NETCALS: NETWORK OF COHORT ASSESSMENT IN ALS

Report of a JPND Working Group on Longitudinal Cohorts

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JPND research

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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

ALS is one of the most devastating diseases in neurology. Differential phenotypic and genetic observations indicate that ALS should no longer be considered a single disease entity. A challenge in ALS research will be to identify subgroups of patients based on phenotypic, genetic, environmental or lifestyle characteristics or molecular biomarker, neuroimaging and neurophysiological data. Only transnational collaborations of ALS centres will be able to create a sample size with sufficient power to analyse multiple layers of data.

NETCALS established pan-European 'best practice' guidelines and a methodological framework for data sharing/handling, cognitive/functional outcome measures (including training & certification), standards for neuro-imaging/neurophysiology, and clinical data linkage in population-based cohorts of ALS patients.

The workshops and first transnational training session for harmonization and certification of clinical, imaging and neurophysiological assessments of disease status resulted in:

1) IDALS a unique patient identifier as uniform identifier avoiding duplication and facilitating multicentre, multilayer, data analyses;
2) Drafts and guidelines on data sharing agreements enabling efficient transnational data sharing;
3) Standardization of cognitive and functional outcome measures for multicentre cohort studies;
4) Standardization of neuro-imaging and neurophysiology outcome measures for multicentre cohort studies;
5) Training and certification program on outcome measures to minimize intra- and intercentre variability in multicentre studies.

NETCALS capitalises on existing pan-European infrastructure based on the European Network for the Cure of ALS (www.ENCALS.eu), an FP7 systems biology project EuroMOTOR, 4 JPND initiatives (SOPHIA (biomarkers), STRENGTH (risk factors), ALS-CarE and Needs in ALS (both on healthcare pathways and decision making)), and community-led projects (The Neuroimaging Society in ALS (www.NISALS.org), Project MinE (whole-genome sequencing initiative for ALS, www.projectmine.com) and TRICALS (trials)). Therefore NETCALS offers a unique opportunity to

disseminate our results into on- going projects involving a wider community of ALS researchers. The results of NETCALS are already being incorporated successfully in these projects.

Introduction

Amyotrophic Lateral Sclerosis is one of the most devastating diseases in neurology affecting some 50,000 individuals at any time in Europe, and causing around 10,000 deaths each year. There are currently no effective disease-modifying therapies for ALS. Management is palliative and is aimed at maximizing quality of life and minimizing the burden of disease. The clinical presentation and progression of ALS varies considerably. It affects otherwise healthy people at any adult age. Initial manifestations are weakness of limbs or abnormalities of speech. Muscle weakness may be mild at first but gradually progresses, spreading to other regions of the body, and the patient eventually becomes paralysed. Over 65% of patients die within 3 years, and 90% die within 5 years of their first symptom, usually of respiratory failure. In addition to the inexorable motor decline, up to 50% of those with ALS develop mild cognitive impairment and 15% develop frontotemporal dementia (FTD). In about 5% of cases there is a family history of ALS (fALS), which leaves 95% of the cases as non-familiar or sporadic ALS (sALS).

Multiple genes have been identified responsible for about 60% of such cases. Whole genome/exome sequencing efforts in larger patient cohorts need to determine whether all mutations are indeed pathogenic. This is important for gene therapy studies that are being planned. The recently discovered repeat expansions in the C9orf72 gene, which appears to be the most frequent mutation in ALS (about 30% fALS, 5% sALS), enables us to assess the presymptomatic stages of ALS for the first time on a larger scale. In sALS a number of common genetic variants with small effect have been identified in GWASs. A new (mainly European) project to generate arger scale GWASs and whole-genome sequencing in sporadic ALS has been initiated (Project MinE).

Context

Population- based ALS cohort studies are on-going in many European countries aiming to identify environmental or lifestyle factors (= exposome) that increase susceptibility for ALS or modify the disease course. The most consistent lifestyle factors associated with ALS are a low premorbid BMI and beneficial serum lipid profile despite a high caloric diet, all pointing towards a hypermetabolic state. Larger incident population-based cohorts using a validated exposome questionnaire are required for replication and studying gene-environment interaction. This can only be achieved by linkage across cohorts.

