AWARD NUMBER: W81XWH-18-1-0648

TITLE: Study of immune-based biomarkers using the longitudinal CLIMB dataset

PRINCIPAL INVESTIGATOR: Tanuja Chitnis

CONTRACTING ORGANIZATION: Brigham and Women's Hospital
BOSTON MA 02115

REPORT DATE: Sept 2019

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
Sept 2019	Annual	1 Sep 2018 - 31 Aug 2019

4. TITLE AND SUBTITLE
Study of immune-based biomarkers using the longitudinal CLIMB dataset

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-18-1-0648

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Tanuja Chitnis

E-Mail: tchitnis@rics.bwh.harvard.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Brigham and Women's Hospital,
Inc., The
75 FRANCIS ST
BOSTON MA 02115-6110

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

During this period, we have evaluated proteomic biomarkers and their associations with relapses and/or new gadolinium enhancing lesions (GD+) and 10-year outcomes in MS patients. We have specifically identified that serum neurofilament light chain (sNFL) is elevated within a 3-month window of new GD+ MRI lesions, which informs the frequency at which this biomarker may be surveyed in the clinical setting. We have also found that at a relapse or GD+ lesion that sNFL decreases with increasing age, with the opposite in remission samples. We have screened over 1100 other proteins and have identified a multivariate proteomic signature that more strongly associates with GD+ lesions than serum NFL alone. We have found that serum NFL levels within the first 3-5 years after disease onset strongly correlate with 10-year brain atrophy and deep grey matter volumes including thalamic volumes. These findings can inform the uses of sNFL and a multivariate proteomic biomarkers in clinical MS settings.

15. SUBJECT TERMS
multiple sclerosis, longitudinal, predictive, biomarker, proteomic, metabolomic, lipid, neurofilament, relapse, MRI, disability, treatment, microRNA, antibody, biosamples, responder, inflammation, innate, adaptive immunity, neurodegeneration

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	30	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

1. **INTRODUCTION:** The goal of this study is to evaluate proteomic, metabolomic and lipidomic biomarkers in the prediction of disease course in multiple sclerosis patients using data and biosamples from the Comprehensive Longitudinal Investigations in MS at the Brigham (CLIMB) Study. We have selected promising biomarkers identified from preliminary studies representing adaptive immunity, innate immunity and neurodegeneration. These will be evaluated in well characterized cohorts identified from the CLIMB study. Our intent is then to develop multivariable predictive models of disease course including relapses, disability and radiological outcomes.

2. **KEYWORDS:** multiple sclerosis, longitudinal, predictive, biomarker, proteomic, metabolomic, lipid, neurofilament, relapse, MRI, disability, treatment, microRNA, antibody, biosamples, responder, inflammation, innate, adaptive immunity, neurodegeneration.

3. **ACCOMPLISHMENTS:**
   - **What were the major goals of the project?**

   Aim 1: Development of biomarkers for MS staging
   Aim 2: Development of predictive algorithms for short and long-term outcomes
   Aim 3: Development of composite panels and predictive models

   There were no significant changes to this project.
   The specific Aims during the first 12 months, along with their completion dates are listed below:

Specific Aim 1 (specified in proposal)	Timeline (months)	Site 1 – responsible key personnel	Date completed
Aim 1: Development of biomarkers for MS staging	12		
a) Local IRB/IACUC Approval (all aims)	0-1	Dr. Chitnis	October 2018
b) Preparation of datasets for nested cohorts	1-6	Dr. Chitnis	January 2019
c) MRI validation – T1Gd+ lesions, T2 lesion accrual	3-9	Dr. Bakshi	May 2019
d) Biomarker panel conduct	6-12	Dr. Chitnis + Dr. Weiner	March 2019
e) Statistical analysis	12-15	Dr. Healy + Patsopoulos	March-September 2019
f) Milestone(s) Achieved – reporting final results Aim 1	15	Dr. Chitnis	See abstracts and manuscripts below

   - **What was accomplished under these goals?**

   1) Major activities: The major activities during the first 12 months of this project are as follows,
   a) Local IRB approval – completed
   b) Preparation of datasets – completed
   c) MRI validation – T1Gd+ lesions, T2 lesion accrual – completed
   d) Biomarker panel conduct – initiated and will be completed by month 26 of project
   e) Statistical analysis – conducted for abstracts/manuscripts below

   2) Specific objectives: Our specific objectives for the first 12 months are listed below. We have completed these objectives including IRB approval and preparation of datasets. We have completed MRI validation in nested cohorts for this study. We have initiated conduct of biomarker panels including studies of serum neurofilament light chain and inflammation-associated proteomics.

