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Outcomes of endovascular thrombectomy in patients selected by computed tomography perfusion imaging — a matched cohort study comparing nonagenarians to younger patients

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ABSTRACT

Background Endovascular thrombectomy (EVT) is efficacious for appropriately selected patients with large vessel occlusions (LVO) up to 24 hours from symptom onset. There is limited information on outcomes of nonagenarians, selected with computed tomography perfusion (CTP) imaging.

Methods We retrospectively analyzed data from a large academic hospital between December 2017 and October 2019. Patients receiving EVT for anterior circulation LVO were stratified into nonagenarian (≥90 years) and younger (<90 years) groups. We performed propensity score matching on 18 covariates. In the matched cohort we compared: primary outcome of inpatient mortality and secondary outcomes of successful reperfusion (TICI ≥2B), symptomatic intracranial hemorrhage (sICH), and functional independence. Subgroup analysis compared CTP predicted core volumes in nonagenarians with outcomes.

Results Overall, 214 consecutive patients (26 nonagenarians, 188 younger) underwent EVT. Nonagenarians were aged 92.8±2.9 years and younger patients were 74.5±13.5 years. Mortality rate was significantly greater in nonagenarians compared with younger patients (43.5% vs 10.4%, OR 9.33, 95% CI 2.88 to 47.97, P<0.0001) and a greater proportion of nonagenarians developed sICH (13.0% vs 3.0%, OR 6.00, 95% CI 1.34 to 55.20, P=0.02). There were no significant differences for successful reperfusion (P=1.00) or functional independence (P=0.75). Nonagenarians selected with smaller ischemic core volumes had decreased mortality rates (P=0.045).

Conclusions Nonagenarians were noted to have greater mortality and sICH rates following EVT compared with matched younger patients, which may be ameliorated by selecting patients with smaller CTP core volumes. Nonagenarians undergoing EVT had similar rates of successful reperfusion and functional independence compared with the younger cohort.

INTRODUCTION

In an aging population, cases of ischemic stroke are steadily increasing in number with high post-stroke morbidity and mortality rates. ^{1 2} Endovascular thrombectomy (EVT) is a well-established modality

of treatment in appropriately selected patients, but older patients such as nonagenarians were excluded from the major randomized controlled clinical trials. $^{3-7}$ The Highly Effective Reperfusion evaluated in Multiple Endovascular Stroke Trials (HERMES) meta-analysis demonstrated favorable effect in patients undergoing EVT without heterogeneity of effect across patient age subgroups including nonagenarians, although only five nonagenarians were included in this meta-analysis.5 Recent landmark endovascular trials with an extended time window of 16-24 hours reported similar findings in the nonagenarian subgroup, but again their underrepresentation failed to level up the recommendation in the stroke guidelines.6-8 Previous cohort and observational studies have examined outcomes of EVT in nonagenarians, but found conflicting results.9-16 Most of these studies have examined data within the nonagenarian group. 9 11 12 15-17 A smaller set have compared nonagenarians to other age groups. 10 13 14 Thus, there remains uncertainty among treating clinicians when offering EVT treatment to nonagenarians.

Recent EVT trials demonstrated the use of perfusion imaging for better selection of patients, including the nonagenarians.⁴⁷ Computed tomography perfusion (CTP) provides physiological information to generate maps, determining who would benefit the most from EVT in the proper clinical context.¹⁸ CTP has been demonstrated to successfully assist in selecting patients for EVT but the extent to which it can improve patient selection in nonagenarians is unclear. We sought to examine whether CTP could assist in selecting nonagenarians for EVT and how the outcomes of nonagenarians selected for EVT with the aid of CTP compared with a younger matched cohort that was also selected for EVT using CTP. Additionally, we aimed to examine whether considering CTP-predicted ischemic core volumes when selecting nonagenarians may lead to better clinical outcomes.

METHODS

Patient selection

We retrospectively analyzed prospectively collected data from an academic hospital between December



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2017 and October 2019. Patients receiving EVT for anterior circulation large vessel occlusion (LVO) selected using CTP within 24 hours of last known normal were stratified into nonagenarian (≥90 years) and younger (<90 years) groups. The study was reviewed and approved by the institutional review boards at Allegheny Health Network, Pittsburgh PA, USA, which waived the need for written informed consent. Demographics, clinical characteristics, imaging data and outcomes were collected.

