Triage of stroke patients in the chain of acute stroke care

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T. Truc My Nguyen

geboren te Almere

in 1990

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TABLE OF CONTENTS

INTRODUCTION

Chapter 1

General introduction

INTRODUCTION

Stroke is one of the leading causes of case fatality and long-term disability worldwide and has a high socioeconomic burden. In the Netherlands, the incidence of stroke is estimated 45.000 each year, while this number is exceeding 11 million worldwide.[1, 2] In the next few decades, the burden of stroke will increase due to ongoing demographic changes, including ageing of the population.[3]

Stroke can be divided into two subtypes: ischemic stroke (87%) and hemorrhagic stroke (13%).[4] Hemorrhagic strokes are caused by a ruptured arterial blood vessel into the brain and the hemorrhage causes mass effect and mechanical compression on the surrounding tissue, followed by a cascade of physiological responses that lead to brain tissue injury. Depending on location of hemorrhage, hemorrhagic strokes are either intracerebral hemorrhages (ICH) or subarachnoid hemorrhages (SAH). Ischemic strokes are caused by a sudden disruption of blood flow to a focal area of the brain due to a blood clot. The impaired blood flow causes brain tissue to be deprived of oxygen and other essential nutrients, in particular glucose, which leads to brain tissue injury within seconds.

DIAGNOSIS AND TREATMENT

Currently, the diagnosis of acute stroke and treatment decisions rely on clinical assessment and neuro-imaging techniques.

Both stroke subtypes result in acute neurological dysfunction and can present with clinical symptoms that are a direct consequence of the territory of the vessels involved. For example, deficits in motor function (paresis of face and/or limb) or language (aphasia) can be the result of stroke of the anterior circulation and deficits in vision (diplopia) or vertigo can occur when the posterior circulation is affected. Clinical assessment can indicate which brain area is affected, but distinction between subtypes cannot be made on clinical assessment alone.

Neuroimaging is necessary to differentiate intracerebral hemorrhage from ischemic stroke as treatments differ for both stroke subtypes. While non-contrast computed tomography (CT) is highly sensitive for the presence of hemorrhage, in the acute phase, a non-contrast CT-scan cannot reliably distinguish between ischemic stroke or a stroke-mimicking condition (such as epilepsy or migraine). In addition, stroke due to a large anterior vessel occlusion requires a CT-angiography/-perfusion to confirm the presence of such an occlusion.

Acute ischemic stroke patients need rapid reperfusion treatment to ensure early recanalization of the blood vessel before more neuronal cells die and deficits become permanent. Reperfusion treatments consists either of dissolving the clot systemically by intravenous thrombolysis (IVT) with recombinant tissue plasminogen activator or by removing the clot mechanically with endovascular treatment (EVT). The clinical benefit of these treatments is highly time-dependent with a limited treatment window of 4.5 hours after symptom onset for IVT and 6 hours for EVT.[5, 6] Recent trials, however, have shown that these windows can be expanded to 9 hours after stroke onset or on awakening with stroke (<9 hours from the midpoint of sleep)[7] and up to 24 hours for EVT, in patients selected with neuroimaging.[8, 9] Although treatments will continue to include a more individual approach as opposed to the fixed treatment window, the sooner recanalization can occur, the better the outcome for the patient will be: time is brain.[10]

We are facing several challenges in clinical practice due to these difficulties to establish the correct diagnosis within a short amount of time. This can result in erroneous diagnosis and treatment in clinical practice.

In clinical practice, it is difficult to distinguish patients with acute ischemic stroke from stroke mimics. A study found that of all IVT-treated patients, approximately 20% consisted of stroke mimics.[11] Although administering thrombolytics appear to have a low complication rate in the

stroke mimic population, these patients were unnecessarily exposed to an added risk of symptomatic intracerebral hemorrhage.[12, 13] Identifying these patients beforehand will also prevent overburdening of emergency departments and concomitantly socio-economic costs.

Since EVT has become part of standard care, another dilemma arises: how to reveal the subset of patients with ischemic stroke due to large anterior vessel occlusion who are eligible for EVT. A review indicated that 11-20% of the ischemic stroke patients are eligible for EVT, while only 3%-7% receive treatment.[14, 15] Moreover, EVT is such an elaborate and costly treatment that it is restricted to comprehensive stroke centers with EVT facilities, in contrast to IVT which is available in most primary stroke centers. Since large anterior vessel occlusion cannot be reliably identified in the ambulance, inter-hospital transfers lead to a median of 60-109 minutes delay with associated unfavorable functional outcomes.

Indeed, the most important reason for patients being excluded from reperfusion treatment is delayed hospital presentation.[14, 16] Improving diagnosis and timely access of stroke patients to specialized care can have a tremendous impact on current daily clinical practice and improve patient outcome.[17, 18]

Time delays to treatment

Most time delays occur in the prehospital phase, as in-hospital delays have been reduced substantially in the last decades, especially for IVT.[14, 19] These phases are summarized in the chain of acute stroke care. This stroke chain starts with the stroke event and ends when treatment is initiated, or hospital admission is required (Figure 1).

Various healthcare providers are involved in this chain of acute stroke. Hence, potential time delaying factors are also abundant. By improving existing stroke triage systems, mainly in the prehospital phase, it is expected that more patients will arrive in the hospital in time. As a consequence, more patients will receive earlier treatment leading to improved clinical outcome.

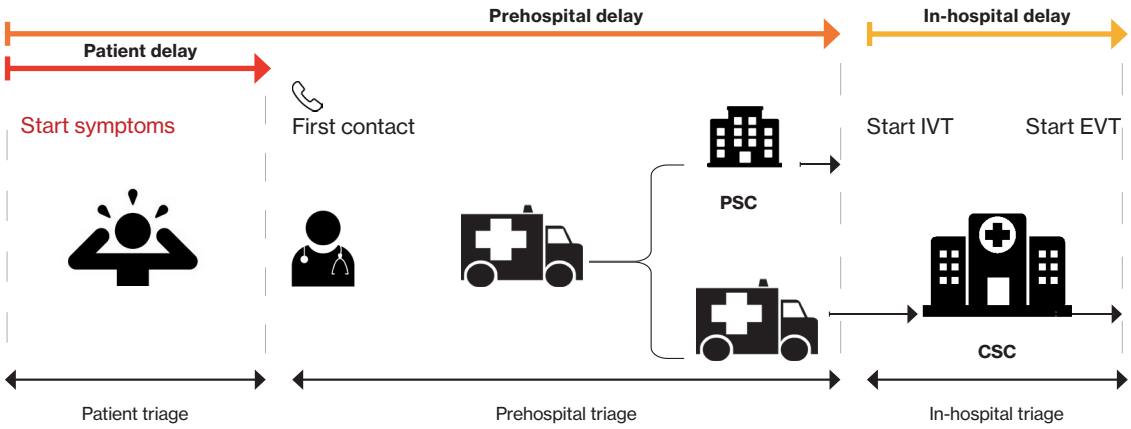

Figure 1.
The chain of acute stroke care: prehospital and in-hospital delays

PSC: primary stroke center; CSC: comprehensive stroke center, IVT: intravenous thrombolysis; EVT: endovascular treatment.

TRIAGE OF ACUTE STROKE PATIENTS

I. Patient triage

In the prehospital phase, triage is challenging for patients as well as for primary healthcare providers. In the Dutch healthcare system, patients can either directly call the emergency medical services with the emergency number 112, or first alarm the general practitioner. Guidelines state that the emergency medical services should immediately be called if acute stroke is suspected, and the public is educated to directly call 112 in case of suspected stroke by stroke awareness campaigns. Patients, however, often do not perceive stroke symptoms as a medical emergency that requires immediate action.[20] In addition, healthcare providers face difficulties in the triage of stroke patients in whom diagnosis is not apparent. This applies specifically to general practitioners as they function as gate keepers to refer patients that need immediate medical care as well as prevent emergency departments from overburdening.

II. Prehospital triage

The emergency medical services play an important part in the triage of stroke patients and transferring them to a hospital as fast as possible. Since stroke due to a large anterior vessel occlusion cannot be reliably identified in the ambulance, suspected acute stroke patients are often transferred to the nearest hospital, often a primary stroke center, in order to start IVT as soon as possible. Prehospital triage tools that could rapidly identify patients with a large anterior vessel occlusion in the ambulance would enable direct allocation of these patients to a comprehensive stroke center with EVT facilities and would greatly reduce delays to EVT treatment and improve clinical outcomes.

In addition, if stroke biomarkers could differentiate between acute ischemic stroke, intracerebral hemorrhage and stroke mimics, the clinical added value would be earlier initiation of treatment, for example in the ambulance, and preventing mimics from unnecessary treatment with IVT and emergency departments from overburdening. However, such a diagnostic tool to accurately differentiate between stroke subtypes and stroke mimics is not yet available in clinical practice.

III. In-hospital triage, treatment and patient outcome

Although in-hospital delays are reduced drastically, we can still achieve progress to improve the patient's outcome by continuing our efforts to reduce in-hospital delays, as well as investigate other factors that are known to have an adverse effect on patient outcome.

In some hospitals, weight assessment of the patient prior to IVT in order to accurately titrate the recombinant tissue plasminogen activator dose, is based on an estimation of the weight rather than measuring the patient's weight with a weighing scale, as the latter is thought to be more time-consuming. However, it remains unclear which weight assessment is more accurate. This is important since an overdose increases the risk of symptomatic intracerebral hemorrhage. Of all IVT-treated patients, symptomatic intracerebral hemorrhage occurs in 3-8%. It has a high morbidity and mortality and is therefore associated with poor patient outcome.

Another important factor that is associated with poor outcome is hyperglycemia on admission. Hyperglycemia is often present in the acute phase of ischemic stroke and is associated with poor functional outcome, higher risk of intracerebral hemorrhages and lower recanalization rates in IVT and EVT treated patients. However, these associations have not yet been widely investigated in patients treated with EVT outside a trial setting.

OBJECTIVE AND OUTLINE OF THIS THESIS

The main objective of this thesis is to investigate opportunities to improve triage of acute stroke patients to achieve timely access to specialized care for stroke patients.

The first part of this thesis focuses on triage of acute stroke patients.

In **Chapter 2,** I assessed the patient's choice of primary healthcare provider after acute onset of stroke symptoms. I identified factors that differ between patients who first alarm the general practitioner and those who directly alarm the emergency medical services.

The second part focuses on diagnostic tools that can be used as prehospital triage of acute stroke patients. First, several clinically relevant questions with regard to the use of stroke triage tools in the prehospital phase were addressed. In **Chapter 3,** I systematically reviewed prehospital prediction scales that have been used to identify patients with stroke due to large anterior vessel occlusion. In **Chapter 4,** I investigated the external validity and feasibility of these prehospital prediction scales in the emergency setting. Secondly, I explored the role of biomarkers as a diagnostic tool for prehospital triage and improvement of stroke diagnosis. In **Chapter 5,** I aimed to discover promising biomarkers to distinguish stroke subtypes in the prehospital phase to aid in timely clinical decision making.

In the third part, in-hospital contributors of unfavorable patient outcomes were studied. In **Chapter 6,** I investigated two different methods that are implemented into daily clinical practice for weight assessment in ischemic stroke patients prior to receiving intravenous thrombolysis. I studied whether these two methods were associated with differences in outcome after ischemic stroke. In **Chapter 8,** I assessed whether a higher glucose on admission was associated with outcome in EVT-treated patients.

Finally, in **Chapter 9,** the main findings of my research presented in this thesis are summarized and discussed, together with implications for clinical practice and future research.

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Part I

PATIENT TRIAGE

Chapter 2

Stroke patient's alarm choice: general practitioner or emergency medical services

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and Heleen M den Hertog.

ABSTRACT

Background and purpose

Stroke patients should be treated as soon as possible since the benefit of reperfusion therapies is highly time-dependent. The proportion of patients eligible for reperfusion therapy is still limited, as many patients do not immediately alarm healthcare providers. The choice of healthcare system entrance influences the time of arrival in the hospital. Therefore, we assessed differences in these choices to obtain insight for strategies to reduce time delays in acute stroke patients.

Methods

Patients with suspected acute stroke admitted to the participating hospitals received a questionnaire. We assessed differences between patients who initially alarmed the general practitioner (GP) and patients who directly alarmed the emergency medical services (EMS). Additionally, we assessed regional differences and patient trajectories after medical help was sought.

Results

We included 163 patients. Most patients alarmed the GP as primary healthcare provider (n=104; 64%) and median onset-to-door times were longer in these patients (466 minutes [IQR 149 –1586]) compared to patients directly alarming the EMS (n=59; 36%) (90 minutes [IQR 45 –286]). This was even more pronounced in less densely populated areas. Patients who alarmed the GP first, more often had patient delay >15 minutes, hesitated to burden healthcare providers and underestimated symptomatology.

Conclusion

Our results showed that patients who alarmed the GP first instead of the EMS differed in several factors that are potentially modifiable. Strategies to achieve reduction of vital pre-hospital time delays and to improve patient outcome are optimizing public awareness campaigns and GP triage along with adjusting current guidelines by enabling and focusing on immediate involvement of the EMS once acute stroke is suspected.

INTRODUCTION

In patients with acute ischemic stroke, intravenous thrombolysis (IVT) and endovascular treatment (EVT) are effective, and the clinical benefit of both reperfusion therapies is highly time-dependent.[1, 2] Therefore, it is essential to assess and treat acute stroke patients as soon as possible. Previous studies have shown that most treatment delays occur prior to hospital arrival (prehospital delay). Most studies focused on the help seeking behavior of patients in terms of recognition, interpretation and severity perception of symptoms.[3-5] Limited data are available on factors that influence the patient's choice of primary healthcare provider to be alarmed first. This, however, is important as studies have shown that delays to hospital arrival increase when other healthcare providers than the Emergency Medical Service (EMS) are initially alarmed after stroke onset.[6-10] In the Dutch healthcare system, patients can either directly call the EMS with the emergency number 112, or first alarm the General Practitioner. Guidelines state that the EMS should immediately be called if acute stroke is suspected and the public is educated to directly call 112 in case of suspected stroke by stroke awareness campaigns.[11, 12] The aim of this study was to identify factors that determine the patient's choice to enter the healthcare system. Knowledge of these factors can help to develop focused interventions to reduce vital time delays for acute stroke patients. In addition, we assess possible regional differences and patient trajectories after medical help was sought.

METHODS

Study design and population

We performed a qualitative study involving a questionnaire, carried out in three hospitals in the Netherlands including Leiden University Medical Centre (LUMC), Medisch Spectrum Twente (MST) and Isala hospital in the period between October 2018 and May 2019. All have well-established acute stroke programs including intravenous thrombolysis, endovascular treatment and a comprehensive stroke unit. The LUMC is an academic medical center situated in a densely populated urban region whereas the other centers (MST and Isala) are large non-academic teaching hospitals situated in less densely populated regions with subsequent longer travel distances to hospitals and between hospitals. The time window for treatment was <4.5 hours for IVT and <6 hours for EVT.

Patients aged 18 years or older admitted with suspected stroke, received a questionnaire during admission on the stroke unit. Patients unable to comprehend the questionnaire (i.e., due to aphasia, language barrier or cognitive impairment) could be included if a partner or relative was able to help. Patients were excluded if they were presented to the emergency department (ED) on their own initiative without first alarming the GP or the EMS. Additional clinical data were collected from hospital registries, including patient characteristics, prior ischemic stroke/Transient Ischemic Attack (TIA), time of symptom onset, time of hospital arrival, onset-to-door-time (OTD: defined as the time between symptom onset and hospital arrival), stroke severity (assessed with the National Institute of Health Stroke Scale (NIHSS) score) and discharge diagnosis.

The questionnaire aimed to assess factors that differ between patients who initially alarmed the GP as primary healthcare provider (the GP group) and those immediately alarming the EMS (the EMS group). First, a pilot study (n=57) was performed with preliminary in-depth semi-structured interviews based on the available literature. Second, experts of various disciplines (stroke neurologists and stroke research nurses) were asked for content validity prior to the start of the study. The final questionnaire includes 30 closed-ended questions on the following topics: (1) socio-demographic factors, (2) stroke symptoms and stroke knowledge, (3) external factors, (4) healthcare system trajectory and (5) emotional aspects.

Socio-demographic factors included: ethnicity and level of education. Stroke symptom knowledge was assessed by asking patients to specify Face-Arm-Speech-Time (FAST) or FAST+ symptoms (e.g. FAST symptoms with accompanying symptoms such as dizziness). External factors involved place of the event (at home or outdoors), presence of bystanders and alarming the primaryhealthcare provider during office

hours (defined as hours between 8 a.m. and 5 p.m. on weekdays). The healthcare system trajectory involves the process after patients alarmed the primary healthcare provider. The time between symptom onset and alarming a healthcare provider (patient delay) was noted. Patients could also state their extent of (dis)agreement on various proposed statements to assess emotional aspects.

In secondary analyses, we assessed if regional differences were present between the GP and the EMS group by comparing densely and less densely populated regions. In addition, we studied patient trajectories starting after alarming the primary healthcare provider by examining the referral pattern after initial alarming of the GP. Since we know that involvement of the GP is associated with delays to reperfusion therapy, we took the time window for treatment into account as well. We defined GP triage as the action undertaken by the GP that follows after the patient initially alarmed the GP. Evaluation by the GP occurs after triage and the GP is the first healthcare provider that the patients has seen after initial alarming.

Statistical Analysis

Patient characteristics are presented as mean (standard deviation, SD) or median (interquartile range [IQR]) depending on whether the variables were normally distributed. Differences between the GP and EMS groups were assessed by unpaired t-test (normal distribution) or Mann-Whitney U test (non-normal distribution) for continuous variables and Pearson X^2 test or Fisher exact test (if expected cell count is less than 5 in >20% of the cells) for categorical variables as appropriate. A p-value <0.05 was determined as statistically significant. Data were analyzed with SPSS version 25 (SPSS Inc., Chicago, IL).

RESULTS

Study population and patient characteristics

In total, 179 patients were initially included. Of these, 16 were excluded because entry into the healthcare system remained unknown (n=11) or patients had referred themselves to the emergency department (n=5). This left 163 patients for the analyses. Mean age was 69 years (±14 SD) and 94 patients (58%) were men (Table 1). Median NIHSS score was 3 [IQR 1–4] with a median onset-to-door time (OTD) of 255 minutes [IQR 90–928]. The primary healthcare provider that was alarmed first was the GP in 104 patients (64%) and the EMS in 59 patients (36%) (Figure).

Patient characteristics and socio-demographic factors

We found no differences in age, educational level and prior history of ischemic stroke/TIA or discharge diagnosis between the GP and the EMS groups (Table 1). Patients in the GP group had a lower median NIHSS score (2 [IQR 1–4]) than patients in the EMS group (3 [IQR 2–6]) and median OTD was longer in the GP group (477 [IQR 149–1586]) than in the EMS group (90 [IQR 45–286]). Forty-two of 163 ischemic stroke patients (26%) received reperfusion therapy, 18 out of these 42 (43%) patients alarmed the GP first versus 24 out of 42 (57%) that alarmed the EMS first.

Stroke symptoms and stroke knowledge

Of all 163 patients, 16 (10%) perceived FAST symptoms only and 103 (63%) perceived FAST+ symptoms without differences between the GP and EMS groups (Table 2). Prior stroke experience and knowledge of stroke symptoms did not differ between the GP and EMS groups (Table 2). The GP was alarmed first more often if patient delay was ≥15 minutes or if patients were familiar with public stroke awareness campaigns.

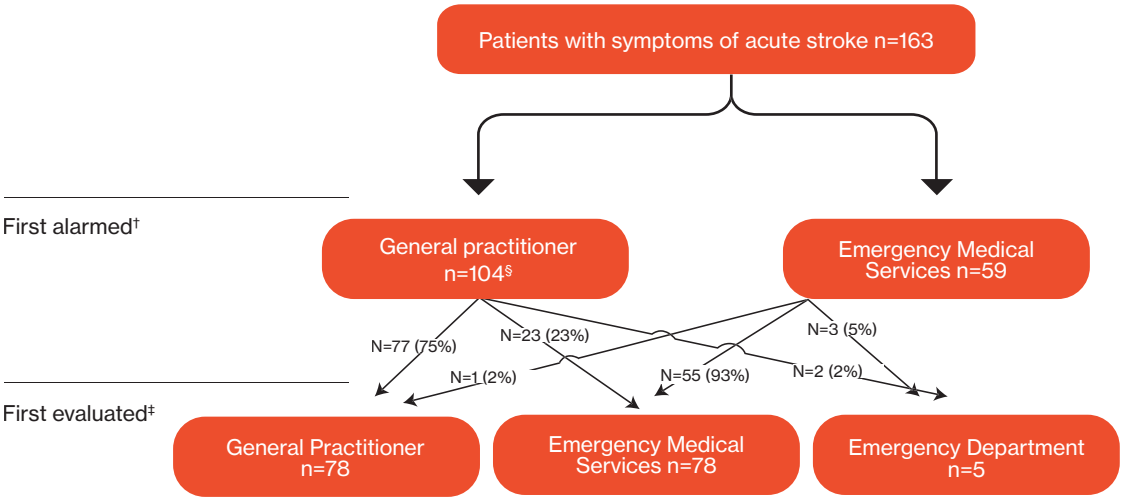

Figure 1.
Flowchart patient's choice of alarm

† First alarmed: first healthcare provider that was alarmed by the patient
‡ First evaluated: first healthcare provider that the patient has seen after initial alarming
§ No. of missing values: 2 (2%) GP patients were unable to recollect the healthcare provider by whom they were evaluated after initial alarming.

Table 1.
Patient characteristics and socio-demographic factors

	Total No. (%) *n=163* *n (column %)*	General practitioner n=104 n (row %)	Emergency medical services n=59 n (row %)	p-value
Age[†]	*69 ±14 y*	70 ±12 y	68 ±17 y	0.38
Sex				
Men	*94 (58)*	54 (57)	40 (43)	0.04
Women	*69 (42)*	50 (72)	19 (28)	
Level of Education[‡]				
Low	*65 (41)*	42 (65)	23 (35)	0.88
Medium-high	*93 (59)*	59 (63)	34 (37)	
Prior ischemic stroke /TIA[§]				
Yes	*47 (29)*	32 (68)	15 (32)	0.46
No	*116 (71)*	72 (62)	44 (38)	
Diagnosis				
Ischemic stroke	*127 (78)*	79 (62)	48 (38)	0.38
Intracerebral hemorrhage	*10 (6)*	5 (50)	5 (50)	
TIA	*10 (6)*	7 (70)	3 (30)	
Stroke mimic	*16 (10)*	13 (81)	3 (19)	
Treatment				
Reperfusion treatment	*42 (26)*	18 (43)	24 (57)	<0.01
No reperfusion treatment	*121 (74)*	86 (71)	35 (29)	
NIHSS score[†‖]	*3 [1,4]*	2 [1,4]	3 [2,6]	<0.01
OTD[†±]	*255 [90, 928]*	477 [149, 1586]	90 [45, 286]	<0.01

[†] Continuous variables are given as mean ± standard deviation (SD) if normally distributed, or median [25th percentile, 75th percentile] if non-normally distributed.

No. of missing values: [‡]: 5 (3%); [§]: 1 (1%); [‖]: 1 (1%); [±]: 23 (14%).
TIA = transient ischemic attack;
NIHSS = National Institutes of Health Stroke Scale;
OTD = onset-to-door time in minutes.

Table 2.
Stroke symptoms and stroke knowledge

	Total No. (%) n=163	General practitioner n=104	Emergency medical services n=59	p-value
	n (column %)	n (row %)	n (row %)	
Stroke symptoms				
Only FAST	*16 (10)*	13 (81)	3 (19)	0.45
FAST+	*103 (63)*	63 (61)	40 (39)	
No FAST	*32 (20)*	21(66)	11 (34)	
No FAST and No FAST+	*12 (7)*	7(58)	5 (42)	
Patient delay†				
<15 min	*51 (32)*	18(35)	33 (65)	<0.01
15 min – 1 hour	*41 (26)*	27(66)	14 (34)	
1 – 6 hours	*32 (20)*	26(81)	6 (19)	
>6 hours	*34 (22)*	30(88)	4 (12)	
Knowledge of stroke symptoms‡				
Yes	*106 (67)*	67(63)	39 (37)	0.97
No	*52 (33)*	33(63)	19 (37)	
Familiar with public stroke awareness campaigns§				
Yes	*87 (54)*	60(69)	27 (31)	0.03
No	*59 (37)*	30(51)	29 (49)	
Not Sure	*15 (9)*	12(80)	3 (20)	
Prior experience with stroke¶				
Yes	*115 (71)*	74(64)	41 (36)	0.89
No	*41 (26)*	25(61)	16 (39)	
Not Sure	*5 (3)*	3(60)	2 (40)	

No. of missing values: †: 5 (3%); ‡: 5 (3%); §: 2 (1%); ¶: 2 (1%).
FAST = face-arm-speech-time symptoms; FAST+ = comprising of FAST symptoms as well as other accompanying symptoms, such as dizziness.

Table 3.
External factors and the healthcare system

	Total No. (%) *n=163*	General practitioner n=104	Emergency medical services n=59	p-value
	n (column %)	n (row %)	n (row %)	
Presence of bystander†				
Yes	*139 (87)*	87 (63)	52 (88)	0.71
No	*21 (13)*	14 (66)	7 (12)	
Location of event				
At home	*132 (81)*	89 (67)	43 (33)	0.04
Outdoors	*31 (19)*	15 (48)	16 (52)	
Office hours event				
During office Hours	*83 (50)*	65 (78)	18 (22)	<0.01
Outside office hours	*80 (50)*	39(49)	41 (51)	

No. of missing values: †:3 (2%); ‡: 4 (2%); §: 2 (1%).

External factors

The GP was alarmed more frequently if the event occurred at home (n=89; 67%) and if the event's onset was during office hours (n=65; 78%) (Table 3).

Healthcare system trajectory

Most patients were immediately assessed (n=125; 79%), once a primary healthcare provider was alarmed.

The majority of the patients in the GP group was also evaluated by the GP before admission to the hospital (n=77; 75%) (Figure). Out of these patients, 58 (75%) reported FAST(+) symptoms versus 19 (25%) non-FAST symptoms. Of the 77 patients, 55 (71%) did not arrive in the hospital within the time window for reperfusion therapy (<6 hours) compared to 9 out of 23 (39%) patients in the GP group who were immediately referred to the EMS (data not shown). Moreover, patients from the GP group evaluated by the GP first, had slightly lower median NIHSS scores compared to patients directly referred to the EMS by the GP (median NIHSS 2 [1 –4] vs. 3 [1-4]) and these patients had a higher prevalence of non-FAST symptoms compared to the patients directly referred to the EMS by the GP (25% vs. 4%) (data not shown).

Emotional aspects

Patients who agreed with the following statements, were more frequently present in the GP group compared to the EMS group: (1) the GP knows best what I need (73% vs. 27%), (2) I rather not burden healthcare providers (80% vs. 20%) and (3) I did not perceive my symptoms serious enough to call the EMS directly (92% vs. 8%) (Table 4). Statements regarding anxiety/panic or embarrassment were similar in both groups.

Regional differences

Most patients were residents of the less densely populated region (114 out of 163; 70%).

Secondary analyses for region type showed that, in less densely populated regions, patients alarmed the gp more often than the ems (72% vs. 28%), Whereas in the densely populated region this was reversed (45% vs 55%) (data not shown).

Table 4.
Emotional aspects

	Total No. (%) *n=163*	General practitioner n=104	Emergency medical services n=59	p-value
	n (column %)	n (row %)	n (row %)	
I prefer to call my GP, he/she knows best what I need†				
Agree	*105 (66)*	77 (73)	28 (27)	<0.01
Neutral	*32 (20)*	14 (44)	18 (56)	
Disagree	*22 (14)*	10 (45)	12 (55)	
I rather not burden healthcare providers‡				
Agree	*43 (27)*	34 (80)	9 (20)	0.03
Neutral	*23 (15)*	15 (65)	8 (35)	
Disagree	*91 (58)*	51 (56)	40 (44)	
I did not perceive my symptoms serious enough to call the EMS§				
Agree	*49 (32)*	45 (92)	4 (8)	<0.01
Neutral	*24 (16)*	20 (83)	4 (17)	
Disagree	*80 (52)*	31 (39)	49 (61)	

No. of missing values: †: 4 (2%); ‡: 6 (4%); §: 9 (6%); ⁋: 9 (6%); ±: 9 (6%).
GP = general practitioner; EMS = emergency medical services.

DISCUSSION

In this study, we showed that the majority of acute stroke patients alarm the GP rather than the EMS as primary healthcare provider and patient-related factors seem to have an important contribution in the choice of healthcare provider. Patients alarming the GP more often had patient delays longer than 15 minutes, more often hesitated to burden healthcare providers or did not perceive their symptoms serious enough to call the EMS directly. These factors are potentially modifiable. Furthermore, patients in the GP group had longer onset-to-door times, a lower stroke severity score and more often had knowledge of public stroke awareness campaigns compared to patients in the EMS group.

We also showed that three-quarter of patients in the GP group were evaluated by the GP first before admission to the hospital. This could possibly result in longer OTD times and subsequent denial of access to reperfusion therapies. These observations appear to be even more pronounced in less densely populated areas, also with longer OTD times in the GP group compared with the EMS group, suggesting that education of the public and triage by the GP could be regionally and nationally improved.

Our findings are in line with previous studies that showed that involvement of the GP leads to prehospital time delays compared with alarming the EMS directly.[6-10] Our study, however, is the first to assess factors that influence the patient's choices of healthcare provider that was first alarmed, the trajectory thereafter and differences between more and less densely populated areas.

Our finding that patients more often alarm the GP first, could partially be explained by the Dutch healthcare system that might differ from other healthcare systems in that the GP has a more pivotal role as a gate keeper for hospital referrals. Patients therefore might typically seek medical advice from their GP first. Our study also showed that three-quarter of patients in the GP group was evaluated by the GP first instead of being immediately referred to the EMS. A possible explanation for this could be that these patients more often had clinical presentation of non-FAST symptoms. Therefore, these patients might not be triaged as possible acute stroke suggesting that triage by the GP could be improved, for example, by incorporating other stroke symptoms in addition to FAST symptoms (e.g. dizziness, diplopia, visual disturbances) in the current triage tools and public campaigns as well. Another explanation could be that the guidelines instructing to refer acute stroke patients immediately to the EMS might not be followed thoroughly. The guidelines further states that calling the EMS immediately is not warranted if the diagnosis of acute stroke is uncertain or if the patient is not eligibility for acute therapy and

the GP is advised to urgently visit and evaluate the patient instead. This could possibly lead to a higher threshold for GPs to immediately call an ambulance without evaluating the patient first, with subsequent time delays to treatment.

To help optimize GP triage, GPs should be able to immediately call the EMS when the slightest possibility of acute stroke is suspected in a patient without having to physically evaluate the patient first, this should be adopted into current guidelines to improve rapid hospital arrival of these patients and to minimize time delays that are now caused by the physical evaluation by the GP before the EMS is called. Consistent with a review,[3] a previous stroke experience or stroke knowledge did not lead to alarming the EMS more frequently. Another interesting finding is that 54% of the patients were familiar with public stroke awareness campaigns that advices to call the EMS directly. Despite this knowledge, patients still alarmed the GP first. A previous review showed that public campaigns to raise stroke awareness had limited impact on behavior, however, reasons for these findings were not studied.[13] Thus, it seems that other factors (such as perceived severity of symptoms) are deemed more important by patients than stroke knowledge.

Our study has some limitations. First, we could not include all consecutive patients admitted to the hospital for practical reasons. Second, we excluded patients unable to comprehend the questionnaire resulting in a patient group with relatively mild strokes. Therefore, it is unclear whether our results are generalizable to very severe strokes. Nevertheless, since more severe strokes generally lead to shorter pre-hospital delays, we think that insights in delays are particularly relevant in mild to moderate severe strokes.[3, 8] Therefore, patients with milder strokes are precisely the population who can benefit most from our findings. Moreover, prior studies found that more than half of the acute ischemic stroke patients had a mild stroke (i.e., NIHSS ≤3) at hospital presentation, this is similar to our study. Therefore, our study resembles daily practice, which makes our study relevant[14] Third, we could not entirely rule out that some patients with non-FAST symptoms tried to call 112 but were subsequently referred to their GP. However, we do not believe that this occurred often since these patients were admitted on the stroke unit, which indicates that their symptoms were severe enough to justify hospital admission and calling 112 also indicates a higher sense of urgency, therefore it is less likely that patients were referred to their GP once the call to 112 has been made. Fourth, although questionnaires were taken at a median of 1 day after admission, we cannot rule out that some patients already received stroke education. Finally, due to the retrospective design, patients might have recalled events less accurately. To minimize this, patients were always included within seven days after stroke.