3) **Significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative):** We have completed the following studies which have been submitted for publication and/or major congresses:

A. Neurofilament light chain serum levels correlate with 10-year MRI outcomes in multiple sclerosis. Chitnis T, Gonzalez C, Healy BC, Saxena S, Rosso M, Barro C, Michalak Z, Paul A, Kivisakk P, Diaz-Cruz C, Sattarnezhad N, Pierre IV, Glanz BI, Tomic D, Kropshofer H, Häring D, Leppert D, Kappos L, Bakshi R, Weiner HL, Kuhle J. Ann Clin Transl Neurol. 2018 Oct 16;5(12):1478-1491. doi: 10.1002/acn3.638. eCollection 2018 Dec. PMID: 30564615; PMCID: PMC6292183.
   - This work was also presented at the 2019 ACTRIMS meeting in Dallas, TX.
   - Major findings: This study found in 125 MS patients with annual serum sampling that averaged serum NFL levels drawn during the first 3-5 years of MS disease onset correlated with brain atrophy at 10 years.

B. Temporal association of sNfL and gad-enhancing lesions in multiple sclerosis. Mattia Rosso, MD; Cindy T. Gonzalez, MPH; Brian C. Healy, Ph. D Shrishti Saxena, MS Anu Paul, Ph. D Kjetil Bjornvik, MD; Jens Kuhle, MD, Ph. D; Pascal Benkert, Ph. D; David Leppert, MD, Charles Guttmann, MD, Rohit Bakshi, MD, MA, Howard L. Weiner, MD Tanuja Chitnis, MD.
   - This work was submitted for publication and was also presented at the 2019 ECTRIMS Congress in Stockholm, Sweden (platform presentation: Serum neurofilament light chain levels are increased within three months of new gadolinium enhancing lesions in multiple sclerosis)
   - Major findings: This study of 94 MS patients found that sNFL was elevated 90 days pre and post-gadolinium enhancing lesions; which may inform its use in clinical practice. sNFL was note significantly elevated at a relapse without Gd+ lesions. We also found that sNFL was elevated in spinal cord lesions and brainstem lesions compared to other lesion locations.

C. Differential association of Age and Serum neurofilament light chain in remission and after Gd+ lesions in MS. Mattia Rosso; Brian C. Healy; Shrishti Saxena; Anu Paul; Kjetil Bjornevik; Jens Kuhle; Pascal Benkert; David Leppert, Charles Guttmann, Rohit Bakshi, Howard L. Weiner; Tanuja Chitnis.
   - This work was submitted to a journal for publication and for consideration as an abstract and/or presentation at a 2020 academic meeting (submitted September 2019).
   - Major findings: sNFL levels during remission (non-relapse, non-Gad) sample showed an increasing association with patient age) adjusted estimate=1.2% yearly increase, 95% CI=0.3%-2.0%, p=0.008). However, sNFL levels taken within 90 days prior to a Gd+ lesion showed no age-associated increase. Further, we report a negative interaction between age and gadolinium-enhancing lesion status (adjusted estimate=1.7% annual decrease, 95% CI= -2.9% – -0.6%, p=0.003).

D. Serum NFL levels in first five years correlate with 10-year Deep Gray Matter volumes in multiple sclerosis. Hrishikesh Lokhande, Shahamat Tauhid, Renxin Chu, Cindy Gonzalez, Brian C. Healy, Shrishti Saxena, Mattia Rosso, Christian Barro, Zuzanna Michalak, Anu Paul, Neda Sattarnezhad, Bonnie I. Glanz, David Leppert, Ludwig Kappos, Jens Kuhle, Howard L. Weiner, Rohit Bakshi, Tanuja Chitnis.
   - This work was submitted for consideration as an abstract and/or presentation at a 2020 academic meeting (submitted September 2019).
   - Major findings: A negative association was seen between averaged annual NFL and 10-year GMF values, which included years 1-5 and 1-6 values (unadjusted $p<0.05$; adjusted analysis $p<0.05$). The 10-year WMF analysis showed a similar negative association for all averaged year (1-10) combinations (unadjusted $p<0.05$; adjusted analysis $p<0.05$). Negative associations were also seen for the 10-year thalamus analysis and all averaged year (1-10) NFL combinations (unadjusted $p<0.05$; adjusted analysis $p<0.05$). Similar negative associations were also seen for both the 10-year Caudate and Globus Pallidas analysis with

averaged NFL values for years 1-5, 1-6, 1-7 and 1-10 respectively (unadjusted $p<0.05$; adjusted analysis $p<0.05$).

