Statistical and Epidemiological Analysis Plan for Protocol titled

Safe Implementation of Treatments in Stroke (SITS) - Intravenous thrombolysis in acute ischaemic stroke patients over 80 years, SITS-IVT>80 years study

(BI protocol study no. 0135-0344)

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2 RESEARCH QUESTIONS AND OBJECTIVES

2.1 Primary objective:

The SITS (Safe Implementation of Treatment in Stroke) Intravenous thrombolysis (IVT) with alteplase >80 years study will primarily investigate the safety and outcome of IVT in acute ischaemic stroke in patients >80 years collected during the post- approval period and to compare the safety and outcome with

existing pre-approval patients >80 years collected in routine clinical practice and recorded in the SITS-ISTR (International Stroke Thrombolysis Registry).

2.2 Secondary objectives:

We will describe patient's characteristics, management time: onset of symptoms to time of treatment initiation, and its components: onset of symptoms to door; door to treatment initiation.

3 Definitions and Outcome Measurements/Variables

3.1 Definitions:

- **Index event** is defined as the date/time of initiation of stroke onset
- **Treatment:** IV thrombolysis with alteplase (Actilyse®).
- **Baseline visit:** day of hospital admission for ischaemic stroke
- The **3 month follow-up data point** for patients is 90 (+/- 10) days after the index event.
- **Post-approval:** Patients >80 years old presenting with acute ischaemic stroke symptoms for which thrombolysis treatment was initiated within 4.5 hours after stroke onset according to the SmPC during the study period July 2018 to June 2021.
- **Pre-approval:** Patients >80 years presenting with acute ischaemic stroke symptoms for which thrombolysis treatment was initiated within 4.5 hours after stroke onset otherwise according to SmPC during the period of approximately 3 years prior to approval (June 2015 to June 2018)

3.2 Primary Outcome measurements/variables

- Safety:
  1.SICH per SITS-MOST definition
  2. Mortality (modified Rankin score, mRS=6) within 90 days
- Functional Outcome:
  Functional Independency as defined by mRS 0-2 within 90 days

  A modified Rankin score, mRS, is used to evaluate daily activity of the patient at day 90 (described in Attachment 8.3). The scores are entered in the on-line entry form. The score 0-1 is defined as favourable outcome and 0-2 as independence of the patient.

Symptomatic intracerebral haemorrhage, SICH per SITS-MOST, is defined as 'Intracerebral haemorrhage' (parenchymatous haemorrhage type 2, local PH2 or remote PHr2), at the 22-36 hours post-treatment scan or earlier if clinically indicated combined with neurological deterioration leading to an increase of 4 points on the NIH Stroke Scale' from baseline to 24h or death within 24 hours(SITS-MOST definition).

The evaluation of post-treatment haemorrhage is done using the CT/MRI scan performed between 22-36 hours after start of treatment or earlier if clinically indicated.

Death within 90 days as classified by the investigator according to cause: cerebral infarct, cerebral haemorrhage, cerebral infarct and haemorrhage unspecified, myocardial infarct, pulmonary embolism, pneumonia, other vascular cause, unknown and other cause of death (ICD 10 to be provided).

3.3 Secondary outcomes measurements/variables

Patient characteristics at baseline including stroke severity (NIHSS)

Outcomes: Favourable outcome as defined by mRS 0-1 within 90 days

Secondary safety: SICH per ECASS 2 (defined as any Intracerebral haemorrhage including haemorrhagic infarctions at the 22-36 hours post-treatment scan combined with neurological deterioration leading to an increase of 4 points on the NIH Stroke Scale' or death within 7 days)

Delays of management

o Time from onset of symptoms–start of treatment
o Time from onset of symptoms–door (or as captured in the registry arrival at hospital)
o Door to needle(treatment) time

4 Study design

The SITS-IVT>80 years is an observational, multinational, multicentre study in patients with acute ischaemic stroke treated with IV thrombolysis (0.9 mg/kg Alteplase) according to the revised and approved Summary of Product Characteristics (SmPC) in European countries. The data will be extracted from the existing SITS –ISTR database.