CONCLUSION

Our results shows that most patients first alarm the GP instead of the EMS and patient related factors are of influence on the choice of healthcare provider. Patients alarming the GP have a threshold to burden EMS or underestimate their symptoms. Thus, significant and relevant gains can still be achieved by employing strategies to reduce prehospital delays, including optimization of public awareness campaigns, GP triage and adjusting current guidelines by enabling and focusing on immediate involvement of the EMS once acute stroke is suspected.

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Part II

PREHOSPITAL TRIAGE

Chapter 3

Clinical prediction of thrombectomy eligibility: A systematic review and 4-item decision tree

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ABSTRACT

Background and purpose

A clinical large anterior vessel occlusion (LAVO)-prediction scale could reduce treatment delays by allocating endovascular treatment (EVT)-eligible patients directly to a comprehensive stroke center. The study aim was to subtract, validate and compare existing LAVO-prediction scales, and develop a straightforward decision support tool to assess EVT-eligibility.

Methods

We performed a systematic literature search to identify LAVO-prediction scales. Performance was compared in a prospective, multicenter validation cohort of the Dutch acute Stroke study (DUST) by calculating area under the receiver operating curves (AUROC). With group lasso regression analysis, we constructed a prediction model, incorporating patient characteristics next to National Institutes of Health Stroke Scale (NIHSS) items. Finally, we developed a decision tree algorithm based on dichotomized NIHSS items.

Results

We identified seven LAVO-prediction scales. From DUST, 1316 patients (35.8% LAVO-rate) from 14 centers were available for validation. FAST-ED and RACE had the highest AUROC (both >0.81, $p<0.01$ for comparison with other scales). Group lasso analysis revealed a LAVO-prediction model containing seven NIHSS items (AUROC 0.84). With the GACE (Gaze, facial Asymmetry, level of Consciousness, Extinction/inattention) decision tree, LAVO is predicted (AUROC 0.76) for 61% of patients with assessment of only two dichotomized NIHSS items, and for all patients with four items.

Conclusion

External validation of seven LAVO-prediction scales showed AUROCs between 0.75 and 0.83. Most scales, however, appear too complex for Emergency Medical Services use with prehospital validation generally lacking. GACE is the first LAVO-prediction scale using a simple decision tree as such increasing feasibility, while maintaining high accuracy. Prehospital prospective validation is planned.

INTRODUCTION

Time is the most crucial factor limiting clinical effectiveness of endovascular treatment (EVT) in stroke due to large anterior vessel occlusion (LAVO).[1,2] With every minute of delay, 4.2 days of disability-free life are lost, and chances of undergoing EVT are reduced by 2.5%.[3,4] A clinical scale to identify LAVO in the prehospital Emergency Medical Services (EMS) setting could reduce treatment delays by allocating EVT-eligible patients directly to a comprehensive stroke center (CSC).[5,6] Ideally, such a scale should be straightforward, widely applicable, have high interrater reliability and high accuracy in terms of LAVO-prediction.[7] Various scales have been designed, but it is unclear which performs best in clinical practice.[8-14] The National Institutes of Health Stroke Score (NIHSS) retains the highest overall accuracy predicting LAVO,[15,16] but is too extensive for EMS personnel. The Face-arm-speech-time (FAST) score is widely used by EMS personnel but was primarily developed to distinguish stroke from non-stroke rather than stroke subtype.[17,18]

LAVO-prediction scales were compared before, but never systematically, and in different datasets with radiological endpoints not reflecting current clinical practice.[14,19,20]

Patient characteristics may improve a LAVO prediction model but were not included in previous scales.[21,22]

We aimed to (i) systematically identify published LAVO-prediction scales designed for prehospital use, (ii) assess these scales in terms of feasibility, (iii) assess predictive value in a large, multicenter, prospective dataset with EVT-eligible LAVO as a well-defined radiological outcome measure, (iv) compare these scales to NIHSS and FAST, and finally, (v) develop a prediction model assessing both NIHSS items and patient characteristics associated with LAVO.

METHODS

A computerized literature search was performed in the following databases: MEDLINE, EMBASE, EMCARE and Web of Science from October 1991 to June 2017 using the following search terms: "stroke," "cerebrovascular accident," "scales," "scores," "large vessel occlusion," "large artery occlusion," "Emergency Medical Services," "prehospital" and "triage." Two reviewers (GTK and TTMN) independently screened titles and abstracts for eligibility. Full-text versions were obtained from all studies that were considered to be potentially relevant by one or both reviewers. After a first selection, bibliographies of all relevant studies were searched manually for additional studies and this method of crosschecking was continued until no further publications were found. Authors of relevant articles were contacted for supplementary information.

Cohort studies were reviewed with the STROBE (Strengthening the Reporting of OBservational studies in Epidemiology) statement and had to comply with the following inclusion criteria: (1) original data report on an inception cohort or a clinical trial; (2) a clinical score had to be assessed within 6 hours from stroke onset; (3) it had to be clear from the paper at what moment and by whom (e.g. EMS personnel, neurologist) a clinical score was assessed; (4) assessment of LAVO had to be done with either CT angiography (CTA), magnetic resonance angiography, or digital subtraction angiography; (5) data available on the performance of clinical score(s) used had to be expressed as: area under the receiver operating characteristics curve (AUROC), sensitivity/specificity or likelihood ratio, and (6) the clinical score had to be retrievable from NIHSS. Because studies had to fulfill these strict inclusion criteria, no further formal quality assessment was undertaken.

We estimated and/or retrieved the following characteristics from identified studies: feasibility for EMS use, interrater reliability and external validity (i.e., applicability to the unselected population of suspected acute stroke patients).

Validation cohort

To assess validity, we used the Dutch acute Stroke study (DUST) cohort.[23] DUST is a multicenter, prospective, observational cohort study conducted in six university and eight non-university hospitals in the Netherlands. From May 2009 to August 2013, consecutive patients >18 years presented at the emergency department with a suspicion of acute (<9 h) ischemic stroke (based on clinical assessment and non-contrast CT (NCCT) imaging) and NIHSS >1 and/or considered eligible for intravenous thrombolysis (IVT) were included. All patients received CTA within 9 hours after symptom onset as part of the CT stroke workup including NCCT, CT perfusion and CTA. The DUST imaging protocol has been described before.[24]

ANALYSIS

Patients with CTA yielding insufficient diagnostic quality to assess LAVO were excluded. We defined LAVO according to current EVT-eligible criteria: proximal middle cerebral artery (MCA: M1- and/or M2-segment), proximal anterior cerebral artery (ACA: A1- and/or A2-segment), intracranial carotid artery (ICA) or tandem (ICA plus MCA) occlusion.[25] Patients with incomplete admission NIHSS were excluded from analyses related to validation of existing scales, since NIHSS was required to reconstruct these.

Descriptive statistics were used to determine baseline characteristics of the validation cohort. Categorical variables were compared with the X^2 test and presented as number (percentage). Continuous variables are compared using the t test or Mann–Whitney U test and are presented as mean ± standard deviation or median (interquartile range, IQR) if appropriate. To assess predictive value, we computed AUROC and respective 95% confidence intervals (CIs) per identified LAVO-prediction scale, and for the NIHSS and FAST score.

Having data from 14 sites participating in DUST, we performed external validation by excluding one site at a time (cross-validation) for every scale. This is an important advantage, because external validation gives a better indication of the generalization error. We performed all pairwise comparisons of the cross-validated AUROCs of the various scales using the DeLong's test.[26]

For the development of a new prediction model, we did not exclude patients with NIHSS items that could not be assessed since this reflects clinical practice. In addition, we introduced (combinations of) patient characteristics into the model that we considered to be predictive of LAVO provided that these also differed on baseline between LAVO and non-LAVO patients. These include: history of atrial fibrillation (AF), AF without the use of anticoagulation, and AF without diabetes mellitus and/or hyperlipidemia.

Group lasso regression analysis was used to reveal (a combination of) NIHSS items and patient characteristics yielding the highest predictive value for LAVO.[27] The lasso is a popular method for penalized regression and classification that also performs variable selection.[28] The group lasso is a variant where the user can specify groups of variables (e.g. all variables within one NIHSS item) that are either all in or all out of the model.[29] We used the R package "grpreg" with default settings to fit the group lasso.[30]

In addition, a decision tree algorithm and diagram based on dichotomized NIHSS items ((1) 'yes/present/abnormal', or (0) 'no/absent/normal') was developed. A decision tree works by consecutively assessing

the item with the highest predictive value in the (remaining) cohort, as such leading to a minimum number of items to be assessed to reach an outcome (i.e., LAVO or non-LAVO), with the highest possible predictive value. Cross-validation (as described before) will determine the number of knots in the decision tree. The decision tree was fitted using the R-package "rpart" using default settings. In particular, this means that the default priors are proportional to the data counts, the losses default to 1, and the split defaults to the Gini index.

Statistical analysis was performed using SPSS software (version 23, IBM, New York, USA), and R software (version 3.4.1).

RESULTS

Systematic literature search

The MEDLINE search yielded 185 citations, the EMBASE search 263 citations, the EMCARE search 58 citations, and the Web of Science search 163 citations. After removal of duplicates, 522 records remained; 446 records were excluded based on title and abstract; 28 additional relevant studies were found by searching the bibliographies. Screening reference lists and a search of the Science Citation Index yielded 12 additional studies. One-hundred-and-sixteen citations remained for full text assessment. A total of seven clinical scales meeting pre-defined criteria were identified (see Figure 1). Clinical scale characteristics and methods of validation are shown in Supplementary Material I. Except for the RACE scale, all validations were performed retrospectively and/or in-hospital and validation cohorts ranged between 62 and 3505 patients. Generally, patients with intracerebral hemorrhage (ICH) were excluded. Definition of LAVO varied substantially between studies (ranging from 'MCA occlusion' to 'anterior or posterior circulation occlusion'), and LAVO-rate ranged between 21 and 73%. .

Validation cohort

A total of 1393 patients were included in DUST. Of these, 59 (4%) were excluded because of incomplete NIHSS and 18 (1%) because CTA was of insufficient diagnostic quality to assess LAVO. This left 1316 patients for analysis.

LAVO was present in 471 patients (35.8%). Demographic details of the validation cohort, stratified by presence of LAVO, are presented in Table 1. LAVO-patients were similar in age and sex compared to non-LAVO patients. AF was more prevalent in LAVO-patients, whereas other cardiovascular risk factors (previous stroke, hyperlipidemia) and antiplatelet therapy were more prevalent in non-LAVO patients. LAVO-patients had higher baseline NIHSS compared to non-LAVO patients; NIHSS 12 [IQR 7–17] versus NIHSS 4 [2–7] were more frequently treated with IVT, and onset-to-needle time was shorter; 97 [72–140] min in LAVO-patients versus 115 [85–170] in non-LAVO patients. Median systolic blood pressure was lower in LAVO-patients: 150 mmHg [133–167] versus 157 mmHg [140–180] in non-LAVO patients.

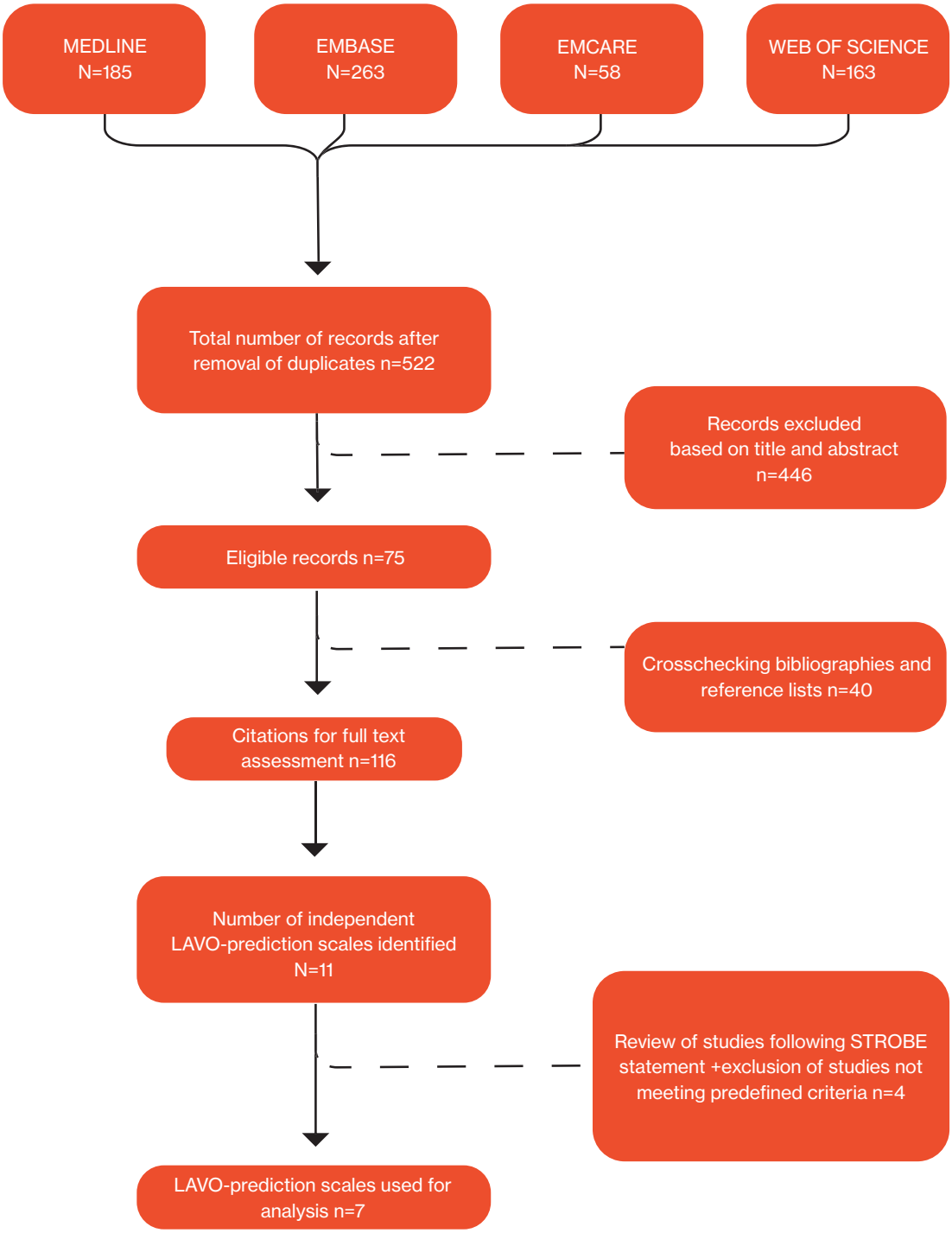

Figure 1.
Flowchart systematic literature search

LAVO: large anterior vessel occlusion; STROBE: strengthening the reporting of observational studies in epidemiology.

Table 1.
Baseline characteristics of DUST validation cohort

	Total patients n=1316	LAVO n=471 (36%)	non-LAVO n=845 (64%)	p-value
Demographics				
Age (years)	69 [57-78]	68 [55-77]	69 [58-78]	0.14
Male sex	752 (57)	264 (56)	488 (58)	0.55
Medical history				
Atrial fibrillation, 18 missings,	168 (13)	72 (15)	96 (11)	0.04
Atrial fibrillation without anticoagulation, 27 missings	88 (7)	44 (9)	44 (5)	<0.01
Diabetes mellitus, 11 missings	198 (15)	59 (13)	139 (16)	0.05
Previous stroke, 12 missings	316 (24)	82 (17)	234 (28)	<0.01
Hypertension, 16 missings	680 (52)	238 (51)	442 (52)	0.43
Hyperlipidemia, 41 missings	433 (33)	135 (29)	298 (35)	0.01
Coronary artery disease, 42 missings	242 (18)	79 (17)	163 (19)	0.48
Medication on admission				
Anticoagulation, 9 missings	154 (12)	49 (10)	105 (12)	0.54
Antiplatelet therapy, 10 missings	443 (34)	141 (30)	302 (36)	0.07
Clinical parameters on admission				
Systolic blood pressure (mmHg), 11 missings	154 [138-177]	150 [133-167]	157 [140-180]	<0.01
Diastolic blood pressure (mmHg), 11 missings	85 [75-95]	82 [72-96]	85 [75-95]	0.02
Glucose (mmol/L), 20 missings	6.6 [5.8-8.1]	6.6 [5.9-7.8]	6.6 [5.7-8.1]	0.43
NIHSS at admission	6 [3-12]	12 [7-17]	4 [2-7]	<0.01
Reperfusion therapy				
Intravenous thrombolysis	815 (62)	331 (70)	484 (57)	<0.01

Values are expressed as median [interquartile range] for continuous variables unless stated otherwise and as absolute counts (percentage) for categorical variables. LAVO: large anterior vessel occlusion; NIHSS: National Institutes of Health Stroke Scale.

Comparison of clinical scales

The FAST-ED (AUROC 0.83, 95% CI 0.80–0.85), RACE scale (AUROC 0.82, 95% CI 0.79–0.84) and NIHSS (AUROC 0.81, 95% CI 0.79–0.84) showed the highest AUROC for detecting LAVO in comparison with other scales (p<0.01). FAST-ED showed a comparable AUROC to RACE but a significantly higher AUROC than NIHSS (p<0.01). The FAST score showed the lowest specificity, and the 3I-SS scale showed the lowest sensitivity (see Figure 2 and Table 2).

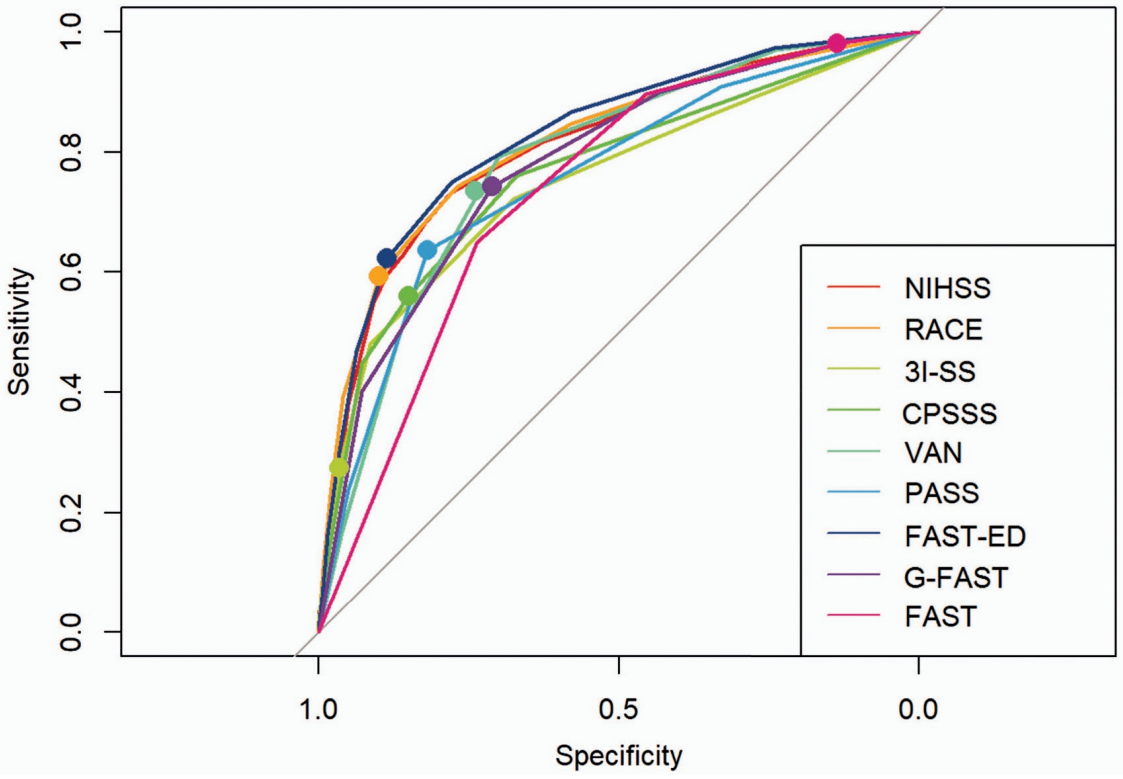

Figure 2.
Receiver operating characteristics (ROC) curves of identified LAVO-prediction scales, and the NIHSS and FAST score.

For every LAVO-prediction scale, the marked point in the ROC indicates the combination of sensitivity and specificity at the original authors' recommended cut-off point. 3I-SS: 3-item stroke scale; CPSSS: Cincinnati prehospital stroke severity scale; FAST: Face-arm-speech-time; FAST-ED: Face-arm-speech-time-eye deviation-denial/neglect; G-FAST: Gaze-face-arm-speech-time; NIHSS: National institutes of health stroke scale; PASS: Prehospital acute stroke severity; RACE: Rapid arterial occlusion evaluation; VAN: Vision aphasia neglect.

Table 2. AUROCs and respective 95%-CIs with corresponding p-values comparing identified LAVO-prediction scales, NIHSS and FAST

Clinical Scale	AUC (95% CI)	FAST	3I-SS	PASS	CPSSS	G-FAST	VAN	NIHSS	RACE	FAST-ED
FAST	0.74 (0.71-0.76)	X								
31-SS	0.75 (0.72-0.78)	0.25	X							
PASS	0.76 (0.73-0.78)	0.10	0.55	X						
CPSSS	0.76 (0.74-0.79)	0.04	0.08	0.31	X					
G-FAST	0.78 (0.76-0.81)	<0.01	<0.01	<0.01	0.12	X				
VAN	0.78 (0.76-0.81)	<0.01	<0.01	<0.01	0.09	0.79	X			
NIHSS	0.81 (0.79-0.84)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	X		
RACE	0.82 (0.79-0.84)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.34	X	
FAST-ED	0.83 (0.80-0.85)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.17	X

3I-SS: 3-item stroke scale; CPSSS: Cincinnati prehospital stroke severity scale; FAST: Face-arm-speech-time; FAST-ED: Face-arm-speech-time-eye deviation-denial/neglect; G-FAST: Gaze-face-arm-speech-time; NIHSS: National institutes of health stroke scale; PASS: Prehospital acute stroke severity; RACE: Rapid arterial occlusion evaluation; VAN: Vision aphasia neglect.

Group lasso LAVO-prediction model

Group lasso analysis showed a prediction model containing a combination of the following NIHSS items (AUROC 0.84, 95% CI 0.81–0.87): (1) level of consciousness (LOC) questions, (2) gaze, (3) visual fields, (4) facial asymmetry, (5) arm motor function, (6) aphasia, and (7) extinction/ inattention. Whereas AF was more prevalent in LAVO-patients, it did not contribute to the prediction model as a separate variable or in combination with other patient characteristics as outlined before.

Decision tree

Figure 3 displays the GACE (Gaze, facial Asymmetry, level of Consciousness, Extinction/inattention) decision tree. The GACE decision tree enables prediction of LAVO by assessment of a maximum of only four predefined dichotomized NIHSS items with an AUROC of 0.76 (95% CI 0.68–0.83). 'Gaze', the item with the highest predictive value for LAVO in our cohort, is the first item to be assessed. For both the group of patients with an abnormal gaze (27%, left side of the diagram) as for the group of patients with a normal gaze (73%, right side of diagram), the following item with the highest predictive value for LAVO is determined. For both subgroups, this item is 'facial asymmetry'. Assessment of this second item leads directly to an outcome (i.e., LAVO or non-LAVO) in 61% of all patients (scoring (a) gaze 'yes' plus facial asymmetry 'yes', (b) gaze 'yes' plus facial asymmetry 'no', or (c) gaze 'no' plus facial asymmetry 'no'). Only for the remaining 39% of patients, the full 4-item decision tree (adding 'LOC questions', followed by 'LOC commands' or 'extinction/ inattention' has to be completed (see Figure 3 and Table 3)

Table 3. Number of patients (%) reaching a LAVO/ non-LAVO outcome per number of completed items within GACE

Number of completed Items	Total patients (n=1370)
2 items *ie (i)gaze, (ii) Facial asymmetry*	830 (60.6%)
4 items *ie (i)gaze, (ii) Facial asymmetry (iii) LOC questions,* *(iv) LOC commands or extinction/inattention*[a]	540 (39.4%)

Abbreviations: LOC = level of consciousness
[a] *dependent on result of assessment of item*

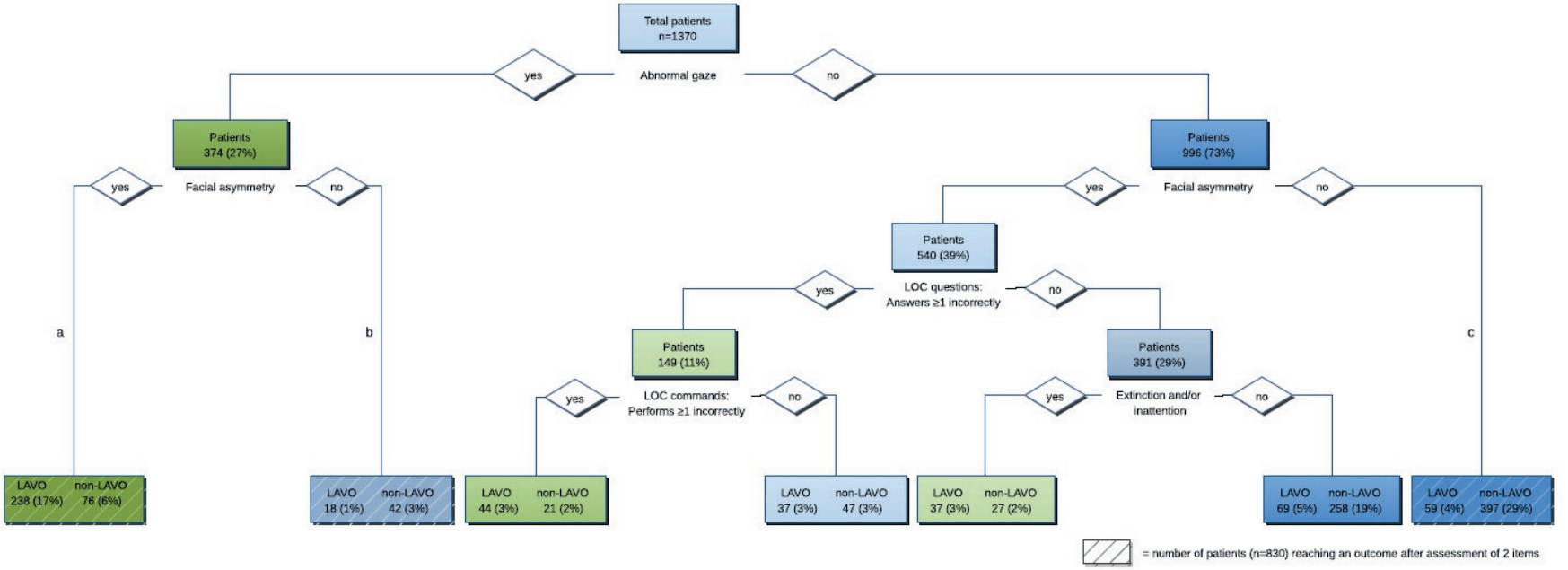

Figure 3.
GACE decision tree diagram based on dichotomized NIHSS items (assessed in DUST)

Numbers on the left side of each bottom box indicate the number of patients (percentage) with a LAVO outcome, whereas numbers on the right side of each bottom box indicate the number of patients (percentage) with a non-LAVO outcome. The color of each box indicates the ratio of LAVO (green) and non-LAVO patients (blue): the higher intensity of the color, the higher the ratio. 830 patients (61%, group a, b and c) reach an outcome (i.e. LAVO or non-LAVO) after assessment of only 2 items. LAVO: large anterior vessel occlusion; LOC: level of consciousness.

DISCUSSION

Our systematic search revealed seven LAVO-prediction scales designed for use in the prehospital phase. However, the majority was retrospectively validated in (small) monocenter cohorts in an in-hospital setting, making it difficult to determine which scale outperforms the other in prehospital clinical practice.

In our large multicenter validation cohort, we found that FAST-ED and RACE had the highest AUROC for prediction of LAVO. A seemingly important advantage of RACE over FAST-ED is that it was validated in the prehospital setting. Nevertheless, RACE appears too complex for prehospital EMS use, comprising a 5-item, 9-point assessment in which the decision to use or omit certain scale items (i.e. agnosia, aphasia) depends on the assumed involved hemisphere.[9] Indeed, during the validation phase, the scale was not performed in 40% of suspected stroke patients.[9] Although FAST-ED is based on the widely used FAST score and outperforms the NIHSS for prediction of LAVO in our database, it has potential drawbacks as it (a) imposes scoring a 'partial', 'mild' or 'moderate' deficit in most items, hampering interrater reliability; and (b) uses complex items (e.g. extinction/inattention) which are difficult for EMS personnel to assess.[13,31]

G-FAST seems more feasible for EMS use. However, in the original G-FAST study (i) vessel imaging modality to detect LAVO is unclear, and more importantly, (ii) definition of LAVO does not meet current clinical EVT-criteria (excluding ACA and M2 occlusion).[14]

In our cohort, as expected, AF was more common in LAVO-patients. Although neither this nor other patient characteristics improved the group lasso model, the model including seven NIHSS-items had a higher AUROC (0.84) than the scales derived from the literature.

Despite significant differences in performance of the scales, it should be pointed out that many of these differences are associated with small absolute differences in AUROC (i.e., 0.02). We accepted a small reduction in AUROC for the GACE decision tree (compared with the group lasso model), as we estimate that the prehospital feasibility is high since EMS personnel only need to take two steps to rule out transportation to a CSC for a substantial proportion of patients (61%, see Table 3), and only four for the remainder.

From a clinical perspective, it seems remarkable that facial asymmetry is such an important scoring item for GACE since it appears to have little localizing value.[32] It is important, however, to bear in mind that it is not this separate item, but the combination with gaze assessment that leads to a high predictive value for LAVO in our cohort.

In addition to LAVO prediction, allocation decision also depends on

(1) the impact of delay on clinical efficacy of both IVT and EVT, (2) patient characteristics (e.g. medical history, time from symptom onset, course of the disease) and (3) logistic factors (e.g. urban/rural area, number of comprehensive and primary stroke centers (PSC) and distance to scene of stroke, interhospital distance, in-hospital door-to-needle and door-to-groin times).[33] Therefore, we chose to display ROCs, enabling determination of a clinically relevant cut-off point considering local circumstances.

Moreover, allocation decision highly depends on what kind of error one is willing to allow: (a) having more patients come to a CSC accepting that some of these may not have LAVO and incorrectly bypass a PSC delaying IVT (false-positives); or (b) being focused on only allocating LAVO-patients to a CSC accepting that some LAVO-patients will primarily be transported to a PSC without EVT-facilities (false-negatives).

For example, a 75-year-old patient presenting with a partial gaze palsy, facial asymmetry, dysarthria and moderate left hemiparesis is assessed by EMS personnel 2.5 hours after symptom onset. Scores for this patient on the best performing LAVO-prediction scales in our validation cohort are: RACE 4/9, FAST-ED 2/13 and G-FAST 4/4. When applying the original authors' cut-off point, the patient has a moderate to high chance of LAVO, advising direct transport to a CSC with G-FAST, and a transport to the nearest PSC with RACE and FAST-ED. These scales, however, do not take local circumstances into account.

Consider that a PSC is located 10 min and a CSC 20 min from scene of stroke (with equal door-to-needle times). Bypassing the PSC is associated with a 10 min delay to IVT but a more substantial time delay to EVT is avoided by preventing inter-hospital transfer. Keeping in mind that IVT has limited efficacy in LAVO-patients,[25,34-39] a scale with a high sensitivity (such as G-FAST) seems the more desirable for this specific situation.

However, when transport time to a PSC is only 10 min and to a CSC is 50 min, a scale with a high sensitivity is less desirable. Most patients (including false-positives) will then be transported to the CSC with a more substantial time delay to IVT (40 min) for non-LAVO patients and, in addition, overloading the CSC with a large volume of patients. Therefore, in this situation, a scale with a high specificity (such as RACE or FASTED) would probably be the more desired choice.

Overall, time is brain, but since LAVO-patients appear to clinically benefit more substantially from earlier EVT than overall stroke patients benefit from earlier IVT,[3,40] a moderate to high likelihood of LAVO seems to allow a fair time delay to IVT. How much delay exactly, however, remains complex, as logistics (which are dynamic as resources shift over time) determine the amount of accepted time delay at expense of the number of false-positively referred patients. To what extent implementation of a clinical LAVO-prediction scale affects local logistics and health care-related costs must be estimated,

since no formal cost-effectiveness analysis was performed.