The differential phenotypic and genetic observations strongly indicate that ALS should no longer be considered a single disease entity, which may have important consequences for development of effective therapy. The factors increasing and decreasing risk, modulating phenotype, altering age of onset, or triggering disease remain largely unknown, thus there is a high need for further research. A major challenge in ALS research is to identify different subgroups of ALS based on phenotypic characteristics, genetics, exposomics, molecular CSF/peripheral blood biomarkers, neuroimaging (marker for upper motor neuron degeneration) and neurophysiology (marker for lower motor neuron degeneration). The optimal strategy requires large, unbiased data sets with these multiple layers of data collected in a standardized manner associated with bioinformatics expertise to handle

sharing and analysing of big data. Thus only transnational collaborations of ALS centres will be able to create a sample size with sufficient power to analyse multiple layers of data.

Standard assessments for the detection of cognitive impairment/dementia are not appropriate in patients with ALS due to the range of physical problems (speech, writing, drawing), all of which form an important part of most cognitive screening batteries. The Edinburgh Cognitive and Behavioural ALS Screen (ECAS), a brief assessment designed specifically for ALS patients, is a useful tool that is currently under (further) validation in an international approach (STRENGTH).

In clinical trials the functional measures are used to evaluate treatment effects. Besides respiratory measures and muscle strength, the functional rating scale ALS Functional Rating Scale (ALSFRS) is used to measure the progressive loss of functions such as speech, swallowing, mobility, and respiration.

However, ALSFRS may not fully capture the functional characteristics of later-stage ALS progression and there is no agreed-upon threshold at which a change in ALSFRS is viewed as a meaningful transition point in functional status. Definition of discrete stages of disease helps to provide a universal and objective measure for disease progression, prognosis, research classification, clinical trial design, therapeutic decision-making and assessment of quality of care. A staging system also allows patients and caregivers to understand the disease and its clinical course better. Staging systems for ALS has been set up. The system is currently optimized and validated in prospective patient cohorts.

Besides enabling more and faster data sharing and data handling with guidelines overcoming legal, ethical and logistical constraints and the use of unique patient identifier, harmonization on outcomes measures is needed to standardize what is measured and how it is measured. Only then the validity is improved and inter- en intra-centre variability minimized.

A program of training and frequent retraining on cognitive, functional, multimodal and neurophysiological outcomes leads to certified ALS centres and assures standardised data collection in multicentre cohort-studies.

Terminology

ALS - Amyotrophic Lateral Sclerosis
fALS – Familial form of ALS
sALS - Sporadic form of ALS
IDALS – patient identifier in ALS
ECAS - Edinburgh Cognitive and Behavioural ALS Screen
ALS-FRS - ALS Functional Rating Scale
EIM – Electrical Impedance Myography
MUNIX - Motor Unit Neuron Index
DSA - Data sharing agreements

Methods

The Working Group NETCALS first convened in Brussels on 5 December 2014 (**Workshop 1**). A general outline of the plans for NETCALS was decided upon by all representatives and tasks were divided. Four NETCALS sub groups (or workpackages) were defined and representatives of all NETCALS partners were divided over the groups:

A = Data handling, integration and sharing (data sharing agreements, unique patient identifier) Ammar Al-Chalabi (leader) (King's College London), Julian Grosskreutz (Jena U.), Peter Andersen (Umea U.), Magdalena Kuzma (Med. U. of Warsaw), Thomas Meyer (Char. U. Berlin), Leonard van den Berg (UMCU)

B = Clinical data linkage & cohort alignment; patients & pre-symptomatic carriers

Kevin Talbot (leader) (U. of Oxford), Leonard van den Berg (UMCU), Philippe Corcia (CHU Tours), Francois Salachas (Hop. de Paris), Vincenzo Silani (IRCCS Milan)

