NINDS Multiple Sclerosis Common Data Elements (CDE) Recommendations
Biospecimens Subgroup

Summary of MS Biospecimens Subgroup Discussions

Note: These recommendations were reviewed in 2019 and are considered current.

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1) Introduction and Background to Decisions

Two aspects of this topic were reviewed by the Subgroup – biospecimen collection and clinically relevant biomarkers in MS derived from those specimens. Specifically not addressed in terms of biomarkers, was the aspect of imaging biomarkers as this is addressed by the Imaging Subgroup. Indeed, achieving the level of success for soluble/cellular biomarkers that exists currently with imaging biomarkers in MS would be a major accomplishment for the MS community.

The Subgroup decided to review, as an initial step, the biospecimen recommendations from other disease areas that have undergone a similar process with NINDS, beginning with procedural issues related to obtaining biospecimens. Subsequently the subgroup would examine individual biomarkers in part because the biomarker issues are often situation-specific. The biomarker elements may be, in many instances, different for disease-related studies (looking for markers of disease progression) versus therapeutic studies (looking for markers that demonstrate differential responsiveness to specific therapies but which may not necessarily be markers of disease progression). Many however may serve dual purposes. The Subgroup reviewed available information for standardizing the sample collection methodology and was charged to recommend revisions as appropriate. A problem with MS biomarker research is the quality of the laboratory work as many studies/centers do not have the resources to follow Good Laboratory Practice (GLP). Thus, recommendations would by default be targeted towards R01 grantees and would try to adequately address quality control and validation issues.

Review of other disease area efforts revealed that functional biomarkers or biomarkers related to treatment were not part of the output of most groups because the groups had focused on general biospecimen collection information with one exception (Stroke). One approach considered by the MS Subgroup was to provide collection methodology information as well as a list of biomarkers to consider, which are then characterized by level of general acceptance as relevant in the MS field. Others thought the Subgroup's purview was to take more of an approach of what needs to be collected to be part of a useful bio-repository. Given that no biomarker in MS is robust or proven, the Subgroup could simply focus on sample collection. The Subgroup agreed it made sense to first address bio-repository elements and then give careful thought to suggestions for specific markers which may be particularly useful to explore as secondary recommendations.

DNA is important to capture and is of modest burden (although rigorous SOPs must be in place to protect confidentiality). Plasma/ serum, CSF (cell pellet/fluid) and PBMC seem reasonable to collect. The Subgroup was divided on whether these samples must be collected in all studies. The approach taken by most other disease area bio-specimen groups was to provide guidelines (i.e. if you are going to collect a

certain sample, this is how it should be collected). The Subgroup agreed it makes sense to provide standardized protocols for collecting the various samples but would further discuss mandating such collection while in parallel explore what samples to acquire, how best to handle the samples and provide some input on recommended testing.

2) Mandatory vs. Not Mandatory Collection of Biological Samples in all NIH-funded trials in MS

The Subgroup reviewed whether mandatory collection of samples should be recommended in order to foster research in bio-specimens in MS both within specific study protocols but also by making samples available widely to MS researchers. The Subgroup consensus was that bio-specimens (e.g. blood, CSF) should ideally be part of every NIH-funded study and that guidance could be given on how to collect and store samples. However, the view was that due to a lack of definitive soluble biomarkers in MS, it was not appropriate to specify which tests to perform. Which biomarkers to test should remain hypothesis-driven. If the mandatory requirements for obtaining samples deemed as Core elements remained modest, most study sites would be capable of participating.

However, there are many issues surrounding this requirement. If the investigator's study does not include plans for analyzing DNA, how can the NINDS mandate storage of the DNA/cells? Will a central lab (NIH) be needed for storage? How will one build the collection and storage of samples into the protocol? Patient confidentiality, research oversight and sample ownership are other key issues to solve. However, the Subgroup thought that this current initiative was an important opportunity to institute bio-specimen collection in MS studies and felt that practical solutions could be found for all the ethical and logistical issues created by such a policy.