Diagnostic and interventional procedure

Neuroimaging studies (CT, CT angiography, and CTP) were completed on admission to evaluate patients for thrombolysis or EVT and used to aid in selection of these patients. All endovascular procedures were performed by three experienced neurointerventionalists under conscious sedation preferably or general anesthesia when clinically necessary. Stent-retriever and/or aspiration device were used. In general, aspiration catheters were used as the first device and stent-retrievers were used only when an aspiration catheter failed to achieve reperfusion.

Statistical analysis

Data were analyzed with SPSS (IBM SPSS Statistics for Windows, Version 26.0; IBM Corp., Armonk, NY) and R (R Foundation for Statistical Computing, Version 4.0.3; R Core Team, Vienna, Austria). Proportional data in the unmatched group were analyzed using chi square or Fisher's exact tests, as appropriate. Parametric measures are described as mean±SD and nonparametric data as median (IQR). Nonparametric data were analyzed using Mann-Whitney test. In order to reduce risk of bias due to confounding, we conducted propensity score matching. Patients aged 90 years and above were matched to patients younger than 90 years old by variable ratio pair matching up to 1:4, without replacement, using a caliper width of 0.2 of the SD of the logits of the propensity score. We estimated the propensity score using a logistic regression model in which age was regressed on 18 covariates. We aimed to balance groups on the following patient demographics and comorbidities: sex, atrial fibrillation, coronary artery disease, chronic kidney disease, diabetes, heart valve disease, hyperlipidemia, hypertension, mechanical valve, previous myocardial infarction, smoking status; the following patient characteristics: initial National Institutes of Health Stroke Scale (NIHSS), if the patient initially presented to a telestroke spoke hospital, intravenous (IV) alteplase administration prior to EVT; ischemic volume data prior to EVT: CTP predicted core volume defined as the relative cerebral blood flow (rCBF) <30% compared with normal tissue, mismatch volume (Tmax >6 seconds volume - rCBF <30% vol); and procedural data: door to groin puncture and if recanalization was achieved after first pass (table 1). We considered groups balanced on covariates after matching when an absolute standardized difference was <30% and P>0.15. In the final matched cohort, we compared our primary outcome of inpatient mortality and secondary outcomes of successful reperfusion (Thrombolysis in Cerebral Infarction (TICI) ≥2B), symptomatic intracranial hemorrhage (sICH) using SITS-MOST criteria¹⁹ and modified Rankin Scale (mRS) in patients surviving to discharge between nonagenarians and younger patients using McNemar's tests. Subgroup analysis used Mann-Whitney to test CTP predicted core volume in the matched nonagenarian group for each of the primary and secondary outcomes. Sensitivity analyses examined dichotomous CTP predicted core volume at 20 mL in the matched nonagenarian group for mortality and sICH using Fisher's exact test. Post hoc analyses examined CTP core volume at 5 mL in the matched nonagenarian group, which utilized Fisher's

exact test and were corrected for multiple comparisons using the false discovery rate.

RESULTS

A total of 214 consecutive patients (26 \geq 90 years and 188 <90 years) with anterior circulation LVO were selected by CTP and underwent EVT. After matching, 89 patients were included. (23 ≥90 years and 66 <90 years). The mean age of the nonagenarians was 92.8 ± 2.9 years and the younger group was 74.5 ± 13.5 years. Baseline demographic characteristics, comorbidities, and clinical data before and after propensity score matched analysis are summarized in table 1. Matching successfully reduced variance in covariates between nonagenarians and younger patients to target measures. There were no differences in the location of the LVO including hemisphere affected or vessel occluded between nonagenarians and younger patients (all P>0.05) (table 1). There was no difference in the proportion of matched nonagenarians that experienced a complication during EVT compared with younger patients (4.3% vs 1.5%, odds ratio (OR) 2.96, 95% CI 0.18 to 49.25, P=0.45). Similarly, there was no difference in failed EVT attempts in nonagenarians matched to younger patients (0.0% vs 4.5%, P=0.57).