E. Classification of High Versus Low Annualized Relapse Rate Status in Subjects with Relapsing-Remitting Multiple Sclerosis Using Multivariate Serum Protein Biomarker Models. Neda Sattarnezhad, Shrishti Saxena, Cindy Gonzalez, Hrishikesh Lokhande, Bonnie Glanz, Ferhan Qureshi, Michael Becich, Remus Osan, Howard Weiner, Tanuja Chitnis. Poster presentation, 2019 ECTRIMS Congress, Stockholm, Sweden.
   - Major findings: This study evaluated over 1000 proteomic markers in in RRMS patients with high (≥1 relapses/year) and low (≤0.2 relapses/year) relapse rates, and found that sNFL as well as several other inflammatory markers were associated with a higher relapse rate (CD6, IL-1RT2, COL4A1, LEPR, BSCAN).

F. Multivariate Protein Biomarker Models More Accurately Predict Multiple Sclerosis MRI Disease Activity Compared to Serum Levels of Neurofilament Light Chain Alone. Tanuja Chitnis, Hajime Yano, Shrishti Saxena, Hrishikesh Lokhande, Neda Sattarnezhad, Maria Claudia Manieri, Anu Paul, Fermisk Saleh, Mikaela Collins, Bonnie Glanz, Charles Guttmann, Rohit Bakshi, Ferhan Qureshi, Michael Becich, Remus Osan, Victor Gehman, Howard Weiner. Poster presentation, 2019 ECTRIMS Congress, Stockholm, Sweden.
   - Major findings: Over 1000 proteomic biomarkers were evaluated in 326 patients including 226 paired samples in patients with a Gd+ and Gd- state. We found that a multivariate classifier that consisted of 10 biomarkers (including sNfL) improved the classification of Gd+ and Gd- samples with 0.896 ± 0.046 Accuracy and 0.959 ± 0.023 AUC, compared to an AUC of 0.686 with sNFL alone. Furthermore, sNfL alone was unable to distinguish samples with 0 vs. 1 lesion ($p = 0.138$) while multivariate biomarker models were able to at a statistically significant level ($p < 0.01$).

4) Other achievements: The following other achievements related to this project include meeting reports and reviews, led by Dr. Chitnis around the theme of this research grant.

- Dr. Chitnis co-chaired the 2019 ACTRIMS Forum meeting with a theme of "Precision Medicine for MS" with over 1200 attendees (Dallas, TX), which featured talks and presentations on precision biomarkers for MS diagnosis and prognosis.
- Dr. Chitnis co-edited a special edition of Multiple Sclerosis Journal (in preparation) which features 12 articles on the theme of Precision Medicine in MS, with coauthors from the ACTRIMS Forum meeting 2019, and featuring articles on biomarkers and predictive algorithms for MS. This include an overview article entitled "A Roadmap to Precision Medicine for MS" authored by T. Chitnis and A. Prat.
- Invited editorial: "Serum NFL levels should be used to monitor multiple sclerosis evolution" by Dr. Chitnis, accepted for publication in Multiple Sclerosis Journal (September 2019).

- **What opportunities for training and professional development has the project provided?**
  - Professional Development:
    - Mattia Rosso, MD, PhD – additional training in biomarker analysis, statistical analysis
    - Neda Sattarnezhad, MD – additional training in biomarker analysis, statistical analysis
  - Opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project:
    - Mattia Rosso, MD PhD – one-on-one work with a mentor; one-on-one work with biostatistician and development of statistical analysis skills; attendance of workshops on biomarker analysis, network medicine and biostatistics; attendance and presentation of abstracts at international conference (ECTRIMS 2019)
    - Neda Sattarnezhad, MD – one-on-one work with a mentor; attendance and presentation of abstracts at international conference (ECTRIMS 2019)

- Hrishikesh Lokhande, MSc – attendance of workshops on bioinformatics tools, one-on-one work with biostatistician.

