BODY FLUID BIOBANKING OF LONGITUDINAL COHORTS IN NEURODEGENERATIVE DISEASES

Report of a JPND Working Group on Longitudinal Cohorts

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JPND research

EU Joint Programme – Neurodegenerative Disease Research

This document is the final report from one of ten working groups commissioned by the EU Joint programme – Neurodegenerative Disease Research (JPND) in 2014 through a peer-reviewed call for proposals. The working groups were established to address methodological challenges preventing current population- and disease-based cohorts being further exploited for ND research. All ten reports are listed below and are available to download on the JPND website by clicking on the website link at the bottom of this page:

- **HD-READy (High-Dimensional Research in Alzheimer's Disease)**
  Coordinator: Professor M. Afran Ikram, Erasmus University Medical Centre, Rotterdam, Netherlands.

- **Harmonization and innovation of cognitive, behavioural and functional assessment in neurodegenerative dementias**
  Coordinator: Dr Alberto Costa, IRCCS Fondazione Santa Lucia, Rome, Italy.

- **NETCALS (Network of Cohort Assessment in ALS)**
  Coordinator: Professor Leonard van den Berg, University Medical Centre Utrecht, Utrecht, Netherlands

- **21st Century EURODEM**
  Coordinator: Professor Carol Brayne, University of Cambridge, Cambridge, UK

- **Multi-centre cohort-studies in Lewy-body dementia: Challenges in harmonizing different clinical and biomarker protocols**
  Coordinator: Professor Dag Aarsland, Stavanger University Hospital, Stavanger, Norway

- **Developing a methodological framework for trials in presymptomatic neurodegenerative disease – the Presymtomatic Neurodegeneration Initiative (PreNI)**
  Coordinator: Dr Jonathan Rohrer, University College London, London, UK

- **BioLoC-PD: Harmonization of biomarker assessment in longitudinal cohort studies in Parkinson's disease**
  Coordinator: Professor Daniela Berg, Hertie-Institute for Clinical Brain Research and German Center for Neurodegenerative Diseases, Tübingen, Germany

- **Dementia Outcome Measures: charting new territory**
  Coordinator: Professor Gail Mountain, University of Sheffield, Sheffield, UK

- **Body fluid biobanking of longitudinal cohorts in neurodegenerative diseases**
  Coordinator: Dr Charlotte Teunissen, VU University Medical Centre, Amsterdam, Netherlands

- **Realising the potential of cohort studies to determine the vascular contribution to neurodegeneration**
  Coordinator: Professor Joanna Wardlaw, University of Edinburgh, Edinburgh, UK

JPND Website link: http://www.neurodegenerationresearch.eu/initiatives/jpnd-alignment-actions/longitudinal-cohorts/

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Summary

The aim of this Working Group was to develop guidelines to enable exchange of biological material between international cohorts of ND patients. This was to be accomplished by a) developing proficiency testing programs to address biospecimen processing in agreement with current biobanking guidelines, b) to develop guidelines for exchange of biomaterial, c) develop datawarehouse framework for biobanked biospecimens, including development of minimal datasets of biobanked biospecimen information, and links to clinical data.
Several guidelines or tools have been developed:

1. First of all, we have established a Neurodegenerative disease Biobanking working group as part of ISBER, to enable international visibility and embedding of the activities in international Biobanking organisation.
2. EQA survey. The External Quality Assessment tool of ISBER was in place for tissue and blood. This survey tool has been expanded with questions pertinent to CSF.
3. Stability testing SOP and StabCalc tool on ISBER site. The JPND projects Biobanking WG and BIOMARKAPD have developed standard procedure for assessing biomarker stability.
4. Reference material of CSF samples treated according to the stability testing SOP. The reference material free to utilise and with known biomarker values to evaluate the robustness of novel biomarkers.
5. Proficiency testing scheme for biosample processing. The working group has developed proficiency testing schemes to test the biobanking procedures according to ISBER standards. Moreover, the proficiency to perform the tests is assessed in the same program.
6. Material transfer agreements for exchange.
7. RedCap Biobanking tool. We have developed a data warehouse tool available across sites to obtain and share information of accessible bio samples using REDCap.

