PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Reperfusion Injury after ischemic Stroke Study (RISKS): single-center (Florence, Italy), prospective observational study protocol
AUTHORS	Piccardi, Benedetta; Arba, Francesco; Nesi, Mascia; Palumbo, Vanessa; Nencini, Patrizia; Giusti, Betti; Sereni, Alice; Gadda, Davide; Moretti, Marco; Fainardi, E; Mangiafico, Salvatore; Pracucci, Giovanni; Nannoni, Stefania; Galmozzi, Francesco; Fanelli, Alessandra; Pezzati, Paola; Vanni, S.; Grifoni, Stefano; Sarti, Cristina; Lamassa, Maria; Poggesi, Anna; Pescini, Francesca; Pantoni, Leonardo; Gori, Anna Maria; Inzitari, Domenico

VERSION 1 – REVIEW

REVIEWER	Philip Meyers Columbia University College of Physicians & Surgeons New York, New York United States
REVIEW RETURNED	26-Jan-2018

GENERAL COMMENTS	Single center prospective observational study designed to evaluate causes of reperfusion injury associated with treatment of acute ischemic stroke. The manuscript requires minor adjustments to English-language syntax and grammar.

REVIEWER	Ayush Batra, M.D., Assistant Professor of Neurology Northwestern University Feinberg School of Medicine United States of America
REVIEW RETURNED	02-Feb-2018

GENERAL COMMENTS	RISKS helps address a large gap in reperfusion injury in ischemic stroke, and is designed to gain valuable clinical and biological information on this growing subset of patients in the new era of aggressive interventional therapies. The lack of control group is a significant limitation, and the authors may consider including patients with unsuccessful large vessel recanalization as one potential control (though the anticipated number is expected to be low). The heterogeneity of the trial may provide novel insights into physiologic and functional differences with thrombolytic vs. mechanical recanalization strategies. ABSTRACT It is no longer widely accepted to define reperfusion injury as

	cerebral edema. Contrary to prior schools of thought, recanalization of large vessel occlusions appears to be associated with less cerebral edema (Irvine HJ et al. Reperfusion after ischemic stroke is associated with reduced brain edema. JCBFM 2017; Kimberly WT et al. Association of reperfusion with brain edema in patients with acute ischemic stroke, a secondary analysis of the MR CLEAN trial. JAMA Neurol. 2018). INTRODUCTION Lines 17-19: I would suggest removing “cerebral edema (CR) may be considered as expression of reperfusion injury”. METHODS A more robust measure of functional outcome outside of mRs and NIHSS may improve the sensitivity of the study question evaluating outcomes due to reperfusion injury. The current measures of mRS and NIHSS may result in an outcome that only distinguishes between hemorrhagic transformation and not. The authors may further strengthen this study by evaluating a marker of cerebrovascular reactivity (such as using transcranial doppler) following reperfusion. STUDY POPULATION Why limit inclusion to within 12 hours from last seen well, study authors could consider using DAWN Criteria to extend up to 24 hours if hospital guidelines allow (Nogueira RG et al. Thrombectomy 6 to 24 hours after stroke with a mismatch between deficit and infarct. NEJM 2018). IMAGING PROTOCOL The authors have designed a robust method of imaging data interpretation and also incorporating clinically significant variables (small vessel disease markers, prior infarctions, and atrophy). LABORATORY PROTOCOL: The authors list a wide array of traditionally described “biomarkers” for observational study, however do not hypothesize or describe clear rationale for assessment specific to reperfusion injury. An additional column in Table 3 titled “Rationale” or “Relevance” with hypothesized role/mechanism or prior studies evaluating effect would improve the methods. STATISTICAL ANALYSIS: Prior to considering any log-transformations of biomarker values, the authors should determine the natural biologic distribution of the measured biomarker before correcting for any skewed deviations. Additionally biomarker data should be separated by those receiving IV tPA vs. those receiving mechanical thrombectomy alone given the known impact of IV tPA on numerous biomarkers proposed to be measured. DISCUSSION: The authors highlight the study significance, and I agree with the potential utility of the planned observational data for use in predicting undesirable complications following recanalization.

VERSION 1 – AUTHOR RESPONSE

Editorial Requirements:

Please revise your title to include the location. This is the preferred format for the journal.

- We changed title according to Editorial Requirements.

Please work to improve the quality of English throughout the manuscript, either with the help of a native speaking colleague or with the assistance of a professional copyediting agency.

- According to reviewer suggestion, we had our paper reviewed by another native speaking colleague

Reviewer(s)' Comments to Author:

Reviewer: 1
Reviewer Name: Philip Meyers
Institution and Country: Columbia University, College of Physicians & Surgeons, New York, New York, United States
Please state any competing interests: No competing interests

Please leave your comments for the authors below

Single center prospective observational study designed to evaluate causes of reperfusion injury associated with treatment of acute ischemic stroke.

The manuscript requires minor adjustments to English-language syntax and grammar.

- According to reviewer suggestion, we had our paper reviewed by another native speaking colleague

Reviewer: 2
Reviewer Name: Ayush Batra, M.D., Assistant Professor of Neurology
Institution and Country: Northwestern University Feinberg School of Medicine, United States of America
Please state any competing interests: None declared

Please leave your comments for the authors below

RISKS helps address a large gap in reperfusion injury in ischemic stroke, and is designed to gain valuable clinical and biological information on this growing subset of patients in the new era of aggressive interventional therapies.
The lack of control group is a significant limitation, and the authors may consider including patients with unsuccessful large vessel recanalization as one potential control (though the anticipated number is expected to be low).