C = Cognitive & functional outcome measures for disease progression

Leonard van den Berg (UMCU), Pamela Shaw (USFD), Sharon Abrahams (U. of Edinburgh), Orla Hardiman (TCD), Adriano Chio (U. of Turin), Albert Ludolph (Ulm U.), Susanne Petri (Hannover Med. School), Katja Kollewe (Hannover Med. School)

D = Standards for multimodal neuro-imaging and neuro-physiology approaches

Markus Weber (Kantonsp. St. Gallen), Julian Grosskreutz (Jena U.), Kevin Talbot (U. of Oxford), Pierre- Francois Pradat (Hop. de la Pitie-Salp.), Mamede de Carvalho (U. of Lisbon), Malgorzata Mgawel (Med.

U. of Warsaw), Susanne Petri (Hannover Med. School), Katja Kollewe (Hannover Med. School), Philippe Corcia (CHU Tours), Adriano Chio (U. of Turin), Martin Turner (U. of Oxford), Andrea Calvo (U. of Turin) E= Training and Certification of cognitive, functional and neurophysiological outcome measures

The sub groups had frequent conference calls and involved experts from the Advisory Reference Group between December 2014 and July 2015 to discuss a workplan and to execute accordingly. NETCALS met during workshop (**Workshop 2**) on 23rd and 24th of May 2015 in Dublin to discuss most of the results including proposed guidelines and plan dissemination of the results. At the same time a training & certification session on clinical outcome measures was held for nursing staff of all ALS research centres, whereas neurologists/neurophysiologists attended a (re)training session on neurophysiological outcomes (MUNIX).

AGREED GUIDELINES

Data handling, integration and sharing

Patient ID, IDALS

Clinical research faces a conflicting set of priorities. On the one hand, patient information needs to be kept confidential without identifiers, and on the other clinical information needs to be shared between researchers, and duplication avoided. Furthermore, linkage of records between studies enables clinical and research data to be maximised by integration of multiple layers, but only if the same individual is identifiable as having participated in the different studies. Since names are not shared and other identifiers are restricted, a significant benefit of research is lost. To overcome this problem, the NETCALS participants have developed principles, which allow the generation of a unique identifier consistently given the same input information. To achieve this, the patient first and last name, date of birth and age of symptom onset can be used to generate a hash. This can be done retrospectively if needed. The hash process cannot be reversed to find the patient details used to generate the hash (see figure 1). With this system, it remains possible to breach confidentiality using a brute force approach. The weakness is at stages 2 and 3 in Figure 1.

Figure 1.
1. Patient identifiers →→ 2. SHA1 hash generated →→ 3. [Hash ID] available for sharing

If the hash tool is publicly available, and the hash ID is known, it is possible for someone to simply put in the details of someone they suspect of being in the study and the ID generated can be compared with that known to de-identify the subject. There are two possible solutions to this problem. First, the hash generation tool could be restricted. While this is possible, it makes the system inconvenient to use since, for example, it would not be possible for patients to self-register on a website since the tool would only be available to the few with registered access. The second possibility is to restrict knowledge of the hash ID (Figure 2).

Figure 2.
1. Patient identifiers →→ 2. SHA1 hash generated →→ 3. [Hash ID] hidden + [Sequential ID] visible

In this scenario, the hash tool is public, but users only see a sequential ID that is tagged internally to the hash ID. The hash ID is visible to registered users along with the sequential ID. This means that patients or research staff can generate IDs for a study, but a brute force attack is not possible since the hash ID is restricted to registered users. Researchers who want to share data across centres can share hash IDs between registered users, and those who want to link study databases within centres are also able to using the hash ID.