Approximately 1000 patients older than 80 years treated with IV Alteplase are expected to be identified for the proposed SITS-IVT>80 year study in the post-approval period of 3 years (1 July 2018 to 30 June 2021) and also approximately 1000 patients above 80 years treated with IV Alteplase within 4.5 hours otherwise according to SmPC in the pre-approval period (June 2015 to June 2018). Of these, at least 500 patients each should be registered from European mutual recognition procedure (MRP) centres (see list of European countries being part of the MRP procedure) during the pre-approval as well as in the post-approval period. If the target 1000 patient is not reached during the 3 year period (post-approval phase), the data exchange will stop provided at least 500 patients registered from European MRP centers have been included into the analysis.

4.1 Inclusion criteria

4.1.1 At individual patient level

- Post-approval: Patients over 80 years old presenting with acute ischaemic stroke symptoms for which thrombolysis treatment with alteplase (Actilyse®) from 1 July 2018 or the first day of the next month after exact approval date, if later, and treatment was initiated within 4.5 hours after stroke onset according to SmPC are included. (see appendix 2)
- Pre-approval: Patients over 80 years who received thrombolysis with alteplase (Actilyse®) otherwise according to SmPC for AIS within 4.5 hours after stroke onset during the period (approximately 3 years) prior to July 2018 or the exact approval date, if later, are included.

4.1.2 At centre level

- A typical experience from acute stroke treatment defined by at least 5 patients treated with IVT and entered in the SITS-ISTR in both pre and post approval groups
- At least 70% data entered at 3m follow up) in both pre and post approval groups
- A willingness to participate in SITS-IVT>80 years registry

4.1.3 At country level

- At least 500 patients registered from European mutual recognition procedure (MRP) countries
- Among MRP countries, one country could contribute a maximum of 30% of individual patient data to the MRP countries and a similar rules also applies for non-MRP countries, e.g. if the total number of patients from MRP countries is 500, then one MRP country could contribute a maximum of 150 patients and the similar rules also applies for non-MRP countries for each period separately. Date of stroke onset will be used to select the closest 30 % of patients around the approval date for pre – and post approval phase from each country. For the post-approval period, first 30% patients from start date and for the pre-approval period, latest 30% from approval date will be used,
- Countries date of approval varies. In most countries start date for prospective cohort is 1 July 2018 but in some countries approval dates are at a later time points. For MRP and Non-MRP countries the first day of the next months of the exact country approval date is used.

4.1.4 SITS data entry protocol

- IVT-standard

4.2 Exclusion criteria

- Missing data on SmPC criteria which are collected in the SITS IVT Registry (see below)
- Contraindication(s) to the use of IV thrombolysis per local SmPC
- Low dose alteplase (e.g. 0.6 mg/ kg)
- Documentation that the patient was enrolled or is planned to be enrolled in an investigational clinical trial at the time of the onset of index event and for the duration of the data collection.
- Treated with endovascular thrombectomy
- Treated with Tenecteplase

4.3 Data collected in the SITS Registry for exclusion of patients outside SmPC criteria

- Stroke Onset to treatment time >270 minutes
- Previous stroke within 3 months
- Previous stroke earlier than 3 months + Diabetes Mellitus
- Anticoagulants, oral
- Heparin for stroke prevention/ high dose
- Glucose baseline by mmol <2.7 or > 22.2, or by mg/dl <50 or> 400
- NIHSS baseline >25
- Minor neurological deficit (baseline NIHSS score<=3)
- SBP baseline > 185 mm Hg
- DBP baseline > 110 mm Hg

5 Information on Data Extraction, Limitations and Bias

Weaknesses/ limitations

This is a non-interventional study based on existing data, therefore its quality relies on accuracy of recorded data. Important data may not be available. In the absence of randomization, it can be difficult to control for bias and confounders.

Power and sample size:

The sample size was not based on any formal power calculation, rather chosen pragmatically in consultation with authorities and based on numbers achievable within a 3-year period. Approximately 2000 acute ischaemic patients who were treated with IV thrombolysis within 4.5 hours within symptom onset constitute the main analysis population. Data from a minimum of 500 European patients are expected within the database during post approval phase as well as equal number during the pre-approval phase in patients > 80 years old acute ischemic stroke patients.

Selection bias

There is a risk of selection bias at the patient level. Sites might preferentially include "interesting" cases to the SITS study. To minimize selection bias at the patient level, centres were reminded by e-mail every 3 months that all consecutive patients from each site who meet entry criteria must be included in the SITS-Registry.

Lost to follow up

Lost to follow up at 3 months after intravenous alteplase treatment could lead to under-identification of clinical outcome events.