Our study has several limitations. First, our study was performed retrospectively which could have led to selection bias. Data, however, were collected prospectively minimizing such an effect. Second, our cohort does not represent an unselected prehospital cohort. For example, ICHs were not included, leading to an artificially high prevalence of LAVO and IVT-treated patients, which could result in an overestimation of the prediction scales. To what extent this influences a decision to use a scale depends very much on local circumstances since ICHs are often concentrated in CSCs.

Of note, the retrospective nature and lack of an unselected cohort account for all LAVO-prediction scales included in our analysis and therefore do not diminish validity of between scale comparisons.

Finally, clinical scale assessment was performed in the in-hospital setting, rendering translation to the prehospital setting limited. Indeed, prospective validation in this setting is much warranted and our results should primarily be considered an important step towards a large prehospital prospective validation study which we planned to embed in the ongoing 'A Reduction in Time with Electronic Monitoring in Stroke' (ARTEMIS) trial conducted within three EMS regions, which allows patients to be electronically tracked from the first moment the dispatch office is alarmed up until start of reperfusion therapy (clinicaltrials.gov identifier: NCT02808806).[41]

Nevertheless, clinical LAVO-detection could also be very helpful in order to optimize in-hospital logistics of potential EVT-eligible patients (e.g. pre-notification of neuro-interventional team and preparation of the angio suite can reduce door-to-groin times).[42]

CONCLUSION

We identified seven LAVO-prediction scales of which FAST-ED and RACE performed best and comparable to the NIHSS. An important limitation remains; however, that prospective validation in the prehospital EMS setting is lacking.

We developed a practical and easy-to-use decision tree that utilizes only two dichotomized NIHSS items for LAVO prediction for 61% of patients, and four items for the remaining patients in our cohort. Prospective validation of GACE in the prehospital setting is planned.

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SUPPLEMENTAL

Supplemental 1.
LAVO-prediction scales characteristics

	RACE[43]	3I-SS[8]	CPSSS[1]	VAN[44]	PASS[12]	FAST-ED[45]	G-FAST[14]
Clinical scale							
Scale items	facial asymmetry, arm motor function, leg motor function, gaze, aphasia or agnosia	LOC, gaze, 'hemiparesis'	gaze, LOC questions, LOC commands, arm motor function	arm motor function, 'visual disturbance', aphasia, gaze, extinction/ inattention	LOC questions, gaze, arm motor function	facial asymmetry, arm motor function, aphasia, gaze, extinction/ inattention	gaze, facial asymmetry, arm motor function, aphasia
Scouring range (points)	0-9	0-6	0-4	N/A	0-3	0-13	0-4
Cut-off point	RACE ≥ 5	31-SS ≥ 4	CPSSS ≥2	VAN-positive	PASS ≥2	FAST-ED≥4	G-FAST≥3
Validation cohort							
n	357	83	303	62	1057	727	3505
Multicenter	No	No	Yes	No	Yes	Yes	Yes
Suspected stroke patients potentially eligible for reperfusion therapy	Yes	Yes	Yes	Yes	Yes	Yes	Yes
NIHSS at admission, median [IQR]	8 [3-16]	N/S	>8[N/S]	N/S	N/S	5 [2-12]	9 [6-16]
Reperfusion therapy, % (IVT/EVT)	N/S	N/S	100(IVT)	N/S	100 (IVT)	N/S	100(N/s)

	RACE[43]	3I-SS[8]	CPSSS[1]	VAN[44]	PASS[12]	FAST-ED[45]	G-FAST[14]
Final diagnosis							
Ischemic stroke, n (%)	240 (67.2)	N/S	303 (100)	N/S	N/S	N/S	N/S
TIA, n (%)	20 (5.6)	N/S	Excluded	N/S	N/S	N/S	N/S
ICH, n (%)	52 (14.6)	Excluded	Excluded	N/S	N/S	Excluded	Excluded
Stroke mimic, n (%)	45 (12.6)	N/S	Excluded	N/S	N/S	N/S	N/S
Setting							
Study Design	Prospective	Prospective	retro-spective	Prospective	retro-spective	retro-spective	retro-spective
Setting	Prehospital	in-hospital	in-hospital	in-hospital	in-hospital	in-hospital	in-hospital
Raters	EMS - Personel	Neurolo-gists	physicians	ER nurses	N/S	research personnel	N/S
	N/S	0.95	N/A	1.00	N/A	N/A	N/A
Outcome							
LAVO definition (site of occlusion)	ICA, M1, tandem, basilar artery	MCA	ICA, M1, tandem, basilar artery	ICA, M1, M2	anterior or posterior circulation	ICA, M1, M2 basilar artery	ICA, M1
Imaging modality	trans-cranial duplex, CTA, MRA	MRA	CTA	CTA	CTA, MRA	CTA	i.a. CTA, MRA
Vessel imaging done be-fore IVT	N/S	N/S	Yes	No	Yes	N/S	Yes

	RACE[43]	3I-SS[8]	CPSSS[1]	VAN[44]	PASS[12]	FAST-ED[45]	G-FAST[14]
LAVO rate n (%)	76 (21.3)	27 (32.5)	222(73.3)	14 (22.6)	N/S	240 (33)	827 (23.6)
Performance							
Sensitivity	0.85	0.67	0.83	1.00	0.61	0.60	0.89
Specificity	0.68	0.92	0.40	0.90	0.83	0.89	0.39
accuracy	0.72	0.86	N/S	0.92	N/S	0.79	0.51
AUC (95% CI)	0.82 (0.77-0.87)	N/S	0.67 (N/S)	N/s	0.72	0.81 (N/S)	0.72 (0.71-0.74)

Characteristics of identified LAVO-prediction scales. Abbreviations: 3I-SS = 3-Item Stroke Scale; CPSSS = Cincinnati Prehospital Stroke Severity Scale; CTA = CT angiography; EMS = Emergency Medical Services; ER = Emergency Room; FAST-ED = Face-Arm-Speech-Time-Eye deviation-Denial/neglect; G-FAST = Gaze-Face-Arm-Speech-Time; ICA = intracranial carotid artery; ICH = intracerebral haemorrhage; IVT = intravenous thrombolysis; EVT = endovascular treatment; LAVO = large anterior vessel occlusion; LOC = level of consciousness; MCA = middle cerebral artery; MRA = magnetic resonance angiography; NIHSS = National Institutes of Health Stroke Scale; PASS = Prehospital Acute Stroke Severity; RACE = Rapid Arterial Occlusion Evaluation; VAN = Vision Aphasia Neglect a as defined by original authors b based on cut-off point, as defined by original authors

Supplemental 2.
DUST investigators

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Chapter 4

Comparison of prehospital scales for predicting large anterior vessel occlusion in the ambulance setting

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ABSTRACT

Background and purpose:

The efficacy of endovascular treatment (EVT) for symptomatic large anterior vessel occlusion (sLAVO) sharply decreases with time. Because EVT is restricted to comprehensive stroke centers, prehospital triage of patients with acute stroke codes for sLAVO is crucial, and although several prediction scales are already in use, external validation, head-to-head comparison, and feasibility data are lacking. Study objectives were to conduct external validation and head-to-head comparison of seven sLAVO prediction scales in the Emergency Medical Service (EMS) setting and to assess scale feasibility by EMS paramedics.

Methods

This prospective cohort study was conducted between July 2018-October 2019 in a large urban center in the Netherlands with a population of approximately two million people and included two EMSs, three comprehensive stroke centers, and four primary stroke centers. Participants were consecutive patients aged 18 years or older for whom an EMS-initiated acute stroke code was activated. Of 2812 acute stroke codes, 805 (28.6%) were excluded, because no application was used or no clinical data were available, leaving 2007 patients included in the analyses. Applications with clinical observations filled in by EMS paramedics for each acute stroke code enabling reconstruction of the following seven prediction scales: Los Angeles Motor Scale (LAMS), Rapid Arterial Occlusion Evaluation (RACE), Cincinnati Stroke Triage Assessment Tool, Prehospital Acute Stroke Severity (PASS), gaze-face-arm-speech-time, Field Assessment Stroke Triage for Emergency Destination; and gaze, facial asymmetry, level of consciousness, extinction/inattention. Planned primary and secondary outcomes were sLAVO and feasibility rates (i.e., the proportion of acute stroke codes for which the prehospital scale could be reconstructed). Predictive performance measures included accuracy, sensitivity, specificity, the Youden index, and predictive values.

Results

Of 2007 patients who received acute stroke codes (mean [SD] age was 71.1 [14.9] years; 1021 were men [50.9%]), 158 (7.9%) had sLAVO. Accuracy of the scales ranged from 0.79–0.89, with LAMS and RACE scales yielding the highest scores. Sensitivity of the scales ranged

from 38%–62%, and specificity from 80%–93%. Scale feasibility rates ranged from 78% to 88%, with the highest rate for the PASS scale.

Conclusion

This study found that all seven prediction scales had good accuracy, high specificity and low sensitivity, with LAMS and the RACE being the highest scoring scales. Feasibility rates ranged between 78–88% and should be taken into account before implementing a scale.

INTRODUCTION

In acute ischemic stroke, clinical efficacy of intravenous thrombolysis (IVT) and endovascular treatment (EVT) is highly time dependent.[1-3] Endovascular thrombectomy can only be given to a subset of patients with a symptomatic large anterior vessel occlusion (sLAVO), constituting 4.9% to 14.5% of all patients with suspected stroke.[4, 5] Contrary to IVT, which is available in most primary stroke centers (PSCs), EVT is an elaborate treatment and, therefore, restricted to comprehensive stroke centers (CSCs) with EVT facilities. Because sLAVO cannot be reliably identified in the ambulance, patients suspected of acute stroke are often transferred to the nearest hospital (often a PSC) to start IVT as soon as possible. For patients with sLAVO, this routing leads to a median of 60 to 109 minutes' delay due to interhospital transfers, with associated worse functional outcomes.[6, 7] Prehospital identification of patients with sLAVO enabling direct allocation to a CSC would greatly reduce delays to EVT and improve clinical outcomes. Several clinical prediction scales have been developed with this purpose; however, most scales were validated only in the hospital and not in the field (i.e., prehospital by emergency medical service [EMS] paramedics), and external validation is often lacking.[8, 9] Moreover, to decide which scale is preferred, head-to-head comparison in the field is required, which is currently lacking. Finally, feasibility ratings of the scales have not been investigated systematically although this is an important feature to consider before adopting a scale in clinical practice. The aims of the present study are therefore to (1) externally validate field performance, including head-to-head comparisons, of seven prediction scales and (2) assess feasibility rates (i.e., the proportion of acute stroke codes for which the prehospital scale could be reconstructed) of these scales in the EMS setting.

METHODS

Study design and study population

This is a prospective, multiregional, observational cohort study. All consecutive patients 18 years of age or older for whom an EMS-initiated acute stroke code was activated between July 2018 and October 2019 were included. Patients were recruited from the Leiden and The Hague regions, encompassing two EMSs, three CSCs, and four PSCs, serving a total population of approximately two million inhabitants. Results are reported according to the Standards for Reporting of Diagnostic Accuracy (STARD) reporting guideline for diagnostic accuracy studies.[10] The institutional review boards of Leiden University Medical Center and of the participating hospitals approved this study and waived the need for obtaining informed consent because the extent of the effort required by the large number of health care providers to obtain permission from the participants was disproportionate compared with the relatively limited sensitivity of the collected encoded data and the related limited intrusion to the personal privacy. This study is registered with ClinicalTrials.gov.[11] An acute stroke code was initiated by EMS if there was a prehospital suspicion of acute stroke with a positive face-arm speech test (FAST) or other focal neurologic symptoms. When symptom onset or last seen well was 6 hours or less, it was routine policy to transport these patients to the nearest hospital, and when symptom onset was 6 to 24 hours, it was policy to transport patients to a CSC. The guidelines allow for protocol deviation based on a paramedic's individual judgment. Sample size calculation for external validation of the prediction models was estimated at a minimum of 100 patients with the primary outcome event (sLAVO).[12, 13] To increase power, we aimed to include more than 120 patients with sLAVO. Based on the literature, we estimated that 4.9% to 14.5% of patients receiving an acute stroke code have sLAVO.[4, 5] Considering the lower bound of the estimated sLAVO incidence, we therefore expected that a total of 2000 acute stroke codes were to be included.

Prediction scales and prehospital application

Seven prediction scales were simultaneously assessed and subsequently validated: the Los Angeles Motor Scale (LAMS),[14] the Rapid Arterial Occlusion Evaluation (RACE),[15] the Cincinnati Stroke Triage Assessment Tool (C-STAT; formerly CPSSS),[16] the Prehospital Acute Stroke Severity (PASS) scale,[17] the gaze face-arm-speech-time (G-FAST) test,[18] the Field Assessment Stroke Triage for Emergency Destination (FAST-ED),[19] and thegaze, facial asymmetry, level of consciousness, extinction/inattention (GACE) scale.[8] The scales were selected based on a previous systematic review.[8] All are ordinal scales with a cut point to decide whether or not a patient has sLAVO, except for the GACE scale, which uses a 4-item decision tree. Some scales are already implemented in clinical practice (e.g., FAST-ED and RACE).[20, 21] The EMS paramedics were instructed to fill in an application on site or during transport for each EMS-initiated acute stroke code. The application contained 10 to 13 items to structure neurologic observations, enabling reconstruction of all 7 prediction scales according to the authors' scoring instructions and prespecified cut points. The application was designed and tested in close collaboration with Research and Development (R&D) EMS Hollands Midden and filled in online or directly in the electronic transport record. Because the transport record system is part of standard care and the application merely structures routine clinical observations, it largely fits within the regular workflow. For one of the EMS regions, filling in the application was not mandatory. We anticipated that this would result in acute stroke codes in which the application would not be used at all. To investigate possible selection bias, we collected clinical data from patients with an acute stroke code in this region without a filled-in application for comparison.

Hospital data collection

Clinical data were retrieved from electronic patient records and included demographic characteristics, medical history, medication use, and stroke severity as assessed with the National Institutes of Health Stroke Scale (NIHSS). In cases for which an NIHSS score was not noted, the score was reconstructed from neurologic examination at admission by NIHSS certificated research members with a validated algorithm as described previously.[22] In-hospital performance metrics included symptom- onset-to-door time, door-to-needle time, and door-to-groin-puncture time (the *door* was defined as the door of the first hospital).[23] Finally, data on neuroimaging and diagnoses at admission, discharge, and after 3 months were retrieved from electronic patient records. Clinical outcomes according to the modified Rankin Scale were also retrieved after 3 months (patients with stroke only), which is a mandatory outcome parameter in Dutch stroke centers.

Data privacy

A trusted third party was installed to safeguard privacy, storage, and use of data. Application data and clinical data were coupled, encoded, and then transferred by the trusted third party to a data safe allowing access to investigators only.

Outcomes

The primary outcome was sLAVO clinically assessed by the treating stroke team taking the following radiologic criteria into account: occlusion of the intracranial carotid artery, tandem intracranial carotid artery, middle cerebral artery (M1 or M2 segment), or anterior cerebral artery (A1 or A2 segment). For feasibility, we considered the cutoff reconstruction rate the most important parameter. There construction rate was defined as the proportion of acute stroke codes for which the authors' prespecified scale's cut point could be determined with the available data. This was possible if (1) the cut point was reached with the points scored by EMS paramedics or (2) assigning maximal scores to missing or untestable items would still lead to a total number of points below the authors' prespecified cut point. We also calculated the full-scale feasibility: the proportion of acute stoke codes for which the full scale could be reconstructed. A scale could not be reconstructed and therefore was deemed not feasible when any required item to reconstruct a scale was missing or untestable. We excluded the GACE scale for feasibility analysis because this would always reach 100% owing to its decision tree construction. Finally, for each scale, we assessed the item that was reported missing or untestable most frequently to provide insight to the most important limiting factor for a scale to be feasible. In addition, to estimate how the use of a prediction scale might have influenced patient allocation in our cohort, we provided a hypothetical example by applying the scale with the highest diagnostic accuracy.

Statistical analysis

Categorical variables were compared with Pearson X^2 tests and presented as proportions. Continuous variables were compared using the *t* test or Mann-Whitney test and presented as mean (SD) values or median values and interquartile ranges (IQRs) as appropriate. A 2-sided $P \leq .05$ was considered statistically significant. Scale performance was assessed by calculating diagnostic accuracies (C statistic) of the authors' prespecified cut point as well as sensitivity, specificity, the Youden index, positive predictive value, and negative predictive value, with corresponding 95% CIs.

Accuracy was considered excellent for values 0.9 to 1.0, good for 0.8 to 0.9, fair for 0.7 to 0.8, poor for 0.6 to 0.7, and failed for 0.5 to 0.6.[24] Accuracies between the scales were compared with the McNemar test. The Youden index was used to evaluate the overall discriminative power of a diagnostic test and was calculated by deducting 1 from the sum of the test's sensitivity and specificity. The Youden index equals 0 for poor accuracy and 1 for excellent accuracy.[25] In addition, the accuracy for full-scale range was assessed by the area under the curve (AUC). Since hitherto the in-hospital NIHSS score holds the highest accuracy in predicting sLAVO, we also included this as a reference.[26] Reconstruction rates are provided with 95% CIs for comparison. Data analyses were performed using SPSS, version 24.0, or R, version 3.5.1, CRAN (R-CRAN project).

RESULTS

Patient inclusion

Between July 2018 and October 2019, 2812 acute stroke codes were activated (Figure 1). We excluded 805 acute stroke codes (28.6%), because no application was used (752 [26.7%]) or because no clinical data were available in the electronic patient record (53 [1.9%]). We collected clinical data on 442 of 752 patients with acute stroke codes (58.8%) for whom the application was not used. These patients had similar baseline characteristics, incidence of sLAVO, and stroke severity (median [IQR] NIHSS score, 4 [2-8] vs 4 [2-10]) and more often had hemorrhagic stroke or a stroke mimic compared with patients with application data (eTable 1 in the Supplement).

Patient characteristics

Of 2007 included patients with acute stroke codes, 1021 (50.9%) were men, the mean (SD) age was 71.1 (14.9) years, and the median (IQR) NIHSS score was 4 (2-8) (Table 1). Of 2007 patients with acute stroke codes, 781 (38.9%) first presented in a PSC, and 1226 (61.1%) first presented in a CSC. The final diagnosis after 3 months was ischemic stroke in 842 patients (41.9%), intracerebral hemorrhage in 148 patients (7.4%), transient ischemic attack in 264 patients (13.2%), and stroke mimic in 753 patients (37.5%). In addition, 158 patients (7.9%) with an acute stroke code received a diagnosis of sLAVO. Compared with patients without sLAVO, patients with sLAVO less often had a history of ischemic stroke or transient ischemic attack (33 of 158 [20.9%] vs 638 of 1849 [34.5%]; $P = .001$), had higher median (IQR) NIHSS scores (11 [5-17] vs 3 [2-6]; $P < .001$), and had higher incidence of atrial fibrillation de novo (13 of 158 [8.2%] vs 66 of 1849 [3.6%]; $P = .01$). Other baseline characteristics are given in Table 1.

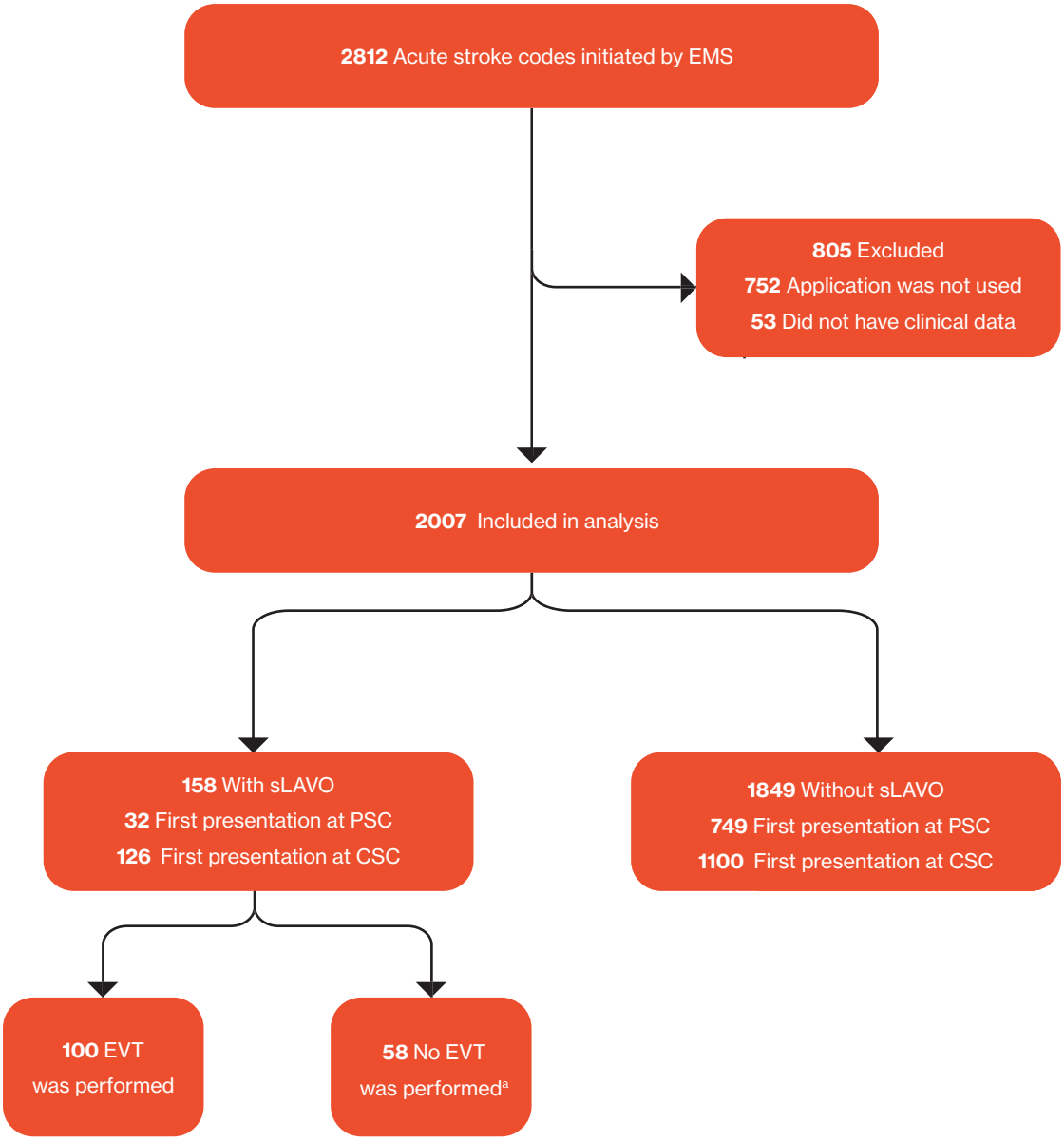

Figure 1. Flowchart of patient recruitment

CSC represents comprehensive stroke center; EMS, emergency medical service; EVT, endovascular treatment; PSC; primary stroke center; and sLAVO, symptomatic large anterior vessel occlusion.

[a] Imaging did not show an occlusion or a perfusion mismatch; the occlusion was technically not accessible. Patients showed clinical recovery or deteriorated neurologic status, or patients participated in the MR CLEAN LATE study randomized for no EVT.

Treatment and logistic metrics

Of 158 patients with sLAVO, 32 (20.3%) first presented in a PSC and 126 (79.7%) in a CSC (vs 1100 of 1849 patients without sLAVO [59.5%]) (Table 1). Median [IQR] symptom-onset-to door time was shorter in patients with sLAVO compared with patients without sLAVO (115 [45-340] vs 142 [62-446] minutes; *P* = .02). More patients with than without sLAVO received IVT (61 of 158 [38.6%] vs 253 of 1849 [13.7%]; P < .001), and EVT was performed in 100 patients with sLAVO (63.3%), with a median (IQR) door-to-groin-puncture time of 72 (54-105) minutes. For patients who presented directly to a CSC, the median (IQR) door-to-groin-puncture time was shorter (61 [51-81] minutes) compared with patients who first presented in a PSC (114 [103-140] minutes; P < .001) (eTable 2 in the Supplement).

Table 1.
Baseline patient characteristics

Characteristic	PATIENTS, NO. (%)		
		sLAVO	
	Total (N = 2007)	Yes (n=158)	No (n = 1849)
Age, mean (SD), y	71.1 (14.9)	72.4 (13.3)	70.9 (14.9)
Male sex	1021 (50.9)	92 (58.2)	929 (50.2)
Stroke logistics			
Primary stroke center	781 (38.9)	32 (20.3)[a]	749 (40.5)
Comprehensive stroke center	1226 (61.1)	126 (79.7)	1100 (59.5)
Medical history			
Ischemic stroke/transient ischemic attack	671 (33.4)	33 (20.9)	638 (34.5)
Intracerebral hemorrhage	83 (4.1)	2 (1.3)	81 (4.4)
Atrial fibrillation	273 (13.6)	30 (19.0)	243 (13.2)
Diabetes Mellitus	421 (21.0)	37 (23.4)	384 (20.8)
Hyperlipidemia	817 (40.7)	48 (30.4)	769 (41.6)
Hypertension	1179 (58.7)	88 (55.7)	1091 (59.0)
Myocardial infarction	223 (11.1)	20 (12.7)	203 (11.0)
Medication use			
Oral anticoagulation	337 (16.8)	24 (15.2)	313 (16.9)
Anti-platelets	690 (34.4)	50 (31.6)	640 (34.6)
Hospital admission			
NIHSS score, median (IQR)[b]	4 (2–8)	11 (5–17)	3 (2–6)
ODT, median (IQR), min[b]	140 (59–441)	115 (45–340)	142 (62–446)
Wake-up stroke[b]	235 (23.7)	46 (29.7)	189 (22.6)
Blood pressure, median (IQR), mm Hg			
Systolic	160 (140–182)	157 (139–182)	160 (140–182)
Diastolic	87 (77–100)	87 (75–99)	87 (77–100)
Glucose level, median (IQR), mg/dL	6.6 (5.7–8.1)	6.8 (5.9–8.6)	6.6 (5.7–8.1)
Atrial fibrillation de novo	79 (3.9)	13 (8.2)	66 (3.6)

Characteristic	PATIENTS, NO. (%)		
	Total (N = 2007)	sLAVO	
		Yes (n=158)	No (n = 1849)
Reperfusion therapy			
IVT[c]	314 (15.6)	43 (27.2)	253 (13.7)
EVT[d]	100 (5.0)	61 (38.6)	NA
IVT + EVT	43 (2.1)	100 (63.3)	NA
In-hospital performance metrics, median (IQR), min			
DNT	25 (19–34)	24 (18–33)	25 (19–34)
DGT	72 (54–105)	72 (54–105)	NA
ICA(-T)	28 (1.4)	24 (15.2)	NA
MCA M1	77 (3.8)	75 (47.5)	NA
MCA-M2	75 (3.7)	73 (46.2)	NA
ACA 1/2	5 (0.2)	5 (3.2)	NA
Other[e]	29 (1.4)	5 (3.2)	24 (1.3)
Final diagnosis after 90 d			
Ischemic stroke	842 (41.9)	155 (98.1)	687 (37.2)
Intracerebral hemorrhage	148 (7.4)	0 (0.0)	148 (8.0)
Transient ischemic attack	264 (13.2)	3 (1.9)	261 (14.1)
Stroke mimic	753 (37.5)	0 (0.0)	753 (40.7)
mRS after 90 days[b,f]	2 (1–4)	3 (2–6)	2 (1–4)

Abbreviations: ACA, anterior cerebral artery; DGT, first-door-to-groin-puncture time for EVT; DNT, door-to-needle time for IVT; EVT, endovascular thrombectomy; ICA(-T), intracranial carotid artery or tandem ICA; IQR, interquartile range; IVT, intravenous thrombolysis; MCA M1 or M2, middle cerebral artery segment M1 or M2; mRS, modified Rankin scale; NA, not applicable; NIHSS, National Institutes of Health Stroke Scale; ODT, onset to hospital door; sLAVO, symptomatic large anterior vessel occlusion.
SI conversion factor: To convert glucose level to mmol/L, multiply by 0.0555.

[a] Of 32 patients with sLAVO, 27 (84.4%) were transferred to a comprehensive stroke center for EVT or observation

[b] Only provided for patients with stroke (ie, ischemic stroke and intracerebral hemorrhage).

[c] Missing data in 3.5%.

[d] No missing data.

[e] Large posterior vessel occlusion locations: basilar artery, vertebral artery, and posterior cerebral artery segments P1 or P2.

[f] Missing data in 36.2%.

Scale performance

Table 2 gives the accuracy for identifying sLAVO with sensitivity, specificity, and predictive values. Accuracies ranged from 0.79 to 0.89, with LAMS (0.89; 95% CI, 0.87-0.90) and RACE (0.88; 95% CI, 0.86-0.89) having the highest accuracies. Head-to-head comparisons showed that these scales significantly outperformed the other scales (eTable 3 in the Supplement). Specificity was high for all scales (range, 80%-93%), whereas sensitivity was low (range, 38%-62%). The Youden index ranged from 0.30 to 0.47, and RACE had the highest index score (0.47; 95% CI, 0.37-0.56). In addition, negative predictive value was high for all scales (range, 95%-96%), and positive predictive value was low (range, 21%-32%). The AUC for the full-range accuracies for sLAVO prediction are shown in Figure 2. Scales showed fair to good performance, with accuracies ranging from 0.70 to 0.80 (eTable 4 in the Supplement). Although FAST-ED (AUC, 0.80; 95% CI, 0.74-0.85) had the highest accuracy, the accuracy was not statistically significantly different from G-FAST (AUC, 0.77; 95% CI, 0.72-0.82; *P* = .46), LAMS (AUC, 0.76; 95% CI, 0.71-0.81; *P* = .10), or RACE (AUC, 0.75; 95% CI, 0.69-0.82; *P* = .53).

Table 2.
Diagnostic performance of the prediction scales according to prespecified cutoff points

Prediction scale[a]	Accuracy (95% CI) [b]	Sensitivity (95% CI)	Specificity (95% CI)	Youden's index (95% CI)	PPV (95% CI)	NPV (95% CI)
C-STAT ≥2	0.79 (0.77-0.81)	0.62 (0.54-0.69)	0.80 (0.78-0.82)	0.42 (0.34-0.50)	0.21 (0.18-0.24)	0.96 (0.95-0.96)
PASS ≥2	0.81 (0.79-0.83)	0.55 (0.47-0.64)	0.83 (0.81-0.85)	0.39 (0.30-0.48)	0.21 (0.18-0.25)	0.95 (0.95-0.96)
G-FAST ≥3	0.82 (0.81-0.84)	0.61 (0.53-0.69)	0.84 (0.82-0.86)	0.46 (0.37-0.54)	0.24 (0.21-0.27)	0.96 (0.95-0.97)
FAST-ED ≥4	0.83 (0.81-0.85)	0.60 (0.53-0.69)	0.85 (0.83-0.87)	0.46 (0.37-0.54)	0.25 (0.22-0.29)	0.96 (0.95-0.97)
RACE ≥5	0.88 (0.86-0.89)	0.56 (0.46-0.65)	0.90 (0.89-0.92)	0.47 (0.37-0.56)	0.32 (0.27-0.38)	0.96 (0.95-0.97)
LAMS ≥4	0.89 (0.87-0.90)	0.38 (0.29-0.46)	0.93 (0.91-0.94)	0.30 (0.22-0.40)	0.28 (0.22-0.34)	0.95 (0.94-0.96)

Abbreviations: C-STAT, Cincinnati Stroke Triage Assessment Tool; FAST-ED, Field Assessment Stroke Triage for Emergency Destination; GACE, gaze, facial asymmetry, level of consciousness, extinction/inattention; G-FAST, gaze-face-arm-speech-time; LAMS, Los Angeles Motor Scale; NPV, negative predictive value; PASS, Prehospital Acute Stroke Severity; PPV, positive predictive value; RACE, Rapid Arterial Occlusion Evaluation.
[a] GACE is not included in this analysis because GACE has no cut point.
[b] Accuracy at cut point: ([true positives + true negatives]/total number of patients).

Feasibility

The mean reconstruction rate of the scales' cut point was 84.1% (range, 78.1%-87.9%) (Table 3). The PASS scale had the highest reconstruction rate (87.9%; 95% CI, 86.5-89.4). Compared with reconstruction rates of the full scale, calculating the rates for the cut point allowed scale reconstruction of 6.1% to 24.1% more acute stroke codes (e.g., for RACE, 78.1% vs 57.2%). Missing or untestable items that mainly prevented the reconstruction of a scale's cut point were neglect or motor function (Table 3).

Hypothetical example

Applying LAMS to our cohort, an urban region with relatively short distances between PSCs and CSCs and a low prevalence of sLAVO, indicated that 13 patients with sLAVO who first presented to a PSC would have benefited from direct allocation to a CSC, 17 patients with ischemic stroke treated with IVT allocated to a PSC would have unnecessarily bypassed a PSC, and 38 patients without sLAVO (including stroke mimics) allocated to a PSC would have been allocated to a CSC (including six patients with clinically severe intracerebral hemorrhage) (eFigure in the Supplement).