- **How were the results disseminated to communities of interest?**
  - Publications: 1 published; 2 additional publications submitted
  - Abstract presentations at major meetings: 6 presented or submitted.

- **What do you plan to do during the next reporting period to accomplish the goals?**
  - We plan to continue biomarker analysis in the identified cohorts. In the upcoming year, we will focus on metabolomic and miRNA analysis in the nested cohorts.
  - We will begin to develop composite panels and predictive models.
  - In order to accomplish these goals, we will continue to analyze biosamples for the identified biomarkers and continue to conduct statistical analysis for the identified outcome measures.

4) IMPACT:

- **What was the impact on the development of the principal discipline(s) of the project?**
  - Nothing to report
- **What was the impact on other disciplines?**
  - Nothing to report
- **What was the impact on technology transfer?**
  - Nothing to report
- **What was the impact on society beyond science and technology?**
  - Nothing to report.

5) CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**
  - No changes
- **Actual or anticipated problems or delays and actions or plans to resolve them**
  - No problems
- **Changes that had a significant impact on expenditures**
  - No changes that had a significant impact on expenditures
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
  - No changes
- **Significant changes in use or care of human subjects**
  - No changes
- **Significant changes in use or care of vertebrate animals.**
  - NA
- **Significant changes in use of biohazards and/or select agents**
  - NA

6) PRODUCTS:

- **Publications, conference papers, and presentations**
  - **Journal publications:**
    1) Neurofilament light chain serum levels correlate with 10-year MRI outcomes in multiple sclerosis. Chitnis T, Gonzalez C, Healy BC, Saxena S, Rosso M, Barro C, Michalak Z, Paul A, Kivisakk P, Diaz-Cruz C, Sattarnezhad N, Pierre IV, Glanz BI, Tomic D, Kropshofer H, Häring D, Leppert D, Kappos L, Bakshi R, Weiner HL, Kuhle J. Ann Clin Transl Neurol. 2018 Oct 16;5(12):1478-1491. doi: 10.1002/acn3.638. eCollection 2018 Dec. PMID: 30564615; PMCID: PMC6292183.
       - This work was also presented at the 2019 ACTRIMS meeting in Dallas, TX.

- Acknowledgement of federal support – yes

2) Temporal associate of sNfL and gad-enhancing lesions in multiple sclerosis. Mattia Rosso, MD; Cindy T. Gonzalez, MPH; Brian C. Healy, Ph. D Shrishti Saxena, MS Anu Paul, Ph. D Kjetil Bjornvik, MD; Jens Kuhle, MD, Ph. D; Pascal Benkert, Ph. D; David Leppert, MD, Charles Guttmann, MD, Rohit Bakshi, MD, MA, Howard L. Weiner, MD, Tanuja Chitnis, MD.
   - This work has been submitted for journal publication.
   - This work was also presented at the 2019 ECTRIMS meeting in Stockholm, Sweden.
   - Acknowledgement of federal support – yes
3) Differential association of Age and Serum neurofilament light chain in remission and after Gd+ lesions. Mattia Rosso; Brian C. Healy; Shrishti Saxena; Anu Paul; Kjetil Bjornevik; Jens Kuhle; Pascal Benkert; David Leppert, Charles Guttmann, Rohit Bakshi, Howard L. Weiner; Tanuja Chitnis
   - This work is in preparation for journal submission.
   - This work was also presented as a platform presentation entitled, Serum neurofilament light chain levels are increased within three months of new gadolinium enhancing lesions in multiple sclerosis (same authors), at the 2019 ECTRIMS meeting in Stockholm, Sweden.
   - Acknowledgement of federal support – yes

- **Books or other non-periodical, one-time publications:** None.

