Statistical Analysis Plan for ASTER 2 study :

Combined use of contact aspiration and the stent retriever technique versus stent retriever alone for recanalisation in acute cerebral infarction trial.

Version : 2.2 Date: 17/12/2018

This document is written according to Guidelines for the Content of Statistical Analysis Plans in Clinical Trials. (1)

Trial registration: NCT03290885

Protocol version: The SAP has been written based on information contained in the study protocol version 3, dated 12 October 2017.

SAP revision history: No SAP revision after the first SAP version signed.

Roles and Responsibilities of SAP contributors

Julien Labreuche (Statistician Writing the SAP), Univ. Lille, CHU Lille, Department of biostatistics, EA 2694 - Santé publique: Epidémiologie et Qualité des Soins, Lille, France

Alain Duhamel (Supervising Senior Statistician), PhD, Univ. Lille, CHU Lille, Department of biostatistics, EA 2694 - Santé publique: Epidémiologie et Qualité des Soins, Lille, France

Bertrand Lapergue (Principal investigator reviewing the SAP), MD, PhD, Service de Neurologie, Hôpital Foch.

Michel Piotin (Scientific responsible, reviewing the SAP), MD, PhD, Neuroradiologie Interventionnelle, Fondation Ophtalmologique de Rothschild

Claire Prevot (Project Manager reviewing the SAP), DRCI, Hôpital Foch

Signature Page for SAP

Protocol title: Combined use of contact aspiration and the stent retriever technique versus stent retriever alone for recanalization in acute cerebral infarction: the randomized: the randomized ASTER2 study

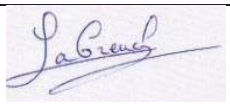
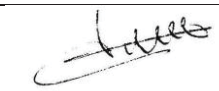
Finalized and Reviewed by	**Signature**	**Date**
Julien Labreuche (Biostatistician)		19-December-2018
Alain Duhamel, PhD (Chief Biostatistician)		19-December-2018
Bertrand Lapergue MD, PhD (Chief investigator)		18-December-2018
Michel Piotin, MD, PhD (Scientific responsible)		21-December-2018
Claire Prevot Project Manager		
Sponsors		
Elisabeth HULIER-AMMAR (Head of DRCI Hospital Foch)		

Table of Contents

List of Abbreviations

ASPECTS	Alberta Stroke Program Early CT Score
CA	Contact Aspiration
ICA	Internal carotid artery
ITT	Intent-to-treat
IV	Intravenous
MCA	Middle cerebral artery
MT	Mechanical thrombectomy
mTICI	Modified treatment in cerebral infarction
mRs	Modified rankin scale
NIHSS	National Institutes of Health Stroke Scale
OR	Odds ratio
PP	Per-protocol
TIA	Transient ischemic stroke
SR	Stent retriever

1 INTRODUCTION

1.1 Background and rational

Briefly, mechanical thrombectomy (MT) with a stent retriever (SR) device is now the standard intervention in anterior circulation ischemic stroke with large vessel occlusion (2-5). Contact aspiration (CA) (a new device of MT) is a promising treatment although they was not superior to SR as a first-line therapy for achieve successful reperfusion. (6) However, the potential synergistic effect of CA and SR devices as first-line endovascular treatment remains to be evaluated.

1.2 Research hypothesis

The null hypothesis is that there is no difference in perfection reperfusion rate the end of the endovascular procedure between the combination of CA and SR first-line thrombectomy versus the standard first-line SR thrombectomy groups. The alternative hypothesis is that there is a difference between the two groups.

1.3 Study Objectives

The primary objective of the ASTER2 trial is to determine the effectiveness (superiority) of combination of CA and SR first-line thrombectomy compared with standard first-line SR thrombectomy for increasing the perfect reperfusion rate at the end of the endovascular procedure.