Primary and secondary outcomes of EVT in nonagenarians are shown in table 2. In the matched cohort, mortality rate during hospitalization was significantly greater in nonagenarians compared with younger patients (43.5% vs 10.4%, OR 9.33, 95% CI 2.88 to 47.97, P<0.0001) and a greater proportion of nonagenarians developed sICH compared with younger patients (13.0% vs 3.0%, OR 6.00, 95% CI 1.34 to 55.20, P=0.02). The frequency of successful reperfusion (TICI 2b or 3) was not significantly different between matched nonagenarians and younger patients (91.3% vs 90.9%, P=1.00). Similarly, there was no difference in achieving functional independence in patients surviving index hospitalization (23.1% vs 32.2%, P=0.75).

Subgroup analyses suggested that nonagenarians surviving to discharge were selected for EVT with smaller CTP predicted ischemic core volumes (0 (IQR 0–10.5) vs 7.5 (IQR 4.5–38.5), P=0.045). Nonagenarians developing sICH following EVT had larger estimated ischemic core volumes prior to EVT compared with nonagenarians who did not develop sICH (37 (IQR 6–43) vs 2.5 (IQR 0–10.8)) but the difference was not significant (P=0.07). There was no difference in the CTP core volume of nonagenarians with successful reperfusion versus unsuccessful reperfusion (6 (IQR 0–17.5) vs 21.5 (IQR 0–43), P=0.69). Nonagenarians achieving functional independence when surviving index hospitalization had lower core volumes than nonagenarians who were discharged without functional independence (0 (IQR 0–0) vs 7 (IQR 0–30.8)) but this difference was not significant (P=0.15).

Sensitivity analyses found no statistical difference in mortality of the nonagenarians selected for EVT with CTP core volume cut-offs of \leq 20 mL and \leq 5 mL (P=0.34 and P=0.07, respectively) (tables 3 and 4). Furthermore, there was no difference in the incidence of sICH after EVT in nonagenarians selected with core volumes limited to 20 mL and 5 mL (P=0.16 and P=0.22, respectively) (tables 3 and 4).

DISCUSSION

Age is not an exclusion criteria for EVT under the current stroke guidelines. A recent survey among vascular neurologists revealed that most physicians do not consider nonagenarians a contraindication for EVT, despite the fact that there is limited evidence to show the benefit of EVT in this subgroup. Previous cohort and observational studies found variable 90-day mortality

Table 1 Cohort demographic characteristics, medical history, and clinical data before and after propensity score matched analysis.

Variable	Unmatched cohort				Propensity score matched cohort			
	Age ≥90 years (n=26)	Age <90 years (n=188)	d*	P value	Age ≥90 years (n=23)	Age <90 years (n=66)	d*	P value
Patient demographics and cor	norbidities							
Sex (female)	21 (80.8)	100 (53.2)	61.3	0.01	18 (78.3)	47 (71.2)	16.3	0.59
Atrial fibrillation	12 (46.2)	69 (36.7)	19.3	0.35	9 (39.1)	29 (43.9)	-9.8	0.69
Coronary artery disease	9 (34.6)	62 (33.0)	3.5	0.87	7 (30.4)	25 (37.9)	-15.7	0.52
Chronic kidney disease	8 (30.8)	38 (20.2)	24.4	0.22	6 (26.1)	19 (28.8)	-6.1	0.80
Diabetes	3 (11.5)	52 (27.7)	-41.5	0.10	3 (13.0)	16 (24.2)	-29.1	0.38
Heart valve disease	2 (7.7)	10 (5.3)	9.6	0.64	1 (4.3)	3 (4.5)	-1.0	1.00
Hyperlipidemia	12 (46.2)	89 (47.3)	-2.4	0.91	10 (43.5)	33 (50.0)	-13.1	0.59
Hypertension	22 (84.6)	145 (77.1)	19.1	0.46	19 (82.6)	56 (84.8)	-6.1	0.75
Mechanical heart valve	0 (0)	10 (5.3)	-33.5	0.61	0 (0)	0 (0)	-	1.00
Previous myocardial infarction	2 (7.7)	11 (5.9)	7.3	0.66	1 (4.3)	3 (4.5)	-1.0	1.00
Smoker (current)	0 (0)	36 (19.1)	-68.8	0.01	0 (0)	0 (0)	-	1.00
Patient characteristics								
NIHSS	14 (10–20)	16 (11–19)	-4.6	0.42	14 (10–18)	14 (10–19)	-7.4	0.76
Transferred from spoke hospital	10 (38.5)	67 (35.6)	5.8	0.78	8 (34.8)	25 (37.9)	-6.4	0.79
IV alteplase prior to EVT	9 (34.6)	47 (25.0)	21.1	0.31	8 (34.8)	22 (33.3)	3.1	0.90
Location and volume								
Hemisphere of LVO (left)	11 (42.3)	102 (54.3)		0.25	9 (39.1)	31 (47.0)		0.52
Vessel of LVO				0.61				0.69
ICA	5 (19.2)	38 (20.2)			5 (21.7)	9 (13.6)		
Tandem	0 (0.0)	9 (4.8)			0 (0.0)	2 (3.0)		
M1	14 (53.8)	104 (55.3)			13 (56.5)	40 (60.6)		
M2	7 (26.9)	37 (19.7)			5 (21.7)	15 (22.7)		
Ischemic core (mL)†	2.5 (0–14)	7 (0–23)	-25.4	0.25	6 (0–24)	0 (0–12)	14.3	0.44
Mismatch volume	72 (45–102)	71 (45–110)	-2.7	0.93	71 (42–81)	66 (45–105)	-2.4	0.91
Procedure								
Door to groin puncture	85 (34–132)	81 (52–109)	23.5	0.49	87 (37–128)	84 (48–116)	20.5	0.46
Recanalization after first pass	18 (69.2)	105 (55.9)	27.9	0.20	15 (65.2)	38 (57.6)	15.7	0.52
Complications	1 (3.8)	2 (1.6)		0.32	1 (4.3)	1 (1.5)		0.45
Failed EVT‡	0 (0.0)	9 (4.8)		0.60	0 (0.0)	3 (4.5)		0.57