Table 3.
Scale feasibility in the field: reconstruction rate

Prediction scale[a]	No./total No. (%) [95% CI][b] Cutoff reconstruction rates	Full reconstruction rates	Most frequent missing or untestable item, No. item/total No. (%)c
PASS	1765/2007 (87.9) [CI 86.5–89.4]	1533/2007 (76.4) [CI 74.5–78.2]	Motor deficits arm, 226/242 (93.4)
G-FAST	1753/2007 (87.3) [CI 85.9–88.8]	1532/2007 (76.3) [CI 74.5–78.2]	Motor deficits arm, 238/254 (93.7)
C-STAT	1750/2007 (87.2) [CI 85.7–88.7]	1267/2004 (63.1) [CI 61.0–65.2]	Motor deficits arm, 224/257 (87.2)
LAMS	1662/2007 (82.8) [CI 81.1–84.5]	1540/2007 (76.7) [CI 74.9–78.6]	Motor deficits arm, 322/345 (93.3)
FAST-ED	1632/2007 (81.3) [CI 79.6–83.0]	1325/2007 (66.0) [CI 63.9–68.1]	Motor deficits arm; Neglect, 301/375 (80.3)
RACE	1568/2007 (78.1) [CI 76.3–79.9]	1148/2007 (57.2) [CI 55.0–59.4]	Motor deficits legs, 418/439 (95.2)

Abbreviations: PASS, Prehospital acute stroke severity; G-FAST, Gaze-face-arm-speech-time; C-STAT, Cincinnati stroke triage assessment tool; LAMS, Los Angeles Motor Scale; FAST-ED, Face-arm-speech-time-eye deviation-denial/neglect; RACE, Rapid arterial occlusion evaluation.
[a] GACE is not included in this analysis because the feasibility would always reach 100% owing to its decision tree construction.
[b] Total number of reconstructed scales/all acute stroke codes.
[c] Number of highest missing or untestable item/total missing or untestable items.

DISCUSSION

In this prospective cohort study assessing more than 2000 patients with acute stroke codes, we found that several established sLAVO prediction scales had good accuracy when used in the EMS setting, with RACE and the LAMS showing the highest accuracies. Feasibility rates were relatively high for all scales, with the highest feasibility for PASS. We also found that feasibility rates could increase by using all available information to reconstruct the scale's cut point, thereby enabling additional inclusion of acute stroke codes with incomplete or untestable items. The prevalence of sLAVO in our cohort is in line with previous reports using a similar reference group of acute stroke codes (i.e., also including hemorrhagic strokes and stroke mimics), indicating that our cohort was a good reflection of patients in general clinical practice.[4, 5] The accuracies in our study (0.79- 0.89) were at the higher end of the spectrum of accuracies presented in earlier studies (0.51-0.91) and were comparable with a recent report investigating a novel clinical prediction scale in a similar cohort (including all acute stroke codes), although additional teleconsultation with a stroke neurologist was incorporated in that study.[9, 27-29] In practice, the preferred sLAVO prediction scale will depend on the local context, which will include such factors as prevalence of sLAVO, differences in transport times between hospitals, in-hospital performance metrics, and local policies.[30, 31] Although differences among the scales in accuracies were small, in larger populations, these small differences may result in clinically meaningful outcomes. Taking into account median delays associated with transferring patients with sLAVO between a PSC and a CSC (i.e., 53 minutes in our cohort) against a background of relatively small distances between a PSC and CSC (approximately 10 min driving time), our hypothetical example showed a clear benefit for reducing delays to reperfusion treatment when using a sLAVO prediction scale in the ambulance setting. Despite local policy to always allocate a patient with an acute stroke code to the nearest hospital, in our cohort, a relatively high proportion of patients with sLAVO were allocated directly to a CSC (79.7% vs 59.5% of patients without sLAVO). In addition, the use of LAMS would have resulted in meaningful improvements in patient logistics. Our study also adds important data on feasibility of sLAVO prediction scales. We show that by using all available information, additional acute stroke codes for patients with items that were missing or untestable could still be included, which resulted in higher feasibility rates for all scales. The PASS scale had the highest reconstruction rate, probably as a result of fewer items that needed to be assessed compared with the other scales. The RACE scale had the lowest feasibility rates, although the cutoff

reconstruction rate was much higher than the full reconstruction rate (78.1% vs 57.2%). The feasibility of RACE was shown to be low (40%) in the first study published using this scale,[15] but increased to 78% in a later study.[22] This suggests that feasibility may improve once EMS paramedics become more familiar with a scale. Focused training aimed at the items missing most frequently (i.e., motor deficits in arm or legs) would improve this rate substantially.

Limitations

Our study has some limitations. First, the application was not used in 26.7% of acute stroke codes. However, acute stroke codes without application data differed only in final diagnosis, whereas stroke severity and percentage of sLAVO were comparable between both groups, therefore, we do not think that this lack of application data biased our results. Second, we selected the clinical sLAVO prediction scales based on a previous report,[8] but other scales have been developed since our study onset. We do not believe that performance outcomes will differ greatly because clinical assessment of the scale items showed considerable overlap with the scales that we tested and reported accuracies are comparable to our findings.[32-35] Third, we used the presence of sLAVO as assessed on computed tomography angiography performed by the radiologist in the local hospital although we did not have these images available for centralized review. Therefore, it is possible that we missed some patients with sLAVO. However, sLAVO detection is high across all levels of radiologist training according to a previous report,[36] and during the present study, computed tomography angiography was part of the routine workup for patients with acute stroke codes in all hospitals. Fourth, to reconstruct all scale items for each separate scale according to our instructions, the applications we used contained more items than the original scales, which could have negatively influenced the scale's feasibility.

CONCLUSION

Our study is the first to our knowledge to provide external validation in the field as well as to offer head-to-head comparisons of several established sLAVO prediction scales. Our results indicate that these scales had good diagnostic accuracies, with LAMS and RACE showing the highest accuracies. Scale feasibility rates ranged from 78% to 88%, and it is important to take feasibility into account before implementing a prediction scale in the field because focused training could substantially increase these rates.

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SUPPLEMENTAL

eTable 1.
Comparing patients with and without application data

	PATIENTS, NO. (%)			
		Application Data		
Characteristic	Total (N = 2449)	Yes (n = 2007)	No (n = 442)	P value
Age, mean (SD), y	70.9 (14.7)	71.1 (14.9)	70.4 (14.3)	.26
Male sex	1247 (50.9)	1021 (50.0)	226 (51.1)	.92
Hospital admission				
NIHSS score[a], median (IQR)	4 (2–9)	4 (2–8)	4 (2–10)	.31
sLAVO	198 (8.1)	158 (7.9)	40 (9.0)	.41
Final diagnosis				
Ischemic stroke	978 (39.9)	831 (41.4)	147 (33.3)	<.001
Intracerebral hemorrhage	202 (8.2)	149 (7.4)	53 (12.0)	
Transient ischemic attack	319 (13.0)	274 (13.7)	45 (10.2)	
Stroke mimic	950 (38.8)	753 (37.5)	197 (44.6)	

Abbreviations: NIHSS, National Institute of Health Stroke Scale; sLAVO, symptomatic large anterior vessel occlusion.
[a] Only provided for stroke patients (i.e. ischemic stroke and intracerebral hemorrhage).

eTable 2.
Stroke logistics and in-hospital performance metrics in PSC vs. CSC presented patients

	Total, No. (%) (n = 158)	sLAVO presented in PSC, No. (%) (n = 32)	sLAVO presented in CSC, No. (%) (n = 126)	*P* value
Stroke logistics				
Transferred from PSC to CSC for EVT	26/158 (17)	26/32 (81)	NA	NA
In-hospital performance metrics				
DNT, median (IQR), min	24 (18–33)	26 (19–34)	24 (18–33)	.79
DGT, median (IQR), min	72 (54–105)	114 (103–140)	61 (51–81)	<.001

Abbreviations: sLAVO, symptomatic large anterior vessel occlusion; PSC, primary stroke center; CSC, comprehensive stroke center; NA, not applicable; EVT, endovascular thrombectomy. DNT, door-to-needle time; DGT, *first*-door-to-groin puncture time.

eTable 3.
Comparing accuracies of the prediction scales according to prespecified cutoff points

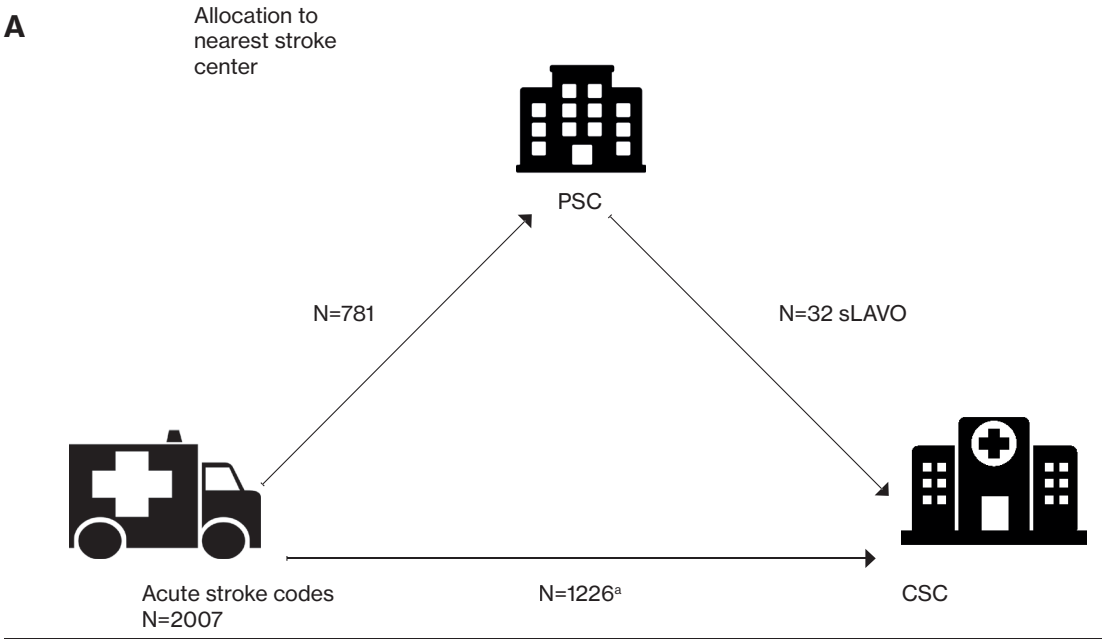
Prediction scale	Accuracy[a]	C-STAT ≥2	PASS ≥2	G-FAST ≥3	FAST-ED ≥4	RACE ≥5	LAMS ≥4
C-STAT ≥2	0.79 (0.77-0.81)	NA					
PASS ≥2	0.81 (0.79-0.83)	0.03	NA				
G-FAST ≥3	0.82 (0.81-0.84)	0.76	0.13	NA			
FAST-ED ≥4	0.83 (0.81-0.85)	0.06	0.49	0.19	NA		
RACE ≥5	0.88 (0.86-0.89)	<0.001	0.02	<0.001	0.001	NA	
LAMS ≥4	0.89 (0.87-0.90)	<0.001	<0.001	<0.001	<0.001	0.51	NA

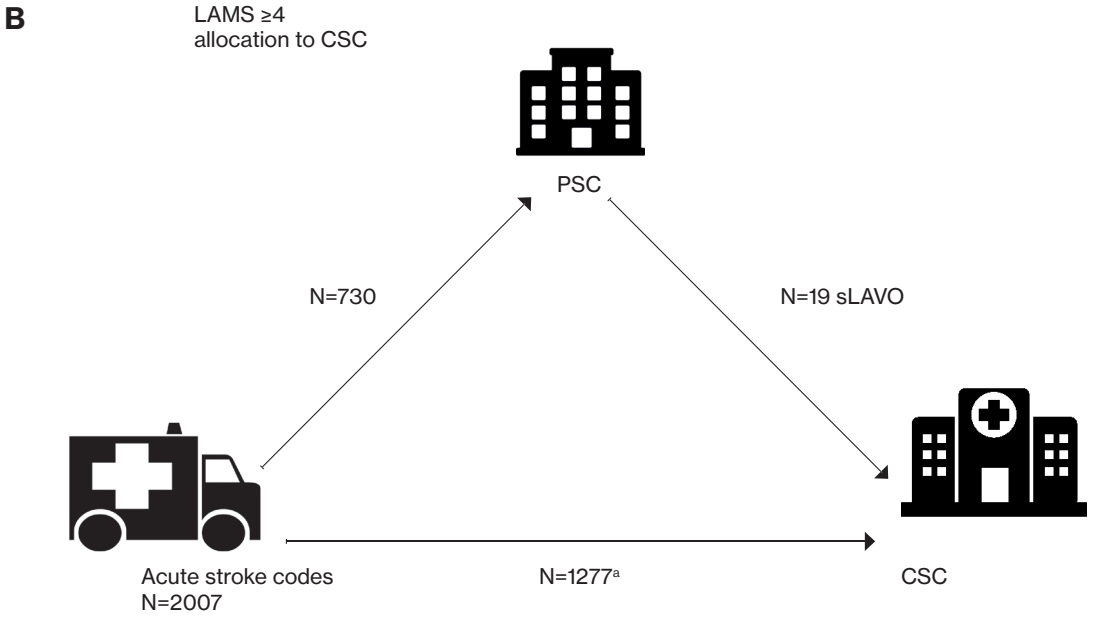
Abbreviations: NA, not applicable; C-STAT, Cincinnati stroke triage assessment tool; PASS, Prehospital acute stroke severity; G-FAST, Gaze-face-arm-speech-time; FAST-ED, Face-arm-speech-time-eye deviation-denial/neglect RACE, Rapid arterial occlusion evaluation; LAMS, Los Angeles Motor Scale.
[a] Accuracy at cutoff point: ([True positives + true negatives])/total number of patients).

eTable 4.
Comparison of full range accuracy of the prediction scales

Prediction scale	AUC 95% CI	C-STAT	PASS	GACE	RACE	LAMS	G-FAST	FAST-ED	NIHSS
C-STAT	0.70 (0.64-0.76)	NA							
PASS	0.73 (0.68-0.78)	0.19	NA						
GACE	0.73 (0.69-0.78)	0.27	0.85	NA					
RACE	0.75 (0.69-0.82)	0.05	0.08	0.04	NA				
LAMS	0.76 (0.71-0.81)	0.05	0.07	0.18	0.40	NA			
G-FAST	0.77 (0.72-0.82)	0.04	0.01	0.01	0.51	0.48	NA		
FAST-ED	0.80 (0.74-0.85)	0.01	0.001	0.02	0.53	0.10	0.46	NA	
NIHSS	0.82 (0.78-0.85)	<0.001	<0.001	<0.001	0.001	0.01	0.001	0.20	NA

Abbreviations: NA, not applicable; C-STAT, Cincinnati stroke triage assessment tool; PASS, Prehospital acute stroke severity; G-FAST, Gaze-face-arm-speech-time; FAST-ED, Face-arm-speech-time-eye deviation-denial/neglect RACE, Rapid arterial occlusion evaluation; LAMS, Los Angeles Motor Scale.
[a] Accuracy at cutoff point: ([True positives + true negatives])/total number of patients).





eFigure.
Allocation of acute stroke code patients

A: Real scenario for patient allocation in our cohort.
B: Real scenario for patient allocation based on LAMS score in our cohort.
[a] Thirteen patients with sLAVO would directly be allocated to a CSC, 17 IVT-treated patients would have unnecessary by-passed a PSC and 38 patients without sLAVO would have been allocated to a CSC (including six patients with clinically severe ICH)

Chapter 5

Circulating tRNA fragments as a novel biomarker class to distinguish acute stroke subtypes

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ABSTRACT

Background and purpose

Early blood biomarkers to diagnose acute stroke could drastically reduce treatment delays. We investigated whether circulating small non-coding RNAs can serve as biomarkers to distinguish between acute ischemic stroke (IS), intracerebral hemorrhage (ICH) and stroke mimics (SM).

Methods

In an ongoing observational cohort study, we performed small RNA-sequencing in plasma obtained from a discovery cohort of 26 patients (9 IS, 8 ICH and 9 SM) presented to the emergency department within 6 hours of symptom onset. We validated our results in an independent data set of 20 IS patients and 20 healthy controls.

Results

ICH plasma had the highest abundance of ribosomal and tRNA-derived fragments, while microRNAs were most abundant in plasma of IS patients. Combinations of four to five tRNAs yielded diagnostic accuracies (areas under the receiver operating characteristics curve) up to 0.986 (ICH vs. IS and SM) in the discovery cohort. Validation of the IS and SM models in the independent data set yielded diagnostic accuracies of 0.870 and 0.885 to distinguish IS from healthy controls.

Conclusion

We identified tRNA-derived fragments as a promising novel class of biomarkers to distinguish between acute IS, ICH and SM, as well as healthy controls.

INTRODUCTION

The diagnosis of acute stroke currently relies on clinical assessment and neuroimaging. While neuroimaging can rule out intracerebral hemorrhage (ICH), acute ischemic stroke (IS) can often not be confirmed and, therefore, diagnostic uncertainty can persist. Moreover, about 40% of suspected stroke patients presented to the emergency department as possible reperfusion candidates (so-called 'stroke codes') appear to have symptoms based on a different pathology than stroke: stroke mimics (SM).[1] It is critical to establish the diagnosis as quickly as possible, as the different types of stroke require completely different interventions. Particularly in acute IS speed is of the essence, as efficacy of reperfusion therapies sharply declines with time[2]. An early biomarker that reliably distinguishes stroke subtypes (IS or ICH) and SMs would therefore be of substantial clinical value.

Circulating small non-coding RNAs (sncRNAs) potentially fit this profile and especially microRNAs have been studied intensively in this context.[3] Recently, sncRNAs derived from transfer RNAs (tRNAs) have been described as an emerging biomarker class.[4] tRNAs are encoded in the human genome codes by more than 400 genes, which can be divided into 48 *isodecoders*: tRNAs with the same anticodon. Although tRNAs are best known for their role in protein synthesis, tRNAs and tRNA-derived fragments (tRFs) also play a role in regulatory processes such as gene expression and translational control.[5] Importantly, tRFs are found in liquid biopsies such as plasma and serum and show promise as biomarkers for several other pathologies, such as cancer and epilepsy.[6, 7]

We investigated whether sncRNAs in plasma, especially tRFs, can be used as early biomarkers to differentiate between acute IS, ICH and SM.

MATERIAL AND METHODS

Study design

This is a preplanned preliminary analysis of the MicroRNA in Acute Stroke (MIRAS) study, an ongoing prospective observational cohort study. Enrollment started on November 2018. This study is registered at AsPredicted.org with unique identifier #44134. Reporting was done in accordance with the STROBE Statement (see Supplementary Material).[8] The study was conducted in accordance with the with the Declaration of Helsinki, the principles of Good Clinical Practice, the Dutch Agreement on Medical Treatment Act (WGBO) and the European General Data Protection Regulation and was approved by the Medical-Ethical Committee Leiden-Den Haag-Delft (P18.030; NL63060.058.17, 18 May 2018). Written informed consent was obtained from all patients or their legal guardian.

Patient selection

Consecutive stroke code patients aged 18 years or older who presented primarily at the emergency department of the Leiden University Medical Center within 6 h of symptom onset were included during office hours. Stroke was categorized according the American Heart Association/American Stroke Association.[9] Stroke mimics (SM) were defined as stroke code patients for whom a stroke as the underlying pathology was excluded. Additional inclusion criteria for stroke patients were admittance to the neurology ward and a NIHSS score of ≥4. Patients were excluded if they had an active malignant disease or used heparin within 24 h prior to symptom onset. In case of doubt about eligibility, blood samples were processed according to protocol. For all patients, the study coordinators (TTMN and MLvdB) verified that patients complied with the in- and exclusion criteria using the electronic patient record. Samples and clinical data of non-eligible patients or patients that did not provide informed consent were destroyed. For this discovery analysis, we selected 27 patients (n = 8–10 per group) from a total of 68 eligible patients who were included between November 2018 and August 2019. Criteria for selection included technical quality of the sample (i.e., sufficient amount and absence of hemolysis) and a confirmed final diagnosis of IS, ICH or SM. One SM sample was excluded post hoc from our discovery cohort because of an uncertain onset-to-door time, leading to a final sample size of 26 (9 IS, 8 ICH and 9 SM). According to the standard stroke protocol, all patients underwent clinical assessment at presentation at the emergency department followed by non-contrast CT with CT-

angiography if indicated. Final diagnosis was established three months after discharge by an experienced stroke neurologist (NDK) based on all available clinical and neuro-imaging data and blinded to blood biomarker outcome.

Clinical data

Clinical data, neuro-imaging data and diagnosis at admission, discharge and after three months were retrieved from electronic patient records. Clinical data included demographic characteristics, medical history, medication use and stroke severity as assessed with the NIHSS score. In case the NIHSS score was not available, this was reconstructed from neurological examination at admission by NIHSS certified research members with a validated algorithm as described previously.[10] In-hospital performance metrics included onset-to-door time. In case of a wake-up stroke, we included patients if the time of last seen well was within 6 hours.

Sample collection

Non-fasting venous blood was collected at the earliest possibility upon admission to the emergency department, prior to the administration of therapy or medication, using a 18G infusion needle in two 9 mL VACUETTE 9NC Coagulation sodium citrate tubes (Greiner Bio-One, Alphen aan den Rijn, the Netherlands). To prevent platelet activation and cell lysis, agitation of the blood collection tubes was kept to a minimum, tubes were kept vertical at all times after inverting carefully to mix the blood with the citrate buffer and they were delivered to the laboratory by one of the researchers, rather than through standard pneumatic tube delivery.[11] Blood was processed at room temperature within 60 min. Sodium citrate tubes were centrifuged for 15 min at 2500× g, after which plasma was transferred to a clean plastic 15 mL conical bottom centrifuge tube (Greiner) and centrifuged once more for 15 min at 2500× g. After each centrifugation step, plasma was pipetted off carefully from the top and at least 200 µL of plasma was left untouched in order to isolate platelet-poor plasma. Plasma aliquots were snap frozen in liquid nitrogen, then stored at -80 °C. Hemolysis was monitored visually and by spectrophotometry,[12] as well as by RT-qPCR of the erythrocyte-specific microRNA miR-451a relative to the stable miR-23a-3p, prior to sequencing.[13]

Ultracentrifugation and RNA-sequencing

To deplete plasma of extracellular vesicles (EVs), 2.5 mL of plasma was thawed and diluted once with phosphate-buffer saline (PBS). The plasma was then depleted of EVs by two rounds of ultracentrifugation

using a swinging-bucket rotor in a Beckman Coulter XE-90 instrument (Beckman Coulter, Woerden, The Netherlands). To remove large vesicles, samples were first centrifuged for 70 min at 4 °C at 17,500× g, using maximum acceleration (0) and slow deceleration (5). The upper 4.5 mL was transferred to a new tube and filled up to 5 mL with PBS, so that the final dilution of the plasma was approximately 2.2-fold. In the second step, samples were centrifuged for 70 min at 4 °C at 166,400× g to remove small vesicles, using the same acceleration and deceleration settings. Three fractions were separated: The top 0.5 mL, the middle 3.5 mL and the bottom 1 mL These fractions, as well as the bottom 0.5 mL that remained after the first round of ultracentrifugation, were aliquoted and snap-frozen on dry ice, then stored at -80 °C. The upper 0.5 mL of EV-depleted plasma was sent to QIAGEN (Venlo, The Netherlands) for small RNA-sequencing (sRNA-seq). Briefly, RNA was isolated using the miRNeasy Serum/Plasma Kit (QIAGEN) according to the manufacturer's instructions. Five microliters of total RNA per sample were used for library preparation using the QIAseq miRNA Library Kit (QIAGEN). Adapters containing unique molecular identifiers (UMIs) were ligated to the RNA, followed by reverse transcription. cDNA was amplified by 22 cycles of PCR and indices were added during the PCR. PCR samples were purified, and libraries were pooled in equimolar ratios. These pools were quantified using qPCR and sequenced on a NextSeq500 instrument (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Raw data were de-multiplexed and FASTQ files were generated using the bcl2fastq software. Data quality was assessed using the FastQC tool.[14]

Publicly available sRNA-seq data of an independent study cohort were retrieved from the NCBI Sequence Read Archive (project identifier SRP133275)[15]. In this cohort, the authors included 20 IS patients (≤24 h of symptom onset; mean 3.9 h ± 3.6 h) and 20 matched healthy controls. Briefly, the authors collected nonfasting blood samples at the emergency department in EDTA tubes using a 18G needle and tourniquet, after which platelet-poor plasma was isolated by two rounds of centrifugation (10 min at 2000× g followed by 15 min at 2500× g). RNA was isolated from the plasma using the miRCURY RNA Isolation Kit-Biofluids (Exiqon, Aarhus, Denmark); sRNA-seq libraries were prepared using the TruSeq Small RNA sample prepkit v2 (Illumina) and sequenced on an Illumina HighScan-SQ sequencer.

Data analysis

Clinical data were analyzed using SPSS (v24.0, IBM, New York, NY, USA). Continuous data were presented as mean ± standard deviation (SD) if normally distributed, and as median with interquartile range (IQR) if skewed. Categorical data were presented as number and percentage.

Continuous data were compared using One-way ANOVA or Kruskal–Wallis tests, as appropriate. Descriptive categorical data were analyzed using Fisher's exact test.

Both sRNA-seq datasets were analyzed using the virtual machine of the sRNAtoolbox.[16] Briefly, the sRNAbench module was used to (i) trim the Illumina adapter sequence from the raw reads in the fastq files, (ii) identify and deduplicate reads based on UMIs, and (iii) map the reads to the human genome using bowtie. Several libraries were used for annotation, including miRbase, gtRNAdb, RNAcentral, and NCBI ncRNA and cDNA libraries. Then, the sRNAde module was used to investigate differential expression of various classes of small RNAs and variants. Data were visualized using Prism 8 (GraphPad) and R version 3.5.1.5.[17]

After mapping the sequencing reads, we calculated the levels of tRFs for each isodecoder by taking the sum of the multi-mapping adjusted total RPM (Reads Per Million, normalized to the total number of genome mapped reads). Isodecoder levels were then compared between the three groups by one-way ANOVA, followed by Tukey's post hoc tests for pairwise comparisons. Least absolute shrinkage and selection operator (LASSO) regression analysis was performed to select optimal statistical models for the prediction of pathology (IS, ICH or SM), using the glmnet package in R.[18] Only isodecoders with a minimum expression of 1 read count in each sample and at least 10 RPM in at least 8 samples in our own dataset (resulting in 46 predictors) or at least 5 RPM in at least 20 samples in the independent dataset (resulting in 24 predictors) were considered in the LASSO analysis. Due to the small sample size per group in our own dataset, each group was compared to the other two groups using binomial analysis. The optimal model was defined as the model with the log (lambda) value that corresponded to the lowest binomial deviance. Generalized linear models were calculated for the optimal combination of predictors using the R stats package. Finally, using the pROC package in R, receiver operating characteric (ROC) curves were generated to assess diagnostic accuracy for each separate predictor, as well as for the generalized linear models of the combinations of predictors.[19]

RESULTS

Patient characteristics

Our discovery cohort included 9 IS, 8 ICH and 9 SM patients, 58% of whom were men. The mean age was 71 ± 14 years, median onset-to-door-time was 78 min [IQR 57-116] and the median National Institutes of Health Stroke Scale (NIHSS) score of stroke patients was 8 [IQR 4-18] (Table 1). Patients with intracerebral hemorrhage more often had diabetes (n = 4; 50%) and hypertension (n = 7; 89%) compared to the ischemic stroke and stroke mimic group ($p < 0.05$).

Small RNAs in plasma

We detected RNAs from various sncRNA classes, including microRNA, ribosomal RNA and tRNA (Figure 1A). The distribution of the fraction of sequencing reads from these sncRNA classes differed between the groups. Notably, plasma of ICH patients contained the highest relative abundance of reads deriving from ribosomal RNAs and tRNAs (ICH: 22.89% and 1.65%; IS: 17.08% and 1.12%; SM: 18.39% and 1.46%, respectively), while microRNAs were relatively most abundant in IS plasma (IS: 44.13%; ICH: 32.52%; SM: 38.17%). tRFs showed the largest differences in abundance between the groups (ICH vs. IS: 1.47-fold; ICH vs. SM: 1.13-fold; SM vs. IS: 1.30-fold).

tRFs as biomarkers

In addition to overall differences in tRF abundance, we found that several isodecoders were differentially expressed between the groups ($p < 0.05$; Figure 1B and Supplementary Table S1). LASSO analysis yielded models consisting of four to five isodecoders. By combining these different isodecoders in a single model, diagnostic accuracy was markedly increased over each of the individual isodecoders, reaching an area under the ROC curve (AUC) of 0.986 (95% CI, 0.953–1.000) for ICH, 0.915 (95% CI, 0.809–1.000) for IS and 0.928 (95% CI, 0.832–1.000) for SM vs. the other two groups (Figure 1C).

Table 1.
Patient baseline characteristics of the discovery cohort

	Total No. Patients (n=26)	Ischemic Stroke (n= 9)	Intracerebral Hemorrhage* (n=8)	Stroke Mimics† (n=9)	p-value
Age, mean (SD), y	71 (14)	76 (8)	71 (14)	65 (16)	0.25
Male sex, n (%)	15 (58)	6 (67)	3 (38)	6 (67)	0.43
Medical history, n (%)					
Ischemic stroke/TIA	8 (31)	3 (33)	3 (38)	2 (22)	0.87
Intracerebral hemorrhage	2 (8)	0 (0)	2 (25)	0 (0)	0.09
Atrial fibrillation	5 (19)	1 (11)	2 (25)	2 (22)	0.84
Diabetes Mellitus	5 (19)	1 (11)	4 (50)	0 (0)	0.02
Hyperlipidemia	12 (46)	6 (67)	3 (38)	3 (33)	0.38
Hypertension	15 (58)	6 (67)	7 (89)	2 (22)	0.02
Medication use, n (%)					
Oral anticoagulation	5 (19)	1 (11)	3 (38)	1 (11)	0.38
Antiplatelets	8 (31)	3 (33)	2 (25)	3 (33)	1.00
Hospital admission					
ODT, median (IQR), min	78 (57-116)	72 (45-144)	95 (63-203)	68 (60-102)	0.76
NIHSS‡, median (IQR)	8 (4-18)	5 (4-19)	11 (6–18)	-	-
LAVO§, n (%)		4 (44)	-	-	-
Neuro-imaging‖					
Lesion confirmed, n (%)		8 (89)	8 (100)	-	
Reperfusion therapy					
IVT, n (%)	-	7 (78%)	-	-	-
EVT, n (%)	-	4 (44%)	-	-	-
DNT, median (IQR), min	-	20 (17-40)	-	-	-
DGT, median (IQR), min	-	56 (51-65)	-	-	-

TIA: transient ischemic attack; ODT: onset-to-door time; NIHSS: National Institute of Health Stroke Scale; LAVO: large anterior vessel occlusion; IVT: Intravenous thrombolysis; EVT: Endovascular treatment; DNT: door-to-needle time in IVT-treated patients; DGT: door-to-groin puncture time in EVT-treated patients.
* Location of hemorrhage: 4 (50%) basal ganglia, 4 (50%) lobar.
† Stroke mimics: 2 (22%) epilepsy, 5 (56%) acute vestibular syndrome (all with positive head impulse test), 1 (11%) intoxication, 1 (11%) partial oculomotor nerve palsy.
‡ Stroke only (i.e. ischemic stroke and intracerebral hemorrhage).
§ Occlusion of the intracranial carotid artery(-tandem) (ICA/ICA-T), middle cerebral artery (M1/M2), or anterior cerebral artery (A1/A2).
‖ Computed tomography, -angiography/-perfusion, magnetic resonance imaging.

Validation of tRF models

We validated these models using data from an independent cohort of IS patients and healthy controls (hereafter referred to as the validation cohort).[15] Our models to distinguish IS and SM yielded comparable AUCs of 0.870 (95% CI 0.756–0.984) and 0.885 (95% CI 0.781–0.989) (Figure 2A,B and Supplementary Table S2). The validation cohort did not include ICH patients and accordingly, our ICH model had much lower diagnostic accuracy in this dataset (AUC: 0.728; 95% CI 0.561–0.894; Figure 2C).