Secondary objective are:

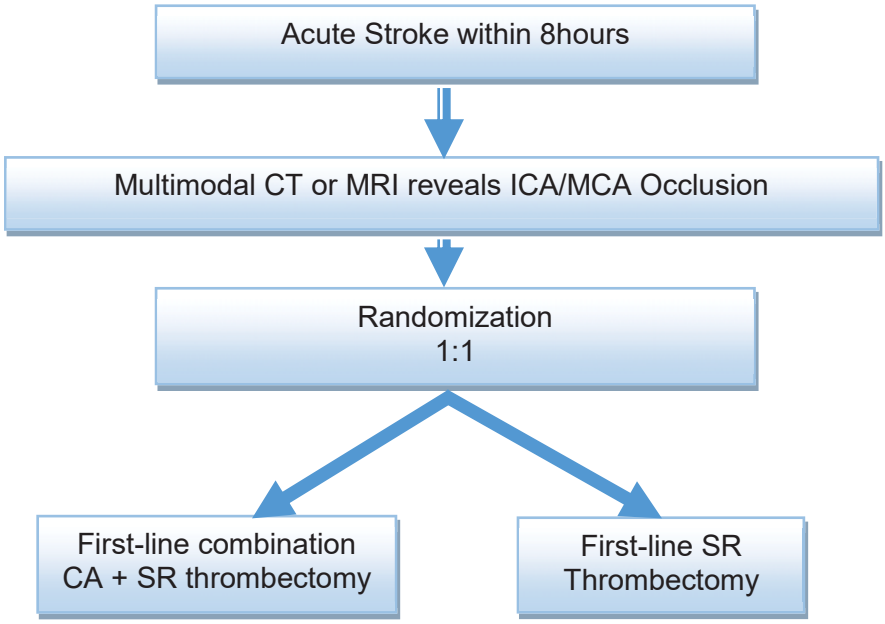
1) To determine the effectiveness of the combination of CA and SR first-line thrombectomy compared to the use of stent with standard first-line SR thrombectomy to improve :
    - angiographic efficacy outcomes after the fist-line strategy and at end of procedure
    - clinical efficacy outcomes
2) To compare the safety of the combination CA and SR first-line thrombectomy compared to the use of stent with standard first-line SR thrombectomy.
3) To determine the cost effectiveness combination CA and SR first-line thrombectomy compared to the use of stent with standard first-line SR thrombectomy based on the primary outcome

2 TRIALS METHODS

2.1 Trial design

The ASTER2 trial, is a multicenter, randomized, parallel group, controlled, open-label, with blinded endpoint evaluation (PROBE design). It as an academic trial designed to answer the question to: is combination CA and SR first-line thrombectomy is superior for increasing the perfect reperfusion rate compared to standard first-line SR thrombectomy. Patients are recruited from 11 high-volume, comprehensive stroke centers in France. Adults patients admitted with suspected ischemic stroke secondary to large vessel proximal occlusion of the anterior circulation, within 8 hours of the onset of symptoms are randomized to be treated either by first-line combination CA and SR thrombectomy or standard first-line SR thrombectomy with a treatment allocation ratio of 1:1.

Overview of Study Flow



2.2 Randomisation

The randomization process is described in full within the clinical trial protocol. To be brief, a dynamic randomization procedure using the Pocock and Simon minimization method (7) incorporates the following factors: age (70≤ vs. >70 years), prior use of IV thrombolysis, occlusion site (isolated middle cerebral artery (MCA) versus tandem MCA and internal carotid artery (ICA)). The center will be also considered in the minimization method.

2.3 Sample size

Full details of the sample size is described within the clinical trial protocol. A sample size of 204 per group will have 80% power to detect a difference in perfect reperfusion rate of 15% (absolute difference) assuming a reperfusion rate in control group (standard first-line SR thrombectomy) of 55% using a two-group Z-test (normal approximation) with a 0.05 two-sided significance level and taking into account an anticipated rate of spontaneous recanalization and catheterization failures of 20%.

2.4 Framework

Primary and secondary objectives of ASTER2 trials are testing for superiority.

2.5 Statistical interim analyses and stopping guidance

None statistical interim analysis is planned on the ASTER 2 trial.

2.6 Timing of analysis

Final analysis is planned to take place in two separate stages. The first main report/publication of the ASTER2 trial will be prepared when all patients had reached the 3-month follow-up and the database including primary endpoint and all secondary endpoints collected within the 3-month follow-up is cleaned and frozen (anticipated to be Janvier 2018) as described in data-management plan. 12-month outcomes (functional and all-cause mortality) and cost-effectiveness will be analyzed when all patients had reached the 12-month follow-up and the modified Rankin score/ EuroQol EQ-5D-3L have been collected and cleaned in the database (anticipated to be July 2019).