All values are n (%) or median (IQR).

Proportion P values assessed by chi square or Fisher's exact test as appropriate. Nonparametric measures assessed by Mann-Whitney test.

EVT, endovascular thrombectomy; ICA, internal carotid artery; IQR, interquartile range; IV, intravenous; LVO, large vessel occlusion; MI, myocardial infarction; NIHSS, National Institutes of Health Stroke Scale.

rates in nonagenarians who underwent EVT, with mortality rates ranging from 34% to 70%. 9-13 15 16 In contrast, Sweid *et al* compared nonagenarians and younger patients aged 60–89 years undergoing EVT and found no difference in the rate of inpatient mortality in nonagenarians compared with younger patients. 14 In our study, we observed significantly higher mortality rates in matched nonagenarians compared with younger patients, despite utilizing CTP to aid in selecting for EVT. This may be due to our propensity to pursue palliative measures in this age group who have sICH or disabling strokes despite successful reperfusion. There also appears to be less enthusiasm for pursuing prolonged rehabilitation by the families of nonagenarian stroke patients though this evidence is anecdotal. A prior study examining EVT in nonagenarians compared with nonagenarians that selected

patients for EVT with CTP found a trend toward a higher mortality rates at 90 days in nonagenarians (63%) compared with octogenarians (41.9%). Although nonagenarians may have a higher mortality rate after EVT, there are some factors that may reduce this. A previous study noted lower mortality rates and better outcomes in nonagenarians with successful reperfusion. Our study found high rates of recanalization after EVT in nonagenarians (91.3%), which were similar to the younger cohort (90.9%); but, regardless of this, we found increased mortality rates in nonagenarians. A prior study examining octogenarians and younger patients also found high rates of recanalization following EVT (88%) in both groups, but increased mortality in the octogenarians.

^{*}Standardized difference.

 $ext{†Defined}$ as volume of blood flow <30% by computed tomography perfusion imaging.

[‡]Defined as Thrombolysis in Cerebral Infarction (TICI) score of 0.

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 Table 2
 Primary and secondary outcomes before and after propensity score matched analysis.

Unmatched cohort				Propensity score matched cohort				
Outcome	Age ≥90 years (n=26)	Age <90 years (n=188)	OR (95% CI)	P value*	Age ≥90 years (n=23)	Age <90 years (n=66)	OR (95% CI)	P valuet
Primary								
Death during initial hospitalization	11 (42.3)	20 (10.6)	6.16 (2.49 to 15.24)	< 0.001	10 (43.5)	17 (10.4)	9.33 (2.88 to 47.97)	<0.0001
Secondary								
Successful reperfusion‡	23 (88.5)	164 (87.2)	1.12 (0.31 to 4.02)	1.00	21 (91.3)	60 (90.9)	1.20 (0.31 to 4.97)	1.00
Symptomatic ICH	3 (11.5)	7 (3.7)	3.37 (0.82 to 13.96)	0.11	3 (13.0)	2 (3.0)	6.00 (1.34 to 55.20)	0.02
Functional independence in patients surviving discharge§	4 (26.7)	53 (31.5)	0.79 (0.24 to 2.59)	0.78	3 (23.1)	19 (32.2)	1.00 (0.23 to 4.35)	0.75

All values are n (%).