Beyond the NETCALS process, a real life tool (IDALS) was developed by one contributor (JG), which includes a number of measures to ensure the integrity of the hash tool. First, the hashing algorithm uses a number of salts, which are not accessible for non-authorized personnel. It is customised so that only those with access to the tool can generate hash IDs. This means that an attacker who obtained a list of hash IDs would not be able to de-identify data by brute force unless they also

obtained the tool. Second, it is important that the tool generates consistent IDs across countries and languages. We therefore set up a standard operating procedure to transliterate letters and sounds across languages into a UTF-8 encoded 26 letter alphabet of upper case Latin characters; this allows use of IDALS across Europe.

The transliteration system and hash-generating tool IDALS have been tested by partners both using individual entries and large systematic data transfers. The tool is currently being integrated into the UK National MND Register database and will be used to generate retrospective IDs for samples across Europe under an academic use software license agreement. IDALS lends itself well to use in other diseases and countries and is a generic solution to the data linkage and integration problem. The outcome has been disseminated at ENCALS consortium meetings in Utrecht, and will be presented in an upcoming international meeting in Florida, as well as by publication in a peer-reviewed journal.

Data sharing agreements

Data sharing agreements (DSA) within Europe are dealt with by legal teams from each University, Hospital or Institution. In general, the legal document on which data sharing will be based originates in the coordinating country and is sent to collaborating countries for modification and approval. Although standard documents exist, they differ between countries. For example, the UK uses a different template from much of Europe.

The DSA for a project should ideally include an agreement on foreground and background intellectual property, a material transfer agreement for DNA or similar samples, a description of the governance of the consortium, a publication policy, a method for data sharing that respects confidentiality and ethical restrictions, particularly as they apply across countries, a method for restricting or controlling access including a Data Access Committee, and a description of what should happen to the data or samples after use. In contrast to one time cross sectional projects which allow full anonymization of data from different sources, longitudinal studies most often rely on continuous data acquisition. In this case, the ability to collect data through pseudonymization and on-going data upload must be balanced against the need to remove patient identifying information. For international collaboration, DSA should provide an exact description of how this process is handled to not conflict with national laws. One way of realizing such collaboration is by generating project specific internal patient identifiers which allow synchronization of databases without disclosing information which may allow real life identification of the patient having contributed to the data collection.

Whereas the technical aspect of data sharing has been resolved using the principles of a hashing algorithm outlined within NETCALS, we concluded that the legal aspect does not have a common solution, since even the principles will depend on the law in each nation state, and the stipulations of the funding body.

Nevertheless, we determined the principles on which further agreements should be based that will be useful for future projects:

1) The purpose of the data sharing should be stated clearly;
2) The existing intellectual property should be defined where possible;
3) The expected outcome for generated intellectual property should be defined;

4) The governance structure of the collaboration should be clearly stated, including the names and contact details of the coordinator, steering group, management group and technical committees;

5) The agreement should include material transfer agreements for biological and other samples, including what should happen after use (return, destruction, storage, etc);

6) Collaborators should feel secure that data remains within the contributor's control, either through use of electronic or other means of data protection, and through representation on a Data Access Committee where relevant;

7) A publication policy should clearly state whether all consortium members are to be represented in some way for every paper, or only a subset, and whether consortium names are acceptable as authors. Examples of successful papers using various authorship systems exist

8) The agreement must of course comply with the ethical and legal restrictions of the countries involved, and European law;

9) The DSA needs to state clearly the nature of the data collection (i.e. cross sectional vs. longitudinal on- going) and outline the principles of data handling between the contributing and the analysis groups.

These guidelines have been distributed through ENCALS to all partners.

Clinical data linkage & cohort alignment; patients & pre-symptomatic carriers

Consensus was reached on core set of clinical data that will be collected European-wide. A database has been established successfully for efficient online data collection (Progeny) including genotype. This will facilitate cohort alignment and will be applied to on-going population-based patient cohorts and controls.

Experience was shared among groups on studying pre-symptomatic gene carriers. Policies on ethical issues appear to be highly variable in Europe. Discussions on combining datasets of C9orf72 positive families will be continued and will become more important as C9orf72 specific therapy is being developed by several companies.