2.7 Timing of outcome assessments

The time points at which outcomes are measured in provided in table 1. Full detail of the schedule of the study procedures including expected visit dates and visit windows are described within the clinical trial protocol.

Table 1. The schedule of study procedures related to outcome measures

Outcomes	**End of first-line thrombectomyc**	**End of endovascular procedure**	**24 hrs Post-Randomization**	**90 days Post-Randomization**	**12 months Post-Randomization**
mTICI	X	X			
mRs				X	X
NIHSS			X		
Intracerebral hemorrhage (ECASS3 classification)			X		
Periprocedural complications	X	X			
Adverse Event assessment	X	X	X	X	X
EuroQol EQ-5D-3L				X	X

3 STATISTICAL PRINCIPLES

3.1 Confidence intervals and p-values

All applicable statistical tests will be 2-sided and will be performed using a 5% significance level.

No correction for multiple comparisons will be applied; all secondary objectives will be considered as exploratory.

All confidence intervals (CI) presented will be 95%CI and 2-sided.

3.2 Adherence and Protocol Deviations

Adherence to the intervention is defined as the use of at least 3 passes of first-line strategy before changing to use of other adjunctive devices to achieve at successful reperfusion defined as a mTICI 2b to 3. The number and % of patients with adherence to the intervention will be provided by treatment group, with no formal statistical comparison.

The following protocol deviations are pre-defined as major protocol violations with a direct bearing on primary outcome:

1) Patients without adherence to the intervention (as defined above)
2) Patients who did not received the allocated intervention

Protocol deviations will be identified and classified as major or minor in blind reviews before the database freezing. The number and % of patients with major and minor protocol deviations will be provided by treatment group, with details of the type of deviation. No formal statistical comparison will be done.

3.3 Analysis population

Intent-to-treat (ITT): The ITT population will include all randomized patients, regardless of their eligibility and any protocol deviations, according to the treatment group to which they were assigned at randomization. The ITT population will be the primary analysis population for primary and secondary efficacy outcomes, as well as for any safety outcomes and cost- effectiveness outcomes.

Per-protocol (PP): The PP population will be included all randomized patients excluding:

1) Patients who did not received treatment for any reason (spontaneous recanalization, catheterization failure or investigator decision)
2) Patients with treatment cross over, defined as receiving, as initial first-line thrombectomy strategy, the non-assigned first-line thrombectomy strategy

3) Patients with major protocol deviations

4) Patients without cor-lab blinded evaluation for primary endpoint.

PP analysis will be considered only for primary endpoint as a secondary analysis.

4 TRIAL POPULATION

4.1 Screening data

The overall recruitment period will be provided in months. The number of screened patients, number of randomized patients and the reason for non-randomization will be reported for overall population according to consort flow diagram (figure 1) compliant with the CONSORT 2010 standard.

4.2 Eligibility

The trial inclusion and exclusion criteria are full detailed in clinical trial protocol. The number of ineligible patients screened and not randomized will provided. The number of ineligible patients randomized will be reported by treatment group according to consort flow diagram (figure 1) compliant with the CONSORT 2010 standard.