- **Other publications, conference papers, and presentations:**
  1. Serum NFL levels in first five years correlate with 10-year Deep Gray Matter volumes in multiple sclerosis. Hrishikesh Lokhande, Shahamat Tauhid, Renxin Chu, Cindy Gonzalez, Brian C. Healy, Shrishti Saxena, Mattia Rosso, Christian Barro, Zuzanna Michalak, Anu Paul, Neda Sattarnezhad, Bonnie I. Glanz, David Leppert, Ludwig Kappos, Jens Kuhle, Howard L. Weiner, Rohit Bakshi, Tanuja Chitnis.
     - Submitted in September 2019 for a 2020 national meeting/academic conference.
     - Acknowledgement of federal support – yes
  2. Classification of High Versus Low Annualized Relapse Rate Status in Subjects with Relapsing-Remitting Multiple Sclerosis Using Multivariate Serum Protein Biomarker Models Authors: Neda Sattarnezhad, Shrishti Saxena, Cindy Gonzalez, Hrishikesh Lokhande, Bonnie Glanz, Ferhan Qureshi, Michael Becich, Remus Osan, Howard Weiner, Tanuja Chitnis.
     - Submitted in September 2019 for a 2020 national meeting/academic conference.
     - Acknowledgement of federal support – yes
  3. Classification of High Versus Low Annualized Relapse Rate Status in Subjects with Relapsing-Remitting Multiple Sclerosis Using Multivariate Serum Protein Biomarker Models. Neda Sattarnezhad, Shrishti Saxena, Cindy Gonzalez, Hrishikesh Lokhande, Bonnie Glanz, Ferhan Qureshi, Michael Becich, Remus Osan, Howard Weiner, Tanuja Chitnis. Poster presentation, 2019 ECTRIMS Congress, Stockholm, Sweden.
     - Acknowledgement of federal support – yes
  4. Multivariate Protein Biomarker Models More Accurately Predict Multiple Sclerosis MRI Disease Activity Compared to Serum Levels of Neurofilament Light Chain Alone. Tanuja Chitnis, Hajime Yano, Shrishti Saxena, Hrishikesh Lokhande, Neda Sattarnezhad, Maria Claudia Manieri, Anu Paul, Fermisk Saleh, Mikaela Collins, Bonnie Glanz, Charles Guttmann, Rohit Bakshi, Ferhan Qureshi, Michael Becich, Remus Osan, Victor Gehman, Howard Weiner. Poster presentation, 2019 ECTRIMS Congress, Stockholm, Sweden.
     - Acknowledgement of federal support – yes

- **Website(s) or other Internet site(s)**
  - www.climbstudy.org

- **Technologies or techniques**
  - Pending technology on Proteomic markers associated with a Gd+ lesion (in collaboration with Octave Biosciences).

- **Inventions, patent applications, and/or licenses**
    - None.

- **Other Products**
    - data or databases; This grant supports in part the CLIMB database infrastructure required for this project.
    - biospecimen collections; This grant has supported the identification of nested cohorts of MS patients in the CLIMB study which can be used for future studies.
    - audio or video products; None
    - software; None
    - models; Multivariate predictive models of Gd+ lesions were developed as a result of this project; Predictive models of 10-year outcomes were developed as a result of this project.
    - educational aids or curricula; Abstracts and talks presented at major congresses (ACTRIMS 2019, ECTRIMS 2019) were developed as a result of this project.
    - instruments or equipment; NA
    - research material (e.g., Germplasm; cell lines, DNA probes, animal models); NA
    - clinical interventions; NA
    - new business creation; no
    - other; NA

7) PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	Mark Anderson
Project Role:	MRI Analyst
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2.64 CM
Contribution to Project:	Mr. Anderson is an MRI analyst and will conduct the processing of MRIs for volumetric analysis
Funding Support:	

Name:	Rohit Bakshi
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0001-8601-5534
Nearest person month worked:	1.90 CM
Contribution to Project:	He will supervise the conduct of MRI analysis for this project including the derivation of brain volumetrics and lesion volumes. He will work with Dr. Chitnis on data analysis and manuscript preparation.
Funding Support:	

Name:	Tanuja Chitnis
Project Role:	PD/PI
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0002-9897-4422
Nearest person month worked:	1.80 CM
Contribution to Project:	She will oversee all aspects of the project including the management of the dataset, subject identification and phenotyping, and biosample analysis. She will oversee data analysis and manuscript development. She will oversee the postdoctoral fellows, technicians and will coordinate meetings with the bioinformatician and collaborators. Dr. Chitnis will be primarily responsible for all study design and manuscript preparation
Funding Support:	