Introduction

There is a strong interest in research for identification of new biomarkers for neurodegenerative diseases (NDs), for which body fluids (serum, plasma, CSF) are commonly stored for many years in biobanks. Longitudinal cohorts are necessary to establish the predictive value of biomarkers and to monitor biomarker change during

the disease course or upon treatment. Usually, several longitudinal cohorts are needed to be able to validate research findings and confirm the value of biomarkers in independent populations and different environments. Use of samples from more than one biobank for biomarker research is often hampered by variability in pre-analytical procedures, which are an importance source of variation in analytical and study outcomes [1]. Pre-analytical variation during biobanking is defined as variation in all aspects of the total biospecimen process, ranging from correct labelling of samples or patient misidentification; patient-related factors, such as dietary intake or circadian rhythms; variation in the biological fluid collection procedures, such as delayed blood processing, and use of different anticoagulants or tubes. The effect of pre-analytical variation on CSF and blood constituents is largely unknown but is body fluid specific [2]. To minimize pre-analytical variation, standardised protocols for body fluid and tissue collection and biobanking are of utmost importance. Such protocols have been established within international networks for Multiple Sclerosis biomarkers (www.bioms.eu [2]), Alzheimer's disease and other dementias [3,4] and Amyotrophic Lateral Sclerosis [5]. These protocols are all based on the original BioMS-eu protocol developed under guidance of the PI [2], having undergone refinements and no substantial change. Moreover, the participating centers in these networks have adopted the above protocols. This functional network now corresponds to >80 biobanks worldwide.
Usually, the exact contents of the biobanks and related information are not accessible to third parties, and upon sharing of samples, researchers have to reinvent to wheel with respect to legal, ethical and procedural issues to address during exchange. Thus, formation of data warehouses with uniform sample information and annotation, and guidelines for exchange are needed to allow access of researchers to biospecimens from various ND patient cohorts. The aim of the Working Group is therefore to develop guidelines to enable exchange of biological material between international cohorts of ND patients.

Context

Guidelines are applicable for people responsible for biobanking for body fluids of neurodegenerative diseases. However, since all tools and protocols are publicly available, and are generic for blood and CSF studies, they can be implemented beyond the neurodegenerative disease area.

Other groups that can benefit from our tools are researchers studying novel biomarkers, as the stability testing SOP is relevant for those persons. They can apply the tools for any novel biomarker in blood or CSF, whether for neurological diseases or not. Tools are of benefit for the global biobanking community through the ISBER Forum.

Terminology

ELISA: Enzyme Linked Immunosorbant Sandwich Assay
EQA: External Quality Assessment

Methods

We organised two major face to face meetings. The first was on October 28 2014, held in Amsterdam. The program included an introduction to the working group, and discussion on the criteria of sentinel biomarkers, discussion on the guidelines of exchange. Drafts of these criteria and guidelines of exchange had been prepared by Fay Betsou and Charlotte Teunissen that had intensive discussions to prepare the meeting. Furthermore, a possible datastructure using RedCAp was introduced by Reinhard Schneider that was also discussed during the meeting. After the meeting, tasks were executed. A major task was the building of the REDCAP tool that was built by Kirsten Roomp and revised and optimised after discussions with C. Teunissen, Eline Willemse and Fay Betsou. In between the meetings, we had several TCs and e-mail discussion to prepare the guidelines and tools.

For the final meeting, we invited all participants of BIOMARKAPD, who are strongly interested in the biobanking procedures. We organised an interactive training, in order to present all our tools, and to let them get acquaintanced with the tool (hands-on practice). The completion of the EQA survey by the participants enabled obtaining reference data for the survey outcomes. Further activities included the participation in the meeting of HD-READY by PI Afram Ikram.

AGREED GUIDELINES

We have developed 6 guidelines or tools:

Table 1. Tools developed during the Biobanking WG

No	Name	Character	Web-link
1	Neurodegenerative diseases Working Group established under umbrella of ISBER	Working group, webforum	http://www.isber.org/?page=ND#
2	EQA survey	Biobanking procedure quality assessment survey	http://www.isber.org/?page=EQAsurvey
3a	Stability testing SOP	Consensus on procedure for pre-analytical stability testing	**See below for contents**
3b	Stability testing calculation tool	Calculation sheet to be completed with raw data obtained during performance of the Stability testing SOP	http://www.isber.org/?page=STABCALC
4	Reference material of mistreated samples		http://www.vumc.nl/afdelingen/klinische-chemie/laboratoria/htw-Neurochemisch-Laboratorium/htw-Collaboration-CSF-Stability/
5	Proficiency testing scheme for biosample processing.	Procedure and scheme developed embedded in framework of similar procedures of the IBBL.	http://biospecimenpt.ibbl.lu/
6	MTAs for exchange	Template forms to be used during exchange of samples	http://www.isber.org/?page=ND#
7	RedCap Biobanking tool	Webbased datawarehouse and biobank information system for use of biobanks	http://www.isber.org/?page=ND#

Tool1: Neurodegenerative disease Biobanking working group as part of ISBER
Established to enable international visibility and embedding of the activities in international Biobanking organisation.