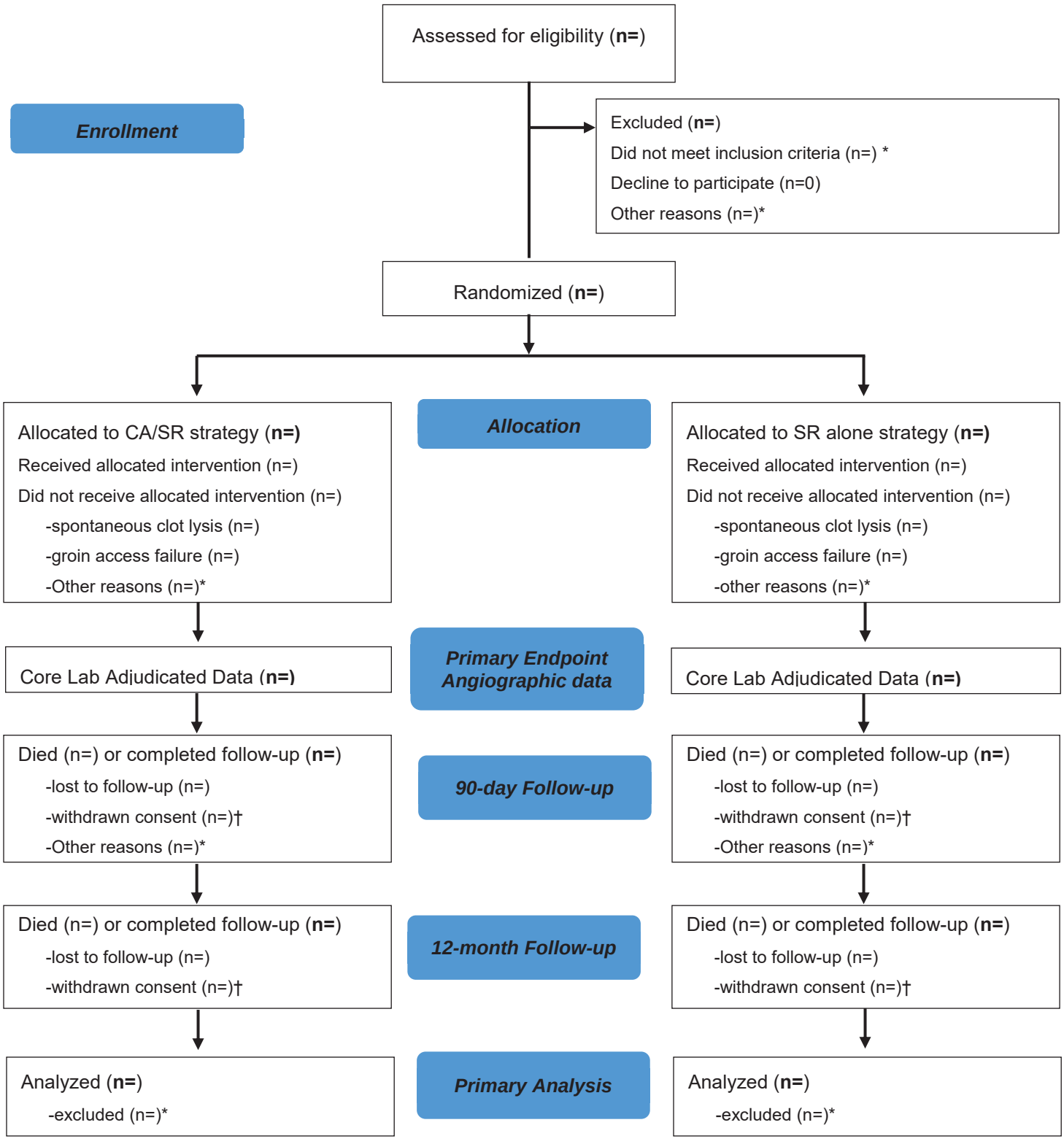
4.3 Withdrawal/Follow-up-level of withdrawal

The level of withdrawal will be tabulate and classified as:

1) Withdraw consent from follow-up but allow data collected to date be used
2) Withdraw consent from follow-up and withdraw consent for data collected to date to be used
3) Withdraw due to lost to follow-up
4) Withdraw due to investigator decisions

The timing of withdrawal and reasons for withdrawal will be provided by treatment group according to consort flow diagram (figure 1) compliant with the CONSORT 2010 standard.

Figure 1. Flow of participation in the ASTER2 trial.



* Reasons will be provided
† Level of consent withdrawal will be provided

4.4 Baseline patient characteristics

Detail of baseline characteristics are reported in table 2. Baseline characteristics will be described, in overall and according treatment groups. Quantitative variables will be expressed as mean (standard deviation) or median (interquartile range) for non-Gaussian distribution. Categorical variables will be expressed as frequencies and percentages. Normality of distribution will be assessed graphically and using the Shapiro-Wilk test. The number of missing data will be also reported. No formal statistical comparisons will be done; clinical importance of any imbalance will be noted.