Cognitive & functional outcome measures for disease progression

To date, all methodological studies on the ALSFRS (ALSFRS, ALSFRS-R and ALSFRS-EX, used classical test theory procedures and all have demonstrated good internal consistency, reproducibility and convergent validity. Recent research on ALSFRS-R using classical test theory procedures as well as item response theory (Rasch analysis) for examining the psychometric properties of the scale suggests that the ALSFRS-R has some shortcomings and we decided that these will need to be, at least in part, revised by our groups. Dimensionality analysis of the ALSFRS-R revealed that the items cannot be simply summed to an overall functional score since this score does not represent a single dimension. Patients with the same score are, in fact, not comparable in a disease like ALS with heterogeneous clinical presentation and progression.

The ECAS is a brief multi-domain cognitive screening test including assessment of a) ALS-Specific functions (executive functions, fluency and language) known to be affected in ALS, b) ALS non-specific functions (memory and visuospatial) included to differentiate impairment from that typically associated with Alzheimer's Disease and c) a separate carer behaviour screen based on diagnostic criteria for bvFTD. It is designed to determine which patients have cognitive and/or behavioural impairment and what type of cognitive and/or behavioural impairment each individual has. The

ECAS has been successfully translated into 8 languages with several further translations underway. Validation of the German/Swiss version of the ECAS has been undertaken in 136 ALS patients without dementia and abnormality cut-offs were adjusted for age and education (Lule et al. 2014).

Standards for multimodal neuro-imaging and neuro-physiology approaches

With respect to imaging, we made two important achievements: a) analysis of variability amongst the centres b) recommendation for standards (e.g. how should (routine) MRI be performed in ALS patients

Multi modal neuro-imaging

The establishment of biomarkers for ALS is essential to improve therapeutic trials, which currently rely on survival, or a significant change in the slope of decline of a questionnaire-based disability score, both of which necessitate studies typically 12-18 months in length. In addition, significant heterogeneity in rate of disease progression in ALS has a major confounding effect. Thus, biomarkers sensitive to disease activity, and those that improve patient stratification would be of great value. The diagnosis of ALS is currently clinical, and based on the presence of signs of mixed upper and lower motor neuron degeneration. Electromyography detects lower motor neuron (LMN) demise so biomarkers sensitive to occult upper motor neuron (UMN) degeneration may reduce the current mean diagnostic delay of 1 year from symptom onset, and allow LMN-predominant ALS patients to be considered for therapeutic trials at an earlier stage. The brain is fundamentally part of ALS pathogenesis, though the precise temporal and physiological relationship to downstream LMN degeneration in ALS is not entirely understood. Clinical, histopathological and genetic overlap of ALS with frontotemporal dementia (FTD) is relevant in up to 15% of ALS patients, with subtle cognitive and behavioural deficits affecting at least a third. Histopathology shows loss of Betz cells of the motor cortex, degeneration of the corticospinal tracts and interhemispheric callosal fibres, and widespread loss of interneurons and neuroinflammatory infiltrates, so that relevant portions of the brain carry ALS related changes. Advanced MRI techniques capable of generating biomarkers in ALS have developed through group-based comparisons, not single-subject studies. It is expected that the combination of biomarkers will allow translation at the level of the individual. Meanwhile there is a desire to pool datasets across centres to increase the power to detect pathology which is complex due to a large number of factors that introduce systematic variability between centres. These include magnetic field strength, precise MRI acquisition parameters, scanner manufacturer, and image analysis software. Some MRI sequences have greater problems than others, and the leading ones in ALS are considered below.