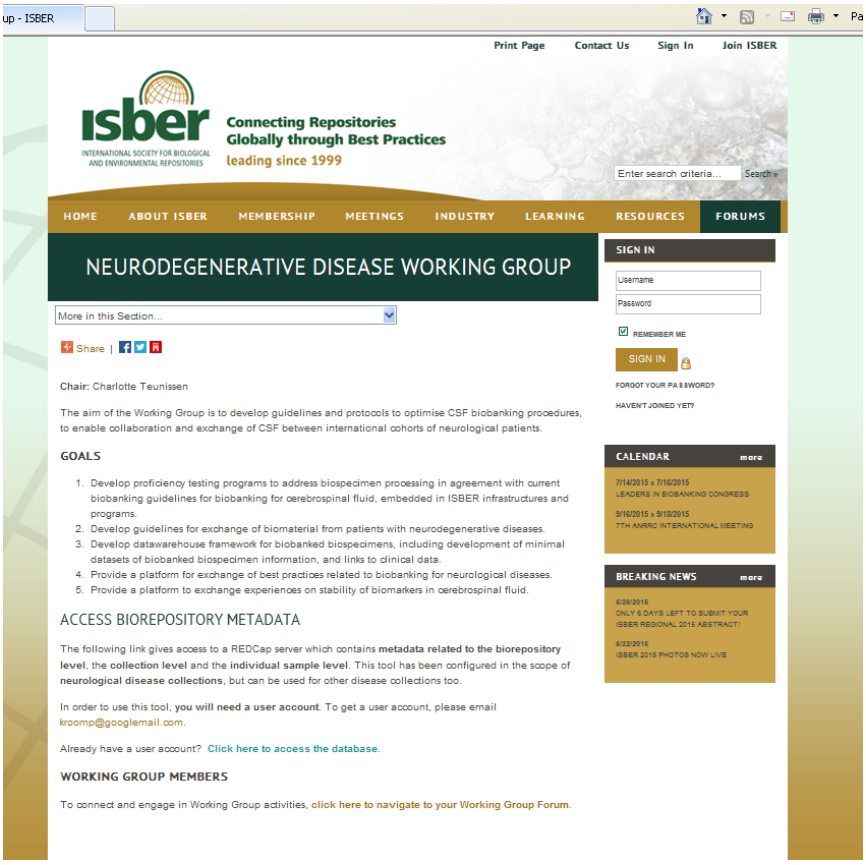


Figure 1: opening page of Neurodegenerative Disease working group on ISBER site: http://www.isber.org/?page=ND

Tool2: **EQA survey**
The External Quality Assessment tool of ISBER was in place for tissue and blood. This survey tool has been expanded with questions pertinent to CSF. The tool is open for external users. It is a tool via which centers can assess the quality of their procedures, and receive a report of their performance relative to other EQA participants. See http://www.isber.org/?page=EQAsurvey

Tool3: Stability testing SOP and StabCalc tool on ISBER site.
The stability of novel identified biomarkers under different pre-analytical conditions (a.o. processing delay. repeated freezing/thawing, long-term storage) is usually unknown. The JPND projects Biobanking WG and BIOMARKAPD have developed standard procedure for assessing biomarker stability (see http://www.isber.org/?page=STABCALC).

Tool 4: Reference material of CSF samples treated according to the stability testing SOP.

This material is free to utilise and with known biomarker values to evaluate the robustness of novel biomarkers. Reference material can be requested via this site: http://www.vumc.nl/afdelingen/klinische-chemie/laboratoria/htw-Neurochemisch-Laboratorium/htw-Collaboration-CSF-Stability/

SOP for sample stability test

1. To define the sample- or biomarker- stability, perform the following steps for three independent samples, preferably with different concentrations of the measurand (low, medium, high). For a full validation we recommend to use all variables indicated below, however, dependent on the purpose of the stability testing some (in-between) steps may be left out.
2. Divide the sample into nineteen aliquots with equal sample volume.