Table 2. Baseline patient's characteristics

Characteristics	**Overall (N=)**	**First-line combination CA and SR** (N=)	**First-line SR alone** (N=)
Baseline demographics and medical history			
Age, years			
Men			
Medical history			
Hypertension			
Diabetes			
Hypercholesterolemia			
Current smoking			
Coronary artery disease			
Previous stroke or TIA			
Previous atrial fibrillation			
Previous antithrombotic medications			
Antiplatelet			
Anticoagulant			
Current stroke event			
Admission Systolic blood pressure, mmHg			
Admission Glucose, mmol/l			
Admission NIHSS score			
Pre-stroke mRS			
0			
1			
2			
3			
>3			
Admission ASPECTS			
Site of occlusion [1]			
M1-MCA			
M2-MCA			
Intracranial ICA			
Clot burden score [1]			
Clot length, mm, [1]			
AOL score			
0			
1			
2			

3	
Favorable collaterals [2]	
Suspected stroke cause	
Large artery atherosclerosis	
Cardioembolic	
Other/Unknown	
Directly admitted to a comprehensive stroke center	
IV rt-PA	
General anesthesia	
Onset to groin puncture time, min	
Onset to imaging	
Imaging to randomization	
Randomization to groin puncture	

[1] assessed angiographically by independent core lab. [2] favorable collateral defined as ASITN/SIR grading system, with grade 3-4.

5 Analysis

Data on primary and secondary efficacy/safety outcomes will be performed by the Biostatistics Department of University of Lille under the responsibility of Professor Alain Duhamel. For data analysis, statisticians will be aware of the treatment group allocation.

Data on cost effectiveness will be described in a detail in specific document.

5.1 Ouctome definitions

- Primary efficacy outcome (angiographic outcome) is:

the percentage of patients with perfect reperfusion defined as a mTICI score (as graded by an independent cor laboratory) of 2c [near-complete perfusion except for slow flow in a few distal cortical vessels or presence of small distal cortical emboli] or 3 [complete perfusion with normal filling of all distal branches] at the end of angiography done after the endovascular treatment (i.e. after the assigned first-line MT strategy and any further treatment deemed necessary “rescue therapy).

- Secondary angiographic efficacy outcomes are:

a) successful reperfusion (mTICI 2b/2c/3 graded by an independent cor laboratory) at the end of angiography done after the endovascular treatment

b) complete reperfusion (mTICI 3 graded by an independent cor laboratory) at the end of angiography done after the endovascular treatment

c) successful reperfusion (mTICI 2b/2c/3 graded by an independent cor laboratory) after the first-line thrombectomy strategy

d) perfect reperfusion (mTICI 2c/3 graded by an independent cor laboratory) after the first-line thrombectomy strategy

e) complete reperfusion (mTICI 3 graded by an independent cor laboratory) after the first-line thrombectomy strategy

f) perfect reperfusion (mTICI 2c/3 graded by an independent cor laboratory) after the first pass (i.e first pass effect)[1]

[1] No pre-specified in final version of protocol

e) complete reperfusion (mTICI 3 graded by an independent cor laboratory) after the first pass (i.e first pass effect)[1]

g) time from groin puncture to achieve perfect reperfusion, among patients achieved perfect reperfusion (mTICI 2c/3 graded by an independent cor laboratory) at the end of angiography done after the endovascular treatment

h) time from clot contact to maximum reperfusion

- Secondary clinical efficacy outcomes are:

a) global disability assessed by overall distribution of the mRs at 90 days (shift analysis combining scores of 5 and 6) (8)

b) global disability assessed by overall distribution of the mRs at 12 months (shift analysis combining scores of 5 and 6) (8)

c) the rate of favorable functional outcome at 90-day defined by a mRs ≤2

d) the rate of favorable functional outcome at 12-month defined by a mRs ≤2

e) the 24-hours change in NIHSS from baseline defined as the difference between NIHSS score at 24 hours and NIHSS score at admission

- Safety outcomes are:

a) any intracerebral hemorrhage on brain imaging (MRI or CT scan) at 24±12h after thrombectomy (according to ECASS3 classification)

b) the rate of parenchymal hematoma 2 on brain imaging (MRI or CT scan) at 24±12h after thrombectomy (according to ECASS3 classification)

c) all-cause mortality at 90-day

d) all-cause mortality at 12-month

e) procedure-related serious adverse events defined as arterial perforation, arterial dissection, embolization in a new territory and subarachnoid hemorrhage

- Cost-effectiveness outcomes are detailed in a separated file.