T1-weighted volumetry and cortical thickness

Voxel-based morphometry (VBM) involves automated segmentation of the grey matter from a T1-weighted sequence. The regional volume of this segment is then compared voxel-by-voxel across groups of images. In cross-sectional analyses it reveals consistent, though modest volume reductions in primary motor cortex. Longitudinally, the changes appear more extensive, and involve clinically silent areas such as the thalamus. Surface-based morphometry on the other hand allows the measurement of cortical thickness which is potentially more sensitive, applicable to the individual, and has revealed additional changes in the temporal lobes linked to more rapid rates of disease progression. Both techniques have shown more extensive frontal lobe changes in relation to the cognitive deficits in ALS. The T1-weighted isometric whole-brain volume is one of the simplest MRI sequences that can be rapidly acquired, potentially from a routine hospital diagnostic scanner. Early attempts at data-pooling were highly successful with more than 450 datasets pooled

from 15 centers from Europe and North America through the NiSALS Network. VBM associated tools now allow extensive quality control on sample inhomogeneity, noise and near edge resolution which allow to identify datasets which do not meet minimum quality standards, and include software correction routines to compensate for some of the scanner specific distortions.

Diffusion tensor imaging

Diffusion tensor imaging (DTI) allows the detection of large white matter tract degeneration, by exploiting the sensitivity of MRI to the directionality of protons. This has revealed a core signature of involvement in ALS, comprising the corticospinal tracts and interhemispheric fibres of the corpus callosum. As for volumetric analyses, frontal and temporal lobe projection tracts have been shown to be disrupted in relation to cognitive deficits in ALS. DTI requires data acquisition in multiple directions, involving fast gradient shifts, ideally with a higher field strength than many hospital scanners e.g. 3 Tesla. It is very sensitive to subject movement, and the interpretation of white matter tract changes in regions of crossing fibres remains controversial, and several analysis platforms exist. Diffusion-weighted imaging has become more routine in the investigation of acute stroke, but DTI is not a standard sequence available on routine hospital scanners. Within the NiSALS network, a new DTI multicentre pooling approach was developed which is based on single-adjacent slice noise analyses and allows to identify grossly aberrant single slices to be identified, removed and replaced by adjacent averaging if they exceed anatomical and noise limits expected from the underlying DTI model. This approach has allowed the pooling of 250 DTI data sets from ALS patients resembling a smaller ALS clinical trial.

Functional imaging

Blood oxygen level-dependent (BOLD) functional MRI (fMRI) exploits the differences in paramagnetic properties of oxygenated and deoxygenated blood, which can be used as a surrogate marker for regional brain activation. This technique largely superseded activation PET and, in ALS, confirmed a widened area of activation in response to motor and cognitive tasks during the scan. Such tasks are required to be simple enough to duplicate reliably, both within and between participants, and involve sequences not found on routine hospital scanners. It would be very challenging to attempt to match the conditions for even a simple motor task across multiple centres, in addition to the physical challenges of a disease with progressive motor disability. BOLD data can also be acquired in a task-free, so-called resting-state which reports coherence of spontaneous brain activity in discrete networks. Reduced network activity can be identified in the sensorimotor network in ALS, but subsequent studies have demonstrated increased 'functional connectivity' in more disparate regions. The sequence itself would not be challenging to add to a routine hospital scanner, but the analysis of such data is still in evolution.

Meeting the challenges of multi-centre MRI studies

In 2010 the Neuroimaging Society in ALS (NiSALS) was formed with the aim of a forum for international scientists focused on ALS neuroimaging to discuss developments and methodological challenges. Consensus guidelines for 'essential' and 'desirable' criteria for MRI studies in ALS were developed. A secure data repository for anonymised data-sharing was established at the University of Jena. Subsequently, a European Union-based consortium project 'SOPHIA' (Sampling and biomarker OPtimization and Harmonization In ALS and other motor neuron diseases) began the process of eventually developing a quality assurance framework across neuroimaging, neurochemical and electromyographic studies. For neuroimaging biomarkers, a multi-centre study of cerebral DTI was conducted through NiSALS involving 253 patient and 189 control datasets from 8

centres. This confirmed both the feasibility and investigative power of large dataset pooling. Structural grey and white matter changes have now matured to the point where they are contenders as biomarkers to inform therapeutic trials. The experience from multi-centre data-pooling studies is positive for these measures, and it is expected that harmonization around a standard set of sequences will build further on this early success.