NB:It is important that every aliquot contains the same sample volume and to use the same kind of reaction vials, since unequal sample volumes may affect the concentration of the measurand due to adsorption. We recommend to use 500µl in e.g. Sarstedt 1.5 mL PP tubes (#72.703) and close the cap carefully.

- **Freeze-thaw stability** -

3. Place aliquots #1-6 at -80°C.
4. Thaw aliquots #2-6 and store again at -80°C.

Note: Thaw for 2 hours at room temperature and next store the sample at least 12 h at -80°C for each freeze/thaw cycle.

5. Thaw aliquots #3-6 and store again at -80°C (see fig.2).
6. Thaw aliquots #4-6 and store again at -80°C.
7. Thaw aliquot #5-6 and store again at -80°C.
8. Thaw aliquot #5-6 and store again at -80°C.
9. Thaw aliquot #6 and store again at -80°C.
10. Thaw aliquot #6 and store again at -80°C.

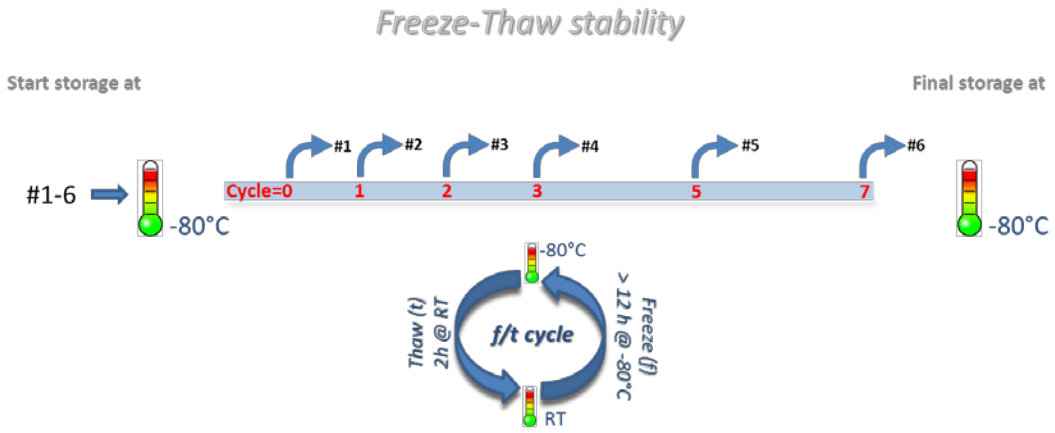


Figure 1. Workflow freeze-thaw stability.

- **Storage stability –**

11. At time point 0, store aliquots #7-12 at room temperature and another six aliquots #13-18 at 4ºC.
12. At time points t=1h, t=2h, t=4h, t=24h, t=72h, t=168h, transfer one sample stored at each temperature, RT and 4ºC, to -80ºC (see fig. 1).

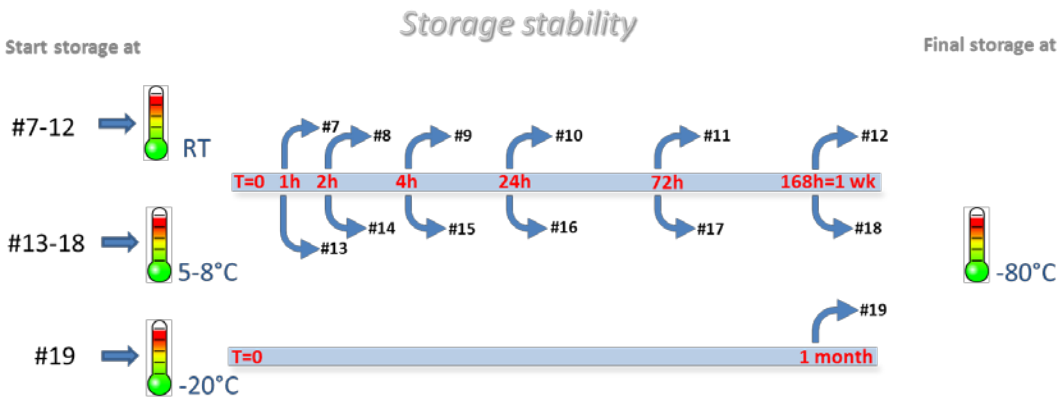


Figure 2. Workflow storage stability

13. Store aliquot #19 at -20ºC during one month before transfer to -80ºC.
14. Thaw all aliquots for a given sample simultaneously and analyse them in the same run (in duplicates for standard ELISA assays)..

Note: All samples should be analysed in a randomised order using the same lot.