Name:	Brian Healy
Project Role:	Biostatistician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.60 CM
Contribution to Project:	He will conduct the biostatistical analysis for the project in concert with Dr. Patsopoulos and the bioinformatician. He will participate in manuscript preparation.
Funding Support:	

Name:	Hrishikesh Lokhande
Project Role:	Bioinformatician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2.40 CM
Contribution to Project:	He will participate in bioinformatics analysis for this project. He will meet weekly with Dr. Chitnis and her team.
Funding Support:	

Name:	Anu Paul
Project Role:	Research Technician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2.40 CM
Contribution to Project:	She will assist in sample procurement, processing, and conduct of proteomic assays.
Funding Support:	

Name:	Mattia Rosso
Project Role:	Postdoc
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6.0 CM
Contribution to Project:	He will participate in the conduct patient selection, data validation and data analysis. He will work with Dr. Chitnis and the team bioinformatics data analysis and manuscript preparation.
Funding Support:	

Name:	Shrishti Saxena
Project Role:	Laboratory Technician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6.0 CM
Contribution to Project:	She will participate in the conduct patient selection, data validation and data analysis. She will work with Dr. Chitnis and the team bioinformatics data analysis and manuscript preparation.
Funding Support:	

Name:	Howard Weiner
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.6 CM
Contribution to Project:	He will provide input on the biological assays and immune markers being assessed. He will work with Dr. Chitnis on development of manuscripts
Funding Support:	

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
    - Nothing to report.

- **What other organizations were involved as partners?**
    - **Organization Name:** University of Basel
        - **Location of Organization:** Basel, Switzerland
        - **Partner's contribution to the projec**t conduct of biosample analysis
        - **Financial support -** N/A
        - **In-kind support** - N/A
        - **Facilities** – the University of Basel conducted biomarker analysis (serum NFL) using the SIMOA machine on a subset of patients from the CLIMB cohort.
        - **Collaboration** – Investigators from the University of Basel who participated in this biomarker analysis are included as coauthors in several manuscripts.
        - **Personnel exchanges** – N/A
        - **Other -** N/A
    - **Organization Name:** Octave Biosciences
        - **Location of Organization:** Menlo Park, California
        - **Partner's contribution to the projec**t contribution to statistical analysis
        - **Financial support –** Provided funding for biomarker analysis
        - **In-kind support** - N/A
        - **Facilities** – provided biomarker analysis platform through OLINK (located in Boston, MA) for nested cohorts within the CLIMB Study.
        - **Collaboration** – Investigators from Octave Biosciences who participated in this biomarker analysis are included as coauthors on abstracts of these results.
        - **Personnel exchanges** – N/A
        - **Other** – N/A

8) SPECIAL REPORTING REQUIREMENTS:

- **COLLABORATIVE AWARDS:** NA
- **QUAD CHARTS:** NA

9) APPENDICES:

1. Differential association of Age and Serum neurofilament light chain in remission and after Gd+ lesions (Rosso, et al.) – submitted abstract, under review
2. Serum NFL levels in first five years correlate with 10-year Deep Gray Matter MRI volumes in multiple sclerosis (Lokhande, et al.) – submitted abstract, under review
3. Classification of High Versus Low Annualized Relapse Rate Status in Subjects with Relapsing-Remitting Multiple Sclerosis Using Multivariate Serum Protein Biomarker Model (Sattarnezhad, et al.) – poster presentation, 2019 ECTRIMS Congress, Stockholm, Sweden
4. Serum neurofilament light chain levels are increased within three months of new gadolinium enhancing lesions in multiple sclerosis (Rosso, et al.) – poster presentation, 2019 ECTRIMS Congress, Stockholm, Sweden
5. Multivariate Protein Biomarker Models More Accurately Predict Multiple Sclerosis MRI Disease Activity Compared to Serum Levels of Neurofilament Light Chain Alone (Chitnis, et al.) – poster presentation, 2019 ECTRIMS Congress, Stockholm, Sweden
6. Neurofilament light chain serum levels correlate with 10-year MRI outcomes in multiple sclerosis. Chitnis T, Gonzalez C, Healy BC, Saxena S, Rosso M, Barro C, Michalak Z, Paul A, Kivisakk P, Diaz-Cruz C, Sattarnezhad N, Pierre IV, Glanz BI, Tomic D, Kropshofer H, Häring D, Leppert D, Kappos L, Bakshi R, Weiner HL, Kuhle J. Ann Clin Transl Neurol. 2018 Oct 16;5(12):1478-1491. doi: 10.1002/acn3.638. eCollection 2018 Dec. PMID: 30564615; PMCID: PMC6292183.