Common tRF model

When an optimal LASSO model to distinguish IS from healthy controls was determined in the validation cohort, three out of nine isodecoders overlapped with our IS and SM models: tRNA-TyrGTA, tRNA-ThrCGT and tRNA-ValCAC (Figure 3A). The combination of these three isodecoders yielded an AUC of 0.875 (95% CI, 0.759–0.991) to distinguish IS from healthy controls in the validation cohort (Figure 3B and Supplementary Table S2). This common tRF model showed poor diagnostic accuracy in our discovery cohort to distinguish between ICH and SM (AUC 0.653; 95% CI, 0.359–0.947), but high diagnostic accuracy for distinguishing between IS and ICH (AUC 0.847; 95% CI, 0.656–1.000) and between IS and SM (AUC 1.000) (Figure 3C and Supplementary Table S2).

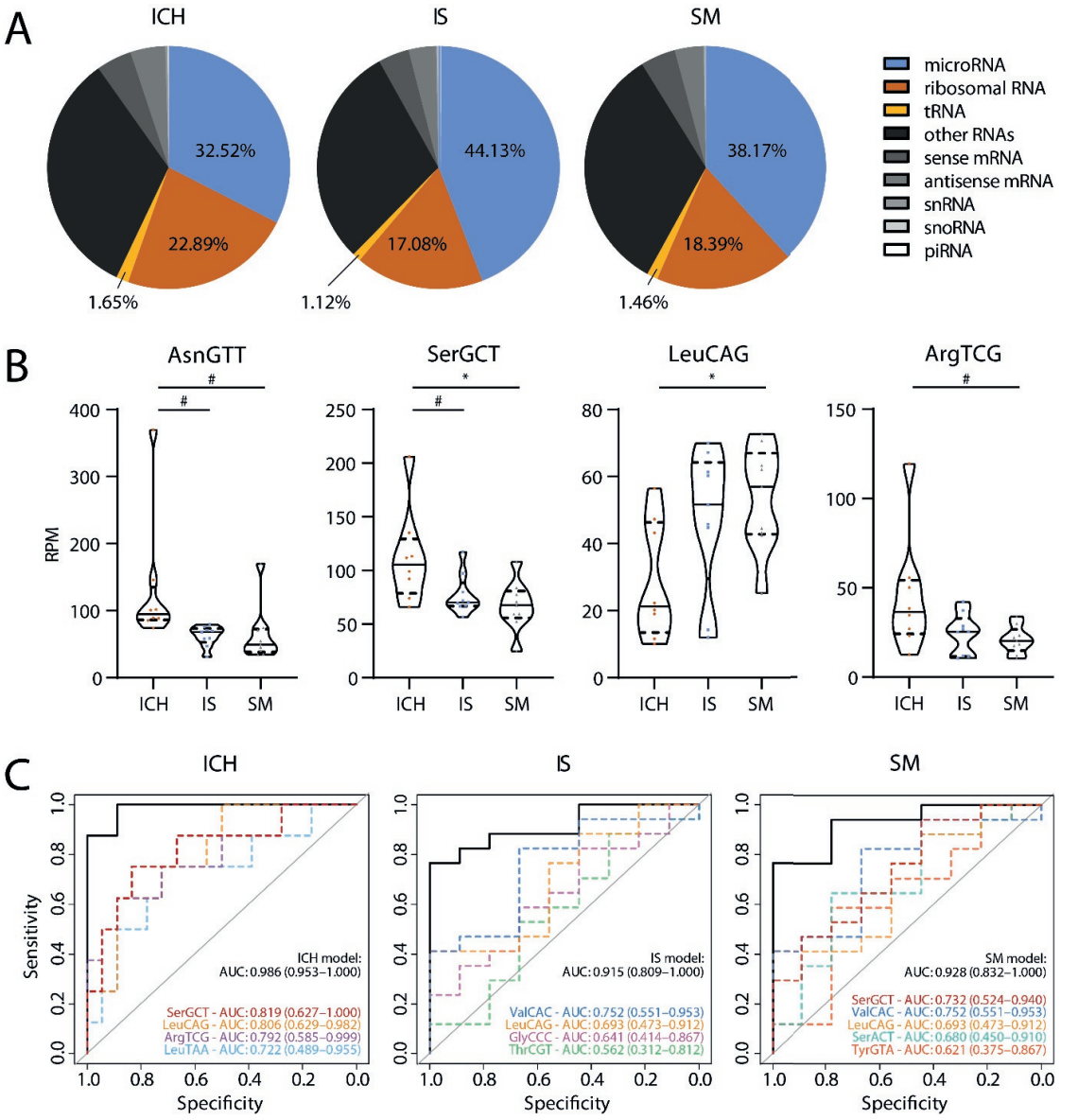

Figure 1.
sRNA-seq results in the discovery cohort

(A) Distribution of reads assigned to various small non-coding RNAs (sncRNA) classes. (B) Summed tRNA-derived fragments (tRF) abundance of differentially expressed isodecoders. Tukey's post-hoc test: # $p < 0.10$; * $p < 0.05$. (C) ROC analysis of diagnostic accuracy for each of the three groups from the other two groups. Dashed colored curves: Separate isodecoders in each optimal least absolute shrinkage and selection operator (LASSO) model. Black curves: Combination of these isodecoders. n = 8–9 per group. RPM: Reads per million; AUC: Area under the curve.

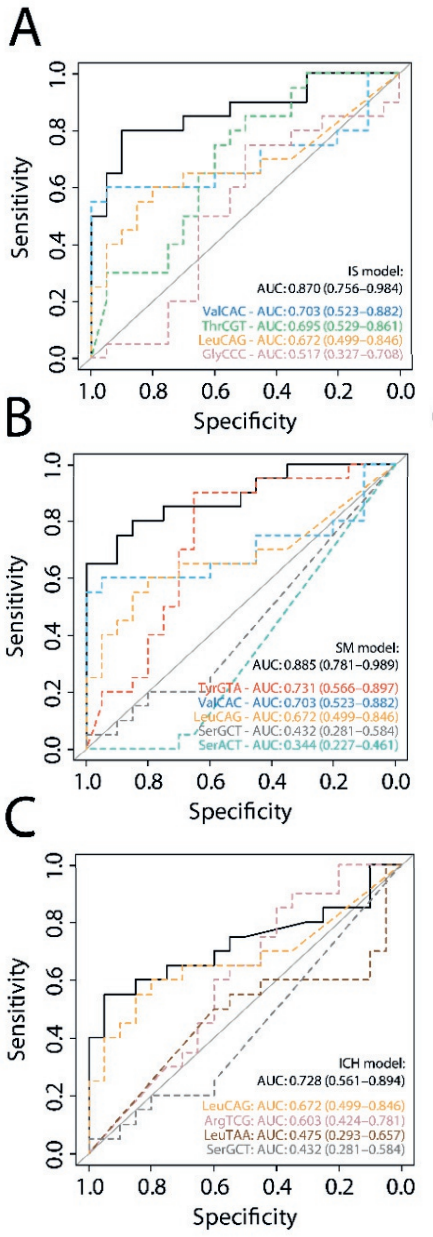

Figure 2.
ROC analyses on the validation cohort

(A) Ischemic stroke (IS) model, (B) stroke mimic (SM) model and (C) intracerebral hemorrhage (ICH) model, as determined in our discovery cohort, applied to the sRNA-seq dataset from the validation cohort (IS vs. healthy controls; n = 20 per group). Dashed colored lines show the diagnostic potential of the separate isodecoders; black curves show the diagnostic potential of the combination of these. AUC: Area under the curve. HC: Healthy controls.

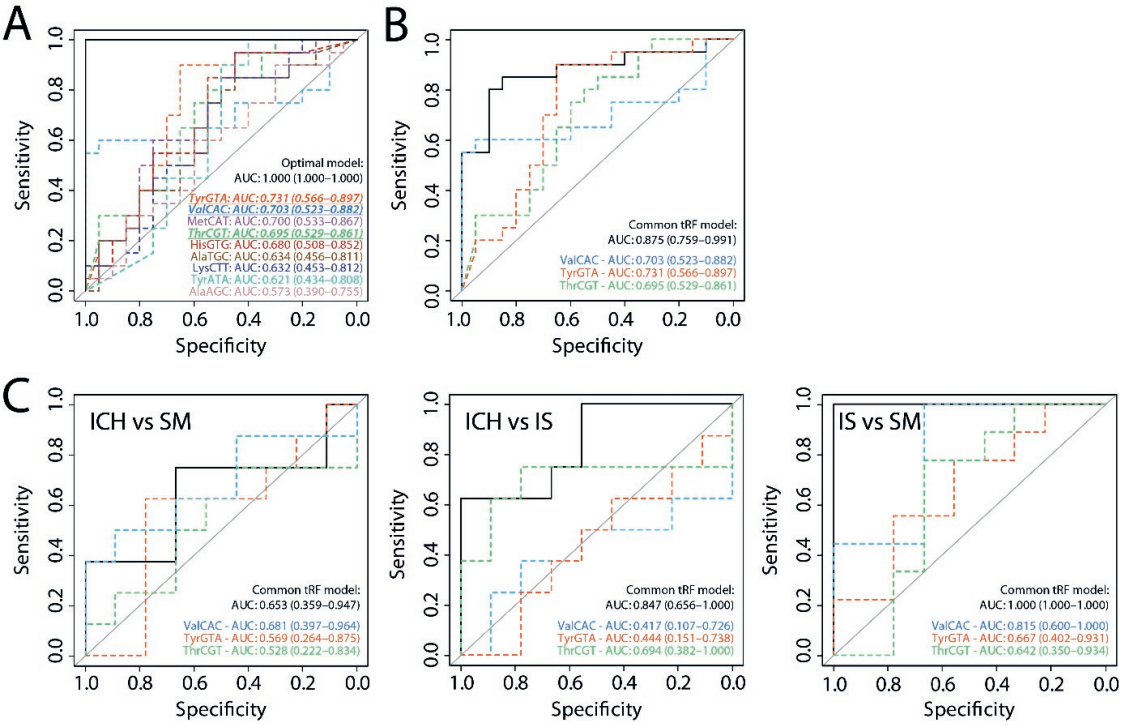

Figure 3.
Common tRF model

(A) Optimal LASSO model determined in the validation cohort; the three tRF isodecoders that were identified in both the discovery and the validation cohort are italicized and underlined. (B) ROC analysis of these three common tRFs in the validation cohort. (C) Common tRF model applied to the discovery cohort (n = 8–9 per group) to distinguish between ICH and SM (left), ICH and IS (middle), and IS and SM (right). Dashed colored lines show the diagnostic potential of the separate isodecoders; black curves show the diagnostic potential of the combination of these. AUC: Area under the curve. HC: Healthy controls.

DISCUSSION

We show that tRFs hold potential as biomarkers for the early diagnosis of acute stroke. Importantly, we found that tRF abundance from specific isodecoders can be used to (1) differentiate acute ICH from IS and SM, and (2) distinguish acute IS from SM and healthy controls. In all cases, combining different isodecoders in a single model yielded substantially higher diagnostic accuracies than individual isodecoders.

By combining the tRFs that were identified in both the discovery and the validation cohort, we formulated a model consisting of three tRFs that had good diagnostic potential for differentiating IS from healthy controls, SM and ICH. As there were no ICH patients in the validation cohort, it was not surprising that the common tRF model showed only poor diagnostic accuracy for the differentiation between ICH and SM. In fact, this confirmed the validity of the data obtained from the discovery cohort of the MIRAS study in distinguishing between IS and ICH.

To our knowledge, this is the first study on sncRNAs that includes all acute stroke codes, thereby reflecting clinical practice. Furthermore, we demonstrate the diagnostic potential of tRFs in stroke patients, which, to our knowledge, has only been reported in in vitro and in vivo models before.[20-22]

For stroke biomarkers to be of clinical use, rapid detection is crucial. A recent paper demonstrated that rapid detection of low concentrations of tRFs in plasma is indeed feasible.[23] We found that tRFs were also detectable in plasma depleted of extracellular vesicles, suggesting that circulating tRFs are mainly present outside vesicles. Therefore, we hypothesize that they can also be directly detected in whole blood, without needing to disrupt cellular or vesicular membranes. Importantly, these characteristics will absolve the need for labor- and time-intensive sample processing steps such as lysis, ultracentrifugation, RNA isolation and PCR, which is essential for point-of-care test development. Such a point-of-care test could potentially be used in the pre-hospital setting, for example in the ambulance or at the general practitioner. Thereby, the time to treatment could be drastically reduced, especially for ischemic strokes, which require reperfusion treatment as quickly as possible. Future studies need to confirm the feasibility, as well as the added diagnostic value of tRFs on top of clinical assessment (i.e., based on medical history, symptoms and neuroimaging), although the latter is known to be unreliable.[24]

The prognostic potential of tRNA-derivatives in general has been shown by Ishida et al. in plasma collected from IS patients after seven days and has also been suggested in other diseases.[4, 25] Apart from their potential as diagnostic biomarkers, it will therefore also be highly

interesting to determine whether specific circulating tRFs have prognostic value for clinical outcome. Moreover, elucidating how tRFs are released into the circulation will be crucial to understand the pathophysiological link with stroke subtypes. Interestingly, a recent paper showed that tRNA-derived small RNAs in the brain may have a functional effect after experimental ICH induction in rats.[26]

Our study is limited by the explorative design and small sample size. These limitations should be taken into account when interpreting the data. Due to the small sample size, we did not adjust for the fact that the groups were not completely matched for the vascular risk factors DM-II and hypertension, which may have confounded the results. Our results will therefore be validated in a larger cohort from the ongoing MIRAS study, allowing for multivariate analysis to adjust for vascular risk factors. Furthermore, the addition of tRFs as biomarkers to a clinical score could be investigated, for example using diagnostic measures such as the Net Reclassification Index or using likelihood-based methods.[27, 28] Finally, our results have not yet been validated using an independent detection method such as RT-qPCR. Nonetheless, the IS and SM models yielded comparable AUCs in the validation cohort. As expected, the ICH model had much lower predictive value in the validation cohort, as this cohort did not include ICH patients. These findings provide additional support for our results and underline the specificity of the models for each stroke subtype.

In conclusion, we identified tRFs as a promising novel biomarker class to distinguish between acute IS, ICH and SM. New detection technologies may facilitate swift translation into a point-of-care test that can eventually be implemented in clinical practice to reduce treatment delays and improve patient outcomes.

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SUPPLEMENTAL

SUPPLEMENTAL 1.
STROBE Statement. Checklist of items that should be included in reports of cohort studies

	Item No	Recommendation	Page No
Title and abstract	1	*(a)* Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/ rationale	2	Explain the scientific background and rationale for the investigation being reported	2
Objectives	3	State specific objectives, including any prespecified hypotheses	2
Methods			
Study design	4	Present key elements of study design early in the paper	10
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	10-11
Participants	6	*(a)* Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	10
		(b) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	11
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	11
Bias	9	Describe any efforts to address potential sources of bias	9

Study size	10	Explain how the study size was arrived at	10
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	13-15
Statistical methods	12	**Recommendation** *(a) Describe all statistical methods, including those used to control for confounding*	13-14
		(b) Describe any methods used to examine subgroups and interactions	
		(c) Explain how missing data were addressed	
		(d) If applicable, explain how loss to follow-up was addressed	
		(e) Describe any sensitivity analyses	
Results Participants	13*	*(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed*	8
		(b) Give reasons for non-participation at each stage	
Descriptive data	14*	*(c) Consider use of a flow diagram* *(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders*	3
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Report numbers of outcome events or summary measures over time*	3s

Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	3-7
		(b) Report category boundaries when continuous variables were categorized	
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done – eg analyses of subgroups and interactions, and sensitivity analyses	N.A
Discussion			
Key results	18	Summarise key results with reference to study objectives	8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8-9
Generalisability	21	Discuss the generalisability (external validity) of the study results	8
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15

*Give information separately for exposed and unexposed groups.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

Table S1.
Sum of tRF levels for each isodecoder

Isodecoder	Mean	SD	Mean	SD	Mean	SD	p Value
SerGCT	112.2	44.0	77.4	18.7	67.1	23.0	0.0138
LeuCAG	28.8	17.6	47.5	21.3	53.5	15.6	0.0301
AsnGTT	132.0	98.5	62.2	15.3	64.0	42.3	0.0438
ArgTCG	45.2	33.1	24.4	11.3	21.0	7.6	0.0440
ValCAC	145.0	56.7	128.9	30.2	179.0	47.3	0.0800
ProAGG	77.6	83.3	30.1	9.8	32.9	18.7	0.0959
IleAAT	204.3	106.5	140.3	35.8	145.7	48.0	0.1302
LeuTAA	48.4	20.0	32.9	11.1	37.7	15.3	0.1385
ThrTGT	47.3	26.7	33.0	14.5	30.1	13.3	0.1567
GlyCCC	698.2	516.9	392.1	109.8	701.9	450.1	0.1894
HisGTG	49.4	21.3	53.5	27.3	68.7	22.1	0.2270
GlyTCC	220.4	434.8	41.2	19.0	44.3	24.1	0.2420
SerACT	7.1	4.9	9.9	8.8	13.6	8.9	0.2544
ProCGG	37.1	41.3	18.0	5.4	21.3	14.3	0.2551
GlyGCC	1016.6	744.5	535.6	239.0	1262.2	1376.3	0.2560
ArgTCT	162.1	23.9	134.9	46.3	136.6	52.0	0.3693
ValAAC	77.2	48.5	51.8	18.8	69.6	43.0	0.3877
TyrATA	6.4	3.2	8.4	3.5	6.7	2.8	0.3945
ArgCCG	21.3	12.9	15.4	6.4	18.1	7.3	0.4208
AlaAGC	156.7	55.4	136.2	22.5	136.3	26.1	0.4361
CysGCA	454.3	475.0	271.7	120.0	352.8	196.5	0.4566
AspGTC	932.5	325.4	783.0	108.1	888.2	286.6	0.4681
LeuAAG	192.8	165.6	231.8	122.2	272.2	101.9	0.4706
ThrCGT	27.2	14.4	20.8	6.4	24.7	10.5	0.4723
AlaTGC	152.5	68.1	168.4	39.8	185.9	60.0	0.4894
Undet???	6.2	3.5	8.2	3.7	7.7	3.8	0.5230
LeuTAG	74.2	48.2	79.1	42.0	95.8	36.9	0.5441
ProTGG	259.5	281.2	178.8	78.6	184.2	59.3	0.5487
TyrGTA	90.8	23.1	84.8	21.5	97.3	26.9	0.5525
LysCTT	464.4	436.5	343.7	158.2	342.3	57.2	0.5563
LysTTT	337.0	162.9	270.8	84.8	308.4	124.0	0.5620
SerCGA	61.4	51.6	65.7	47.7	45.6	17.3	0.5639
GluTTC	185.4	201.8	172.6	79.5	236.3	103.5	0.5829
SupTTA	18.5	10.4	14.1	3.4	17.0	11.0	0.5928
MetCAT	117.4	66.7	99.8	27.3	98.9	15.4	0.5930
TrpCCA	8.7	6.2	12.1	10.5	9.2	4.1	0.6025
GlnTTG	21.0	38.2	10.2	6.8	15.2	9.6	0.6130
SerAGA	96.2	37.5	102.4	21.7	90.7	26.3	0.6956
AlaCGC	51.8	44.8	41.8	8.1	50.4	18.1	0.7138
ValTAC	304.8	151.4	263.2	83.0	280.2	94.4	0.7465
GluCTC	278.0	110.0	278.1	115.2	249.1	44.7	0.7627
LeuCAA	59.1	36.1	50.6	14.6	55.8	19.6	0.7705
GlnCTG	11.6	8.5	9.7	5.8	11.3	4.6	0.8017
PheGAA	110.6	38.2	102.4	32.8	101.7	38.3	0.8605
ArgACG	20.7	27.2	16.5	10.1	16.8	10.6	0.8634
SerTGA	72.0	12.0	75.4	16.1	72.8	21.2	0.9083
SeC(e)TCA	31.9	23.1	31.1	23.6	34.1	21.3	0.9586
ArgCCT	28.5	10.6	29.1	7.9	28.0	8.0	0.9662

Mean and standard deviation of the sum of the multi-mapping adjusted total RPM. One-way ANOVA was used to test for differences between group means.

Table S2.
Diagnostic parameters determined by ROC analysis on generalized linear models of combinations of isodecoders

	Sensitivity	Specificity	AUC (95% CI)
Optimal LASSO models (discovery cohort; Figure 1C)			
ICH vs other groups (ICH model)*	1.000	0.889	0.986 (0.953-1.000)
IS vs other groups (IS model)†	0.889	0.941	0.967 (0.907-1.000)
SM vs other groups (SM model)‡	0.765	1.000	0.928 (0.832-1.000)
Previous models (validation cohort; Figure 2)			
ICH model*	0.550	0.950	0.728 (0.561-0.894)
IS model†	0.800	0.900	0.870 (0.756-0.984)
SM model‡	0.800	0.850	0.885 (0.781-0.989)
New models (validation cohort; Figure 3A and 3B)			
Optimal LASSO model§	1.000	1.000	1.000 (1.000-1.000)
Common tRF model‖	0.800	0.900	0.875 (0.759-0.991)
Common tRF model‖ (discovery cohort; Figure 3C)			
ICH vs SM‖	0.750	0.667	0.653 (0.359-0.947)
ICH vs IS‖	0.625	1.000	0.847 (0.656-1.000)
IS vs SM‖	1.000	1.000	1.000 (1.000-1.000)

*LeuCAG, ArgTCG, LeuTAA and SerGCT; †ValCAC, ThrCGT, LeuCAG and GlyCCC; ‡TyrGTA, ValCAC, LeuCAG, SerGCT and SerACT; §TyrGTA, ValCAC, MetCAT, ThrCGT, HisGTG, AlaTGC, LysCTT, TyrATA and AlaAGC; ‖ValCAC, TyrGTA and ThrCGT.

Part III

IN-HOSPITAL TRIAGE

Chapter 6

Thrombolysis related symptomatic intracranial hemorrhage in estimated versus measured body weight

International Journal of Stroke, 2019

T. Truc My Nguyen; Stephanie IW van de Stadt; Adrien E. Groot; Marieke JH Wermer; Heleen M. den Hertog; Hanneke M. Droste; Erik W. van Zwet; Sander M. van Schaik; Jonathan M. Coutinho and Nyika D. Kruyt.

ABSTRACT

Background and purpose

In acute ischemic stroke, under- or overestimation of body weight can lead to dosing errors of recombinant tissue plasminogen activator (rt-PA) with consequent reduced efficacy or increased risk of hemorrhagic complications. Measurement of body weight (MBW) is more accurate than estimation of body weight (EBW) but potentially leads to longer door-to-needle times (DNT). Our aim was to assess if weight modality (EBW versus MBW) is associated with (i) symptomatic intracranial hemorrhage (sICH) rate (ii) clinical outcome and (iii) DNT.

Methods

Consecutive patients treated with IVT between 2009-2016 from 14 hospitals were included. Baseline characteristics and outcome parameters were retrieved from medical records. We defined sICH according to the ECASS-III definition and clinical outcome was assessed with the modified Rankin Scale (mRS). The association of weight modality and outcome parameters was estimated with regression analyses.

Results

A total of 4801 patients were included. Five hospitals used MBW (n=1753), six hospitals used EBW (n=2325) and three hospitals (n=723) changed from EBW to MBW during the study period. In 2048 of the patients (43%) MBW was used and in 2753 (57%) EBW. In the MBW group, an inbuilt weighing bed was used in 1094 patients (53%) and a patient lift scale in 954 patients (47%). In the EBW group policy regarding estimation was similar. Estimation of body weight was not associated with increased sICH risk (adjusted OR= 1.16; 95% CI 0.83-1.62) or favourable outcome (adjusted OR= 0.99; 95% CI 0.82-1.21), but it was significantly associated with a longer DNT compared to MBW using an inbuilt weighing bed (adjusted B= 3.57; 95% CI 1.33–5.80) and a shorter DNT compared to MBW using a patient lift scale (-3.96; 95% CI -6.38– -1.53).

Conclusion

We did not find evidence that weight modality (EBW versus MBW) to determine rt-PA dose in IVT eligible patients is associated with sICH or clinical outcome. We did find that EBW leads to longer DNTs compared to MBW using an inbuilt weighing bed and to shorter DNTs compared to MBW using a patient lift scale.

INTRODUCTION

Acute ischemic stroke patients should receive intravenous thrombolysis (IVT) with recombinant tissue plasminogen activator (rt-PA) as quickly as possible for optimal clinical efficacy.[1-5] The most feared complication of IVT is symptomatic intracranial hemorrhage (sICH) occurring in 2.7%–5.7% of patients.[6]

Dose finding trials for rt-PA indicate that 0,9 mg per kilogram body weight has an optimal safety and efficacy profile: a lower dose resulted in reduced efficacy and a higher dose in increased sICH risk. The patient's weight is therefore essential, but exact measurement can be time consuming leading to increased door-to-needle times (DNT) with less clinical IVT efficacy. Therefore estimation of body weight (EBW), rather than exact measurement of body weight (MBW), is often used with potential under- or overestimation.[7-11] Indeed, overestimation due to EBW was shown to result in increased rt-PA dose and increased sICH risk.[8, 9, 12, 13] In contrast, other studies did not confirm this observation, indicating that EBW is acceptable since dosing errors did not influence outcomes.[10, 14] However, the sample sizes of these studies so far are small (n=222; n=308) and were therefore underpowered to detect differences in sICH rate. Hence, based on available evidence, it is not possible to draw conclusions on the best weight modality.

National and the American Stroke Association (ASA) guidelines lack recommendations regarding weight modality, thus both EBW and MBW are being used in clinical practice.[15] [16] We used this disparity to assess, if weight modality is associated with (i) sICH rate (ii) clinical outcome and (iii) DNT.

METHODS

Study design and patient selection

We derived data from prospective IVT registries of 14 centers and included consecutive adult patients with acute ischemic stroke (AIS) treated with IVT between January 2009 and December 2016. Patients were excluded if weight modality was unknown or if no clinical data were available.

The ethical standards committee of the Leiden University Medical Center approved the protocol and waived the need for written informed consent from individual patients.

Patient data

The following data were collected: patient characteristics including demographics, vascular risk factors and history, medication use, admission blood pressure and baseline stroke severity assessed with the National Institute of Health Stroke Scale (NIHSS) score. In case data were missing, these were complemented from the medical records. In case NIHSS score was not noted, this was reconstructed from neurological examination at admission with a validated algorithm as described previously.[17]

Weight assessment

Mode of weight assessment during the inclusion period was acquired by asking the stroke neurologist involved and by assessing local protocols of each participating center. In all centers, either estimation or exact measurement of body weight was done before the CT scan. None of the EBW centers measured body weight during (infusion of) IVT, so possible discrepancies between reported and estimated weight did not led to alteplase dose adjustments.

In the EBW group, policy was similar in all centers: i) weight was assessed first by asking the patient; ii) in case this was not possible (e.g., due to aphasia) by asking a relative and iii) if this was not possible estimation was always done by the treating physician, but in case another health care worker had a different estimation, consensus was reached.

In the MBW group, weight was measured: i) by transferring the patient to a bed with an inbuilt weighing option or a stretcher standing on a ground scale or ii) by using a patient lift scale, requiring lifting the patient in a sling.

Outcome measures

Our primary outcome measure was the sICH rate. We defined sICH according to the ECASS-III definition i.e.: any apparently extravascular blood in the brain or within the cranium that was associated with clinical deterioration, as defined by an increase of four or more points on NIHSS score, or that led to death and that was identified as the predominant cause of the neurological deterioration.[3] In our study, we included all sICH within seven days after stroke onset. Secondary outcome measures included favorable outcome at 90 days (defined as a score of 0-2 on the modified Rankin Scale (mRS)) and DNT (which was defined as the time between patient arrival at the hospital and intravenous rt-PA initiation).[18] In case of missing data on clinical outcome at 90 days, the mRS was derived using available follow up data before three months and ≥1 month after hospital discharge. Both sICH and clinical outcome were retrieved from medical records, including neuro-imaging data by two independent reviewers (TTMN and AEDG). Discrepancies were solved by discussion. Time of symptom onset, time of center arrival and time of IVT initiation were extracted to calculate the DNT.

Statistical analysis

Descriptive statistics were used to compare patient characteristics. Categorical variables were compared with X^2 *test.* Continuous variables were compared using the *t* test or Mann-Whitney U test and are presented as mean ± standard deviation (SD) or median and interquartile range (IQR).

We used logistic regression to assess the association of separate outcomes (sICH and clinical outcome) in relation to weight modality, expressed as odds ratios (ORs) or adjusted OR's (aOR) with corresponding 95% confidence interval (CI). Linear regression analysis was performed to assess the association between weight modality and DNT, presented as regression coefficient (B) and corresponding 95% CI. In secondary analysis, we adjusted for baseline characteristics associated with outcomes (P<0.1) except for the analysis related to the outcome DNT where we adjusted for variables known to have an association with the DNT: availability of a CT in the emergency department (ED), blood pressure above the threshold for IVT (>185/110 mmHg), NIHSS score at baseline[19, 20], onset-to-door time (defined as the time between stroke onset and patient arrival at the hospital) and for annual IVT-volume divided as follows: low-volume (≤24), medium-volume (25-49) or high-volume (≥50) as described previously, with low-volume as reference category.[21] In subgroup analyses we investigated if differences in methods within the EBW or the MBW group, could have affected the association between weight modality and the outcome measures.

Missing data

For missing data, we performed multiple imputation with the fully conditional specification method with five sets of imputations. The predictive mean matching model type was used for scale variables. Then, we compared the results of the analysis of the imputed data set with the non-imputed dataset to assess if this leads to consistent parameter estimates. Additionally, we performed *post hoc* sensitivity analyses to assess the effect of missing data with regards to mRS score after 90 days, by recalculating the estimates while omitting patients with missing mRS score after 90 days.

Statistical analysis was performed using SPSS software (version 23, IBM, New York, USA).

RESULTS

Baseline characteristics

Data from 5066 patients with AIS were collected. A total of 4801 (95%) patients met the inclusion criteria (Figure 1). Five centers used MBW, six centers EBW and three centers changed from EBW to MBW during our inclusion time window. In 2048 of the patients (43%) MBW was used and in 2753 patients (57%) EBW. EBW-patients were slightly older, and they had more cardiovascular risk factors (atrial fibrillation, diabetes mellitus, hypertension and hyperlipidemia) (Table 1). Other known predictors for sICH (sex, NIHSS score, blood pressure and onset-to-door time) did not differ between the EBW and the MBW group. More EBW-patients were treated in high-volume centers (n=2181; 79%) compared to MBW-patients (n=1121; 55%) and a CT in the ER was present for 407 EBW-patients (15%) and for 927 MBW-patients (45%).

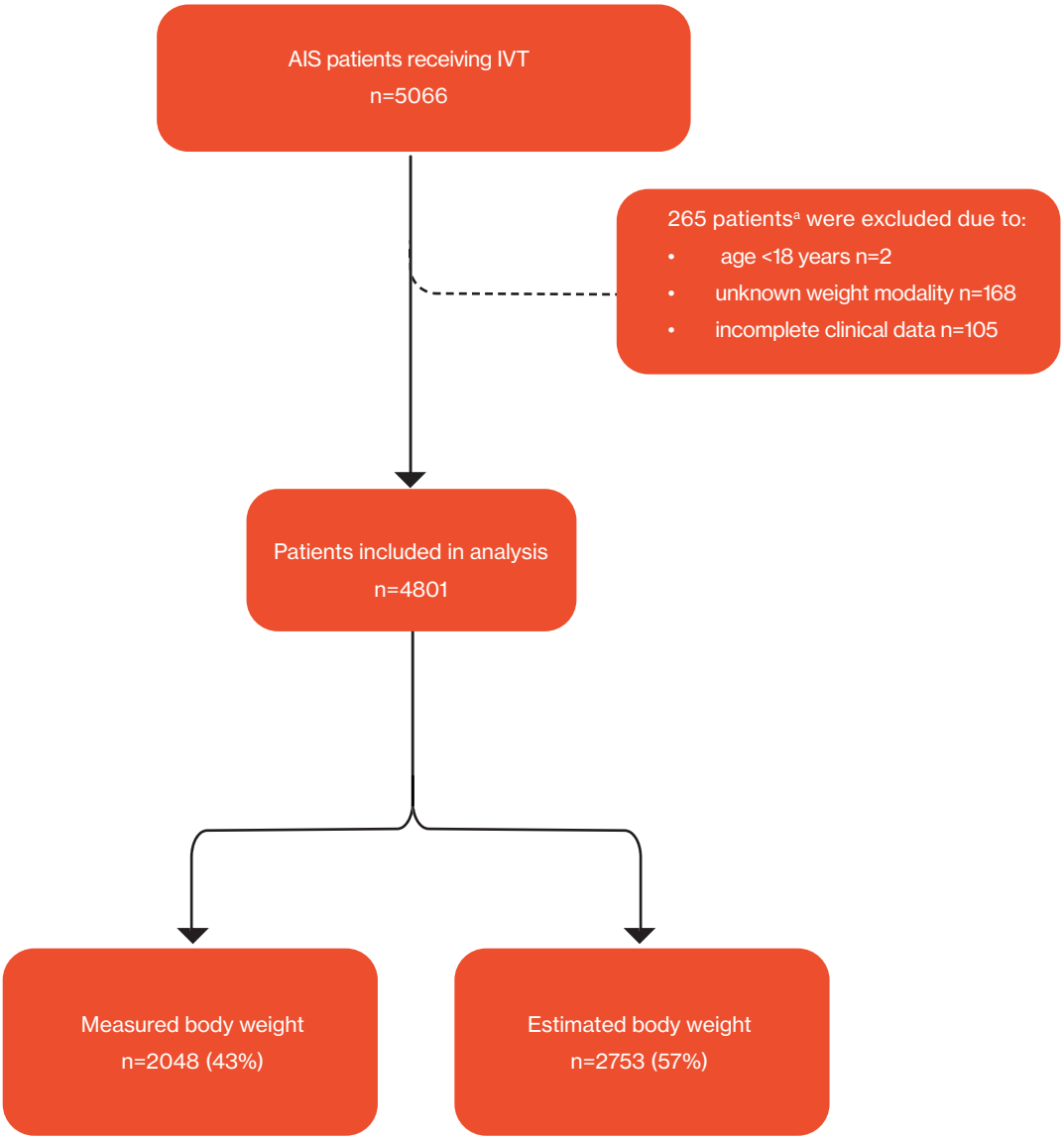

Figure 1.
Flowchart of the study

AIS: acute ischemic stroke; IVT: intravenous thrombolysis. [a] Ten patients had incomplete data and an unknown weight modality.

