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Cerebrospinal fluid-biomarkers-based diagnostic and prognostic models for multiple sclerosis

Multi-center study:

- Washington University in St. Louis (WUSTL): Dr. Anne H. Cross, Dr. Laura Piccio, Dr. Salim Chain, Dr. Laura Ghezzi
- NIAID/NDS: Dr. Bibiana Bielekova, Dr. Peter Kosa
- University of Colorado: Dr. Enrique Alvarez
- University of Ottawa: Dr. Mark Freedman
- University of Montana: Dr. Mark Greenwood

Supported by:

The National Multiple Sclerosis Society (NMSS-RG-1907-34348**)**

Version 3
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SYNOPSIS

Study title

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Summary and Objectives

Using machine learning, we have developed from SOMAScan:

1. A molecular diagnostic test that differentiates MS from other inflammatory and non-inflammatory central nervous system (CNS) diseases (area under receiver-operator characteristic curve-AUROC of 0.98);
2. A molecular test that differentiates relapsing-remitting MS from progressive MS variants (AUROC of 0.91); and
3. A molecular test that predicts future rates of disability progression, concordance coefficient of 0.425 ($p<0.001$).

Because these results are derived from a single research center (NIAID/NDS), it is imperative to determine their performance in real clinical practice settings as a necessary step for their potential regulatory approval. Consequently, this application has 2 specific aims:

AIM 1. To independently validate afore-mentioned CSF-biomarker-based tests for their clinical value within the multicenter Spinal fluid Consortium for MS (SPINCOMS). In Aim 1, each of the 3 defined tests will be validated in 100 new SPINCOMS patients. To validate the prognostic test, 100 MS patients with CSF collected at least 3 years from the date of a follow-up study visit will be evaluated at follow-up examination with standardized clinical outcomes. CSF will be analyzed blinded using pre-defined statistical models.

AIM 2. To explore whether collected CSF-biomarkers point towards pathogenic heterogeneity that may predict patient-specific efficacy for different disease-modifying treatments (DMTs) or identify pathogenic mechanisms not targeted by current DMTs. In Aim 2, clustering analysis will assess pathogenic heterogeneity and explore potential predictors of response to therapy.

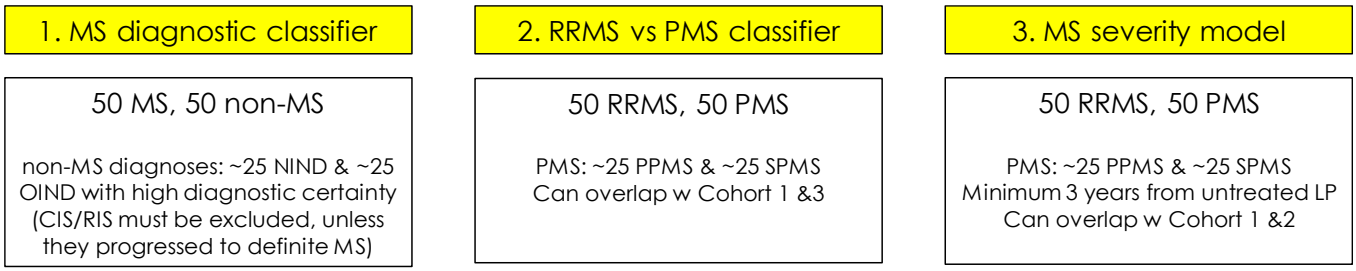
1. STUDY DESIGN

Cross-sectional cohort studies (Figure 1.).

1. For the MS diagnostic test, we will select 50 patients with definite MS (CIS/RIS excluded) and 50 controls, including 25 non-inflammatory neurological diseases (NIND) that mimic MS (such as patients with vascular-ischemic changes, complex migraines, leukodystrophies, etc.) and 25 other inflammatory neurological diseases (OIND) such as NMO, Susac's syndrome, sarcoidosis, CNS vasculitis.
2. For validating the RRMS versus progressive MS classifier, we will select CSF from 50 RRMS and 50 progressive MS patients (25 SPMS and 25 PPMS).

3. Cryopreserved CSF samples from these patients will be analyzed blindly using SOMAscan assay. Probability of MS diagnosis and progressive MS diagnosis will be calculated based on published models[2]. Results will then be unblinded and analyzed for accuracy of diagnostic conclusion.
4. To evaluate the MS severity score, we will select 50 RRMS and 50 progressive MS (25 PPMS and 25 SPMS).