Another key issue regarding mandating sample collection is funding, which is not trivial, particularly given the current economic and research environment. Mandating collection would require provision of funding to investigators who include sample collection in the protocols. The NINDS noted this could make it more difficult to get grant approval as this aspect may in some cases, in the eye of reviewer, rate a lower study score if not properly incorporated with adequate scientific rigor. An alternate method would be provision of supplementary funding to investigators as a means to encourage voluntary biospecimen collection. In fact, there exist already parallel application procedures at NIH for bio-specimen collection as stand-apart from the main grant application which allows for investigators to potentially obtain funds for this purpose, without impacting overall study protocol rating.

The Subgroup raised the idea of mandating collection at the general meeting of all CDE subgroups and while generally favorably perceived, the concept also generated discussion around most of the limitations discussed above. Subsequent to that meeting, the Subgroup received feedback from the NINDS on the concept of mandatory specimen collection. The NINDS agrees it is valuable to collect samples and store in a repository; however, it must weigh the practical considerations of mandating this and would not be supportive of such a recommendation at this time. Rather NINDS preferred that the Subgroup focus its recommendations on standards for collection, shipping and storage of samples. Decisions regarding

NINDS-funded sample collection will continue to be addressed by NIH on a case-by-case basis. The Subgroup agreed in the absence of funds it is not reasonable to mandate this sample collection, while highlighting that this approach may continue to allow the soluble/cellular biomarker field in MS to languish. It was suggested by the Subgroup that if a study did in fact receive NIH funding, any stored samples from that study should be made available to others in the MS community, assuming the request is based on a scientifically valid question, presumably requiring an adjudication/review committee.

The Subgroup then focused further work on identifying which samples are important to collect in MS and how the specific specimens should be collected and stored. Additionally, a list of current investigational biomarkers would be provided as "for information" for interested investigators.

3) What to collect, how to handle and what to analyze

a) *Samples*

There are limited numbers of options of samples to collect including blood, other body fluids (CSF, urine), and tissues (biopsies, autopsy). It was felt that some of these are of greater utility than others and some pose greater logistical challenges. Therefore, the Subgroup ranked various samples on these two aspects to provide some guidance to investigators wishing to include bio-specimen sampling in their study. The grading system, as currently envisaged, was from 1 to 3, with 1 being most relevant on the utility scale and 1 being the easiest to perform/obtain on the feasibility scale. Based on these rankings, biospecimens could be grouped in 3 overall categories; (i) easy to obtain and highly relevant (serum, plasma, whole blood for RNA/DNA, FACS), (ii) harder to obtain but highly relevant (CSF, CSF cells) and (iii) hard to obtain or less relevant (autopsy material, biopsy specimens [CNS, skin, bone marrow etc.], urine). A listing of biospecimen samples that could be acquired in clinical studies is provided in Appendix A with a ranking on utility and feasibility. Clearly appropriate specific handling for samples (e.g. PBMC) will depend on the nature of biomarker studies being planned to determine if samples must be processed immediately, or frozen and shipped etc.

The Subgroup discussed whether coding and data management is within its purview. Coding of this material is very complicated. The TBI and Stroke groups have provided basic high-level guidance on how the data should be documented. To properly approach this key topic would require agreement on core elements to collect and then agreement on a universal coding system, presumably driven by those maintaining a central repository. This larger effort could be subsequently implemented by individuals collecting samples even if not part of a collaborative group to ensure uniformity should such samples later become more generally available. This effort is beyond the scope of the Subgroup.

The Subgroup discussed the importance of collecting associated phenotype data (e.g. demographics, disease measures, MRI data) for each bio-specimen collected. The clinical and para-clinical elements to collect will be aligned with the CDEs proposed by the relevant sub-groups. Demographic data should include age, gender, race while the samples themselves should have recorded key collection information

(e.g. date, time, amount collected, number of aliquots, volume per aliquot, storage location, ID number). The Subgroup feels strongly that without a well-documented clinical/demographic dataset linked to the specimens, the effort will lose much of its potential.