Table 1.
Patient Characteristics

Variables	Measured body weight (n=2048)	Missing data %	Estimated body weight (n=2753)	Missing data %	P-values
Patient characteristics					
Age at stroke, years – mean ±SD)	70 (±14)	0%	71 (±14)	0%	<0.01
Male sex – n (%)	1122 (54.8)	0.1%	1502 (54.6)	0%	0.86
Vascular risk factors					
Atrial fibrillation – n (%)	175 (8.6)	1.2%	376 (14.1)	2.9%	<0.01
Diabetes mellitus – n (%)	309 (15.3)	1.1%	469 (17.5)	2.8%	0.04
Hypertension – n (%)	840 (41.5)	1.2%	1355 (50.7)	2.9%	<0.01
Hyperlipidemia – n (%)	238 (11.8)	1.3%	841 (31.6)	3.2%	<0.01
Coronary artery disease – n (%)	410 (20.3)	1.2%	507 (19.0)	2.9%	0.27
Peripheral vascular disease – (n %)	112 (5.6)	1.6%	156 (5.8)	2.8%	0.69
Prior TIA/stroke – n (%)	528 (26.2)	1.5%	660 (24.7)	2.9%	0.25
Medication					
Antiplatelets – n (%)	449 (37.9)	42.2%	638 (37.0)	37.4%	0.62
Anticoagulation – n (%)	35 (3.0)	42.2%	65 (3.8)	37.3%	0.24
Admittance					
Systolic BP, mmHg, mean (±SD)	156 (±25)	5.9%	156(±26)	15.4%	0.43
Diastolic BP, mmHg, mean (±SD)	86 (±17)	5.9%	85 (±27)	15.4%	0.02
NIHSS, median [IQR]	7 [4–12]	1.3%	6 [3–12]	0.7%	0.08
ODT, min – median [IQR]	69 [45–115]	13.5%	69 [45–112]	7.8%	0.89
IVT-volume (IVT/year)					
High volume (≥50) – n (%)	1121 (54.7)	0%	2181 (79.2)	0%	<0.01
Medium volume (25-49) – n (%)	656 (32.0)	0%	470 (17.1)	0%	<0.01
Low volume (≤24) – n (%)	271 (13.2)	0%	102 (3.7)	0%	<0.01
CT available in the ED	927 (45.3)	0%	407 (14.8)	0%	<0.01

TIA: transient ischemic attack; BP: blood pressure; NIHSS: National Institute of Health Stroke Scale; ODT: onset-to-door time; IVT: intravenous thrombolysis; CT: computed tomography scan; ED: emergency departmentroom.

OUTCOMES

We found no significant differences for the primary or secondary outcomes between the EBW and the MBW group (Table 2). The rate of sICH was 4.4% in EBW versus 4.1% in the MBW group, clinical outcome was favorable in 60% of the EBW and 56% of the MBW group and DNT was 33 minutes (IQR 24-50) in the EBW and 32 minutes (IQR 23 – 47) in the MBW group. We did find significant differences for the DNT, when the MBW group was divided into subgroups according to exact weight measurement method. The DNT was 28 minutes (IQR 20–40) for the MBW group with inbuilt weighing bed and 38 minutes (IQR 28–53) for the MBW group with a patient lift scale. Weight modality (in this case EBW versus MBW) was not significantly associated with increased risk of sICH (aOR= 1.16; 95% CI 0.83-1.62), favourable outcome (aOR= 0.99; 95% CI 0.82-1.21) or with DNT (adjusted B= 0.28; 95% CI -1.69–2.25) (Table 3). [insert Table 3] We also did not find a significant association with EBW versus either of the MBW subgroups (inbuilt weighing bed and patient lift scale) with an increased risk of sICH or favourable outcome (supplementary data, Table 4.). We did, however, find a significant association for the DNT. The DNT was longer in the EBW group compared to the MBW group with inbuilt weighing bed (adjusted B= 3.57; 95% CI 1.33–5.80) and the DNT was shorter in the EBW group compared to the MBW with patient scale sling (adjusted B= -3.96; 95% CI -6.38– -1.53) (Table 3).

Missing data

Baseline characteristics did not show a relevant difference in patients with or without a known clinical outcome and missing outcome data were also evenly distributed between the groups. Results of the analysis of the imputed dataset were essentially the same as the results of the analysis without imputed data (supplementary data Table 5). Furthermore, *post hoc* sensitivity analysis excluding patients with an unknown clinical outcome yielded similar robustness of the primary analysis (supplementary Table 6).

Table 2.
Outcome measures.

Outcome	Measured Body Weight (n=2048)	Missing data %	Estimated body weight (n=2753)	Missing data %	P Value
sICH – n (%)	83 (4.1)	0%	122 (4.4)	0%	0.52
mRS 0–2 at 90 days – n (%)	599 (56.2)	48.0%	920 (59.9)	44.2%	0.06
DNT, min – median [IQR]	32 [23–47]	2.7%	33 [24–50]	2.8%	0.15
• Inbuilt weighing bed	28 [20–40]	1.6%			<0.01[a]
• Patient lift scale	38 [28–53]	3.9%			<0.01[b]

sICH: symptomatic intracranial hemorrhage; mRS: modified Rankin Scale; DNT: door-to-needle time; min: minutes.
[a] DNT for EBW versus inbuilt weighing bed scale
[b] DNT for EBW versus patient lift scale

Table 3.
Logistic and linear regression analysis for the association between weight modality (EBW versus MBW) and the outcome measures.

Outcome	**Logistic regression analyses OR (95%)**	**aOR (95%) [a]**
sICH	1.09 (0.83–1.46)	1.16 (0.83–1.62)
mRS 0–2 at 90 days	1.01 (0.88–1.16)	0.99 (0.82–1.21)
	Linear regression analysis B (95% CI)	**B (95% CI) [b]**
DNT in minutes • EBW vs. MBW	0.06 (-1.59–1.71)	0.28 (-1.69–2.25)
DNT in minutes [c] • EBW vs. inbuilt weighing bed	4.01 (1.99–6.01)	3.57 (1.33–5.80)
DNT in minutes [d] • EBW vs. patient scale sling	-4.47 (-6.58– -2.36)	-3.96 (-6.38– -1.53)

EBW: estimated body weight; OR: odds ratio; aOR: adjusted OR; B: unstandardized regression coefficient.
[a] aOR, adjusted for: age; atrial fibrillation; diabetes mellitus; hypertension; hyperlipidemia; admission NIHSS, CT in the ER and IVT-volume.
[b] B, adjusted for: blood pressure exceeding threshold for IVT; admission NIHSS, CT in the ER, onset-to-door time and IVT-volume.
[c] DNT in minutes for EBW versus MBW, inbuilt weighing bed.
[d] DNT in minutes for EBW versus MBW, patient scale sling.

DISCUSSION

Our findings did not demonstrate an association between weight modality and sICH rates or clinical outcome. While previous prospective studies have shown that EBW leads to dosing errors, our results showed that this does not translate into a different safety and efficacy profile of intravenous rt-PA in clinical practice. Interestingly, we found that EBW leads to a longer DNT compared to MBW using an inbuilt weighing bed, but to a shorter DNT compared to MBW using a patient lift scale.

Our main results are in line with some previous studies.[10, 14] However, our study has a much larger study population and unlike the previous studies it concerns a multicenter study. Therefore, it is unlikely that we missed a difference in outcome related to weight modality rendering our results more generalizable to routine clinical practice. In contrast, two studies showed a difference in clinical outcome related to weight modality. One retrospective mono-center study (n=164) found that EBW led to rt-PA overdose in 13 (16%) patients. Of those 13 patients, four had an intracranial hemorrhage (however, it remained unclear whether these were symptomatic or not).[9] Another prospective mono-center study (n=128) found that EBW lead to rt-PA overdose in 52% of the patients with more sICH in the first 24 hours.[12] The overall sICH rate for the whole group was 7.8% in the first 24 hours which is much higher than one would expect from previous studies with this sICH definition.[22] This may have influenced the results limiting generalizability. A possible explanation for the high sICH rate is the predominantly Asian population in this study as Asian ethnicity is associated with increased risk of sICH.[23-25] Furthermore, a follow-up brain CT scan was performed as part of standard clinical care at 24 hours. Therefore, researchers could have been more prone to attribute clinical symptoms to a hemorrhage seen on these standard imaging protocols. Finally, in our study weight modality was not associated with DNT even after adjusting for factors such as IVT volume, CT availability on the ER, baseline NIHSS and blood pressure above IVT threshold.[21, 26-28] Nevertheless, other unknown factors related to the DNT we could not adjust for could possibly explain this lack of an association.

Somewhat surprisingly, the median DNT was shorter in the MBW group using an inbuilt weighing bed compared to the EBW group. An explanation for this could be that in practice weight estimation can require multiple steps (asking the patient or relative and estimation by the treating physician), whereas an inbuilt weighing bed scale only requires one step (transfer of the patient), which is also done in the EBW group (e.g., from ambulance stretcher to hospital bed).

Of note, this difference in DNT does not affect the finding that

weight modality is not associated with an increased risk of sICH or clinical outcome, since we adjusted for the DNT in these analyses.

Our study has several limitations. First, a cluster-randomized trial would be a more suitable design for our research question, but in practice this does not seem feasible since clinics using MBW are not likely to change this to EBW. Due to the retrospective nature of our design, extraction of (outcome) data could have led to bias. However, assessment of our primary outcome, sICH, was done according to strict definitions by two independent reviewers and sICH rates are similar to previous studies using the same definition criteria.[3, 6] Secondly, clinical outcome was missing for a substantial proportion of patients. We investigated the possible influence of missing data on our parameter estimates, by performing different methods of handling missing data in our cohort. Results of the primary analysis remained consistent after imputing missing data (supplementary data Table 5) and after *post hoc* sensitivity analysis (excluding patients with unknown clinical outcome), indicating that missing data was not of significant influence on our outcome parameters (supplementary data Table 6). Additionally, missing outcome data was evenly distributed between the groups and baseline patient characteristics did not show a relevant difference in patients with or without a known clinical outcome (data not shown). Of note, even when excluding patients with unknown clinical outcome our cohort still remains the largest so far investigating weight modality in IVT treated patients. As for the DNT, this is an obligatory practice parameter in all centers and is therefore not likely to be affected by retrospective assessment. Furthermore, data on actual rt-PA dosage and (measured) body weight were lacking. Although this would have enabled us to determine exactly in which patient body weight was over- or underestimated and whether this resulted in over- or under dosing rt-PA, it apparently does not translate into an increased risk of sICH or a clinically meaningful difference. Our data indicates that EBW was not associated with increased risk of sICH (aOR = 1.16; 95% CI 0.83-1.62), therefore a possible effect of weight modality on sICH would be smaller than 1.62 with 95% certainty, independent of whether there is a difference between estimated or measure bodyweight. Finally, a limitation is that centers, with or without a certain weighing modality, could differ in local policies which could lead to a bias related to outcomes. However, all centers treat IVT patients according to the same national guidelines, including pre-hospital notification of potential IVT patients and since patients in each group came from at least five centers and the outcomes are evenly distributed in both groups, we consider this risk minimal.

CONCLUSION

Our study provides the largest multicenter cohort study to date assessing the association between weight modality (EBW or MBW) with sICH, clinical outcome and DNT. We found that MBW with an inbuilt weighing bed leads to shorter DNTs compared to EBW, whereas the latter strategy leads to shorter DNTs compared to MBW with a patient lift scale. We did not find evidence that weight modality for rt-PA titration in IVT eligible patients leads to clinically relevant dosing errors, since it was not associated with an increased risk of sICH or favorable clinical outcome.

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SUPPLEMENTAL

Supplementary Table 4.
Subgroup analysis: logistic regression analysis for the association between weight modality (EBW versus MBW subgroups) and the outcome measures.

Outcome		OR (95% CI)	aOR (95% CI) [a]
sICH			
•	EBW vs. MBW	1.09 (0.83–1.46)	1.16 (0.83–1.62)
sICH			
•	EBW vs. inbuilt weighing bed	1.01 (0.72–1.42)	1.08 (0.74–1.59)
sICH			
•	EBW vs. patient scale sling	1.22 (0.83–1.79)	1.36 (0.87–2.10)
mRS 0–2 at 90 days			
•	EBW vs. MBW	1.01 (0.88–1.16)	0.99 (0.82–1.21)
mRS 0–2 at 90 days			
•	EBW vs. inbuilt weighing bed	1.00 (0.86–1.18)	0.96 (0.75–1.22)
mRS 0–2 at 90 days			
•	EBW vs. patient scale sling	1.01 (0.83–1.24)	1.05 (0.81–1.36)

EBW: estimated body weight; MBW: measured body weight; OR: odds ratio; aOR: adjusted OR. [a] aOR, adjusted for: age; atrial fibrillation; diabetes mellitus; hypertension; hyperlipidemia; admission NIHSS, CT in the ER and IVT-volume.

Supplementary Table 5.
Logistic and linear regression analysis for the association between weight modality (EBW versus MBW subgroups) and the outcome measures with the original dataset (without imputing missing data).

Outcome	Logistic regression analyses	
	OR (95% CI)	**aOR (95% CI)** [a]
sICH		
• EBW vs. MBW	1.09 (0.83–1.46)	1.16 (0.83–1.62)
sICH		
• EBW vs. inbuilt weighing bed	1.01 (0.72–1.42)	1.08 (0.74–1.59)
sICH		
• EBW vs. patient scale sling	1.22 (0.83–1.79)	1.36 (0.87–2.10)
mRS 0–2 at 90 days		
• EBW vs. MBW	1.01 (0.88–1.16)	0.99 (0.82–1.21)
mRS 0–2 at 90 days		
• EBW vs. inbuilt weighing bed	1.00 (0.86–1.18)	0.96 (0.75–1.22)
mRS 0–2 at 90 days		
• EBW vs. patient scale sling	1.01 (0.83–1.24)	1.05 (0.81–1.36)
Outcome	**Linear regression analysis**	
	B (95% CI)	**B (95% CI)** [b]
DNT in minutes		
• EBW vs. MBW	0.06 (-1.59–1.71)	0.28 (-1.69–2.25)
DNT in minutes		
• EBW vs. inbuilt weighing bed	3.99 (1.97– 6.03)	3.74 (1.55–5.93)
DNT in minutes		
• EBW vs. patient scale sling	-4.57 (-6.72– -2.42)	-4.67 (-7.23– -2.09)

EBW: estimated body weight; MBW: measured body weight; OR: odds ratio; aOR: adjusted OR; B: unstandardized regression coefficient.
[a] aOR, adjusted for: age; atrial fibrillation; diabetes mellitus; hypertension; hyperlipidemia; admission NIHSS, CT in the ER and IVT-volume.
[b] B, adjusted for: blood pressure exceeding threshold for IVT; admission NIHSS, CT in the ER, onset-to-door time and IVT-volume.

Supplementary Table 6.
Post hoc sensitivity analysis: logistic and linear regression analysis for the association between weight modality (EBW versus MBW subgroups) and the outcome measures (excluding patients with unknown clinical outcome).

Outcome	Logistic regression analyses	
	OR (95% CI)	**aOR (95% CI)** [a]
sICH		
• EBW vs. MBW	1.49 (0.84–2.67)	1.58 (0.80–3.12)
sICH		
• EBW vs. inbuilt weighing bed	1.13 (0.55–2.30)	1.10 (0.51–2.38)
sICH		
• EBW vs. patient scale sling	1.95 (0.89–4.26)	2.83 (1.03–7.79)
Outcome	**Logistic regression analysis**	
	B (95% CI)	**B (95% CI)** [b]
DNT in minutes		0.28 (-1.69–2.25)
• EBW vs. MBW	0.06 (-1.59–1.71)	
DNT in minutes		7.25 (5.95– 8.55)
• EBW vs. inbuilt weighing bed	7.54 (6.27– 8.82)	
		-2.94 (-4.23– -1.66)
DNT in minutes		
• EBW vs. patient scale sling	-1.98 (-3.12– -0.84)	

EBW: estimated body weight; MBW: measured body weight; OR: odds ratio; aOR: adjusted OR; B: unstandardized regression coefficient.
[a] aOR, adjusted for: age; atrial fibrillation; diabetes mellitus; hypertension; hyperlipidemia; admission NIHSS, CT in the ER and IVT-volume.
[b] B, adjusted for: blood pressure exceeding threshold for IVT; admission NIHSS, CT in the ER, onset-to-door time and IVT-volume

Chapter 7

High admission glucose is associated with poor outcome after endovascular treatment for ischemic stroke

Stroke, 2020

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ABSTRACT

Background and purpose

High serum glucose on admission is a predictor of poor outcome after stroke. We assessed the association between glucose concentrations and clinical outcomes in patients who underwent endovascular treatment (EVT).

Methods

From the MR CLEAN Registry, we selected consecutive adult patients with a large vessel occlusion of the anterior circulation who underwent EVT and for whom admission glucose levels were available. We assessed the association between admission glucose and the modified Rankin Scale (mRS) score at 90 days, symptomatic intracranial hemorrhage (sICH) and successful reperfusion rates. Hyperglycemia was defined as admission glucose ≥7.8 mmol/L. We evaluated the association between glucose and mRS using multivariable ordinal logistic regression and assessed whether successful reperfusion (eTICI 2b-3) modified this association.

Results

Of 3637 patients in the MR CLEAN Registry, 2908 were included. Median admission glucose concentration was 6.8 mmol/L (IQR 5.9-8.1) and 882 patients (30%) had hyperglycemia. Hyperglycemia on admission was associated with a shift towards worse functional outcome (median mRS 4 vs 3; adjusted common OR 1.69; 95%CI 1.44-1.99), increased mortality (40% vs 23%; aOR 1.95; 95%CI 1.60-2.38) and an increased risk of sICH (9% vs 5%; aOR 1.94; 95%CI 1.41-2.66) compared to non-hyperglycemic patients. The association between admission glucose levels and poor outcome (mRS 3-6) was J-shaped. Hyperglycemia was not associated with the rate of successful reperfusion, nor did successful reperfusion modify the association between glucose and functional outcome.

Conclusion

Increased admission glucose is associated with poor functional outcome and an increased risk of sICH after EVT.

INTRODUCTION

In the acute phase of ischemic stroke many patients have hyperglycemia, even if they do not have a history of diabetes mellitus.[1] Hyperglycemia is associated with poor functional outcome[2] and lower recanalization rates[3] in ischemic stroke patients treated with intravenous thrombolysis (IVT). There are various mechanisms by which high glucose concentrations might exert a detrimental effect on the brain: hyperglycemia may induce brain injury due to intracellular acidosis in ischemic brain tissue, which leads to mitochondrial dysfunction.[4] Furthermore, hyperglycemia may stimulate the formation of reactive oxygen and nitrogen species, which can contribute to the development of reperfusion injury including cerebral edema and hemorrhagic transformation.[5]

Previous studies have assessed the association between serum glucose levels on admission and outcome in stroke patients following endovascular treatment (EVT), however these studies either had a small sample size or were performed in selected patient populations from randomized trials. Therefore, we sought to determine whether admission glucose is associated with radiological, functional and clinical outcomes for patients treated in routine clinical practice with EVT.

METHODS

Study design and patient selection

We used data from the MR CLEAN Registry, a prospective, nationwide registry of consecutive patients with acute ischemic stroke treated with EVT in the Netherlands. For the current study, we used data of all patients treated with EVT between March 2014 and November 2017 of whom the glucose concentration on admission was available. Other inclusion criteria were: age ≥18 years, treatment in a center that had participated in the MR CLEAN trial and presence of a large vessel occlusion (LVO) of the anterior circulation (intracranial carotid artery [ICA/ICA-T], middle cerebral artery [M1/M2] or anterior cerebral artery [A1/A2]). No formal power calculation was performed for the purpose of this study.

EVT consisted of arterial catheterization with a micro-catheter to the level of the occlusion, followed by mechanical thrombectomy, thrombus aspiration, or a combined approach, with or without delivery of a thrombolytic agent. The exact strategy for EVT was at the discretion of the treating physician. Patients with reperfusion at first angiography underwent only a diagnostic digital subtraction angiography (DSA) without further intervention. In patients in whom intracranial access was not possible, only DSA was performed. All relevant imaging was analyzed by an imaging core laboratory, whose members were blinded to all clinical data except for the side of symptoms. An adverse event committee, consisting of two vascular neurologists and one neuro-radiologist, evaluated the safety endpoints based on clinical data and reports from the imaging core lab. Detailed methods of the MR CLEAN Registry have been reported previously.[6]

Medical ethic committee statement

The study protocol has been evaluated by the medical ethics committee of the Erasmus University Medical Center in Rotterdam, and permission to carry out the study as a registry was granted (MEC-2014-235). They waived the need for written informed consent.

Outcomes and definitions

The main outcome was functional outcome at 90 days, assessed with the modified Rankin Scale (mRS). The mRS ranges from 0 (no symptoms) to 6 (death).[7] Other outcomes included poor functional outcome at 90 days (defined as mRS 3-6), mortality at 90 days, symptomatic intracranial hemorrhage (sICH) and other complications (new ischemic stroke, extracranial hemorrhage, stroke progression resulting in death or neurological deterioration, pneumonia and other infections). Hyperglycemia was defined as the first glucose on admission of ≥7.8 mmol/L, in accordance with previous studies [8, 9] and the criteria of the American Diabetes Association.[10] sICH was defined as death or neurological deterioration (an increase of ≥ 4 points on the NIHSS, assessed by the treating physician) associated to the hemorrhage (Heidelberg Bleeding Criteria)[11]. Successful reperfusion was defined as extended Thrombolysis in Cerebral Infarction (eTICI) scores of 2b-3.[12] To achieve an eTICI score of 2b-3, DSA needed to include both antero-posterior and lateral views post-EVT. If the lateral view was not available, the eTICI score could be no higher than 2A. The extent of collaterals was graded on baseline CTA by the imaging core laboratory on a 4-point scale, with 0 for absent collaterals (0% filling of the occluded vascular territory), 1 for poor (>0% and ≤50% filling), 2 for moderate (>50% and <100% filling), and 3 for good collaterals (100% filling), as used previously.[13-15] Definitions of complications were as previously described.[6]

Statistical analysis

We compared patients with hyperglycemia on admission with patients without hyperglycemia on admission. We also analyzed glucose as a continuous variable. We performed independent samples t-test, Mann-Whitney U test, Fishers' exact test or chi-square test as appropriate for intergroup comparison. For regression analyses, missing variables were imputed with multivariate imputation by chained equations with 5 imputations.

For the main outcome we used multivariable ordinal logistic regression analysis to evaluate a shift towards poorer functional outcome on the mRS. For the remaining endpoints, we used multivariable binary logistic regression analyses. For the regression analyses with mRS and mortality as outcome, we adjusted for the following prognostic factors: age, sex, NIHSS at baseline, pre-stroke mRS, treatment with IVT, systolic blood pressure, peripheral artery disease, prior stroke, collateral status and onset-to-groin time. For the regression analyses with reperfusion and extracranial or intracranial hemorrhage as outcomes, we adjusted for the following pre-specified prognostic factors: age, sex, NIHSS at baseline, treatment with IVT, anticoagulant use, antiplatelet use, and systolic blood

pressure. The regression analyses for new ischemic stroke, pneumonia and other infections were adjusted for age, sex and NIHSS at baseline. To explore whether an association between hyperglycemia and poor outcome could be explained by an increased risk of sICH, we performed an analysis in which we additionally adjusted for sICH. We performed a sensitivity analysis restricted to patients without a history of diabetes mellitus, as previous studies have suggested that this group of patients has a worse outcome when presenting with hyperglycemia.[16]

We also analyzed admission glucose as a continuous variable (divided into deciles) to determine the association between admission glucose and mRS at 90 days (dichotomized, with mRS 3-6 as poor functional outcome) and the probability of sICH using multivariable ordinal and binary logistic regression models, respectively. We determined whether the association was non-linear by assessing the fit of models with or without restricted cubic splines using the likelihood ratio test. We set out to assess whether successful reperfusion, as a proxy for treatment with EVT, may result in reperfusion injury which would be more severe in patients with higher glucose levels on admission are more severely affected. Therefore, we determined whether successful reperfusion modified the association between admission glucose and functional outcome at 90 days by adding a multiplicative interaction term in the model. We plotted the probability of poor functional outcome (mRS 3-6) and sICH and we reported adjusted (common) odds ratios (ORs) with corresponding 95% confidence intervals (CI). Statistical analyses were performed using R software and R Studio (Version 3.6.1, R Foundation).

Data cannot be made available to other researchers, as no patient approval was obtained for sharing coded data and sharing data would violate Dutch law. However, syntax and output files of statistical analyses will be made available upon reasonable request.

RESULTS

Of the 3637 patients in the MR CLEAN Registry, we excluded 729 patients for the following reasons: age < 18 years (n=9), posterior circulation ischemic stroke (n=172), EVT in a non-MR CLEAN trial center (n=177), and missing admission glucose levels (n=371; Figure 1). The median admission glucose was 6.8 mmol/L (interquartile range [IQR] 5.9-8.1). In total, 882 of 2908 (30%) patients had hyperglycemia on admission.

Patient and procedural characteristics at baseline are described in Table 1. Patients with hyperglycemia on admission were older (73 vs 71 years, p<0.001) and less often male (49% vs 53%, p=0.021) than patients without hyperglycemia. Hyperglycemic patients more often had a history of diabetes (36% vs 7%, p<0.001) or hypertension (62% vs 49%, p<0.001). Patients with hyperglycemia had higher median NIHSS scores at baseline (16 vs 15, p<0.001) and longer median onset-to-groin-times (205 vs 191 minutes, p<0.001). Hyperglycemic patients had worse collateral scores.

The pre-stroke mRS was similar in both groups. Hemicraniectomy was performed in 35/385 (9%) of hyperglycemic patients compared to 20/814 (2%, p<0.001) for non-hyperglycemic patients.

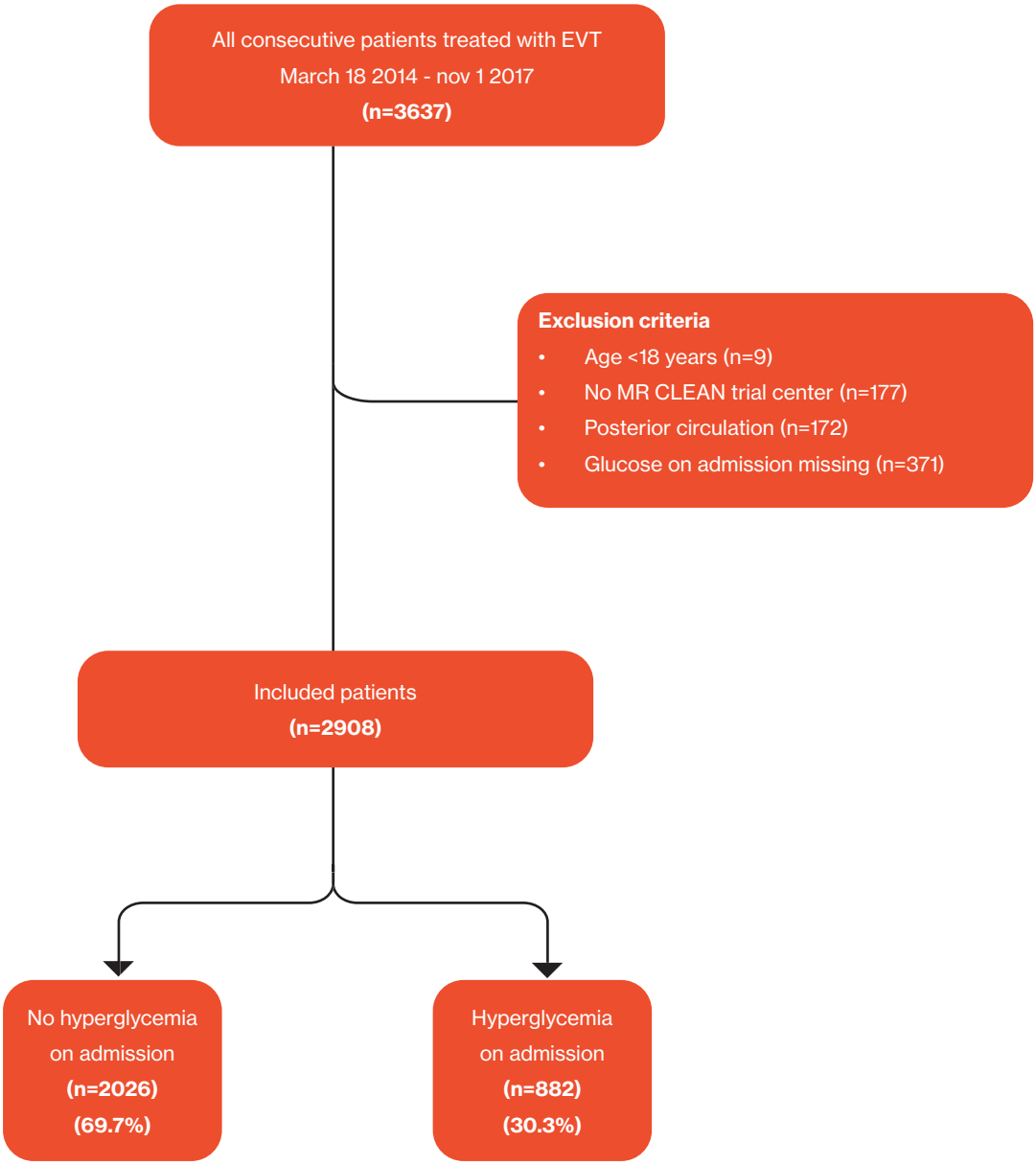

Figure 1.
Flowchart of patient selection

EVT indicates endovascular treatment; and MR CLEAN, Multicenter Randomized Controlled Trial of Endovascular Treatment for Acute Ischemic Stroke in the Netherlands

Table 1.
Baseline and procedural characteristics

	Admission hyperglycemia (n=882)	No admission hyperglycemia (n=2026)	P-value
Median age in years (IQR)	73 (64 – 81)	71 (59 – 80)	**<0.001**
Male, n (%)	430/882 (49)	1082/2026 (53)	**0.021**
Median glucose at admission in mmol/L (IQR)	9.1 (8.3 - 10.8)	6.3 (5.7 – 6.9)	NA
Mean systolic blood pressure (mmHg ± SD)[a]	153 ± 24	149 ± 25	**<0.001**
Mean diastolic blood pressure (mmHg ± SD)[b]	83 ± 16	83 ± 15	0.930
IV thrombolysis, n (%)	664/880 (76)	1522/2023 (75)	0.900
Median NIHSS at admission (IQR) [c]	16 (12-20)	15 (10-19)	**<0.001**
Transfer patients, n (%)	446/882 (51)	1037/2026 (51)	0.759
Medical History			
Stroke, n (%)	163/882 (19)	317/2026 (16)	0.057
Atrial fibrillation, n (%)	205/874 (24)	471/1999 (24)	0.951
Diabetes mellitus, n (%)	315/876 (36)	150/2014 (7)	**<0.001**
Hypertension, n (%)	533/862 (62)	968/1992 (49)	**<0.001**
Peripheral artery disease, n (%)	77/870 (9)	187/1985 (9)	0.628
Pre-stroke mRS, n (%)			0.055
Pre-stroke mRS 0	555/862 (64)	1381/1992 (69)	
Pre-stroke mRS 1	126/862 (15)	242/1992 (12)	
Pre-stroke mRS 2	181/862 (8)	368/1992 (8)	
Pre-stroke mRS ≥ 3	113/862 (13)	216/1992 (11)	
Medication use			
Statin use, n (%)	373/866 (43)	628/1982 (32)	**<0.001**
Anticoagulation use, n (%)	152/868 (18)	356/1991 (18)	0.812
Antiplatelet use, n (%)	306/875 (35)	580/2004 (29)	**<0.001**
Process measures			
General anesthesia, n (%)	202/832 (24)	503/1906 (26)	0.244
Median duration from onset to groin in minutes (IQR)[e]	205 (158-270)	191 (147-255)	**<0.001**
Median duration of procedure in minutes (IQR)[f]	60 (40-85)	56 (35-82)	**0.010**

	Admission hyperglycemia (n=882)	No admission hyperglycemia (n=2026)	P-value
Imaging			
Occlusion location, n (%)			0.397
Intracranial ICA	41/850 (5)	107/1918 (6)	
ICA-T	193 /850 (23)	3851918 (20)	
M1	473 /850 (56)	1126/1918 (58.7)	
M2	135/850 (16)	284 /1918 (15)	
Other †	8 /850 (1)	16 /1918 (1)	
Collateral status - n (%)			**0.029**
Grade 0	61/825 (7)	100/1901 (5)	
Grade 1	298/825 (36)	668/1901 (35)	
Grade 2	324/825 (39)	732/1901 (39)	
Grade 3	142/825 (17)	401/1901 (21)	
Extra-cranial carotid stenosis (50-99%), n (%)	80/783 (10)	158/1767 (9)	0.307
Extra-cranial carotid occlusion, n (%)	91/783 (12)	193/1767 (11)	0.605
Median ASPECTS baseline (IQR)	9 (7-10)	9 (8-10)	0.604

IQR indicates interquartile range; SD, standard deviation; IV, intravenous; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; DSA, digital subtraction angiography; ICA, internal carotid artery; MCA, middle cerebral artery; ACA, anterior cerebral artery; ASPECTS, Alberta Stroke Program Early CT Score;
*Other: local interventionist ended the procedure before a thrombectomy attempt was made, despite a target occlusion being present.
†(M3/A1/A2)
Missing values, n (%): [a] 68 (2), [b]77 (3), [c]40 (1), [e]13 (1), [f]255 (9)

Hyperglycemia and outcome

Patients with hyperglycemia on admission had worse functional outcomes at 90 days than patients without hyperglycemia (median mRS 4 vs 3, p<0.001). After adjustment, hyperglycemia remained associated with poor functional outcome (adjusted common OR (acOR) for a shift towards poor mRS 1.69 (95% CI 1.44-1.99; Figure 2, Table 2).