Planned Analysis

What is the aim of the analyses: To show, if there are differences between pre- and post-approval populations in the safety, outcomes and in the baseline parameters?

Can differences in the outcomes be explained by differences in the baseline parameters?

Primary analyses: Propensity score matching should diminish differences in the outcomes if patients have balanced baseline parameters. If differences persist or an improvement or worsening of outcomes in the post approval phase occurs, other explanations are needed (as processes in the hospital (shorter or longer of door to needle time or increase of experience). This might need further exploration

Secondary analyses:

To confirm the results of the PS matching by adjusting the model parameter “post-approval vs pre-approval patient” for the most important imbalanced confounders for the outcomes of interest.

Descriptive statistics (absolute and relative frequencies for categorical variables, means, standard deviations, medians, inter quartile ranges, minimum and maximum for continuous/ordinal variables) for baseline, demographic characteristics and outcomes for all included patients will be provided separately for pre-approval and post-approval patients. In addition, 95% Wald confidence intervals will be provided for the continuous outcomes and Wilson score interval with continuity correction for binary outcomes. To compare difference among demographic and clinical baseline characteristics between pre-approval and post-approval, standardized differences will be used. Propensity score matching will be the primary statistical approach, multivariable modeling will be used for secondary complementary explorative analysis.

Primary Analysis

The primary analysis will be on propensity score matched patient datasets and we will compare the post-approval with the pre-approval patients regarding baseline demographics, clinical and radiological parameters, as well as treatment logistics, safety outcomes, functional outcome, and death.

Matched patient set constitute propensity score matched pair (1:1 greedy nearest neighbor matching using calipers equal to 0.2*SD of the logit of the propensity score)[1]. The primary propensity score will be based on the variables listed below. These variables were selected based on established clinical significance and relevant SmPC criteria available in the SITS database.
Age, sex, pre-stroke Modified Rankin Scale (mRS) score, baseline NIHSS score, systolic blood pressure and glucose level, antiplatelet treatment at stroke onset, history of hypertension, diabetes, atrial fibrillation, hyperlipidaemia, smoking and previous stroke earlier than 3 months, stroke onset to IVT treatment start time. We will include a baseline covariate only if 85% of the patients have a value reported.

A sensitivity PSM analysis will also be performed based on all baseline characteristics among patients with complete information. To determine whether the propensity score approach achieved balance in all potential confounders, we will compare all baseline characteristics of patients in the post-approval group to their propensity score matched pre-approval patients using standardized difference. If standardized difference >0.2 then propensity score model will be modified with inclusion of interactions and variable transformations to achieve better balance.

Relative risks for binary and mean difference for continuous/ordinal primary and secondary outcomes in post-approval vs pre-approval will be calculated together with 95% profile likelihood confidence intervals and likelihood ratio test p-values for relative risks and 95% Wald confidence intervals and Wald test p-values for mean differences.

Secondary Analysis

Secondary analysis will be on all patients fulfilling the inclusions-exclusions criteria.
Relative risks and mean difference for secondary outcomes in post-approval vs pre-approval will be calculated together with 95% confidence intervals and p-values.
Outcomes among post-approval vs pre-approval will be evaluated with generalized linear multivariable regressions with appropriate response distribution depending on outcome adjusting for potential confounders. Link function will be chosen as to estimate relative risks for binary outcomes and difference for continuous outcomes.
For each primary and secondary outcome covariates in models will be chosen according to the following:

1) Factors that contribute to the outcome of interest in an initial univariable analyses at p values <0.1 and variables known to influence outcomes such as age, sex, baseline NIHSS score and pre-stroke mRS, will be included in the multivariable model as candidate variables.
2) Final variables among the candidate variables with the outcome of interest will be selected by backward stepwise selection procedures using a p value <0.05.

Collinearity will be considered for selection of final list of covariates. Final model will be presented for each outcome with estimates of all final factors together with 95% confidence limits and p-values

Sensitivity Analysis

A sensitivity analysis restricted to European and non-European patients might be considered if minimum sample size required for analysis is met in the matched patient set.

References

1.Austin, Peter C. "Optimal caliper widths for propensity-score matching when estimating differences in means and differences in proportions in observational studies." *Pharmaceutical statistics* 10.2 (2011): 150-161.