- We agree that the lack of a control group is a limitation of our study. Although the number of patients with unsuccessful recanalization is expected to be low, we will consider the possibility to use patients with unsuccessful large vessel recanalization as an internal control

The heterogeneity of the trial may provide novel insights into physiologic and functional differences with thrombolytic vs. mechanical recanalization strategies.

ABSTRACT
It is no longer widely accepted to define reperfusion injury as cerebral edema. Contrary to prior schools of thought, recanalization of large vessel occlusions appears to be associated with less cerebral edema (Irvine HJ et al. Reperfusion after ischemic stroke is associated with reduced brain edema. JCBFM 2017; Kimberly WT et al. Association of reperfusion with brain edema in patients with acute ischemic stroke, a secondary analysis of the MR CLEAN trial. JAMA Neurol. 2018).

INTRODUCTION
Lines 17-19: I would suggest removing “cerebral edema (CR) may be considered as expression of reperfusion injury”.

- Thanks, we followed the reviewer’s suggestion: cerebral edema has been removed as radiological marker of reperfusion injury also due to the fact that the statistical power of the study was calculated considering only hemorragic transformation. ABSTRACT, INTRODUCTION and METHODS have been changed accordingly.

METHODS
A more robust measure of functional outcome outside of mRs and NIHSS may improve the sensitivity of the study question evaluating outcomes due to reperfusion injury. The current measures of mRS and NIHSS may result in an outcome that only distinguishes between hemorrhagic transformation and not. The authors may further strengthen this study by evaluating a marker of cerebrovascular reactivity (such as using transcranial doppler) following reperfusion.

- We thank the reviewer for this observation. Although evaluation of cerebrovascular reactivity may be interesting from a physiopathological point of view, we have some concerns about the feasibility of using transcranial doppler (TCD) as outcome for the following reasons:
- absence of a temporal acoustic window is a considerable problem for routine utilization of TCD. This may result in reducing the sample size and therefore statistical power of the study.
- there are no data about safety and feasibility of using a vasoactive stimulus in acute stroke setting.
- aging and concurrent small vessel disease may heavily affect cerebral perfusion and vasoreactivity, and data could not entirely reflect the vasoreactivity of the ischemic brain [see Immink RV et al, Stroke 2005; Wardlaw JM et al; Cerebrovasc Dis 2011; Shi Y et al; J Cereb Blood Flow Metab 2016; Arba F et al; J Stroke Cerebrovasc Dis 2016].

STUDY POPULATION
Why limit inclusion to within 12 hours from last seen well, study authors could consider using DAWN Criteria to extend up to 24 hours if hospital guidelines allow (Nogueira RG et al. Thrombectomy 6 to 24 hours after stroke with a mismatch between deficit and infarct. NEJM 2018).

- We thank the reviewer for this advice. We fully agree that it would be interesting to update inclusion criteria in view of new evidence from Dawn trial. However a longer time frame for patient enrollment could confound temporal profile of blood biomarkers in study. Moreover, we would have to present a protocol amendment to the ethics committee of the Azienda Ospedaliero Universitaria Careggi (Università degli Studi di Firenze), while we have already enrolled about 2/3 of expected population and the end of patient recruitment is expected within the coming August. At the light of this, we would prefer not to change inclusion criteria

IMAGING PROTOCOL

The authors have designed a robust method of imaging data interpretation and also incorporating clinically significant variables (small vessel disease markers, prior infarctions, and atrophy).

LABORATORY PROTOCOL:
The authors list a wide array of traditionally described “biomarkers” for observational study, however do not hypothesize or describe clear rationale for assessment specific to reperfusion injury. An additional column in Table 3 titled “Rationale” or “Relevance” with hypothesized role/mechanism or prior studies evaluating effect would improve the methods.

- According to Reviewer’s observation, we added a column on Table 3 indicating the general rationale for the choice of biomarkers

STATISTICAL ANALYSIS:
Prior to considering any log-transformations of biomarker values, the authors should determine the natural biologic distribution of the measured biomarker before correcting for any skewed deviations. Additionally biomarker data should be separated by those receiving IV tPA vs. those receiving mechanical thrombectomy alone given the known impact of IV tPA on numerous biomarkers proposed to be measured.

- Thanks for your comment. We agree with a possible effect of IV tPA on biomarkers levels. Therefore, according with your suggestion, we will evaluate patients receiving mechanical thrombectomy and patients receiving IV tPA separately in a sensitive analysis.
As stated in “statistical analysis” section, differences in biomarkers levels between baseline and 24 hours will be evaluated according to their distribution. Only in case of skewed distribution of biomarker values, we will consider the possibility of log-transformation of data

DISCUSSION:
The authors highlight the study significance, and I agree with the potential utility of the planned observational data for use in predicting undesirable complications following recanalization.

VERSION 2 – REVIEW

REVIEWER	Ayush Batra Northwestern University Feinberg School of Medicine, Chicago, IL United States of America
REVIEW RETURNED	02-Apr-2018

GENERAL COMMENTS	Study aims to address an important gap in current stroke literature pertaining to mechanisms and imaging/biological characteristics of reperfusion injury. The prospective study design will for allow for biological sample collection which may serve as the basis for additional scientific inquiry into the precise mechanisms of injury. Authors have addressed major concerns brought up in previous revision, and have addressed comments regarding concerns for using cerebral edema as marker of reperfusion injury.