b) *Biomarkers*

The Subgroup discussed providing a list of those biomarkers being considered in MS. There are currently no biomarkers that are considered sufficiently validated in MS to be considered as Core Data Elements. The Subgroup discussed that if they recommend any biomarkers they would likely be classified as exploratory considering that the biomarkers change quite frequently. An article regarding MS biomarkers was used as a template for items to consider in MS studies [Graber JJ, Dhib-Jalbut S. J Neurol Sci 2011, 305:1-10]. As for the situation with 'Samples' in 3a) above, subgroup members ranked the biomarkers in terms of relevance and degree of consensus of the utility of the biomarker for study in MS. Based both on committee member views and those in the field, no biomarker was viewed as being a required Core data element by the subgroup, and thus the ranking of Core would not apply to any biomarker. Members were then asked to rank biomarkers as either Supplemental or Exploratory. Supplementary would require that the results regarding utility of a biomarker be replicated in at least one additional laboratory than that from which first report was generated. From the very extensive list of potential biomarkers, only a handful are even considered as supplemental (i.e. OCB, CSF IgG index, CXCL13, NCAM and neurofilament H & L chains) with the remainder considered exploratory.

A listing of potential biomarkers (also graded by utility in MS) is provided (Appendix B) for interested investigators when considering which biomarkers to study. The list is quite extensive and clearly no set menu of biomarkers will likely ever be "standard" as the questions asked, disease stage studied, and treatments used will all impact which biomarker(s) to select.

Investigators are encouraged to investigate available bio-specimen facilities, both for logistics of obtaining, processing, and storing samples as well as methodological aspects of testing. This information can be obtained at the respective websites of the bio-repositories listed above. Published reviews on this topic related to CSF sample handling have been developed by BioMS*eu* (http://www.bioms.eu/index.php) and are available in published articles (Teunissen CE et al, Neurol 2009, 73:1914-1922; Teunissen CE et al, MS International 2011, doi:10.1155/2011/246412; Tumani H et al, Neurobiology of Disease 2009, 35:117- 127).

Given the status of biomarkers in MS at present, there can be no specific recommendations about which biomarkers to test and the investigators must determine which biomarker in which sample (blood, CSF, tissue) is most appropriate for their study and the specific questions being addressed.

Over time, greater emphasis may be placed on specific biomarkers for disease course/prognosis as well as treatment-response biomarkers and the aim of the Subgroup is to periodically review and update this document, including the listing.

4) Examples of Bio-specimen Repositories

Several examples of existing bio-specimen repositories exist. Among these are the NIH Coriell Institute, Immune Tolerance Network (http://www.itnbioshare.org), BioMS (CombiRx Study), National Database for Autism Research (NDAR; http://ndar.nih.gov/), Alzheimer's Disease Neuroimaging Initiative (ADNI) as well as Kompetenznetz-multiplesklerose (http://www.kompetenznetz-multiplesklerose.de/en) as an MS-specific example in Europe. As is common in many endeavours, the oncology field is often ahead of others and an extensive amount of information on bio-specimens is available via the NCI including a bio-specimen research database (https://brd.nci.nih.gov/BRN/brnHome.seam) and a best practices document (http://biospecimens.cancer.gov/global/pdfs/NCI_Best_Practices_060507.pdf).

Kompetenznetz-MS is supported by the German health authority and aims to foster clinical and research collaborations. Within the network is a bio-banking task force whose goal is to establish a bio-sample databank to facilitate the search for new biomarkers. Access to the data is expected to be broad. Currently efforts include developing guidelines for collecting, storing and analysing bio-samples as well as defining criteria for quality management and data protection. The bio-samples currently collected are mainly blood samples but other tissues (brain tissue, CSF) will also be archived. The task force will define a minimum set of variables to be applied for all bio-samples.

The NINDS has a bio-repository at the Coriell Institute (http://www.coriell.org/). Coriell stores specimens along with clinical phenotype data and disperses the samples upon request for a fee. For individuals or groups wishing to bio-bank DNA, one needs to apply to the institute and, if approved, support is provided to investigators to ease the process.

BioMS is the bio-repository for the NIH funded COMBI Rx trial (2005 start date and continuing) in which organizers have successfully implemented a process for sample collection and storage. The Combi-Rx study documents do not provide details on test methodology but rather focus on processing and shipment details to the central facility, which either processes the samples or ships them to the appropriate laboratory for testing. The information available from BioMS serves as a good template to follow for multi-center groups wishing to establish sampling protocols within their Phase 3 studies. Information is not currently available via a web site but can be accessed via Dr. S. Jacobson, NIH (JacobsonS@ninds.nih.gov).