Figure 1.



The cohorts of MS patients in 1-3 can overlap.
Select n=100-130 eligible MS patients
Required: ≥ 50 RRMS, ≥ 25 PPMS, ≥ 25 SPMS

2. SELECTION AND ENROLLMENT OF SUBJECTS

2.1 Eligibility criteria

MS Patients selection criteria (n=100; Table 1)

- Lumbar puncture (LP) in the untreated stage with cryopreserved CSF (serum/blood optional) with consent to use it for future research
- ≥ 3 years of follow-up from LP (with respect to the date of the scheduled follow up study visit)
- At time of LP untreated and not treated with steroid or off steroids ≥ one month
- Available/willing to come for in-person follow-up
- Available/willing to sign the NIH 09-I-0032 "Sample processing only" consent form
- Diagnosis of MS based on 2017 McDonald criteria at time of follow-up visit

Non-MS Patients selection criteria (n=50)
Required: 25 NIND, 25 OIND

- Lumbar puncture (LP) in the untreated stage with cryopreserved CSF (serum/blood optional) with consent to use it for future research
- ≥ 3 years of follow-up from LP
- At time of LP untreated and not treated with steroid or off steroids ≥ one month
- Up to date contact information
- Available/willing to sign the NIH 09-I-0032 "Sample processing only" consent form
- Diagnosis:
  NIND: e.g., ischemic-gliotic changes, CADASIL and other leukodystrophies, migraines, ischemic spinal cord lesions etc

OIND: e.g. CNS Sjogren's, SLE, vasculitis, CNS infections, MOG-associated disorders, NMO spectrum disorders (NMOSD)

Data and procedure at follow-up visit for MS patients:

- Signed consent for the present study and procedures.
    - Will include acknowledgment of NIH sample processing consent.
- Collection of a blood sample: one serum tube, four sodium heparin tubes and two potassium EDTA tubes [or one PAXGene tube] (see Figure 2 at the end of the protocol).
- Vital signs, weight, height and BMI.
- Complete neurological examination documented in NeurEx (recorded with an iPAD).
- Complete clinical data questionnaire with the patient.
- 25FW & non-dominant hand 9HPT (required for calculating CombiWISE & MS-DSS).
- Smartphone Apps (include 25FW, SDMT and tests that correlate highly w 9HPT – can be acquired in patient-autonomous manner with minimal assistance).
- Optical Coherence Tomography (OCT).
- Propose optional LP to be done at the NIH.

Data and procedure at phone interview visit for MS patients:

- Phone interview consent for the present study and procedures.
    - Will include acknowledgment of NIH sample processing consent.
- Complete clinical data questionnaire with the patient.
- Propose optional LP to be done at the NIH.

Additional data to be collected after consent is obtained:

MS Patient data collected after follow-up or phone interview visit

Access electronic medical record (EMR) to collect/confirm the following information:

- Transcribing documented neurological examination completed within 1 month of the time of LP to EDSS or NeurEx.
- Detailed DMT history (with estimated date of initiation and termination of all drugs).
- Documentation of proximity of clinical relapse or contrast enhancing lesions (CELs) to the initial LP.
- Estimation of subsequent relapse rate; conversion to clinically-definite MS (for original RIS/CIS) and possibly SPMS (for original RRMS).
- Imaging Data
    - If available, Brain MRI ± 3 month of the **LP date:**
        - If images available transfer de-identified MRI images
        - If images are not available collect the following information:
            1) CEL if MRI done within a month from LP
            2) T2 lesion load (for MRI done within 3 months from LP)
            3) T1 black holes
    - If available, spinal cord MRI ± 3 months of LP date:
        - If images available transfer de-identified MRI images
        - If images are not available collect the following information:
            1) CEL if MRI done within a month from LP
            2) T2 lesion load (for MRI done within 3 months from LP)
    - If available, Brain MRI (within 1 year of follow-up visit):

- If images available transfer de-identified MRI images
- If images are not available collect the following information:
  1) CEL if MRI done within a month from the follow-up visit
  2) T2 lesion load
  3) T1 black holes

- Spinal cord MRI around the time of follow-up visit:
  - If images available transfer de-identified MRI images
  - If images are not available collect the following information:
    1) CEL if MRI done within a month from follow-up visit
    2) T2 lesion load

- Available information for calculating BREMSO score (i.e., symptom onset: sphincter, motor, sensory; number of neurological functional systems involved at onset; incomplete recovery at onset).
- If available, 25FW/9HPT around the time of initial LP.