5.2 Analysis methods

- Primary efficacy outcome

The number and rate of perfect reperfusion at the end of the endovascular procedure will be reported for each treatment group. Primary efficacy outcome will be compared between the treatment groups using a mixed logistic regression model including center as random effects and prognostic factors considered in minimization randomization algorithm as fixed effects (age (70≤ vs. >70 years), IV thrombolysis, occlusion site (isolated MCA versus tandem MCA and /ICA)). Adjusted odds ratio (OR) will be derived from this model as the primary treatment effect size (experimental relative to control strategy). Using the method described by Austin, absolute and relative risk differences will be derived from the marginal probabilities of perfect reperfusion. (9)

- Secondary efficacy outcomes

For each secondary binary outcome (reperfusion rates after first-line strategy and at the end of endovascular procedure, favorable functional rates at 90-day and 12-month, any intracranial hemorrhage, parenchymal hematoma 2, all-cause mortality rate at 90-day and procedure-related serious adverse events), the numbers and percentages will be reported for each treatment group, and compared using a mixed logistic regression model including the same fixed and random effects that for primary outcome; adjusted ORs will be calculated as the treatment effect size. For procedure-related serious adverse events, only the rate of patients with at least one adverse event will be compared between the two groups (based on subject counts and not on event counts). The rate of specific adverse events will be evaluated only descriptively for each treatment group.

The secondary ordinal outcomes (distribution of mRS at 90-day and 12-month, after combining scores of 5 and 6) (8) will be described by the median (IQR) for each treatment group and compared using a mixed ordinal logistic regression model including the same fixed and random effects that previous models; adjusted common OR per 1-point improvement will be calculated as the treatment effect size.

All-cause mortality rate at 12-month will be estimated using the Kaplan-Meier method. The number of mortality and Kaplan-Meier event rate at 12-month will be reported for each treatment group. All-cause mortality will be between the two treatment groups by using a frailty model (Cox proportional hazard

model with center as random effect and prognostic factors considered in minimization randomization as covariates.

The change in NIHSS score at 24h will be calculated and compared between the two treatment group using the constrained longitudinal data analysis (cLDA) model proposed by Liang and Zeger (10) including center as random effect. This model will be used in view of the potential advantages of the cLDA compared to the conventional longitudinal analysis of covariance (ANCOVA) model. (11) In the cLDA, both the baseline and post-baseline values are modeled as dependent variables using a linear mixed model (using an unstructured covariance pattern model), and the true baseline means are constrained to be the same for the 2 treatment groups. Hence, the cLDA provides an adjustment for the observed baseline difference in estimating the treatment effects, using all available baseline and post-baseline values. The between-group mean differences in 24-hour change in NIHSS will be estimated by the time-by-arm interaction as treatment effect size. If normality of model residuals are not satisfied, nonparametric analysis will be used; absolute changes between baseline and 24 hours will be calculated and compared between the 2 treatments groups using non-parametric analysis of covariance adjusted for baseline values. (12, 13)

The others secondary quantitative outcomes (the reperfusion times) will be analyzed using a mixed linear regression model including the same fixed and random effects that previous models; the between-group mean differences in time will be derived from model as effect size. In normality of model residuals are not satisfied (even after a logarithmic transformation), nonparametric analysis will be used; quantitative outcome will be compared using the Mann-Whitney U test.