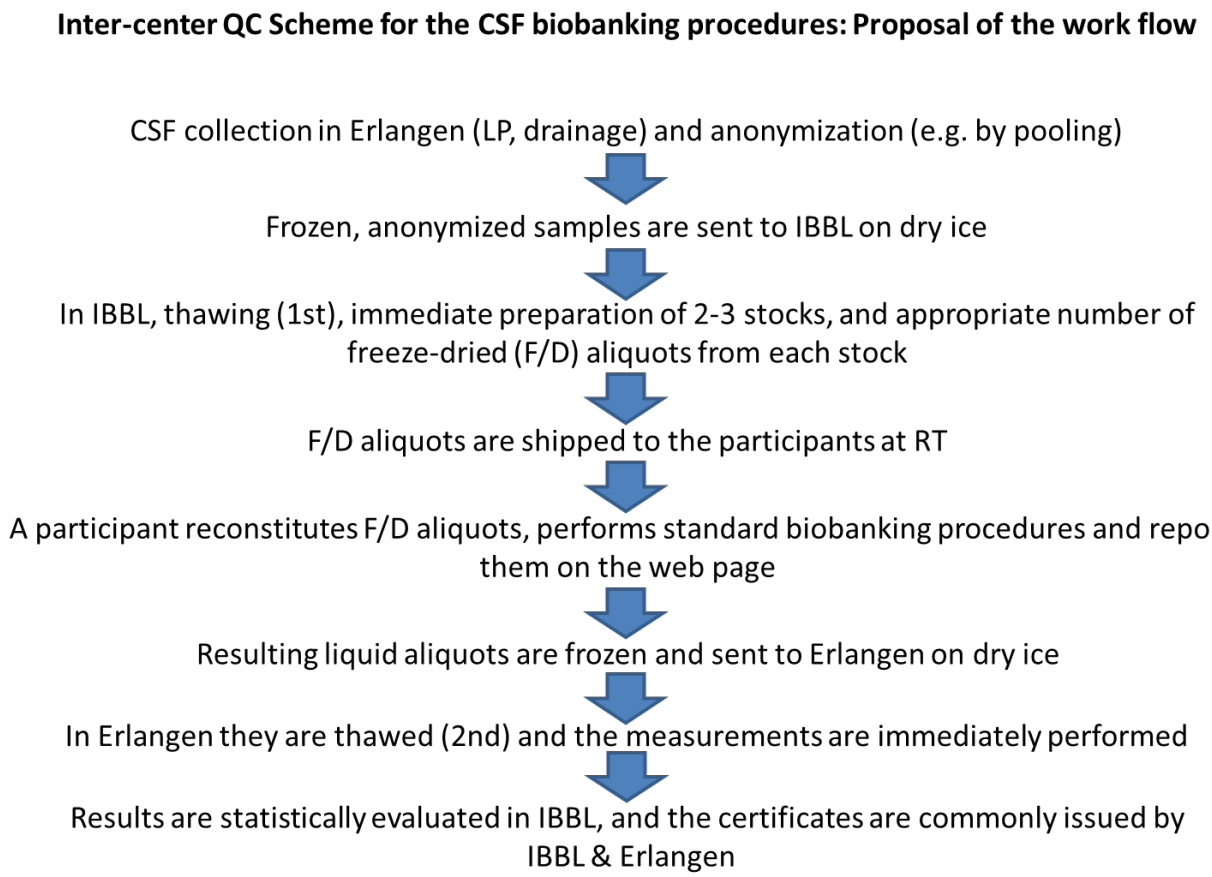
- Stability reporting –

15. Insert raw data of aliquots #1-19 (replicates of observed concentrations) in the Excel file "". The file calculates the mean value, standard deviation (SD), and coefficient of variation (%CV) for both the observed concentration and normalized concentration.

Note: The standard deviation for the storage stability and the freeze-thaw stability should be within the acceptance criteria for the precision defined in the "SOP for fit-for-purpose".