<u>MS Patient data collected under consent waiver</u>

Access electronic medical record (EMR) to collect/confirm the following information:

- Transcribing documented neurological examination completed within 1 month of the time of LP to EDSS or NeurEx.
- Transcribing documented most recently completed neurological examination available in medical record to EDSS or NeurEx.
- Detailed DMT history (with estimated date of initiation and termination of all drugs).
- Documentation of proximity of clinical relapse or contrast enhancing lesions (CELs) to the initial LP.
- Estimation of subsequent relapse rate; conversion to clinically-definite MS (for original RIS/CIS) and possibly SPMS (for original RRMS).
- Available information for calculating BREMSO score (i.e., symptom onset: sphincter, motor, sensory; number of neurological functional systems involved at onset; incomplete recovery at onset).
- If available, 25FW/9HPT around the time of initial LP.

<u>Non-MS Patient data collected after follow-up visit:</u>

- Access electronic medical record (EMR) to confirm the clinical diagnosis.
- Contact the patient on the phone to confirm their availability to be contacted by the NIH to sign their consent.

2.3 Targeted patient number for each SPINCOMS center.

Table 1.

	MS patients All require in-person follow-up visit	**Non-MS patients** Do not require in-person follow-up but require data collection
Wash. U	60 (30 RRMS, 30PMS)	30 (~15 NIND, ~15 OIND)
U. Ottawa	20 (10 RRMS, 10PMS)	10 (~5 NIND, ~5 OIND)
U. Colorado	20 (10 RRMS, 10PMS)	10 (~5 NIND, ~5 OIND)
NIAID/NDS	None budgeted, but will contribute all available that are non-overlapping and unpublished	None budgeted, but will contribute all available that are non-overlapping and unpublished

*University of Montana will not participate in patient recruitment, data analysis only

2.4 Study identification numbers and data entry for enrolled subjects across centers

Each patient will be assigned sequential alphanumeric code and generation of all CSF biomarker data will be performed under this code, by laboratory personnel having no access to any clinical or imaging data.

Study identifiers for the clinical data will be assigned by REDCap as follows: assigned 3-digit Site ID (###) then 3-digit patient ID (001, 002, etc.).

3. SUMMARY OF CLINICAL AND LABORATORY EVALUATIONS

Table 2. List of procedures

Assessment	Follow up visit	Phone Interview	Under Waiver of Consent	Data from time of LP (≥ 3 before follow up)
Informed Consent	X	X		
Check availability to be contacted by NIH and to sign NIAID/NDS consent for "Sample processing only"	X	X		
Vital signs; weight/height and BMI	X	*	*	*
Complete the patient questionnaire	X	X		
Access EMR to obtain complete clinical data (disease history, personal and familial history of autoimmune diseases, detailed DMT history)	X	X	X	
Access EMR to obtain imaging data (MRI images obtained around the time of LP and from the most recent MRI will be used)	X			X
CSF				X (cryopreserved and available)
Check availability to repeat LP at NIH	X			
Blood	X			*
NeuroEx	X			*
25FW/9HPT	X			*
SDMT	X			*
OCT	X			*
Smartphone tests	X			

X=mandatory for the study
* = collected if data available

3.1 Special Instructions and Definitions of Evaluations

Informed Consent
The potential participant and one of the study team members will review the consent together. The potential participant will be given time to read through the consent, ask questions and consult with relatives. The PI or the study coordinator will be responsible for the consent process. They will explain the study consent form in detail, and they will be available for any questions potential study participants may have.

To participate in this study, patients will also need to sign a separate consent for "sample processing only" by NDS/NIAID that includes genotyping of common SNPs using microarrays – this is NOT whole exome or whole genome sequencing. This will be a separate consent, supplied by NIH to research patients for review and once signed, returned directly to NIH through mail using FedEx.

Disease history and treatment history
Information will be obtained from the patient's clinical medical record and from the patient questionnaire. All information related to this study will be kept in an individual study binder for each patient. Enrollment in the study (as well as a possible premature end of the study visit) will be recorded in their clinical charts.

Clinical and Laboratory Assessments

Vital Signs: weight/height (BMI), blood pressure and heart rate

Blood sample collection: 7 tubes of blood (approximately 2.5 tablespoons; Figure 2).

