashimoto J. Mechanical factors in arterial aging: A clinical perspective. *J Am Coll Cardiol*. 2007;50:1-13
25. London GM, Guerin AP, Marchais SJ, Metivier F, Pannier B, Adda H. Arterial media calcification in end-stage renal disease: Impact on all-cause and cardiovascular mortality. *Nephrol Dial Transplant*. 2003;18:1731-1740
26. Lehto S, Niskanen L, Suhonen M, Ronnema T, Laakso M. Medial artery calcification. A neglected harbinger of cardiovascular complications in non-insulin-dependent diabetes mellitus. *Arterioscler Thromb Vasc Biol*. 1996;16:978-983
27. Niskanen L, Siitonen O, Suhonen M, Uusitupa MI. Medial artery calcification predicts cardiovascular mortality in patients with niddm. *Diabetes Care*. 1994;17:1252-1256
28. Teng D, Pannell JS, Rennert RC, Li J, Li YS, Wong VW, et al. Endothelial trauma from mechanical thrombectomy in acute stroke: In vitro live-cell platform with animal validation. *Stroke*. 2015;46:1099-1106
29. Malek AM, Alper SL, Izumo S. Hemodynamic shear stress and its role in atherosclerosis. *JAMA*. 1999;282:2035-2042
30. Gory B, Bresson D, Kessler I, Perrin ML, Guillaudeau A, Durand K, et al. Histopathologic evaluation of arterial wall response to 5 neurovascular mechanical thrombectomy devices in a swine model. *AJNR Am J Neuroradiol*. 2013;34:2192-2198
31. Power S, Matouk C, Casaubon LK, Silver FL, Krings T, Mikulis DJ, et al. Vessel wall magnetic resonance imaging in acute ischemic stroke: Effects of embolism and mechanical thrombectomy on the arterial wall. *Stroke*. 2014;45:2330-2334
32. Woodcock RJ, Jr., Goldstein JH, Kallmes DF, Cloft HJ, Phillips CD. Angiographic correlation of ct calcification in the carotid siphon. *AJNR Am J Neuroradiol*. 1999;20:495-499
33. Subedi D, Zishan US, Chappell F, Gregoriades ML, Sudlow C, Sellar R, et al. Intracranial carotid calcification on cranial computed tomography: Visual scoring methods, semiautomated scores, and volume measurements in patients with stroke. *Stroke*. 2015;46:2504-2509



## SUPPLEMENTAL DATA

**Suppl. Table I.** Characteristics of analysed and patients in the MR CLEAN trial

	Analysed patients (n=344)	MR CLEAN patients (n=500)	p
<b>Allocation to intervention group (%)</b>	156 (45.3)	233 (46.6)	0.22
<b>Age (median [IQR])</b>	66 [56-76]	66 [56-76]	0.64
<b>Sex (male) (%)</b>	204 (59.3)	292 (58.4)	0.85
<b>NIHSS at baseline (median [IQR])</b>	18 [14-22]	18 [14-22]	0.83
<b>Previous stroke (%)</b>	41 (11.9)	54 (10.8)	0.69
<b>Atrial fibrillation (%)</b>	92 (26.7)	135 (27.0)	1
<b>Diabetes mellitus (%)</b>	48 (14.0)	68 (13.6)	0.96
<b>Smoking (%)</b>	101 (29.4)	143 (28.6)	0.87
<b>Myocardial infarction (%)</b>	56 (16.3)	76 (15.0)	0.74
<b>Hypertension (%)</b>	149 (43.3)	227 (45.4)	0.60
<b>Pre-stroke mRS <math>\leq 2</math> (%)</b>	312 (90.7)	479 (95.8)	1
<b>Systolic blood pressure at baseline (mmHg) (median [IQR])</b>	143 [130-160]	143 [130-160]	0.88
<b>Treatment with IV alteplase</b>	304 (88.4)	445 (89.0)	0.86
<b>ASPECTS <math>\geq 8</math> at baseline (%)</b>	269 (78.9)	373 (74.6)	0.25
<b>Location of intracranial occlusion (%)</b>			0.80
ICA	4 (1.2)	4 (0.8)	
ICA-T	90 (26.2)	134 (26.9)	
M1	213 (61.9)	319 (63.9)	
M2	34 (9.9)	39 (7.8)	
A1 or A2	3 (0.9)	3 (0.6)	
<b>Collateral status on baseline CTA (%)</b>			1
Absent collaterals	19 (5.6)	26 (5.2)	
Poor collaterals	94 (27.7)	136 (27.2)	
Moderate collaterals	134 (39.5)	198 (39.6)	
Good collaterals	92 (27.1)	133 (26.6)	
<b>Extracranial ICA <math>&gt; 50\%</math> stenosis at symptomatic side (%)</b>	40 (11.6)	57 (11.4)	1
<b>Extracranial ICA occlusion at symptomatic side (%)</b>	25 (7.3)	39 (7.8)	0.88
<b>Time from stroke onset to randomization (minutes) (median [IQR])</b>	189[145-248]	200 [150-261]	0.08