The Immune Tolerance Network (ITN) has rigorously tested and applied standard operating procedures (SOPs) that are updated annually for the collection and processing of biological samples (http://www.immunetolerance.org/professionals/research/lab-protocols). These provide a valuable resource for clinicians involved in clinical trials for which bio-sampling will occur. They have also done a number of gene expression experiments in MS trials using both whole blood and separated cell subsets from previously frozen PBMCs and have a listing of genes potentially related to disease progression. ITN also performs extensive flow immunophenotyping in their MS studies and is currently conducting T-cell repertoire studies using an ITN core (Adaptive TCR) to do the sequencing.

ITN supported study samples (serum, PBMC, CSF) were initially drawn by sites and processed locally (including T-cell activation) before central shipment for further testing. However, due to issues with yield, delivery and specimen consistency, this has evolved to having sites draw samples and ship, with minimal or no processing at an ITN core facility. For example, to generate PBMCs, whole blood is shipped overnight, at ambient temperature, to Rutgers (Rutgers University Cell and DNA Repository) for isolation of PBMCs, which are then counted, aliquoted and stored in the vapour phase of liquid nitrogen before subsequent batch shipping (in liquid nitrogen dewars) to Fisher Bioservices for longer-term storage. Blood for RNA extraction is drawn directly into a tube that will preserve RNA such as Applied BioSystems Tempus™ tubes, mixed at the sites, frozen at -20ºC or at -80ºC and batch-shipped on dry ice for subsequent processing and storage.

The ITN core laboratories are inspected by accrediting institutions such as the College of American Pathologists (CAP) and adhere to the Clinical Laboratory Improvement Act (CLIA) 1988 quality standards. Monitoring by these accrediting institutions provides additional assurance that assays are performed according to approved standard operating procedures (SOPs) and guidelines and that the results from these laboratories are comparable to those of other laboratories. These Core laboratories are also closely monitored by the Quality Assurance group of the ITN.

The ITN has developed "TrialShare" a clinical trials research web-portal for investigators to gain real-time access to assay data with the clinical and bio-repository information together in simple, easy to use formats that allows data to be sorted, merged, graphed, shared etc. This portal is based on the Labkey application, an extensible framework that allows the ITN software developers and biostatisticians to add new assays, analytical workflows, tools and visualizations as needed over time. The ITN biorepository currently contains over 330,000 specimens, with shipments being received and shipped out on a regular basis.

The ITN repository lends itself to four levels of studies to consider:

Core studies – conducted by all sites in a study.

Pooled studies – subsets of sites, not study-wide, can exchange samples for sub-studies of mutual interest.

Individual studies – single-site, specific studies without involvement of Core or other centers.

Post-facto external studies (ITN BioShare - http://itnbioshare.org/) – other centers/Investigators not involved in a specific ITN study can apply for access to samples via ITN. After scientific review, samples, including phenotypic data, can be provided if deemed appropriate. There is a waiting period on provision of samples until 18 months after last patient/last visit to allow investigators first access at pursuing scientific questions. However, this period can be circumvented if study investigators agree that the proposed work is of scientific merit and not already planned, the ITN mediates these discussions and makes the final call on sample provision.

Informed consent forms (ICFs) are developed for the specific objectives of the various studies. These ICFs are as broad as feasible to encompass future technologies and assays.

The National MS Society (NMSS) supports two biospecimen banks, one that is not MS-specific (www.loni.ucla.edu/uclabrainbank/Research/index.html), although the bulk of samples are MS-related and one that is MS-specific (www.mscenter.org/content/view/153/190/), to which investigators can contribute material and also request material for research purposes. These two banks focus on CNS tissue but also have serum and CSF samples. The NMSS also supports the UCSF MS Genetics Group (http://neurology.ucsf.edu/msdb/index.html) which obtains DNA and relevant information from MS patients and their families as well as sharing samples with researchers.