Complications of EVT are more frequent in elderly patients, and are associated with worse outcomes, including mortality. sICH is the most common complication after EVT. Similar to a previous study comparing nonagenarians to octogenarians, ¹³ we observed nonagenarians to have a significantly higher rates of sICH following EVT compared with the younger matched cohort. Despite the higher rates of sICH in nonagenarians, we observed similar rates of procedural complications and failed thrombectomy attempts in nonagenarians and younger patients. A prior study noted similar rates of complications and post-procedural hemorrhage in octogenarians compared with younger patients, but there was not a difference between parenchymal type 2 hemorrhages between the groups. ²¹

Prior studies have found low but variable rates of functional independence (mRS 0-2) in nonagenarians at 90 days after EVT ranging from 11% to 35%. 9-14 16 17 Studies directly comparing nonagenarians to younger patients found conflicting results. A previous study found nonagenarians to have a significantly lower rate of functional independence at 90 days after EVT compared with a younger cohort (11% vs 48%). 10 Although when restricting the minimum age of the younger cohort to 60 or 80 years, studies found no difference in functional independence at 90 days after EVT in nonagenarians compared with younger patients. 13 14 When we matched nonagenarians to younger patients (aged 18-89 years) we found no difference in the functional independence of patients who were discharged from hospital. A prior study highlighted the importance of the first pass effect of EVT in nonagenarians with a significantly greater proportion of nonagenarians achieving functional independence at 90 days when recanalization was achieved after the first pass. 12 We matched groups on the dichotomous variable as

to whether recanalization was achieved after first pass. Although only three nonagenarians in our study were discharged with functional independence, all three of these patients had recanalization after their first pass.

A previous study found that older patients (≥75 years) with low Alberta Stroke Program Early CT scores (ASPECTS) (0-5) had poor clinical outcomes whether or not patients underwent EVT.²² Another study examining nonagenarians treated with or without IV thrombolytic therapy found higher rates of in-hospital mortality and sICH in treated nonagenarians compared with matched, untreated nonagenarians (27.9% vs 19.7% and 5.9% vs 2.6%), but the differences were not statistically significant.²³ We found higher incidences of in-hospital mortality (43.5%) and sICH (13.0%) in our EVT-treated nonagenarians. Only six patients underwent EVT in the previous study, but stroke severity as measured by median baseline NIHSS was similar in our EVT-treated nonagenarians to the IV thrombolytictreated nonagenarians in the prior study (15 vs 14).²³ We investigated whether selecting nonagenarians for EVT, with the aid of CTP, could improve outcomes. Subgroup analysis examined CTP predicted ischemic core size prior to EVT and found that the nonagenarians who died had a significantly larger core. The median CTP-predicted ischemic core of nonagenarians in this study surviving through index hospitalization was zero and all nonagenarians that survived index hospitalization had a core less than 25 mL. We examined CTP core cut-offs at 20 mL, based on the DAWN trial.6 We observed reduced rates of mortality and sICH in nonagenarians who had an ischemic core of 20 mL or less, but the effects were small to moderate and were not statistically significant. We additionally examined an ischemic core cut-off of 5 mL. The effect size of choosing nonagenarians with

Table 3 Sensitivity analysis examining outcomes in matched nonagenarian group with ischemic core of 20 mL

Outcome	Ischemic core ≤20 mL (n=17)	Ischemic core >20 mL (n=6)	OR (95% CI)	P value*	Phi
Death during initial hospitalization	6 (35.3)	4 (66.7)	0.27 (0.04 to 1.95)	0.34	0.28
Symptomatic ICH	1 (5.9)	2 (33.3)	0.13 (0.09 to 1.75)	0.16	0.36

All values are n (%).