**Suppl. Table II.** Characteristics of patients according pattern of intracranial carotid artery calcification

	<b>Intimal dominance</b> (n=118)	<b>Medial dominance</b> (n=152)	<b>p</b>
<b>Allocated to intervention group</b>	57 (48.3)	65 (42.8)	0.433
<b>Age (median [IQR])</b>	63 [54-70]	76 [66-80]	<0.001
<b>Sex (male) (%)</b>	81 (68.6)	81 (53.3)	0.015
<b>NIHSS at baseline (median [IQR])</b>	18[15-21]	18[14-22]	0.330
<b>Previous stroke (%)</b>	11 (9.3)	22 (14.5)	0.274
<b>Atrial fibrillation (%)</b>	28 (23.7)	56 (36.8)	0.030
<b>Diabetes mellitus (%)</b>	12 (10.2)	31 (20.4)	0.035
<b>Smoking (%)</b>	42 (35.6)	35 (23.0)	0.033
<b>Myocardial infarction (%)</b>	11 (9.3)	40 (26.3)	0.001
<b>Hypertension (%)</b>	43 (36.4)	88 (57.9)	<0.001
<b>Pre-stroke mRS ≤2 (%)</b>	112 (94.9)	130 (85.5)	0.021
<b>Systolic blood pressure at baseline (mmHg) (median [IQR])</b>	140 [128-155]	152 [135-166]	<0.001
<b>Treatment with IV alteplase</b>	107 (90.7)	128 (84.2)	0.087
<b>ASPECTS ≥8 at baseline (%) *</b>	90 (76.9)	121 (79.6)	0.703
<b>Location of intracranial occlusion (%)</b>			0.366
ICA	1 (0.8)	3 (2.0)	
ICA-T	35 (29.7)	33 (21.7)	
M1	67 (56.8)	99 (65.1)	
M2	14 (11.9)	17 (11.2)	
A1 or A2	1 (0.8)	0	
<b>Collateral status on baseline CTA (%) †</b>			0.038
Absent collaterals	6 (5.2)	10 (6.6)	
Poor collaterals	24 (20.7)	53 (35.1)	
Moderate collaterals	56 (48.3)	51 (33.8)	
Good collaterals	30 (25.9)	37 (24.5)	
<b>Extracranial ICA &gt;50% stenosis at symptomatic side</b>	11 (9.3)	24 (15.8)	0.166
<b>Extracranial ICA occlusion at symptomatic side</b>	14 (11.9)	8 (5.3)	0.081
<b>Time from stroke onset to randomization ‡</b>	195 [148-268]	186 [146-239]	0.294
<b>Recanalization status on follow up CTA (%) §</b>			0.622
No recanalization	20 (20.2)	29 (27.1)	
Incomplete recanalization, no distal contrast	4 (4.0)	4 (3.7)	
Incomplete recanalization but any distal contrast	13 (13.1)	10 (9.3)	
Complete recanalization	62 (62.6)	64 (59.8)	
<b>Follow-up (5-7 days) infarct volume (mL) (median [IQR]   </b>	65 [26-115]	83 [30-161]	0.083

\* Alberta Stroke Program Early Computed Tomography Score (ASPECTS) was missing for three patients (1%).

† Data was missing for 5 (1%) patients.

‡ Data was missing for 3 (1%) patients.

§ Data was missing for 64 (19%) patients of which 28 patients died.

|| Data on follow-up infarct volume on NCCT 5-7 days was missing for 24 patients (9%) of which 8 patients died before assessment.

**Suppl. Table III.** Proportions of functional independence stratified by reperfusion grades in EVT-treated patients

mTICI-score *	EVT-patients with intimal ICAC (n=57)		EVT-patients with medial ICAC (n=65)	
	N (%)	mRS 0-2 (%)	N (%)	mRS 0-2 (%)
0-1	9 (19)	0/9	9 (17)	1/9 (11)
2a	13 (27)	2/13 (15)	11 (21)	3/11 (27)
2b-3	26 (54)	10/26 (38)	32 (62)	13/32 (40)
cOR (95%CI)	1.03 (0.89-1.18)		1.21 (1.07-1.38)	

\*Score of 0 indicates no perfusion or antegrade flow beyond occlusion site, 1: penetration of contrast but not perfusion, 2A: some perfusion <50% of vascular territory, 2B: substantial perfusion ≥50, 3: complete perfusion of vascular territory.

To assess impact of reperfusion on functional outcome with ordinal logistic regression, complete scale of dependent (mRS) and independent (mTICI-score) variables were used.

**Suppl. Table IV.** Serious adverse events according pattern of intracranial carotid artery calcification for both treatment groups.

	Patient with intimal calcification pattern		Patient with medial calcification pattern	
	Intervention group (n=57)	Control group (n=61)	Intervention group (n=65)	Control group (n=87)
<b>Any serious adverse event *</b>	26 (46%)	17 (28%)	33 (51%)	44 (51%)
Symptomatic intracerebral haemorrhage	0	4 (7%)	5 (8%)	7 (8%)
Recurrent ischemic stroke	2 (4%)	0	5 (8%)	0
Progression of ischemic stroke	14 (25%)	10 (16%)	13 (20%)	14 (16%)
Pneumonia	6 (11%)	4 (6%)	11 (17%)	23 (26%)
Cardiac ischemia	1 (2%)	1 (2%)	0	3 (3%)
Extracranial haemorrhage	0	0	0	1 (1%)
Allergic complication	1 (2%)	0	0	0
Other complication	9 (16%)	5 (8%)	3 (5%)	16 (18%)

\* Only first events of one type are listed. Patients experiencing multiple events of one type have been counted once.