The above serve as examples of what can be done from the perspective of sample collection and storage and provide information on best practices for bio-specimen work. If SOPs for bio-specimen collection and storage were readily available and agreed upon, it is likely that more investigators would obtain samples and that the pharmaceutical industry would also adopt this approach.

The Subgroup recommends the ITN model to investigators interested in collecting, and analyzing, biospecimen material from MS clinical trials. Further, the ITN is open to assisting investigative groups, including the potential use of ITN resources on a cost-sharing basis.

5) Conclusions

Soluble biomarkers lag considerably behind imaging biomarkers in MS. At present no biomarkers achieve status as Core Date Element and very few even achieve the level of Supplemental, but rather most are simply exploratory. To advance the field, a concerted international effort around this topic is required. However, this requires commitment by many individuals and major financing to be done properly.

Based on discussions with existing groups, although logistic hurdles can be considerable, multi-center sampling, processing, and storage, has been shown to be feasible, but potentially quite costly (e.g. ITN $400,000 per annum for tracking system, storage facility, freezers, generators, and monitoring equipment plus the original infrastructure costs). Such costs exclude sample analysis.

Given the current lack of central funding support for such efforts, and given the existing experience and facilities of groups such as ITN, investigators considering bio-specimen research should consider leveraging this expertise, including partnering with an existing bio-bank facility when establishing biospecimen activities within study protocols.

Large scale projects could be very expensive. However, if the sample size is kept modest, such as MS Phase 2 studies (~ a log scale smaller than the Phase 3 programs), sampling may be done with a reasonable budget. Output from the CombiRx study will provide useful insights on the larger scale study experience.

6) Recommendations:

a) Urge NIH/NINDS to consider mandating biospecimen collection while at the same time providing financial/logistical support to establish protocols/platforms/facilities for biospecimen sample obtention, retention and analysis within MS studies, including dissemination of this information to researchers.

b) Request NIH/NINDS to establish a working group / workshop of relevant stakeholders to set the ground-rules for such an effort, such as the one established by BioMS*eu*

c) Publicize to MS investigators the importance of considering relevant questions to pose in clinical trials that would require and use biospecimen data to enhance understanding of underlying disease course and prognosis as well as treatment-response biomarkers.

d) In the absence of universal guidance, investigators should adhere to already established collection, storage and analytic protocols (such as ITN, BioMS*eu,* BioMS/CombiRx) in an attempt to harmonize data collection while assuring quality control thus potentially allowing for pooling of data and cross-study comparisons

e) As a minimalist first step, encourage all those responsible for MS clinical trials to include collection of DNA samples, with broad consent (including use of samples in future by appropriate 3rd parties) to allow the conduct of important disease-related research in future, even if not as part of the specific study. Linking sufficient biographic, demographic and disease characteristics data with the samples is essential. Institutes such as Coriell and potentially ITN (both cited above), could facilitate storage of samples.

f) No recommendations can be given regarding specific soluble/cellular biomarker testing to conduct given that no biomarker (MR imaging excepted) for disease course or treatment response is currently validated.

g) The initial focus of biomarker work may best be directed to Phase 1 and Phase 2 studies as this may be more feasible, allow more in-depth testing and lead to initial progress that could facilitate broader progress in the field subsequently. Ultimately however, validation will require assessment in large-scale, likely pooled, data sets.

Appendix A – Biospecimen Samples

Utility Rating 1=greatest utility and 3=least utility	
Specimen Type	Average
Serum	1.0
Plasma	1.2
PBMC	1.2
Whole Blood	1.2
CSF*	1.2
CSF cells*	1.5
Whole Blood (ABI Tempus tubes - RNA)	1.5
Tissue - Autopsy	1.7
FACS	2.0
Biopsy - Skin	2.2
Biopsy - CNS	2.2
Urine	2.3
Biopsy - BM	2.3
Biopsy - other (specify)	n.r.
Other (Specify)	n.r.
* Teunissen et al, Neurol 2009, 73:1914-1922	
n.r. Not rated	