6 Appendixes

6.1 Appendix 1

SITS Variables names particularly important for this study

Patient/ file code
Country name
Hospital/ Centre code
Gender
Age At Stroke Onset
Participation In Other Trials
Treatment at baseline
Aspirin
Dipyridamole
Clopidogrel
Other Antiplatelet
Anticoagulants- Yes/ No/ Unknown
If yes, specify Anticoagulants- Not specified/ Warfarin/ Dabigatran/ Apixaban/ Rivaroxaban/ Edoxaban/ Other
Heparin/ Heparinoids for Stroke Prevention
Heparin/ Heparinoids for Prophylaxis Deep Venous Thrombosis
Anti-diabetic, oral
Insulin
Antihypertensive, IV
Antihypertensive, oral
Statin
Other treatment

NSAIDS
Risk factors at baseline
Hypertension
Diabetes
Hyperlipidaemia
Smoking
Previous Stroke
Previous TIA
Atrial fibrillation' (AF)
Congestive Heart Failure
Modified Rankin Scale (mRS) Before Stroke
NIHSS score
Systolic Blood Pressure
Diastolic Blood Pressure
Plasma glucose
Cholesterol
Current Infarct CT/MR
Time logistics
Date Time of stroke onset
Date Time Hospital Arrival
Date Time Of imaging
Date Time Of intravenous thrombolysis
Type of Acute Intervention
Intravenous thrombolysis
Intraarterial thrombolysis
• Dose Of Actilyse
24h follow up
NIHSS
Systolic Blood Pressure
Diastolic Blood Pressure
Current Infarct CT/MR
Local Haemorrhage CT/MR
Remote Haemorrhage CT/MR
Cerebral Oedema CT/MR
At discharge/ 7d
Aspirin
mRS Score
NIHSS
Systolic Blood Pressure
Diastolic Blood Pressure
Dipyridamole
Clopidogrel
Other Antiplatelet
Anticoagulants Oral

Anti Diabetic Oral
Antihypertensive Oral
Statin
Other
Type Of Stroke
Type Of Stroke Ischemic
Type Of Stroke Haemorrhagic
Date Time Of Death
Cause Of Death
At 3-months
Modified Rankin Scale score
If mRS=6=Dead, date/time of death
Cause of death

6.2 Appendix 2

SmPC for Actilyse

Underlined variables are collected in the SITS IVTs registry and will be used to exclude patients from the SITS>80 years study

Contraindications in acute myocardial infarction, acute massive pulmonary embolism and acute ischaemic stroke:

Actilyse is contraindicated in cases where there is a high risk of haemorrhage such as:

- significant bleeding disorder at present or within the past 6 months
- known haemorrhagic diathesis
- patients receiving effective oral anticoagulant treatment, e.g. warfarin sodium (INR > 1.3) (see section 4.4)
- manifest or recent severe or dangerous bleeding
- known history of or suspected intracranial haemorrhage
- suspected subarachnoid haemorrhage or condition after subarachnoid haemorrhage from aneurysm
- any history of central nervous system damage (i.e. neoplasm, aneurysm, intracranial or spinal surgery)
- recent (less than 10 days) traumatic external heart massage, obstetrical delivery, recent puncture of a non-compressible blood-vessel (e.g. subclavian or jugular vein puncture)
- severe uncontrolled arterial hypertension (SBP>185 or DBP>110)
- bacterial endocarditis, pericarditis
- acute pancreatitis
- documented ulcerative gastrointestinal disease during the last 3 months, oesophageal varices, arterial-aneurysm, arterial/venous malformations
- neoplasm with increased bleeding risk
- severe liver disease, including hepatic failure, cirrhosis, portal hypertension (oesophageal varices) and active hepatitis
- major surgery or significant trauma in past 3 months.

Additional contraindications in acute ischaemic stroke:

- symptoms of ischaemic attack beginning more than 4.5 hours prior to infusion start or symptoms for which the onset time is unknown and could potentially be more than 4.5 hours ago (OTT>4.5 hours)
- minor neurological deficit or symptoms rapidly improving before start of infusion (baseline NIHSS score<=3)
- severe stroke as assessed clinically (e.g. NIHSS>25) and/or by appropriate imaging techniques
- seizure at onset of stroke
- evidence of intracranial haemorrhage (ICH) on the CT-scan

- symptoms suggestive of subarachnoid haemorrhage, even if CT-scan is normal
- administration of heparin within the previous 48 hours and a thromboplastin time exceeding the upper limit of normal for laboratory
- patients with any history of prior stroke and concomitant diabetes
- prior stroke within the last 3 months
- platelet count of below 100,000/mm^3
- systolic blood pressure > 185 mm Hg or diastolic BP > 110 mm Hg, or aggressive management (intravenous pharmacotherapy) necessary to reduce BP to these limits
- blood glucose < 50 mg/dl or > 400 mg/dl (< 2.8mM or > 22.2mM).