Of all patients with hyperglycemia on admission, 250/841 (30%) had a good functional outcome (mRS 0-2) compared to 875/1892 (46%) of the non-hyperglycemic patients (aOR 0.60, 95% CI 0.49-0.74, table 2). Mortality at 90 days was higher for patients with hyperglycemia on admission (40% vs 23%, aOR 1.95, 95% CI 1.60-2.38). Hyperglycemic patients had a higher risk of symptomatic ICH (9% vs 5%, aOR 1.94, 95% CI 1.41-2.66). Patients with hyperglycemia on admission also more often had stroke progression (12% vs 8%, aOR 1.44, 95%CI 1.10-1.89), pneumonia (15% vs 10%, aOR 1.48, 95%CI 1.16-1.88), and other infections (5% vs 3%, aOR 1.50, 95%CI 1.03-2.19). There were no differences in the rates of successful reperfusion (60% vs 63%, aOR 0.94, 95%CI 0.79-1.11), or the other complications. Adjusting for sICH yielded similar results for functional outcome at 90 days.

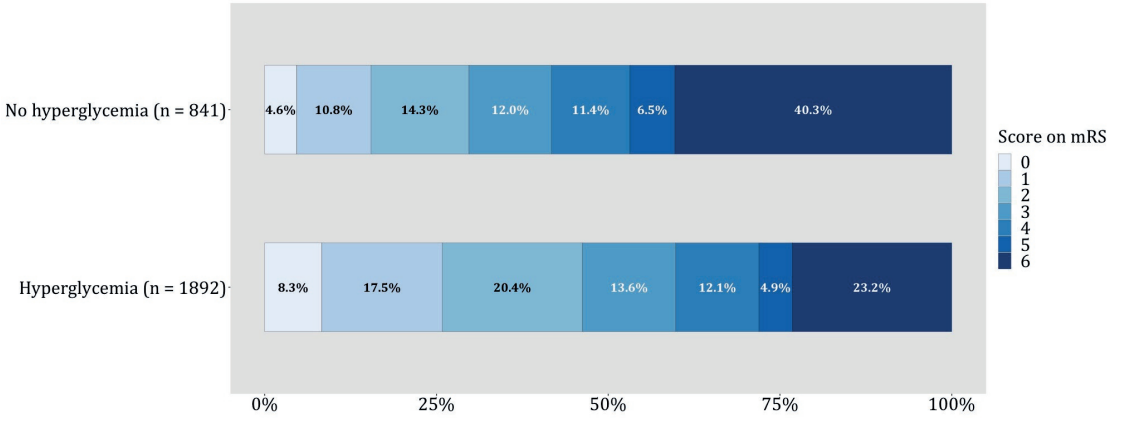

Figure 2.
Functional outcome at 90 days

Modified Rankin Scale (mRS) scores for patients with and without hyperglycemia on admission. A statistically significant difference between the two groups was noted in the overall distribution of mRS scores (adjusted common OR 1.69, 95% CI 1.44-1.99), indicating a shift towards poor functional outcome, with non-hyperglycemic patients as reference group.

Table 2: Outcomes and complications

	Admission hyperglycemia (n= 882)	No admission hyperglycemia (n= 2026)	Unadjusted (c) OR (95% CI)	Adjusted (c) OR (95% CI)
Outcomes				
Median mRS at 90 days (IQR)	4 (2–6)	3 (1–5)	2.03 (1.76-2.36)	1.69 (1.44-1.99)
Good functional outcome at 90 days, n (%)*	250/841 (30)	875/1892 (46)	0.51 (0.43-0.60)	0.60 (0.49-0.74)
Mortality at 90 days, n (%)	339/841 (40)	439/1892 (23)	2.12 (1.79-2.51)	1.95 (1.60-2.38)
Successful reperfusion, n (%)†	513/855 (60)	1241/1973 (63)	0.90 (0.77-1.06)	0.95 (0.80-1.12)
Complete reperfusion, n (%)‡	230/855 (27)	573/1973 (29)	0.91 (0.76-1.08)	0.91 (0.75-1.11)
Complications				
sICH, n (%)	82/882 (9)	92/2026 (5)	2.06 (1.51-2.82)	1.90 (1.40-2.56)
New ischemic stroke, n (%)	15/882 (2)	33/2026 (2)	1.03 (0.55-1.90)	1.05 (0.56-1.97)
Extracranial hemorrhage, n (%)	23/882 (3)	44/2026 (2)	1.24 (0.74-2.12)	1.02 (0.61-1.69)
Stroke progression, n (%)	102/882 (12)	162/2026 (8)	1.49 (1.15-1.95)	1.44 (1.10-1.89)
Pneumonia, n (%)	135/882 (15)	198/2026 (10)	1.61 (1.27-2.05)	1.48 (1.16-1.88)
Other infection, n (%)	47/882 (5)	65/2026 (3)	1.55 (1.07-2.24)	1.50 (1.03-2.19)

(c)OR indicates (common) odds ratio; mRS, modified Rankin Scale; IQR, interquartile range; sICH, symptomatic intracranial hemorrhage; eTICI, extended Thrombolysis In Cerebral Infarction; SD, standard deviation.

*Defined as mRS 0-2 † Defined as eTICI score of 2b – 3 ‡Defined as eTICI score 3.

(aOR 1.62, 95%CI 1.38-1.90). The sensitivity analyses excluding patients with pre-existing diabetes mellitus essentially also yielded the same results (supplemental material Table I, https://www.ahajournals.org/journal/str).

Glucose as a continuous variable and outcome

Overall, higher admission glucose was associated with a shift towards poor functional outcome with an acOR per increase of 1 mmol/L of 1.12, 95% CI 1.08-1.15. However, we observed that admission glucose as a continuous variable was non-linearly associated with poor functional outcome (mRS 3-6) at 90 days (P-value likelihood ratio test < 0.001, Figure 3). Upon further analysis, we found a J-shaped association between admission glucose and functional outcome with a nadir at 6 mmol/L and different associations for patients with admission glucose levels below 6, between 6 and 9 and levels exceeding 9 mmol/L. For patients with admission glucose below 6 mmol/L (n=746), a decrease in admission glucose level appeared associated with a shift towards poorer functional outcome (acOR per 1 mmol/L decrease in glucose 1.16, 95% CI 0.95 –1.41), but this was not statistically significant. For patients with admission glucose between 6 and 9 mmol/L (N=1715) and higher

than 9 mmol/L (N=447), there was a positive association between higher admission glucose levels and poorer functional outcome (acOR per 1 mmol/L increase in glucose 1.27, 95% CI 1.17 – 1.37 and 1.06, 95% CI 1.01 – 1.11, respectively). Successful reperfusion did not modify the association between admission glucose concentration and functional outcome. (P value for interaction = 0.295, supplemental material Figure I, https://www.ahajournals.org/journal/str,)

Admission glucose as a continuous variable was non-linearly associated with sICH in univariate analysis (P-value likelihood ratio test = 0.037). However, after adjustment, the addition of splines no longer improved model fit (P-value likelihood ratio test = 0.068, figure 3). Higher admission glucose was associated with an increased probability of sICH (aOR per 1mmol/L increase 1.11, 95%CI 1.07-1.17, figure 4). In our model, patients with admission glucose levels of 12.0 mmol/L had a 7% (95% CI 5% - 10%) absolute probability of sICH, and in patients with a glucose concentration of 20.0 mmol/L this probability increased to 15% (95% CI 9% - 24%).

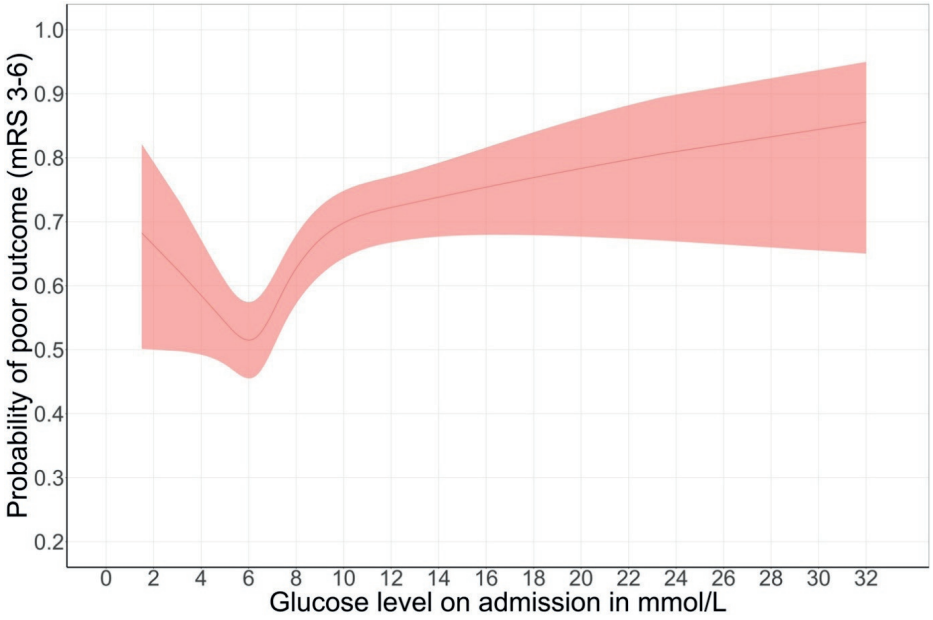

Figure 3.
Admission glucose concentration as a continuous variable and the probability of poor functional outcome (mRS 3-6) at 90 days

The association between glucose on admission and poor outcome (mRS 3-6) including 95% confidence intervals (95% CI) is shown,. We observed a J-shaped curve with different associations based on glucose levels on admission. Overall, glucose level on admission was associated with a shift towards poor functional outcome with an acOR per 1 mmol/L increase of 1.12 (95% CI 1.08-1.15). For patients with admission glucose under 6 mmol/L, glucose appeared associated with a shift towards poor functional outcome (acOR per 1mmol/L decrease 1.16 [95% CI 0.95 –1.41]), but this was non-significant. There was a significant association between glucose levels and poor functional outcome for patients with admission glucose between 6 and 9 (acOR 1.27 per 1 mmol/L increase, 95% CI 1.17 – 1.37) and a less pronounced association for patients with glucose levels on admission higher than 9 mmol/L (acOR 1.06 per 1 mmol/L increase, 95% CI 1.01 – 1.11).

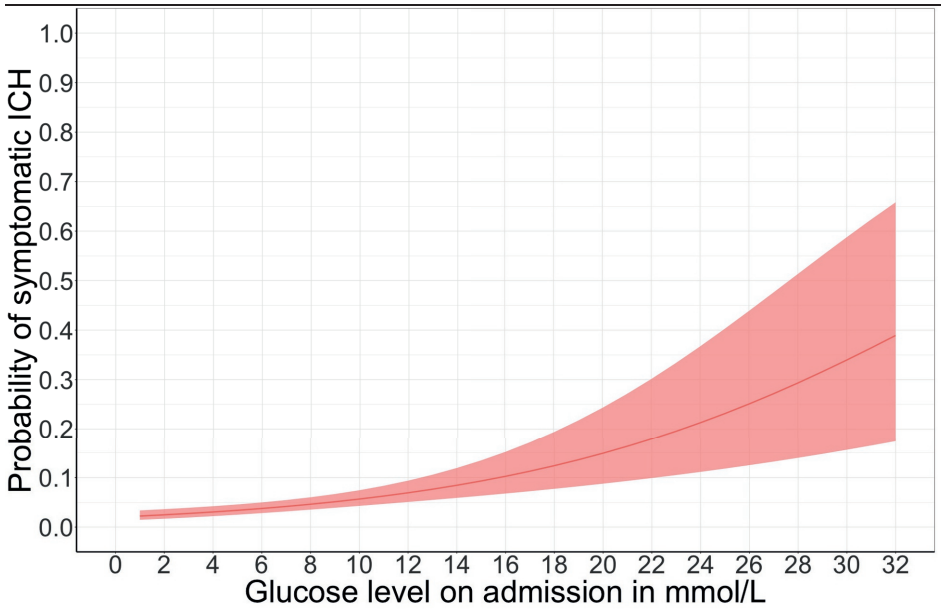

Figure 4.
Admission glucose concentration and the probability of symptomatic ICH

The association between glucose and the probability of symptomatic intracranial hemorrhage (sICH), including 95% confidence intervals with admission glucose levels on the x-axis and the probability of sICH on the y-axis. We observed a linear relationship and an overall significant association between glucose and the probability of sICH (aOR per 1 mmol/L increase 1.11, 95%CI 1.07-1.17).

DISCUSSION

We found that hyperglycemia on admission (≥7.8 mmol/L) is associated with worse functional outcomes at 90 days, increased mortality, and an increased risk of sICH after EVT in stroke patients in routine clinical practice. Further analysis showed a J-shaped association between admission glucose concentration and poor outcome, in which concentrations above 6 mmol/l were associated with poor outcome. By contrast, we found a linear association between admission glucose concentrations and the risk of sICH, with patients with an admission glucose concentration of 12 mmol/L on admission having an absolute risk of sICH of approximately 7% and this risk increased to 15% in patients with an admission glucose concentration of 20 mmol/l.

In our study, about one-third of patients had hyperglycemia on admission, which is comparable to proportions in previous studies.[17] Our findings are in line with those of previous studies that investigated the association between serum glucose on admission and functional outcome after EVT.[17, 18] A subgroup analysis of HERMES showed that higher glucose levels on admission are associated with worse functional outcome and that glucose concentration modified the treatment effect of EVT, with smaller benefit for patients with glucose levels higher than 5.5 mmol/L. The authors suggested that, as patients treated with EVT have a higher rate of successful reperfusion compared to those not treated with EVT, these patients may be more prone to redox-mediated reperfusion injury associated with higher glucose levels resulting in decreased functional outcome. In order to address this in our study population, we analyzed whether successful reperfusion modified the association between admission glucose and functional outcome. Although we observed that the probability of poor functional outcome increased with admission glucose levels above 6 mmol/L, we did not find that successful reperfusion modified the association between admission glucose and functional outcome.

If a causal relation between hyperglycemia and poor outcome after stroke exists, lowering glucose concentrations may improve outcome. Previous trials failed to demonstrate that glucose lowering had a positive influence on outcome in stroke patients. [19,20] The recent randomized SHINE trial which compared intensive glucose lowering with standard treatment in 1151 patients with acute ischemic stroke found no difference in outcome at 90 days.[21] However, in this study only a small proportion (13%) of patients underwent EVT. As patients with a LVO have large ischemic areas, they are potentially more prone to reperfusion injury associated with higher admission glucose levels and may therefore benefit from intensive glucose lowering compared to standard treatment for hyperglycemia. The authors

did not provide a subgroup analysis restricted to patients treated with EVT. Additionally, data on reperfusion rates were not available in SHINE and therefore the authors were unable to perform a subgroup analysis for patients with high glucose levels and successful reperfusion. In contrast to our observation, SHINE did not find a difference between the rates of ICH between the treatment groups. This discrepancy could be due to the fact that the admission glucose levels were similar between the groups and that glucose lowering could have been initiated too late to have an effect on the probability of ICH; because our study included only patients with a LVO and thus more severe ischemic strokes; or because of the smaller sample size of SHINE. Of course, it could also indicate that there is no causal relation between admission glucose and the occurrence of ICH.

We did not find that the association between high admission glucose and poor functional outcome was mediated by the occurrence of sICH, despite the association between higher admission glucose and an increased probability of sICH. All in all, it remains unclear whether high admission glucose levels play a causal role in increasing the probability on sICH or worse clinical outcome, but at least the absolute risk of sICH in patients with hyperglycemia can be taken into account when deciding whether or not perform EVT in a particular patient.

A J-shaped association between admission glucose and functional outcome has been reported in ischemic stroke patients before EVT became routine clinical practice.[22] A similar association has also been described between blood pressure and functional outcome in stroke patients treated with EVT.[23] The association between glucose under 6 mmol/L and functional outcome was not statistically significant in our study, although previous observational studies have suggested that patients with hypoglycemia had worse outcomes compared to normoglycemic patients.[24] The fact that we failed to demonstrate a significant association may be explained by the limited number of patients who presented with low admission glucose levels.

Pneumonia and other infections occurred more frequently in patients with hyperglycemia on admission. The association between hyperglycemia and the occurrence of infections has previously been described in acute ischemic stroke patients.[25] However, it remains unclear whether post-stroke infections explain the higher risk of a poor outcome as the authors of this study did not show evidence that the occurrence of post-stroke infections mediates or explains the association between hyperglycemia on admission and poor functional outcome.

Patients in the hyperglycemia group had a longer median duration of onset-to-groin time. This may be due to a slightly higher age, increased burden of co-morbidities, and a trend towards worse pre-stroke mRS in this group. However, since we adjusted for these variables in the analyses, it is unlikely that this explains the results.

Our study has several strengths. The data presented in this study likely

reflect daily clinical practice as they come from a nationwide, prospective registry of consecutive patients treated with EVT. Another strength is the large sample size of this study with only small numbers of missing data. Finally, all data on imaging and adverse events were centrally adjudicated. There are also several limitations. First, there were no data available on the use of glucose-lowering medication or on follow-up glucose levels. Therefore, we were unable to assess the dynamics of post stroke hyperglycemia while studies have suggested that persisting hyperglycemia is associated with infarct expansion and poor functional outcome[26, 27]. Finally, we could not ascertain whether glucose levels were determined in venous or capillary samples and whether point-of-care testing was applied, which may influenced the measured glucose levels.[28]

CONCLUSION

The presence of hyperglycemia at admission is associated with an increased risk of poor functional outcome and sICH in patients with acute ischemic stroke who undergo EVT. Studies are warranted to determine whether patients treated with EVT may benefit from early intensive glucose lowering therapy.

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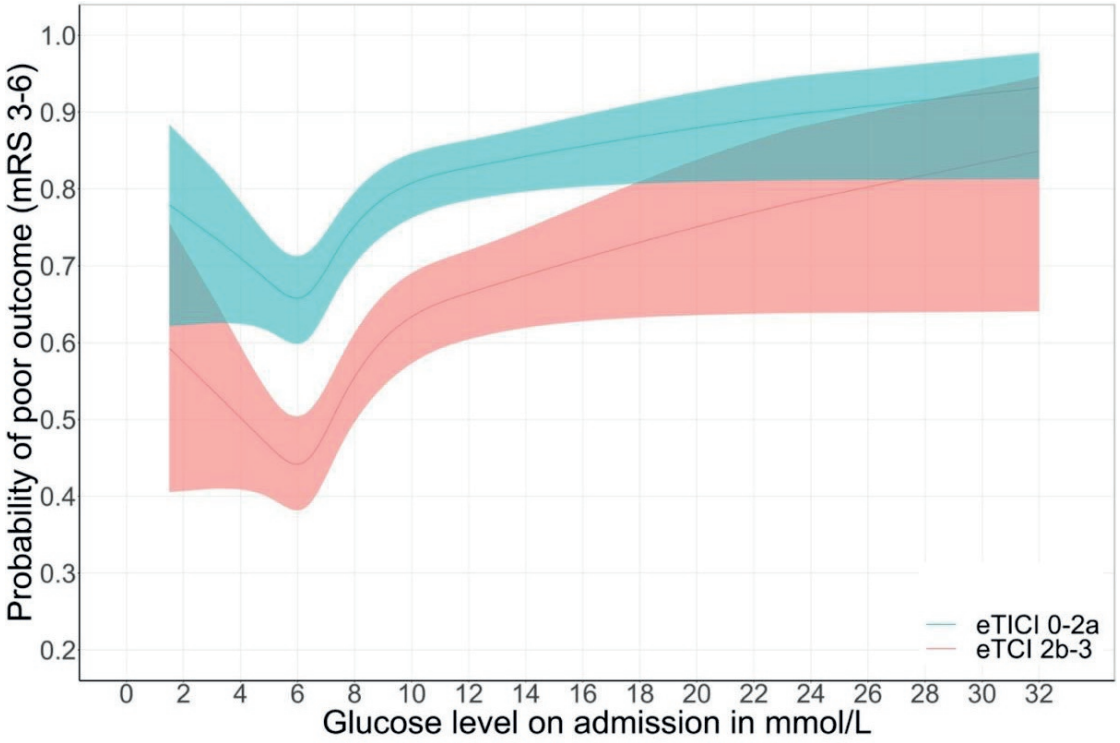
SUPPLEMENTAL



Supplemental Figure I:
Glucose as a continuous variable and the probability of poor functional outcome (mRS 3-6) according to reperfusion status

The effect of glucose on the probability of mRS 3-6, in patients with successful reperfusion (red line) vs no successful reperfusion (blue line), including 95% confidence intervals with serum glucose levels on admission on the x-axis and the probability of poor outcome on the y-axis. Successful reperfusion did not modify the association between glucose and functional outcome (P value for interaction = 0.295).

DISCUSSION & SUMMARY

Chapter 8

General discussion and future directions

DISCUSSION

Acute stroke is a devastating event causing lifelong disabilities that results in a significant decrease in quality of life and immense costs to individuals, their families and the healthcare system. Therefore, we need to seize opportunities to improve outcomes in these patients. The overall aim of this thesis was to assess various ways to improve stroke triage in the chain of acute stroke care. In this final chapter, I put the main findings in a broader context, discuss the clinical implications and make recommendations for future research.

I. Patient triage

Improving stroke triage in the chain of acute stroke care is important in realizing timely hospital arrival and treatment with intravenous thrombolysis (IVT) and/or endovascular treatment (EVT) in ischemic stroke patients, as clinical benefit of both reperfusion therapies sharply declines with time. Currently, the proportion of patients eligible for reperfusion therapy is still suboptimal. An important factor that causes delay appears to be the entry into the chain of acute stroke care once stroke symptoms occur. Patients can alarm the general practitioner (GP) or the emergency medical services (EMS). Alarming the EMS directly after stroke onset has shown to reduce delays to acute treatment.[1,2]

To investigate the patient's motivation in alarm choice after stroke onset, we developed a questionnaire (**Chapter 2)**. Our main finding was that several patient-related factors contribute to the patient's alarm choice. Patients who first alarmed the GP experienced a threshold to burden the EMS or underestimated their symptoms compared to patients who directly called the EMS. We also found that 54% of the patients were familiar with public stroke awareness campaigns that advices to call the EMS directly once stroke symptoms occur and despite this knowledge, patients still alarmed the GP first. Thus, stroke campaigns should also convey the message to the public that I) underestimation of severity of symptoms can be very harmful and II) to directly call 112 even if they only experience mild stroke symptoms.

Another interesting finding was that three-quarters of the patients who initially alarmed the GP, were also first examined by the GP before the EMS was involved for transportation to the hospital. Possible reasons for the GP to examine the patients first instead of directly calling an ambulance could be two-fold. First, these patients might not be triaged as possible acute stroke according to the GP protocols. Indeed, the clinical symptoms leading to stroke diagnosis were less apparent in

patients that were examined by the GP first because they more often presented with non-FAST symptoms compared to patients for whom the GP directly called the ambulance. Second, national guidelines state that involvement of the EMS is not necessary if there is uncertainty regarding the diagnosis of acute stroke or patient's eligibility for reperfusion treatment. Subsequently, GPs could have had a higher threshold to call the ambulance without having examined the patient first. Nevertheless, the majority (60%) of the patients who were first examined by the GP, appeared eligible for reperfusion therapy when they first alarmed the GP. Of these, only 29% arrived in the hospital within 6 hours. We recommend expanding stroke knowledge of the public and GPs, by incorporating other less known stroke symptoms such as vertigo and visual defects into stroke campaigns and education of GPs as well. Additionally, we propose to adjust current guidelines by enabling and focusing on direct involvement of the EMS once stroke is suspected as this can increase patient triage and improve outcome in daily clinical practice.

II. Prehospital triage by EMS paramedics

Another important factor in achieving timely hospital arrival of ischemic stroke patients is to improve the identification of patients eligible for endovascular treatment (EVT). By identifying these patients already in the ambulance, they can be allocated directly to a comprehensive stroke center (CSC) with EVT capabilities instead of first to a primary stroke center (PSC) where only IVT can be given. Thereby, inter-hospital transfers accounting for a median delay of 60-109 minutes can be prevented.[3,4]

Prehospital scales to predict large anterior vessel occlusion

In Chapter 4, we externally validated Prehospital scales have the potential to identify EVT-eligible patients. Numerous prehospital scales have been developed to predict large anterior vessel occlusion (LAVO), however, no optimal scale has yet been widely implemented into clinical practice due to insufficient evidence to recommend one scale over the other as external validation and comparison of scale performances is lacking.[5,6]

Our systematic literature search identified seven scales to predict LAVO in stroke code patients (**Chapter 3**). We validated these scales externally with data from the Dutch acute stroke study (DUST),[7] assessed these scales in terms of feasibility and developed a new scale that includes four predictors: Gaze, facial Asymmetry, level of Consciousness and Extinction/inattention (GACE). We found that FAST-ED (0.83, [95% CI, 0.80–0.85]) and RACE (0.82, [95% CI, 0.79–0.84]) had the highest accuracies to predict LAVO. The GACE can predict LAVO in 61% of patients with assessment of only two clinical items, and for all patients with four items, yielding a high feasibility. In the DUST cohort only a selection of patients eligible for IVT/EVT were included and are therefore not an accurate reflection of stroke code patients in the ambulance. This selection resulted in an artificially higher prevalence of EVT-eligible patients (36% in the DUST cohort versus 15% in stroke code patients),[8] possibly leading to overestimation of the scale performances. However, the study provided the foundation for a subsequent study investigating field performance of these scales.In **Chapter 4,** we externally validated the seven prehospital scales identified in Chapter 3 in the field (i.e., applied by EMS paramedics in the ambulance). In this prospective, multiregional, observational cohort study, a total of 2007 patients with an EMS-initiated acute stroke code were included from a large urban area with approximately two million inhabitants, encompassing two emergency medical services, three comprehensive stroke centers and four primary stroke centers. The scales showed moderate to good scale performance for the prediction of LAVO in the field, with LAMS (0.89, [95% CI, 0.87–0.90]) and RACE (0.88, [95% CI, 0.86–0.89]) showing the highest accuracies. Head-to-head comparison showed that both scales significantly outperformed the other scales. Furthermore, we found that the sensitivities of the scales were low (38% to 62%) and the specificities high (80% to 93%).

The predictive performances in our study were very similar to findings of the prehospital triage of acute stroke patients with suspected stroke symptoms (PRESTO) study.[9] The PRESTO study is a similar Dutch based prospective multicenter observational cohort study, including 1039 stroke code patients.

From a clinical perspective, a scale with a high sensitivity as well as a high specificity would be desirable. A higher sensitivity will improve prevention of delay to EVT by allocating more of these patients directly to a

comprehensive stroke center, whereas a higher specificity will allocate more patients without LAVO to the nearest hospital, resulting in i) less delay to IVT treatment in IVT-eligible patients and ii) less crowding of comprehensive stroke centers with patients that can also be treated elsewhere.

In practice, the preferred LAVO prediction scale will also depend on the feasibility of its use and the local context, including factors such as prevalence of LAVO, differences in transport times between hospitals, in-hospital performance metrics, and local policies.

If we would apply the LAMS scale to our stroke code patient cohort, which is an urban region with relatively short distances between PSCs and CSCs (±10 minutes travel time) and a LAVO prevalence of ±8%, 13 LAVO-patients who were first presented to a PSC would have benefitted from direct allocation to a CSC, whereas 17 ischemic stroke patients treated with IVT would have unnecessarily bypassed a PSC and 38 patients without LAVO who were first presented to a PSC would have been allocated to a CSC, including six patients with clinically severe intracerebral hemorrhage (see Figure 1). When the median inter-hospital transfer delay for EVT is also taken into account (i.e., 53 minutes in our cohort), this example of applying the LAMS scale to our cohort illustrates a clear benefit for reducing delays to EVT when using a LAVO prediction scale in the field. Furthermore, the delay to IVT is only minimal in the studied region considering the relatively short travel times between a CSC and PSC.

Some studies suggest that in patients receiving EVT who are directly allocated to a comprehensive stroke center, shorter delays to EVT correspond with better outcomes compared to patients transferred from a primary stroke center with longer delays.[4,10] Although the potential harm of delaying time to IVT should also be taken into account, a previous study showed a low rate of recanalization with IVT in patients with LAVO.[11] Therefore, we should improve patient triage at the prehospital level. The RACECAT study (ClinicalTrials.gov Identifier: NCT02795962) was the first randomized clinical trial in patients with an EMS-assessed RACE score of ≥5, comparing standard transportation to the nearest hospital to direct transportation to a comprehensive stroke center. Results showed that direct transport was not superior (or inferior) to standard transportation in terms of clinical outcome. Importantly, the median time for inter-hospital transfer was 90 minutes in the RACECAT, whereas this was less than 20 minutes in our region.