Neurological examination/NeuroEx: NeuroEx is a standardized and digitalized neurological examination that automatically calculates most common clinician-generated MS (and related diseases) disability scales. The NeurEx App is developed on FileMaker Pro platform and available in clinics on mobile platforms such as iPad (Kosa et al., 2018).

Cognitive Testing: standardized testing to thinking, memory, hand/arm coordination, and walking ability (Timed 25-FT Walk, 9-Hole Peg Test & Symbol Digit Modality Test (SDMT).

Optical Coherence Tomography (OCT): non-invasive photographing of the retina that will measure the nerve layer and thickness.

Smartphone tests: smartphone apps, employing smart-phone embedded sensors, have been developed by NIH to assess disease-related disability in a patient-autonomous manner. A series of 11 cognitive and motor tests have been implemented to obtain outcomes that better correlate with clinical-assessed disability. The tests will be taken autonomously by the patients in about 30 minutes (Boukhvalova et al., 2019; Boukhvalova et al., 2018).

Laboratory Studies
At follow-up visit we will collect blood that will be used for research purposes.

These are the procedures that are planned:

- Serum collection: serum will be aliquoted and stored at -80°C
- Peripheral blood mononuclear cell isolation to freeze down aliquots in freezing media ($5x10^6$ cells/aliquot) to be stored in liquid nitrogen for future analyses.
- K2 EDTA purple top tube **or** PAXgene Blood DNA tube for collection of genomic DNA

4. DATA COLLECTION

Records to Be Kept

Indicate what information will be retained for each subject and by whom. Describe methods for maintaining confidentiality of subject records.

Paper/hard copy records (hard copy surveys, questionnaires, case report forms, pictures, etc.). Patient data will be maintained in individual study binders. All binders will be maintained in a locked file cabinet in the locked office of the coordinator in a locked suite.

Electronic records (computer files, electronic databases, etc.) - The data will be compiled into a password protected Excel file, where the patients will only be identified by their coded study ID. This file will be stripped of identifiers prior to sending to the statistician for analysis. The master list will be kept in separate password protected file that only the study coordinator and PI will have access to. This list will be destroyed at final analysis. The data will be housed on secure server. Electronic data will also be entered into REDCAP database. This database is password protected.

5. HUMAN SUBJECTS

Institutional Review Board (IRB) Review and Informed Consent

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the IRB or ethics committee responsible for oversight of the study at each site. A signed consent form will be obtained from the subject. For subjects who cannot consent for themselves, such as those below the legal age, a parent, legal guardian, or person with power of attorney, must sign the consent form; additionally, the subject's assent must also be obtained if he or she is able to understand the nature, significance, and risks associated with the study. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject, parent, or legal guardian, and this fact will be documented in the subject's record.

Subject Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified only by the Study Identification Number (SID) to maintain subject confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will

be done using SIDs only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB, the FDA, the NIAID, the OHRP, the sponsor, or the sponsor's designee.

Study Modification/Discontinuation

The study may be modified or discontinued at any time by the IRB, the NIAID the sponsor, the OHRP, the FDA, or other government agencies as part of their duties to ensure that research subjects are protected.

PUBLICATION OF RESEARCH FINDINGS

Results of this trial will be made available to the public.