Criteria for exclusion of patients if treated with rt-PA outside of label

Onset-to-treatment time >270 min
Previous stroke within 3 months
Previous stroke earlier than 3 months + Diabetes
Anticoagulants, oral
Heparin for stroke prevention/ high dose
Glucose baseline by mmol <2.7 or > 22.2, or by mg% <50 or> 400
NIHSS baseline >25
Minor neurological deficit (baseline NIHSS score<=3)
SBP baseline > 185
DBP baseline > 110
Thrombectomy performed

6.3 Appendix 3

Modified Rankin Scale, mRS

Score	Description
0	No symptoms at all
1	No significant disability despite symptoms; able to carry out all usual duties and activities
2	Slight disability; unable to carry out all previous activities, but able to look after own affairs without assistance
3	Moderate disability; requiring some help, but able to walk without assistance
4	Moderately severe disability; unable to walk without assistance and unable to attend own bodily needs without assistance
5	Severe disability; bedridden, incontinent and requiring constant nursing care and attention
6	Dead

Total (0-6): _____

1a.Level of consciousness	**0**	Alert		
	1	Not alert, but arousable with minimal stimulation		
	2	Not alert, requires repeated stimulation to attend		
	3	Coma		
1b. Ask patient the month and their age	**0**	Answers both correctly		
	1	Answers one correctly		
	2	Both incorrect		
1c.Ask patient to open/close eyes and form/release fist	**0**	Obeys both correctly		
	1	Obeyrs one correctly		
	2	Both incorrect		
2.Best gaze (only horisontal eye movements)	**0**	Normal		
	1	Partial gaze palsy		
	2	Forced gaze deviation		
3. Visual field testing	**0**	No visual field loss		
	1	Partial hemianopia		
	2	Complete hemianopia		
	3	Bilateral hemianopia (blind, incl. Cortical blindness)		
4. Facial paresis (Ask patient to show teeth or raise eyebrows and close eyes tightly)	**0**	Normal symmetrical movement		
	1	Minor paralysis (flattened nasolabial fold, asymmetry on smiling),		
	2	Partial paralysis (total or near total paralysis of lower face)		
	3	Complete paralysis of one or both sides (abscence of facial movement in the upper and lower face)		
5a. Motor function - right arm	**0**	Normal (extends arm 90 or 45 for 10 sec without drift)		
	1	Drift		
	2	Some effort against gravity		
	3	No effort against gravity		
	4	No movement		
	9	Untestable (joint fused or limb amputated)		
5b. Motor Function - left arm	**0**	Normal (extends arm 90 or 45 for 10 sec without drift)		
	1	Drift		
	2	Some effort against gravity		
	3	No effort against gravity		
	4	No movement		
	9	Untestable (joint fused or limb amputated)		
6a. Motor Function - right leg	**0**	Normal (holds leg in 30 position for 5 sec without drift)		
	1	Drift		
	2	Some effort against gravity		
	3	No effort against gravity		
	4	No movement		
	9	Untestable (joint fused or limb amputated)		
6b. Motor Function – left leg	**0**	Normal (holds leg in 30 position for 5 sec without drift)		
	1	Drift		
	2	Some effort against gravity		
	3	No effort against gravity		
	4	No movement		
	9	Untestable (joint fused or limb amputated)		
7. Limb ataxia	**0**	No ataxia		
	1	Present in one limb		
	2	Present in two limbs		
8. Sensory (use pinprick to test arms, legs trunk and face, compare side to side)	**0**	Normal		
	1	Mild to moderate decrease in sensation		
	2	Severe to total sensory loss		
9. Best language (describe picture, name items)	**0**	No aphasia		
	1	Mild to moderate aphasia		
	2	Severe aphasia		
	3	Mute		

6.4 Appendix 4: National Institute of Health Stroke Score, NIHSS

6.5 Appendix 5

Global evaluation scale, GES

Has a change in the patient's clinical condition occurred after treatment?

Check one alternative:

Much better	+2
Better	+1
Unchanged	0
Worse	-1
Much worse	-2
Dead	-3