Tool5: Proficiency testing scheme for biosample processing

The working group has developed proficiency testing schemes to test the biobanking procedures (http://biospecimenpt.ibbl.lu/). The participating biobanks will test their blood and cerebrospinal fluid (CSF) sample processing procedures yearly according to this program. Read outs are albumin (typical stable CSF protein), amyloid beta (typical CSF protein with pre-analytical problems) and pTau as a typically stable CSF protein. Moreover, the proficiency to perform to perform a QC test for Hb is assessed in the same program. Figure 2 shows an overview of the scheme:



Tool6: Material transfer agreements for exchange (available on request)

Tool7: RedCap Biobanking tool

We have developed a data warehouse tool available across sites to obtain and share information of accessible bio samples using REDCap (can we insert the isber website link already?). We have utilized existing terminology and standards: 1) Minimum Information About BIobank data Sharing (MIABIS) which gives an overview of the biobank content; 2) Biospecimen Reporting for Improved Study Quality (BRISQ), which focuses on pre analytical factors; 3) Sample PREanalytical Code (SPREC) which generates codes describing preanalytical treatment of bio specimens. Prospective data and cleaned up retrospective data can be locally uploaded into a central REDCap installation, hosted at LCSB in Luxembourg and can be made available to all partners to share information on available biosamples. Link available via the homepage of the Neurodegenerative diseases working group printed at tool 1 (http://www.isber.org/?page=ND).

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References

(1) Pendlebury ST, Rothwell PM. Prevalence, incidence, and factors associated with pre-stroke and post-stroke dementia: a systematic review and meta-analysis. Lancet Neurol 2009; 8(11):1006-1018.

(2) Lodder J. Poststroke cognition and the fight against the hard problem: vascular neurologists, enter the arena! Stroke 2007; 38(1):7-8.

(3) Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. Lancet Neurol 2010; 9(7):689-701.
(4) Hachinski V. Vascular dementia: a radical redefinition. Dementia 1994; 5(3-4):130-132.
(5) JPND Action Group. Longitudinal cohort studies in neurodegeneration. 1-92. 2013.
(6) Sachdev PS, Lipnicki DM, Kochan NA, Crawford JD, Rockwood K, Xiao S et al. COSMIC (Cohort Studies of Memory in an International Consortium): an international consortium to identify risk and protective factors and biomarkers of cognitive ageing and dementia in diverse ethnic and sociocultural groups. BMC Neurol 2013; 13:165.
(7) Lees R, Fearon P, Harrison JK, Broomfield NM, Quinn TJ. Cognitive and mood assessment in stroke research: focused review of contemporary studies. Stroke 2012; 43(6):1678-1680.
(8) Makin S, Turpin S, Dennis M, Wardlaw J. Cognitive impairment after lacunar stroke: systematic review and meta-analysis of incidence, prevalence and comparison with other stroke sub-types. J Neurol Neurosurg Psychiatry 2013; 84:893-900.
(9) Valdes Hernandez M, Booth T, Murray C, Gow A, Penke L, Morris Z et al. Brain white matter damage in aging and cognitive ability in youth and older age. Neurobiol Aging 2013; 34(12):2740-2747.
(10) Kerr GD, Slavin H, Clark D, Coupar F, Langhorne P, Stott DJ. Do vascular risk factors explain the association between socioeconomic status and stroke incidence: a meta-analysis. Cerebrovasc Dis 2011; 31(1):57-63.
(11) Smith EE, O'Donnell M, Dagenais G, Lear SA, Wielgosz A, Sharma M et al. Early cerebral small vessel disease and brain volume, cognition, and gait. Ann Neurol 2015; 77(2):251-261.
(12) Wardlaw JM, Smith EE, Biessels GJ, Cordonnier C, Fazekas F, Frayne R et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration: a united approach. Lancet Neurol 2013; 12(8):822-838.
(13) Staals J, Makin SDJ, Doubal F, Dennis M, Wardlaw JM. Stroke subtype, vascular risk factors and total MRI brain small vessel disease burden. Neurology 2014; 83:1228-1234.
(14) Staals J. Total small-vessel disease score: a new and pragmatic way to assess the full impact of small-vessel disease on the brain. NeuroNews 2015; 16:19.
(15) Del Bene A, Makin SDJ, Doubal FN, Inzitari D, Wardlaw JM. Variation in risk factors for recent small subcortical infarcts with infarct size, shape and location. Stroke 2013; 44(11):3000-3006.
(16) Asdaghi N, Pearce LA, Nakajima M, Field TS, Bazan C, Cermeno F et al. Clinical correlates of infarct shape and volume in lacunar strokes: The Secondary Prevention of Small Subcortical Strokes Trial. Stroke 2014; 45:2952-2958.
(17) Greenberg SM, Salman RA, Biessels GJ, van Buchem M, Cordonnier C, Lee JM et al. Outcome markers for clinical trials in cerebral amyloid angiopathy. Lancet Neurol 2014; 13(4):419-428.

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