Abstract

Full Title: Differential association of Age and Serum neurofilament light chain in remission and after Gd+ lesions

Authors:

Mattia Rosso; Brian C. Healy, Shrishti Saxena, Anu Paul, Jens Kuhle, Pascal Benkert, David Leppert, Charles Guttmann, Rohit Bakshi, Howard L. Weiner, Tanuja Chitnis

Background: Serum neurofilament light (sNfL) is a candidate biomarker of inflammatory activity in patients with multiple sclerosis (MS). This biomarker has high patient-to-patient variability, which has been partly attributed to the effects of age and relapse status. In this study, we aimed to estimate the association between sNfL and age in patients in remission, immediately after a clinical relapse, and immediately after a gadolinium-enhancing (Gd+) lesion.

Methods: This study included 94 patients with MS, enrolled in the Comprehensive Longitudinal Investigation of MS at the Brigham and Women's Hospital (CLIMB) study. sNfL levels were measured at yearly intervals for ten years with a single-molecule array (SIMOA) assay. We used multivariable linear mixed-effects models with random intercept to test the association between age and sNfL separately in subjects during remission, after a clinical relapse, and after a Gd+ lesion. The model was also fit including all subjects with age by relapse/remission status interaction terms to assess the difference in the associations between groups. All analyses were adjusted for medication and sex.

Results: During remission, patients showed a 1.18% annual increase in sNfL levels with increasing age (p=0.008). The fourth age quartile showed 31.2% greater sNfL levels than the first age quartile (p=0.005). During the three months after a clinical relapse, sNfL showed a 2.2% annual increase with increasing age (p=0.049). sNfL was not significantly associated with age during the three months after a Gd+ lesion (p= 0.88). When comparing the association between sNfL and age after a Gd+ lesion to a paired remission sNfL sample, we observed a negative interaction between age and Gd+ lesion status (– 1.72% annual decrease, p=0.004).

Conclusions: Our findings show that there is a direct correlation of sNfL levels with age during remission, whereas this relationship is inversed when a sample is taken at or after a Gd+ lesion.

Serum NFL levels in first five years correlate with 10-year Deep Gray Matter MRI volumes in multiple sclerosis

Authors: Hrishikesh Lokhande[2,3], Mattia Rosso[2,3], Shahamat Tauhid[2,3], Renxin Chu[2,3], Shrishti Saxena[2,3], Brian C. Healy[2,3,4], Christian Barro[5], Anu Paul[2,3], Camilo Diaz-Cruz[2,3], Neda Sattarnezhad,[2,3] Bonnie I. Glanz[1,2,3], David Leppert[5], Ludwig Kappos[5], Jens Kuhle[5], Howard L. Weiner[1,2,3], Rohit Bakshi[1,2,3]Tanuja Chitnis[1,2,3]

Institutional Affiliations
1. Partners Multiple Sclerosis Center, Department of Neurology, Brigham and Women's Hospital, Boston, MA
2. Harvard Medical School, Boston, MA 02115
3. Ann Romney Center for Neurologic Disease, Harvard Medical School, Boston, MA 02115, USA
4. Massachusetts General Hospital Biostatistics Center, Boston, MA 02114
5. Neurologic Clinic and Policlinic, Departments of Medicine, Biomedicine and Clinical Research, University Hospital Basel, University of Basel, Basel, Switzerland

Keywords: neurofilament light chain, multiple sclerosis, CLIMB study, prediction models, gray matter, white matter.