Feasibility Rating 1=most feasible and 3=least feasible	
Specimen Type	Average
Serum	1.0
Plasma	1.2
Whole Blood	1.2
PBMC	1.3
Whole Blood (ABI Tempus tubes - RNA)	1.3
Urine	1.8
CSF*	2.0
CSF cells*	2.3
Tissue - Autopsy	2.3
FACS	2.4
Biopsy - Skin	2.8
Biopsy - BM	2.8
Biopsy - CNS	3.0
Biopsy - other (specify)	n.r.
Other (Specify)	n.r.
* Teunissen et al, Neurol 2009, 73:1914-1922	
n.r. Not rated	

Appendix B – Potential MS Biomarkers

Biomarker*	Source	Ranking Core, *Supplemental, or Exploratory*
Potential serum biomarkers of disease activity in MS		
TNF-α	Serum	Exploratory
IL-10	Serum	Exploratory
IL-12p40	Serum and CSF	Exploratory
IL-17	Serum	Exploratory
IFN-γ	Serum	Exploratory
Osteopontin	CSF	Exploratory
IL-6	CSF	Exploratory
Cell surface biomarkers		
K2P5.1 + T cells	PBMC	Exploratory
IFN-γ Receptor-β	PBMC	Exploratory
IFN-α Receptor-2	PBMC	Exploratory
CD56bright NK cells	PBMC	Exploratory
CD8 + CD25 + FoxP3 + Treg cells	PBMC	Exploratory
Fas/FasL	PBMC	Exploratory
CD80	B cells	Exploratory
CD86	Monocytes	Exploratory
CD40	Monocytes	Exploratory
PD1/PDL1	T-cells/CD19+ B cells	Exploratory
PDL2	PBMC	Exploratory
Survivin	PHA/IL-2 stimulated T-cells	Exploratory
Potential humoral and antibody biomarkers		
OCB	CSF	Supplemental
Aquaporin-4 (NMO) antibody	Serum	Supplemental
IFN-β Neutralizing antibodies	Serum	Supplemental
CD19 + CD138 + B cells	Serum and CSF	Exploratory
MOG/MBP antibodies	Serum	Exploratory
CD46/59 antibodies	Serum	Exploratory
Complement factor H	Serum	Exploratory
C4 fragment	Serum	Exploratory
EBNA IgG	Serum	Exploratory
Ig kappa light chains	CSF	Exploratory

Biomarker*	Source	Ranking Core, *Supplemental, or Exploratory*
Biomarkers related to adhesion and migration		
sVCAM	Serum and CSF	Exploratory
LFA1	Serum and CSF	Exploratory
VLA4	Serum and CSF	Exploratory
MMP9	Serum and CSF	Exploratory
TIMP1	Serum and CSF	Exploratory
MMP-8	Serum and CSF	Exploratory
IL-8	Serum and CSF	Exploratory
IP-10	Serum and CSF	Exploratory
CXCL8	Serum and CSF	Exploratory
CCL2	Serum and CSF	Exploratory
CCL5	Serum and CSF	Exploratory
CXCR3	Serum and CSF	Exploratory
CXCL13	Serum and CSF	Supplemental
CCR5	Serum and CSF	Exploratory
CX3CR1	Serum and CSF	Exploratory
ICAM	CSF	Exploratory
CXCL12	CSF	Exploratory
NCAM	CSF	Supplemental
Biomarkers of tissue damage and repair		
Neurofilament chains	CSF	Supplemental
GFAP	CSF	Exploratory
S100	CSF	Exploratory
NAA	CSF/MRS	Exploratory
Nitric oxide products	CSF	Exploratory
Pentosidine	Serum	Exploratory
BDNF	CSF	Exploratory
CNTF	CSF	Exploratory
GDNF	CSF	Exploratory
NGF	CSF	Exploratory
NT3	CSF	Exploratory
NT4	CSF	Exploratory
Other potential biomarkers		
Myxovirus resistance protein A	CSF	Exploratory
Myoinositol	CSF/MRS	Exploratory
Bri2-23	CSF	Exploratory
Fetuin-A	CSF	Exploratory

Biomarker*	Source	Ranking Core, *Supplemental, or Exploratory*
ILT3	PBMC	Exploratory

* Graber J, Dhib-Jalbut S. J Neurol Sci 2011, 305:1-10