In addition to diagnostic performance, it is important to take feasibility into account before implementing a clinical scale in clinical practice. We found that in our study, without systematically trained EMS paramedics, feasibility rates of the scales ranged from 78%–88%, when only the cut-off of a scale had to be reached instead of the full range scale (57%–75%). This led to a substantial increase in feasibility rates. Furthermore, we demonstrated that reconstruction of the prehospital scales was mostly hampered due to assessments of motor function and/or neglect. While recognition of neglect

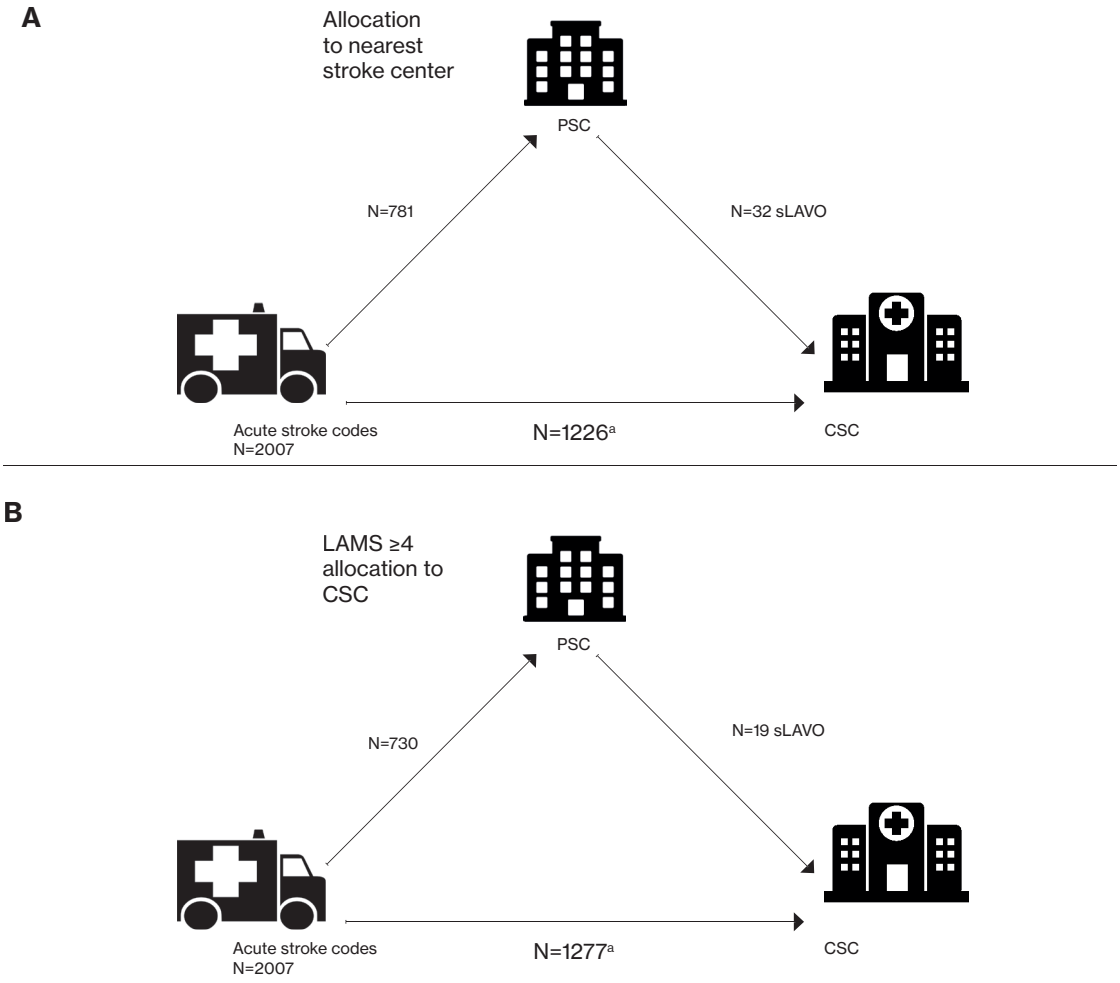


Figure 1.
Allocation of acute stroke code patients

A: Real scenario of patient allocation in our cohort.
B: Hypothetical scenario for patient allocation based on LAMS score in our cohort.
[a] Thirteen patients with LAVO would directly be allocated to a comprehensive stroke center, 17 IVT-treated patients would have unnecessary by-passed a primary stroke center and 38 patients without LAVO would have been allocated to a comprehensive stroke center (including six patients with clinically severe intracranial hemorrhage).

requires a more in-depth understanding of the neurological examination, assessment of motor function can be hampered due to practical reasons (e.g., the patient is strapped in during transport). This is valuable information as focused training of EMS paramedics could increase the scale's feasibility in the field.

Only a few scales (e.g., RACE, LAMS and FAST-ED) are already implemented into clinical practice, although not yet in Dutch clinical practice. The most frequently used scale items are assessment of facial palsy and motor function of the arm, as well as assessment of cortical symptoms such as gaze and neglect.
The LAMS and the RACE show the highest predictive performances in our study. Since cortical symptoms show great localizing value and therefore are often considered to be an important predictor of LAVO, the RACE appears the more rational choice as it incorporates cortical symptoms whereas the LAMS does not.

This, however, is not substantiated by our data. Further implementation of the RACE scale in the Netherlands is needed to compare scale performances in an urban region with relatively short distances between primary stroke centers and comprehensive stroke centers (±10 minutes travel time) and a low prevalence of LAVO (±8%). After the study was completed, the decision was made to start applying the RACE scale in the participating regions, being the first in the Netherlands.

Diagnostic biomarkers in prehospital triage for acute stroke

Next to identifying patients who are likely to benefit from EVT, another approach to improve patient selection in the ambulance is to develop a diagnostic tool (such as a point-of-care-test) to accurately differentiate patients with acute ischemic stroke from intracerebral hemorrhage. This can result in earlier initiation of treatment, for example already in the ambulance by EMS paramedics, thereby reducing delays to treatment and potentially improve clinical outcome. In addition, we should also identify patients who do not benefit from treatment. Our prehospital validation of LAVO prediction scales study showed that up to 38% of all acute stroke codes were stroke mimics (e.g., migraine or epilepsy). By identifying these patients already in the ambulance, it can help prevent erroneous diagnosis, emergency departments from overcrowding, and thereby avoid unnecessary treatment and (costly) use of stroke care facilities.

Biomarkers that are directly released into the circulation after acute brain tissue injury, which are also specific to stroke, are very promising as diagnostic markers to be incorporated into a point-of-care test for use in the ambulance.

In **Chapter 5**, we report on a preplanned discovery analysis of the MicroRNA in Acute Stroke (MIRAS) study. The MIRAS study (AsPredicted.

org with unique identifier #44134) is an ongoing observational cohort study including suspected stroke patients presented to the emergency department within 6 hours of symptom onset. Blood samples were collected on hospital arrival and final diagnosis (ischemic stroke, intracerebral hemorrhage and stroke mimic) was established after three months based on all available clinical and neuro-imaging data. We investigated whether tRNA-derived fragments (tRFs) can be used as early biomarkers in stroke diagnosis using small RNA-sequencing in plasma of a discovery cohort of 26 patients (n=8-10 per group).

We found that combinations of four to five tRFs yielded diagnostic accuracies up to 0.99 (intracerebral hemorrhage versus ischemic stroke and stroke mimic). We validated our results in an independent cohort of ischemic stroke patients and healthy controls which is available online.[12] The prediction models derived from the discovery cohort, also yielded high diagnostic accuracies up to 0.88 (ischemic stroke versus stroke mimic and healthy controls). By combining the tRFs that were identified in both the discovery and the validation cohort, we formulated a model consisting of three tRFs that had good diagnostic potential for differentiating ischemic stroke from healthy controls, stroke mimics and intracerebral hemorrhages.

Previous studies have also assessed biomarkers for stroke diagnosis, but until now none was able to reliable distinguish between stroke subtypes or stroke mimics. Moreover, these studies were often not performed in the acute phase of stroke.[13-15]

Our results will be validated in a larger cohort from the ongoing MIRAS study and is expected to reach the required 120 patients by the end of 2021. However, future studies are needed to confirm the accuracy and feasibility, as well as the added diagnostic value of tRNA-derived fragments to current clinical work process including medical history, clinical- and neuro-imaging assessments. Although our findings are based on a discovery cohort of a small cohort, our results are nevertheless very promising.

A prehospital tool that can reliably predict types of stroke, namely ischemic versus hemorrhagic stroke and LAVO versus non-LAVO, would be of tremendous value to the current workflow in the chain of acute stroke care. However, in clinical practice it seems unlikely that a single test can achieve a degree of accuracy that is sufficient to initiate treatment earlier on in the prehospital phase. In the future, combinations of several modalities to differentiate stroke subtypes will most likely be necessary.

With our current collaboration with the TU Delft, we hope to facilitate swift translation of our discoveries from the MIRAS study into a point-of-care test that can eventually be implemented in clinical practice. The combination of such a point-of-care test with a clinical scale can improve the use of such a prehospital tool in clinical practice. Additional

techniques such as electrode EEG for LAVO-detection, as is currently being investigated with the ELECTRA-STROKE study,[27] can also be of additional value.

Even when the selection of patients eligible for treatment is improved, other important factors such as hospital performance metrics, co-morbidity of patients and real-time traffic times should be incorporated in the decision to which hospital a patient should be allocated. Ideally, an application that incorporates all these factors, can provide the EMS paramedics an informed advice which hospital is most suitable for the individual stroke code patient.

III. In-hospital triage, treatment and patient outcome

In addition to achieving timely hospital arrival of acute stroke patients, as soon as the patient arrives *in the hospital,* it is important to improve patient outcome by continuing our efforts to reduce in-hospital delays, as well as investigate other factors that are known to have an adverse effect on patient outcome in this final part of the chain of acute stroke care.

Association of weight assessment for rt-PA titration and risk of symptomatic intracerebral hemorrhage.

In **Chapter 6**, we discussed the association between mode of weight assessment (i.e., estimated vs. measured weight) to titrate recombinant tissue plasminogen activator (rt-PA) dose for intravenous thrombolysis and several outcomes. In a large patient sample (n=4801) from 11 hospitals, we found that mode of weight assessment was not associated with symptomatic intracerebral hemorrhage or clinical outcome but, interestingly, estimation of body weight was associated with longer door-to-needle times compared with measured body weight using an inbuilt weighing bed. An explanation for this can be that estimation of a patient's body weight requires multiple healthcare providers to reach consensus, which consumes more time, whereas weight measuring requires only one transfer (mostly already incorporated into workflow) of the patient from the ambulance stretcher on to the CT-scanner table which most of the time has an inbuilt weighing scale. While previous prospective studies have shown that estimated body weight leads to dosing errors,[16,17] our results showed that this does not translate into a different safety and efficacy profile of intravenous rt-PA. However, we do recommend hospitals to have an inbuilt weighing scale on the CT-scanner table or otherwise incorporate a weighing scale into workflow, as measuring body weight was associated with shorter door-to-needle times. The rationale for this is that even the slightest reduction of time to treatment can eventually be of clinically importance.

Association of admission hyperglycemia with clinical outcome

A factor associated with worse outcome in acute ischemic stroke patients is hyperglycemia on admission. Hyperglycemia has also been associated with a higher risk of symptomatic intracerebral hemorrhages and, in EVT-treated patients, with lower recanalization rates. However, these associations have not yet been widely investigated in patients treated with EVT outside a trial setting and previous studies were mostly small (N<490), thereby limiting subgroup analysis.

In **Chapter 7**, we assessed the association between hyperglycemia at admission and functional outcome with real world data from the MR CLEAN Registry (Multicenter Registry of Endovascular Treatment for Acute Ischemic Stroke in the Netherlands), a prospective, nationwide registry of consecutive patients with acute ischemic stroke treated with EVT in the Netherlands.[18] We used data of all patients treated with EVT with available admission glucose. We showed that hyperglycemia was associated with worse functional outcome, increased mortality, and an increased risk of symptomatic intracerebral hemorrhage after EVT. There were no differences in the rates of successful reperfusion, nor did we find that successful reperfusion modified the association between admission glucose and functional outcome. Previous trials failed to demonstrate that glucose lowering had a positive influence on outcome in patients with stroke and they also did not take a possible interaction of EVT into account on the association of glucose and clinical outcome.[19,20] A meta-analysis that did investigate whether glucose modified the effect of EVT on clinical outcome, showed that treatment effects were larger at lower glucose levels.[21] However, since the only randomized trial actively lowering blood glucose levels failed to show differences in infarct growth in the treatment group, it remains unclear whether hyperglycemia on admission plays a causal role in clinical outcome after stroke. While this result has to be interpreted with caution since glucose regulation only led to a very small difference in mean glucose levels compared to the control group (0.57 mmol/L), more studies are warranted to investigated whether tight control of glucose improves the efficacy of EVT after large-vessel stroke.

FINAL REMARKS

This thesis highlights various ways to improve stroke triage in the chain of acute stroke care. Our continued efforts in realizing timely hospital arrival and rapid initiation of reperfusion treatment in ischemic stroke patients is important to further improve the prospects of patients with stroke and requires the implementation of various patient, prehospital and in-hospital workflow interventions.

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Chapter 9

Summary
Samenvatting

SUMMARY

By advancing existing stroke triage systems, diagnosis and timely access of stroke patients to specialized care can be improved, which in turn can have a tremendous impact on current clinical practice.

The overall aim of this thesis was to assess various ways to improve stroke triage in the chain of acute stroke. To begin with patient triage by assessing the patient's entrance into the chain of acute stroke care (**Part I**), identifying and validating prehospital triage tools to improve patient selection in the ambulance (**Part II**) and to finalize with investigating in-hospital factors that are known to have an adverse effect on patient outcome in the final part of the chain of acute stroke care (**Part III**).

Part I. Patient triage

The first part of this thesis focuses on the patient's alarm choice after stroke onset. The choice of health care provider that is contacted first by the patient (or bystander) after acute stroke, determines the entrance into the chain of acute stroke care and appears to be an important factor that causes delay. In **Chapter 2**, we assessed differences between stroke patients who first alarmed the general practitioner (GP) and patients who directly alarmed the emergency medical services (EMS) after onset of stroke with data from a questionnaire. Most patients alarmed the GP (64%) and median onset-to-door times were longer (466 min, [95% CI, 149-1586]) compared with patients who directly called the EMS (90 min, [95% CI, 45-286]). Our results showed that patients who alarmed the GP first instead of the EMS differed in several factors that are potentially modifiable. Patients who first alarmed the GP experienced a threshold to burden the EMS or underestimated their symptomatology compared with patients who directly alarmed the EMS. Moreover, three-quarters of the patients who initially alarmed the GP were also first examined by the GP before the EMS was involved for transportation to the hospital. Stroke diagnosis was less apparent in these patients, as they more often presented with non-FAST symptoms compared to patients for whom the GP directly called the ambulance. Strategies to achieve reduction of vital prehospital time delays and to improve patient outcome are optimizing public awareness campaigns and GP triage along with adjusting current guidelines by enabling and focusing on immediate involvement of the EMS once acute stroke is suspected.

Part II. Prehospital triage by EMS paramedics

The second part focuses on identifying stroke code patients with large anterior vessel occlusion (LAVO) in the ambulance. In **Chapter 3**, we described a systematic review on prehospital scales to predict LAVO in stroke code patients. We identified seven scales, validated these scales externally with data from the Dutch acute stroke study (DUST), assessed these scales in terms of feasibility and developed a new scale: the Gaze, facial Asymmetry, level of Consciousness, Extinction/inattention (GACE) decision tree. We found that the FAST-ED (0.83, [95% CI, 0.80–0.85]) and RACE scale (0.82, [95% CI, 0.79–0.84]) had the highest accuracies to predict LAVO. The GACE can predict LAVO in 61% of patients with assessment of only two clinical items, and for all patients with a maximum of only four items, yielding a high feasibility.

These prehospital scales need to be validated in the setting where such a scale would be ideally used: in the field (i.e. applied by EMS paramedics in the ambulance). Therefore, in **Chapter 4**, we performed external validation of the seven prehospital scales identified in Chapter 3. External validation of these scales in the field showed moderate to good scale performance for the prediction of LAVO, with the LAMS and RACE scales showing the highest accuracies (0.89, [95% CI, 0.87–0.90] and 0.88, [95% CI, 0.86–0.89], respectively). Head-to-head comparison showed that both scales significantly outperformed the other scales (P<0.05). Feasibility was relatively high for all scales and ranged between 78%-88%. Furthermore, our results indicate that the use of a prehospital scale in the field can reduce inter-hospital delays (median of 53 minutes) to reperfusion treatment in a large urban area with relatively small distances between a primary stroke center and a comprehensive stroke center.

Another approach to improve patient selection in the ambulance is to develop a diagnostic tool to accurately differentiate patients with acute ischemic stroke from intracerebral hemorrhage. In **Chapter 5**, we reported on a preplanned discovery analysis of the MIRAS (MicroRNA in Acute Stroke) study, an ongoing observational cohort study including suspected stroke patients presented to the emergency department within 6 hours of symptom onset. We investigated whether tRNA-derived fragments (tRFs) can be used as early biomarkers in stroke diagnosis using small RNA-sequencing in plasma in a discovery cohort (N=26). We discovered that tRFs are a promising novel class of biomarkers. An important finding was that combinations of four to five tRFs showed diagnostic accuracies up to 0.99, [95% CI, 0.95-1.00] for intracerebral hemorrhage vs. ischemic stroke and stroke mimic in the discovery cohort. We validated our results in an independent cohort of ischemic stroke patients and healthy controls, which yielded similar diagnostic accuracies.

Part III. In-hospital triage, treatment and patient outcome

In the last part of the chain of acute stroke care, in-hospital factors that are associated with an adverse effect on patient outcome were investigated. In **Chapter 6**, we described the association between mode of weight assessment to titrate recombinant tissue plasminogen activator (rt-PA) dose for intravenous thrombolysis and several outcomes. In a large patient sample (n=4801) from 11 hospitals, we found that weight modality was not associated with symptomatic intracranial hemorrhage or clinical outcome. We showed that patients with estimated body weight had longer door-to-needle times compared to patients with measured body weight. While previous prospective studies have shown that estimated body weight leads to dosing errors, our results indicated that this does not translate into a different safety and efficacy profile of intravenous rt-PA.

Another factor that is associated with worse functional outcome is hyperglycemia on hospital admission. In **Chapter 7**, we performed an analysis with real world data from the MR CLEAN Registry, a prospective nationwide registry of consecutive patients who received endovascular treatment in routine clinical practice. We showed that hyperglycemia was associated with worse functional outcome, increased mortality, and an increased risk of symptomatic intracerebral hemorrhage after endovascular treatment (EVT). There were no differences in the rates of successful reperfusion, nor did we find that successful reperfusion modified the association between admission glucose and functional outcome. Studies are warranted to determine whether tight control of glucose improves efficacy of EVT after large vessel stroke.

In conclusion, this thesis addresses several ways to improve stroke triage in the chain of acute stroke care. To improve patient triage, the focus should be to directly involve the ambulance once stroke is suspected. Furthermore, prehospital triage tools that can help identify patients who are more likely to have LAVO or can differentiate between patients with acute ischemic stroke and intracerebral hemorrhage, will improve patient selection in the ambulance and thereby result in earlier initiation of EVT, thereby improving patient outcomes. Finally, continued efforts need to be made to further reduce in-hospital delays by identifying factors that have an adverse effect on patient outcome.

SAMENVATTING

Verbetering van de diagnostiek en snelle toegang van patiënten met een beroerte tot gespecialiseerde zorg voor acute behandeling kan door het verbeteren van bestaande triagesystemen voor patiënten met een beroerte een grote invloed hebben op de huidige klinische praktijk.

Het doel van dit proefschrift is om verschillende manieren te onderzoeken om de triage van patiënten met een beroerte in de acute zorgketen te verbeteren. Allereerst door het bestuderen van factoren die de keuze van patiënten met een beroerte beïnvloeden bij het inschakelen van spoedeisende hulp (**Deel I**), vervolgens het identificeren en valideren van prehospitale triage-instrumenten om de selectie van patiënten te verbeteren in de ambulance (**Deel II**), en het onderzoeken van factoren in het ziekenhuis waarvan bekend is dat deze een nadelig effect hebben op de uitkomst van de patiënt in het laatste deel van de acute zorgketen (**Deel III**).

Deel I. Patiënt triage

Het eerste deel van dit proefschrift richt zich op de alarmkeuze van de patiënt na het ontstaan van een beroerte, aangezien de ingang in de acute beroerte zorgketen een belangrijke vertragende factor kan zijn. In **Hoofdstuk 2**, hebben we met gegevens uit een vragenlijst de verschillen onderzocht tussen patiënten met een beroerte die de huisarts alarmeerden en patiënten die direct de ambulance belden na het ontstaan van een beroerte. De meeste patiënten vroegen eerst de huisarts om hulp (64%) en de mediane onset-to-door tijden waren langer (466 min, [95% CI, 149-1586]) in deze groep in vergelijking met patiënten die direct de ambulance alarmeerden (90 min, [95% CI, 45-286]). Onze resultaten toonden aan dat patiënten die eerst de huisarts om hulp vroegen in plaats van de ambulancezorg, verschillen in verschillende factoren die mogelijk beïnvloedbaar zijn. Patiënten die eerst de huisarts alarmeerden, ervoeren een drempel om de ambulancezorg te belasten of onderschatten hun symptomen in vergelijking met patiënten die direct de ambulance alarmeerden. Bovendien is driekwart van de patiënten die in eerste instantie de huisarts alarmeerden ook eerst door de huisarts onderzocht voordat de ambulance betrokken was voor vervoer naar het ziekenhuis. De diagnose van een beroerte was ook minder duidelijk bij deze patiënten, omdat zij vaker andere symptomen vertoonden dan de face-arm-speech time (FAST) symptomen in vergelijking met patiënten voor wie de huisarts direct de ambulance belde. Strategieën om belangrijke prehospitale vertragingen te verminderen en de uitkomst van de patiënt te verbeteren,

zijn het optimaliseren van bewustmakingscampagnes en huisartsentriage, samen met het aanpassen van de huidige richtlijnen door onmiddellijke betrokkenheid van de ambulancezorg mogelijk te maken zodra een acute beroerte wordt vermoed.

Deel II. Prehospitale triage door ambulanceverpleegkundigen

Het tweede deel van dit proefschrift richt zich op het identificeren van patiënten met een herseninfarct op basis van een proximale intracraniële occlusie in de voorste circulatie die in aanmerking komen voor endovasculaire behandeling. In **Hoofdstuk 3**, beschrijven we de resultaten van een systematisch review over prehospitale schalen om een proximale intracraniële occlusie te voorspellen bij patiënten die via de ambulance met urgentie worden ingestuurd naar het ziekenhuis vanwege een verdenking op een acute beroerte. We identificeerden zeven schalen, valideerden deze schalen extern met gegevens van een Nederlandse studie naar acute beroertes (DUST),[7] beoordeelden deze schalen op toepasbaarheid en ontwikkelden een nieuwe schaal: the Gaze, facial Asymmetry, level of Consciousness, Extinction/inattention (GACE) beslisboom. Wij ontdekten dat de FAST-ED (0.83, [95% CI, 0.80–0.85]) en de RACE schaal (0.82, [95% CI, 0.79–0.84]) de hoogste nauwkeurigheid hadden om een proximale intracraniële occlusie te voorspellen. De GACE kan een proximale occlusie in 61% van de patiënten voorspellen, met beoordeling van slechts twee klinische items, en voor alle patiënten met een maximum van slechts 4 items dat een hoge toepasbaarheid oplevert.

Deze prehospitale schalen moeten worden gevalideerd in de setting waarin een dergelijke schaal daadwerkelijk zou worden gebruikt: in het werkveld (d.w.z. toegepast door ambulance-medewerkers in de ambulance). In **Hoofdstuk 4** hebben we daarom een externe validatie uitgevoerd van de zeven prehospitale schalen die in Hoofdstuk 3 zijn geïdentificeerd. Externe validatie van deze schalen in het veld toonde matige tot goede schaalprestaties voor de voorspelling van een proximale intracraniële occlusie, waarbij de LAMS en RACE schaal de hoogste nauwkeurigheid lieten zien (respectievelijk 0,89, [95% BI 0,87-0,90] en 0,88, [95% BI 0,86-0,89]). Een onderlinge vergelijking toonde aan dat beide schalen significant beter presteerden dan de andere schalen (P <0,05). De toepasbaarheid was relatief hoog voor alle schalen en varieerde tussen 78%-88%. Bovendien geven onze resultaten aan dat het gebruik van een prehospitale schaal in het veld de vertragingen tussen ziekenhuizen (mediaan van 53 minuten) tot endovasculaire behandeling kan verminderen in een grootstedelijk gebied met relatief kleine afstanden tussen een ziekenhuis waar alleen intraveneuze trombolyse mogelijk is en een interventiecentrum waar ook endovasculaire behandeling kan worden uitgevoerd.

Een andere benadering om de selectie van patiënten in de ambulance te verbeteren, is het ontwikkelen van een snelle en accurate test om onderscheid te maken tussen patiënten met een herseninfarct en een hersenbloeding. In **Hoofdstuk 5** beschrijven we een vooraf geplande exploratieve analyse van de MicroRNA in Acute Stroke studie, een lopende observationele cohortstudie met patiënten met een vermoedelijke beroerte die binnen 6 uur na aanvang van de symptomen op de afdeling spoedeisende hulp werden gepresenteerd. We exploreerden of tRNA-afgeleide fragmenten (tRF's) kunnen worden gebruikt als vroege biomarkers bij de diagnose van een beroerte met behulp van kleine RNA-sequencing in plasma in een klein cohort (N = 26). We ontdekten dat tRF's een veelbelovende nieuwe klasse van biomarkers zijn. Een belangrijke bevinding was dat combinaties van vier tot vijf tRF's diagnostische nauwkeurigheid vertoonden tot 0,99 [95% BI, 0,95-1,00] voor een hersenbloeding versus herseninfarct en stroke-mimics in het preliminaire cohort. We valideerden onze resultaten in een onafhankelijk cohort van patiënten met een herseninfarct en gezonde controles dat een vergelijkbare diagnostische nauwkeurigheid opleverde.

Deel III. In-hospitale triage, behandeling en uitkomst voor de patiënt

In het laatste deel van de acute beroerte zorgketen worden factoren binnen het ziekenhuis onderzocht die zouden kunnen samenhangen met een ongunstig effect op de functionele uitkomst van de patiënt. In **Hoofdstuk 6** hebben we de associatie beschreven tussen de wijze van gewichtsbepaling om de recombinant tissue plasminogen activator (rt-PA) dosis voor intraveneuze trombolyse te titreren en verschillende uitkomsten. In een groot patiëntencohort (n = 4801) afkomstig uit 11 ziekenhuizen vonden wij dat gewichtsmodaliteit niet geassocieerd was met een symptomatische intracerebrale bloeding of klinische uitkomst bij patiënten behandeld met rt-PA. Wij ontdekten dat patiënten met een geschat lichaamsgewicht een langere door-to-needle tijd hadden in vergelijking met patiënten met een gewogen lichaamsgewicht. Hoewel eerdere prospectieve onderzoeken hebben aangetoond dat geschat lichaamsgewicht leidt tot doseringsfouten, toonden onze resultaten aan dat dit zich niet vertaalt in meer bloedingscomplicaties of een slechtere uitkomst.

Een andere factor die wordt geassocieerd met een slechtere functionele uitkomst van de patiënt na een herseninfarct is hyperglycemie bij opname. In **Hoofdstuk 7** hebben we de relatie tussen hyperglycemie en belangrijke uitkomstmaten onderzocht met gegevens uit de MR CLEAN Registry, een prospectieve landelijke registratie van opeenvolgende patiënten die endovasculaire behandeling kregen in de dagelijkse klinische praktijk. We toonden aan dat hyperglycemie geassocieerd was met een

slechtere functionele uitkomst, verhoogde mortaliteit en een verhoogd risico op symptomatische intracerebrale bloeding na endovasculaire behandeling. Er waren geen verschillen in de snelheid van succesvolle reperfusie, noch vonden wij dat succesvolle reperfusie de associatie tussen glucosewaarde bij ziekenhuisopname en functionele uitkomst veranderde. Onze resultaten en eerdere gepubliceerde resultaten, rechtvaardigen het uitvoeren van een gerandomiseerde trial om te onderzoeken of strikte controle van glucose de werkzaamheid van endovasculaire behandeling verbetert na een herseninfarct ten gevolge van een proximale occlusie van de voorste circulatie.

Concluderend behandelt dit proefschrift verschillende manieren om de triage van patiënten met een beroerte in de acute zorgketen te verbeteren. Om de triage van deze patiënten te verbeteren, moet de nadruk komen te liggen op het direct betrekken van de ambulance zodra een beroerte wordt vermoed. Bovendien zullen prehospitale triage-instrumenten kunnen helpen bij het identificeren van patiënten die waarschijnlijk baat zullen hebben bij endovasculaire behandeling of onderscheid kunnen maken tussen patiënten met een acute herseninfarct en hersenbloeding. Hiermee zal de patiëntenselectie in de ambulance verbeteren, de behandeling eerder gestart kunnen worden en uiteindelijk waarschijnlijk de uitkomst van de patiënt verbeteren. Ten slotte moeten er voortdurend inspanningen worden geleverd om de vertragingen in het ziekenhuis verder te verminderen door factoren te identificeren die een ongunstig effect hebben op de uitkomst van de patiënt.

Appendices

ABBREVATIONS

3I-SS	3-item stroke scale
ACA	anterior cerebral artery
acOR	adjusted common odds ratio
AF	atrial fibrillation
AIS	acute ischemic stroke
aOR	adjusted odds ratio
ARTEMIS	A Reduction in Time with Electronic Monitoring in Stroke
ASA	American Stroke Association
AUROC	area under the receiver operating curves
CI	confidence interval
CPSSS	Cincinnati prehospital stroke severity scale
CSC	comprehensive stroke center
CSTAT	Cincinnati Stroke Triage Assessment Tool
CT	computed tomography
CTA	computed tomography angiography
DGT	door-to-groin puncture time
DNT	door-to-needle time
DSA	digital subtraction angiography
DUST	Dutch acute stroke study
EBW	estimation of body weight
ED	emergency department
EMS	emergency medical services
ER	emergency room
eTICI	extended Thrombolysis in Cerebral Infarction
EV	extracellular vesicles
EVT	endovascular treatment
FAST	Face-Arm-Speech-Time
FAST-ED	Face-arm-speech-time-eye deviation-denial/neglect
GACE	Gaze, facial Asymmetry, level of Consciousness, Extinction/inattention
G-FAST	Gaze-face-arm-speech-time
GP	general practitioner
ICA	intracranial carotid artery
ICH	intracerebral hemorrhage

IQR	interquartile range
IS	ischemic stroke
IVT	intravenous thrombolysis
LAMS	Los Angeles Motor Scale
LAVO	large anterior vessel occlusion
LOC	level of consciousness
LUMC	Leiden University Medical Center
MBW	measurement of body weight
MCA	middle cerebral artery
MIRAS	MicroRNA in Acute Stroke
MR CLEAN	Multicenter Randomized Controlled Trial of Endovascular Treatment for Acute Ischemic Stroke in the Netherlands
MIRAS	MicroRNA in Acute Stroke
mRS	modified Rankin score
MST	Medisch Spectrum Twente
NCCT	non-contrast computed tomography
NIHSS	National Institute of Health Stroke Scale
OR	odds ratio
OTD	onset-to-door-time
PASS	Prehospital acute stroke severity
PRESTO	prehospital triage of acute stroke patients with suspected stroke symptoms
PSC	primary stroke center
RACE	Rapid arterial occlusion evaluation
ROC	receiver operating characteristics
rt-PA	recombinant tissue plasminogen activator
SAH	subarachnoid hemorrhage
SD	standard deviation
sICH	symptomatic intracranial hemorrhage
sLAVO	symptomatic large anterior vessel occlusion
SM	stroke mimics
sncRNAs	small non-coding RNAs
STARD	Standards for Reporting of Diagnostic Accuracy
STROBE	Strengthening the Reporting of OBservational studies in Epidemiology
TIA	transient ischemic attack
tRFs	tRNA-derived fragments
tRNAs	transfer RNAs
VAN	Vision aphasia neglect

LIST OF PUBLICATIONS

This thesis:

T.T.M. Nguyen, M.L. van der Bent, M.J.H. Wermer, I.R. van den Wijngaard, B. de Groot, P.H.A. Quax, A.Y. Nossent, N.D. Kruyt. Circulating tRNA fragments as a novel biomarker class to distinguish acute stroke subtypes. International journal of molecular sciences 2020.

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CURRICULUM VITAE

Truc My Nguyen was born on March 16th, 1990 in Almere, the Netherlands. She graduated from high school in 2008 at the Willem de Zwijger College in Bussum. That same year she started her medicine study at the Vrije Universiteit of Amsterdam and in her last year she started her master thesis at the Department of Neurology at the Vrije University of Amsterdam under supervision of dr. M.C. Visser. She graduated in 2015 and shortly thereafter she started as a resident at the Department of Neurology and Neurosurgery (dr. Björn van Geel) at the Noordwest Ziekenhuisgroep in Alkmaar. In 2016 she started a PhD on stroke logistics in acute stroke care at the Department of Neurology under supervision of Prof. Dr. M.J.H. Wermer, Dr. N.D. Kruyt and Dr. M.H. den Hertog. At the end of 2020, she started working as a resident at the Department of Neurology at the Haga Hospital in The Hague (dr. B. de Bruijn and dr. K. de Laat). Truc My is married to José and they live together in Amsterdam.

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