Neurophysiology approaches

The MUNIX protocol has been adopted for a European JPND- funded project called SOPHIA (www.neurodegenerationresearch.eu), which aimed to harmonise and standardise various biomarkers across European ALS centres. Collection of longitudinal 3-months data and test-retest-examinations at the first visit and after 6 and 12 months (intra- and inter-rater) were introduced as part of the protocol. Detailed standard operating procedures including photographs of electrode placement were distributed to centres and posted on the ENCALS website (http://www.encals.eu/). Several full-day training courses were offered including lectures and hands on sessions during annual ENCALS meetings and in between at the coordinating centre in St.Gallen, Switzerland. For all evaluators, attending a training course was mandatory.

All evaluators had to demonstrate a coefficient of variation (COV) of less than 20 % (test-retest) for each individual muscle in four healthy individuals before collecting longitudinal data in ALS patients. Raw data were sent to the coordinating centre where all recordings were checked for quality. A feedback was provided for all evaluators by mail and if deemed necessary repeat tests were requested.

For the on-going longitudinal study at each participating centre the same investigator performs all MUNIX studies twice with a minimum break of 30 minutes between each session (intra-rater-reliability). Electrodes have to be completely removed and any traces of electrode placement have to be deleted/avoided. In centres additionally investigating the inter-rater-reliability, a second investigator performs the investigation alternately with the first investigator. Between each session a minimum rest of 30 minutes must be allowed. All investigations are performed on the same day. According to power calculations a minimum of 12 ALS patients per centre is required to allow statistical analysis of centre-specific intra- and inter-rater reliability. Data are centrally collected in a pan-European database for statistical analysis.

A manuscript "Neurophysiological approaches to measure disease progression in ALS: standardisation and harmonisation" authored by Markus Weber and Mamede de Carvalho is ready for submission.

Over the past 3 years at least one neurologist per centre was trained or re-trained. The last workshop took place on 23rd and 24th of May 2015, Dublin, Ireland. During this refresher course 12 experienced and “certified” investigators measured the same subject twice. This is comparable to a “round robin test” for neurophysiological techniques, which has never been applied to any neurophysiological method and underscores the rigorous quality control of European ALS centres. These data will also be shared within SOPHIA and STRENGTH and published in a paper.

Training & certification

NETCALS met in Dublin, Ireland, on 23rd and 24th of May, where participating centres brought along nursing staff for a training- and certification- session on functional outcome measures such as the ECAS, ALS-FRS, muscle strength, respiratory measures and staging. All 2-hourly sessions were given twice, so at least one of person of each participating ALS centre was able to participate in

three to four training sessions. The sessions were prepared (SOPs) and given by one or more experts in the field:

ECAS: Sharon Abrahams (Edinburgh),

ALS-FRS: Ammar Al-Chalabi (London)

Muscle strength: Pat Andres (Boston, NEALS), Japie Bakers (Utrecht) *Respiratory measures:* (Pamela Shaw, Alison Proctor (Sheffield) *Staging:* Marwa Elamin, Alice Vadja (Dublin)

EIM: Seward Rukove (Boston, NEALS)

Munix: Markus Weber (St Gallen)

ECAS has an already developed training and certification procedure. The same procedure was set-up for the other functional outcome measures. For questionnaires as ECAS and ALSFRS first a clear instruction was given on the background of the questions, how to evaluate and rate. Based on patient vignettes (ALS-FRS) or questioning a volunteer (ECAS) participants could practice. Issues on how to act in certain situations were shared and discussed in. Several clarifying notes were taken and will be added to the next instruction version to ALS-FRS.

For muscle strength measures were practiced in real life, where the emphasis was on e.g. importance of exact position of the hand held dynamometer, short but firm forced measurement with clear positioning of evaluator to the patient muscle in order to measure until able to break. Consensus was gained on measuring preferably four muscle groups (arm, wrist, leg and ankle) measured at two sides, importance of chair, consistency in procedure, same rater per patient when repeating measurements over time.