5.3 Subgroup analyses

As exploratory analyses, heterogeneity in treatment effect size on primary outcome across key subgroups will be evaluated by including the corresponding multiplicative interaction terms in the multivariate mixed logistic regression models (as defined in primary analysis of primary outcome). From these models, treatment effect sizes (adjusted OR) will be estimated in each subgroup. The following key subgroups will be investigated:

- Age (≤70 vs. >70 years)
- Time from onset to randomization (≤ 300 vs. > 300 minutes)

- Baseline site of thrombi on vascular imaging (isolated MCA vs. tandem MCA/ICA as adjudicated by the core lab)
- Prior use of IV alteplase (yes vs. no)
- Clot Burden score (<6 vs. ≥6)
- Collateral status (good versus poor, as adjudicated by the core lab on initial angiogram)
- Morphology of the occlusion (regular or irregular clot)

5.4 Missing data

Since we expected no missing data on primary outcome (assessed immediately after endovascular procedure), no imputation procedure will be applied. In cases of catheterization failure, primary outcome will be considered as failure (no perfect reperfusion) whatever the treatment group. In cases of spontaneous complete recanalization before endovascular treatment, primary outcome will be considered as perfect reperfusion whatever the treatment group. In case of missing core laboratory reading (whatever the reason), the study site evaluation of mTICI grade will be used to handle missing value in primary outcome.

For other secondary outcomes no imputation procedure will be used, except for:

- Reperfusion grades where similar imputation rules for primary outcome was applied.
- Other core laboratory outcomes where missing values were replaced by study site evaluation

5.5 Sensitivity analyses

Two sensitivity analyses will be performed for primary outcome only. A first sensitivity analysis will be conducted in PP population and a second sensitivity will be conducted using the primary outcome as evaluated by the study site.

5.6 Additional analyses

None additional analyses are planned.

5.7 Statistical software

Data will be analyzed using the SAS software (Version 9.4. SAS Institute Inc, Cary, NC, USA). Other package such as R software may be used if necessary.

6 References

1. Gamble C, Krishan A, Stocken D, et al. Guidelines for the Content of Statistical Analysis Plans in Clinical Trials. JAMA. 2017;318(23):2337-2343.
2. European Stroke Organisation Executive C, Committee ESOW. Guidelines for management of ischaemic stroke and transient ischaemic attack 2008. Cerebrovasc Dis 2008;25(5):457-507.
3. European Stroke Organisation (ESO), European Society for Minimally Invasive Neurological Therapy (ESMINT), European Society of Neuroradiology (ESNR). Consensus statement on mechanical thrombectomy in acute ischemic stroke. 2014 [updated 2014; cited Wednesday July 13, 2016]; Available from: http://2014.strokeupdate.org/sites/default/files/Consensus_thrombectomy_ESO_Karolinska_ESMINT_ESNR_final.pdf.
4. Powers WJ, Derdeyn CP, Biller J, et al. 2015 American Heart Association/American Stroke Association Focused Update of the 2013 Guidelines for the Early Management of Patients With Acute Ischemic Stroke Regarding Endovascular Treatment: A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association. Stroke 2015;46(10):3020-35.
5. Jauch EC, Saver JL, Adams HP, Jr., et al. Guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. Stroke 2013;44(3):870-947.
6. Lapergue B, Blanc R, Gory B, et al. Effect of Endovascular Contact Aspiration vs Stent Retriever on Revascularization in Patients With Acute Ischemic Stroke and Large Vessel Occlusion: The ASTER Randomized Clinical Trial. JAMA. 2017;318(5):443-452.
7. Pocock SJ, Simon R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. Biometrics. 1975;31(1):103-15.
8. Savitz SI, Lew R, Bluhmki E, Hacke W and Fisher M. Shift analysis versus dichotomization of the modified Rankin scale outcome scores in the NINDS and ECASS-II trials. Stroke. 2007;38:3205-12.

9. Austin PC. Absolute risk reductions, relative risks, relative risk reductions, and numbers needed to treat can be obtained from a logistic regression model. J Clin Epidemiol. 2010;63(1):2-6.
10. Liang KY ZS. Longitudinal data analysis of continuous and discrete responses for pre–post designs. ankhya. The Indian Journal of Statistics (Series B) 2000;62:134-148.
11. Liu GF, Lu K, Mogg R, Mallick M and Mehrotra DV. Should baseline be a covariate or dependent variable in analyses of change from baseline in clinical trials? Stat Med. 2009;28:2509-30.
12. Vickers AJ. Parametric versus non-parametric statistics in the analysis of randomized trials with non-normally distributed data. BMC Med Res Methodol 2005;5:35.
13. Conover WJ, Iman RL. Analysis of covariance using the rank transformation. Biometrics 1982;38(3):715-24.