Phi: 0.1, small effect size; 0.3, medium effect size; 0.5, large effect size.

*All P values are two-sided Fisher's exact test.

CI, confidence interval; ICH, intracerebral hemorrhage; OR, odds ratio.

^{*}All P values are two-sided Fisher's exact or chi square test, as appropriate, for the unmatched analysis.

[†]All P values are McNemar's test for the matched analysis.

[‡]Defined as Thrombolysis in Cerebral Infarction (TICI) score of 2B or greater.

[§]Defined as modified Rankin Scale score of 0-2.

CI, confidence interval; EVT, endovascular thrombectomy; ICH, intracerebral hemorrhage; OR, odds ratio.

Table 4 Sensitivity analysis examining outcomes in matched nonagenarian group with ischemic core of 5 mL

Outcome	Ischemic core ≤5 mL (n=11)	Ischemic core >5 mL (n=12)	OR (95% CI)	P value*	Phi
Death during initial hospitalization	2 (18.2)	8 (66.7)	0.11 (0.02 to 0.78)	0.07	0.49
Symptomatic ICH	0 (0)	3 (25)	-	0.29	0.37

All values are n (%).

Phi: 0.1, small effect size; 0.3, medium effect size; 0.5, large effect size.

*All P values are two-sided Fisher's exact test, corrected for multiple comparison using the false discovery rate correction.

CI, confidence interval; ICH, intracerebral hemorrhage; OR, odds ratio.

an ischemic core of 5 mL or less was moderate to large, with mortality rates of 18.2% versus 66.7% and there was no sICH in nonagenarians with an ischemic core of 5 mL or less, although after corrections for multiple comparisons this difference was not statistically significant. Interestingly, a prior study examined final ischemic core volumes 24–72 hours post-EVT in nonagenarians and they found that all patients that returned home had final infarct volumes of less than 7 mL. Therefore to select nonagenarians for EVT with a similar chance of a good outcome we may need to be more restrictive in terms of the ischemic core prior to EVT. Though our data demonstrate this trend, more patients would be necessary to power this analysis.

As the elderly population with stroke continues to increase due to an overall aging population, it will be important to identify better selection of nonagenarians. Additionally, it will be helpful to level-set the expectations of the procedure with the patient and family, namely that there are increased chances of poor outcome in nonagenarians. Our study has limitations. This was a single-center, retrospective study, which may limit the generalizability of the results. This dataset is obviously limited by its selection bias for the nonagenarians that were deemed eligible for EVT. A prior study found a relationship between baseline mRS and 90-day outcomes in nonagenarians. 10 We were unable to consider matching on baseline mRS as we had incomplete data. Notwithstanding this, there were no differences in baseline mRS between 14 nonagenarians and 76 younger patients in the unmatched analysis (1 (0-2) vs 1 (0-3), P=0.41). Likewise, median baseline mRS in 12 nonagenarians and 26 younger patients was similar in the matched analysis also (1 (0-3) vs 1 (0-2), P=0.96). We attempted to reduce these biases by meticulously matching nonagenarians and younger patients on a number of covariates. Although we achieved matching targets and groups were balanced on the metrics we chose, there may be inherent differences between the groups for which we were unable to match. Although the nonagenarian group was a relatively small sample, we were able to minimize time-modified cofounding and time-dependent cofounding such as changes in care and clinical practice guidelines over time. All patients in this study were evaluated for EVT up to 24 hours of last known well and had CTP imaging to aid in selection of the patients in a relatively short time span of under 2 years.

CONCLUSIONS

We observed that nonagenarians have higher mortality rates and sICH following EVT compared with matched younger patients. There was no difference in reperfusion or functional independence in patients discharged from the index hospitalization. Age had no influence on treatment decisions for eligible nonagenarians, and CTP was observed to be a reliable determining factor in decision-making. Nonagenarians who died had significantly larger ischemic core volumes prior to EVT. Thus, EVT should

not be withheld for stroke in nonagenarians, but an acute assessment of infarct core is important to consider in this population.

Contributors RR, CTH, and NMF collected data. RR, CTH, RC, and SSR designed the study. CTH analyzed the data. RR, CTH, RC, KM, RW, TH, AHT, and SSR drafted the manuscript and interpreted data. RR, CTH, RC, KM, RW, TH, AHT, and SSR critically reviewed and edited the manuscript.

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