Raters become certified when evaluator has tested 4 normal subjects 2 times with 1 hour to 1 week between testing sessions. If two evaluators are working on a study, inter rater reliability must be established by having the 2 evaluators test the same subject within 1 hour to 1 week of each other with <10% inter- and intra- rater variability

For respiratory measures the most commonly used measures, namely vital capacity (Slow Vital Capacity and Forced Vital Capacity) and Sniff Nasal Inspiratory Pressure (SNIP) were trained and practiced. The measures were demonstrated and trained with the equipment from different suppliers (kindly brought to the workshop from several centres). The pro's and con's per equipment was discussed, as well as lessons-learned based on experience, such as positioning, effect of motivation, use of mouth peace or mouth/nose cap (with bulbar patients). Raters become certified when performed 3 tests on subjects performed twice with <10% inter- and intra- rater variability.

For staging instructions were given, examples were discussed in order to determine thresholds between stages. At the end, the evaluators were requested to stage 10 patients based on case information. The evaluator passed when 8 out of 10 cases were correctly staged.

At least one neurologists per centre was trained or retrained in a parallel session on the execution of neurophysiological outcome measure MUNIX. The training was also used to collect data on the inter and intra-rater variability. These data will be shared within STRENGTH and published in a paper.

All evaluators have to demonstrate a coefficient of variation (COV) of less than 20 % (test-retest) for each individual muscle in four healthy individuals before collecting longitudinal data in ALS patients. Raw data were sent to the coordinating centre where all recordings were checked for quality. A feedback is provided for all evaluators by mail and if deemed necessary repeat tests is requested.

In total 84 research nurses and neuro(physio)logists from 20 ALS centers participated in this first transnational training and certification session. The workshop has been evaluated as useful and successful by the participants.

The standardised documentation with instructions and latest versions of the evaluation tests will be presented on the ENCALS website.

The certification process for ALS research centres is an online process, and is focussed on 'quality assurance'. To become a certified ALS centre, the centre needs a key overall contact/trainer (per outcome measure) with at least one evaluator that is trained and certified for each functional outcome measure. On regular basis a retraining workshops will be organized to have hands-on training and retraining sessions, and to share lessons-learned as input for the expert trainer of outcome measure to optimize its procedure if needed. The certification of raters and evaluators will be administrated centrally in order meet GCP requirements that state proven qualification and retraining at regular intervals (every 2 years). Furthermore, the ALS centre needs to meet criteria on amount of ALS patients followed and newly diagnosed ALS patients per year (>100), availability of calibrated and maintained equipment for respiratory measures, muscle strength and MUNIX, and access to a local reviewing ethical committee (ERB).

This certification process becomes a part of the European ENCALS initiative to create a platform for ALS research centres.

Certified ALS research centres can join in initiatives such as TRICALS, a platform were ALS patients can register for participation in clinical trials. The more certified well-organised centres that are participating and the more ALS patients that are registered on TRICALS, the more attractive Europe becomes for the pharma/biotech industry to start clinical trials for ALS.

Establish plan for dissemination of results to wider ALS and ND community
These deliverables will be of value to the wider ALS/ND research community to overcome barriers to research in population studies. A wide dissemination of results is therefore a key action point for the Working Group.

NETCALS capitalises on existing pan-European infrastructure based on the European Network for the Cure of ALS (ENCALS, www.ENCALS.eu), an EU FP7 funded project EuroMOTOR (systems biology, ~omics data), 4 JPND initiatives (SOPHIA (biomarkers), STRENGTH (risk factors), ALS-CarE and Needs in ALS (both healthcare pathways and decision making)), and community-led projects (e.g. The Neuroimaging Society in ALS (www.NISALS.org), Project MinE (whole-genome sequencing initiative for ALS, www.projectmine.com) and TRICALS (trials)). Therefore NETCALS offers a unique opportunity to disseminate our results into on-going projects involving a wider community of ALS researchers. The results of NETCALS concerning the establishment of pan-European guidelines and a methodological framework for data sharing and data handling, are already being incorporated successfully in many of these projects.

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