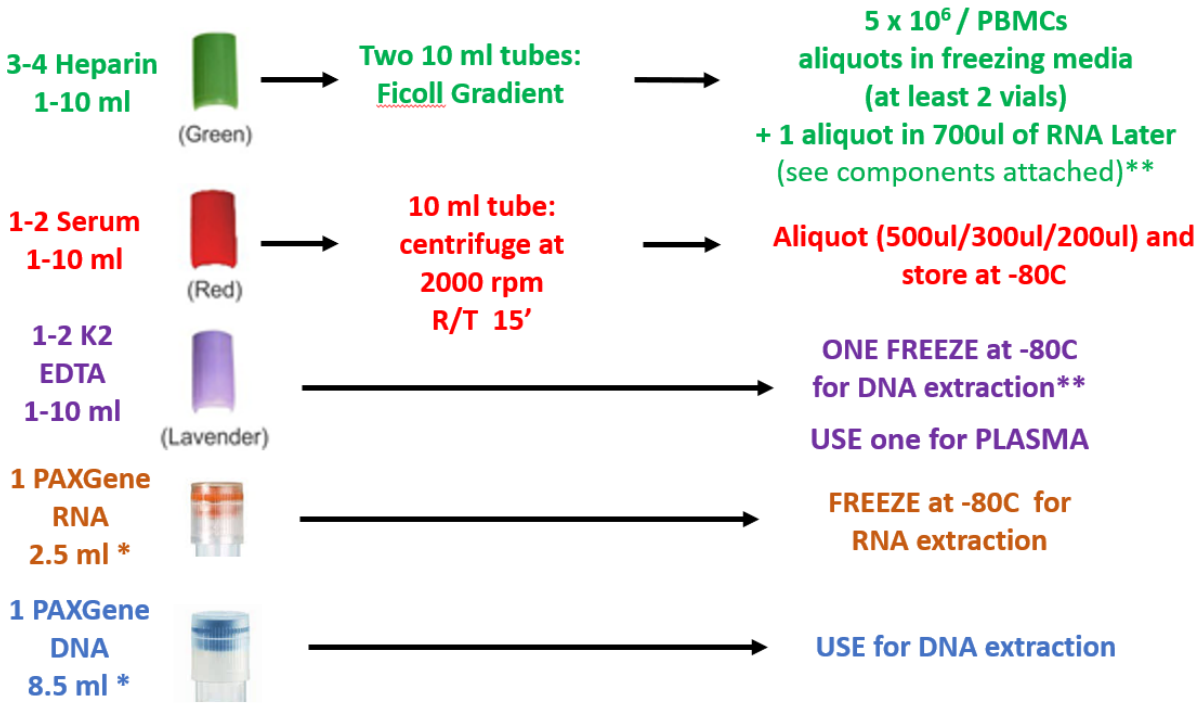
REFERENCES

Kosa P, Barbour C, Wichman A, Sandford M, Greenwood M, Bielekova B. NeurEx: digitalized neurological examination offers a novel high-resolution disability scale. Annals of clinical and translational neurology 2018

Boukhvalova AK, Kowalczyk E, Harris T, Kosa P, Wichman A, Sandford MA, Memon A, Bielekova B. dentifying and Quantifying Neurological Disability via Smartphone. Front Neurol. 2018 Sep 4;9:740. doi: 10.3389/fneur.2018.00740.

Boukhvalova AK, Fan O, Weideman AM, Harris T, Kowalczyk E, Pham L, Kosa P, Bielekova B. Smartphone Level Test Measures Disability in Several Neurological Domains for Patients With Multiple Sclerosis. Front Neurol. 2019 May 28;10:358. doi: 10.3389/fneur.2019.00358

Figure 2. Blood collection tubes.



* Only in Centers using PAXGene Blood tubes for RNA and DNA extraction
** Only in Centers not using PAXGene Blood tubes for RNA and DNA extraction

PROCEDURE FOR BLOOD PROCESSING

FREEZING MEDIA: 75% FBS, 15% RPMI, 10% DMSO
Isolation of PBMCs from whole blood: CONFIRM that the consent for SPINCOM study was collected!!

1. Pour the blood in a 50ml falcon, dilute the sample with an equal volume of PBS. Mix gently.
2. In another 50 ml Falcon add 12-15 ml of Ficoll, add the blood on top of it, really slowly, in order to form two layers.
3. Centrifuge at 3000 RPM for 30 minutes at room temperature, with the brake off.
4. Draw up the ring with a Pasteur and transfer it in a new 50ml falcon.
5. Wash with PBS (full falcon) for two times, centrifuge at 1750 RPM, for 5 minutes at 4°C and decant the supernatant. Resuspend the pellet.
6. Add 10 to 20 ml of PBS and resuspend well the pellet in it. Count the cells:

- If < $15x10^6$ cells Freeze at least 2 aliquots of PBMCs
- If at least $15x10^6$ cells -> take $5x10^6$ cells and transfer in a 15ml tube – for Trizol
- If > $15x10^6$ cells proceed to do more aliquots of frozen PBMCs

7. Wash one more time with PBS, centrifuge at 1200 RPM, for 5 minutes at 4°C and decant the supernatant. Resuspend the pellet. (In the 15 ml tube add 700ul of Trizol. Transfer in a 1.5 ml Eppendorf and store in a -80°C freezer).
8. To freeze the PBMCs add freezing media to the pellet (per cryovial: $5x10^6$ cells in 1ml).
9. Place the vials in the Cryofreezing container and place it in a -80°C freezer.

➢ On the Eppendorf and cryovials write: patient ID, PBMC numbers, date.