ABSTRACT:
Objective To explore the value of annual serum neurofilament light (NfL) measures in predicting 10-year deep gray matter atrophy measured by volumetric MRI in multiple sclerosis (MS).
Methods: The patient population for this study was identified in the Comprehensive Longitudinal Investigations in MS at Brigham and Women's Hospital (CLIMB) study enrolled within 5 years of disease onset, and with annual blood samples up to 10 years (n=122). The measurement of Serum NfL was performed using a single molecule array (SIMOA) assay. A semi-automated pipeline quantified Deep Gray Matter (DGM) in the following structures: Thalamus, Caudate, Putamen and Globus Pallidus from year 10 high-resolution 3T MRI scans. Correlations between averaged annual NfL and 10-year clinical/MRI outcomes were assessed using Spearman's correlation, univariate and multivariate linear regression models.
Results: Linear regression analysis of averaged NFL values revealed several negative associations with different MRI volumetric outcomes. A negative association was seen between averaged annual NFL and 10-year thalamic volumes, with significance reached with year 1-2 measures, (Mean sNFL years 1-2: unadjusted p=0.00005662; adjusted analysis p=0.00011359; multivariate point estimate -1.32) and no further strength of association gained beyond year 5 (Mean sNFL years 1-5; unadjusted p= 0.00000166; adjusted analysis p= 0.00001817; multivariate point estimate, -2.67). Serum NFL in the first five years accounted for approximately 24% of the variance in 10-year thalamic volumes. Similar associations were seen with caudate, putamen and globus pallidus volumes.
Interpretation: Serum NfL measured during the first few years after the clinical onset of MS contributed to the prediction of 10-year deep gray matter volumes.

Classification of High Versus Low Annualized Relapse Rate Status in Subjects with Relapsing-Remitting Multiple Sclerosis Using Multivariate Serum Protein Biomarker Models

Neda Sattarnezhad[1], Shrishti Saxena[1], Cindy Gonzalez[1], Hrishikesh Lokhande[1], Bonnie Glanz[1], Ferhan Qureshi[2], Michael Becich[2], Remus Osan[2], Howard Weiner[1], Tanuja Chitnis[1]

[1]Partners MS Center, Department of Neurology, Ann Romney Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [2] Octave Bioscience, Menlo Park, CA, USA

INTRODUCTION

BACKGROUND:

•Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system with various phenotypes and heterogenous disease course. [1]

•While exact pathophysiology of MS remains elusive, both inflammatory and degenerative processes are believed to play a role in the disease mechanism and disability progression. [1,2]

•Identifying disease-specific biomarkers may assist with predicting the diverse disease course and classifying patients to high risk versus low risk for disease activity and progression. [3,4]

•Use of multivariate models reflecting multiple biological pathways that are involved in the complex pathophysiology of MS will most likely increase predictive accuracy of these biomarkers.[4]

•Annualized Relapse Rate (ARR) is a quantifiable outcome measurement which has been strongly correlated with disability score and disease progression in relapsing forms of MS.[6,7]

•Multivariate biomarker models will most likely correlate strongly with clinical outcome measurements including ARR status.

OBJECTIVE:

To investigate the performance of multiple protein biomarker models to classify samples from Relapsing-Remitting (RR) MS subjects with High and Low ARR from the Comprehensive Longitudinal Investigation of Multiple Sclerosis at Brigham and Women's Hospital (CLIMB) study.

METHODS and MATERIALS

SUBJECTS: Serum samples from 30 RRMS patients with Low ARR (≤0.2 relapses/year) were compared to 30 age, sex and treatment matched subjects with High ARR (≥1.0 relapses/year). All the patients were in the disease remission phase and hadn't received steroids within 30 days prior to the sample collection. All samples were measured for 1104 proteins, including serum levels of neurofilament light chain (sNfL), using Proximity Extension Assays (PEA) from Olink to quantify protein biomarker expression.

STATISTICAL ANALYSIS:

Univariate/multivariate machine learning-driven biostatistical techniques were applied to the proteomic data. Cross-validation and bootstrapping were performed to minimize overfitting and ensure best generalizability for predicting the relapse frequency status of new samples. Area under the curve (AUC) and accuracy were selected as the key metrics for evaluating model performance. Biomarkers that were identified as important features in multivariate models were investigated for biological relevance using pathway and network models to evaluate their involvement in MS pathophysiology.

RESULTS-I

1104 protein biomarkers were measured on all n=60 serum samples to screen for potential biomarkers. Measurements were filtered to exclude quality control warnings, high coefficient of variation, and assays frequently imputed at the limit of detection. To reduce dimensionality, multiple-hypothesis-adjusted univariate statistics and accuracy-weighted multivariate performance across 100,000 simulated models (ranging in type: logistic regression, support vector machine, random forest, stochastic gradient descent) were used to select for the best features. Incremental model-building strategies under regularization optimized for AUC as measured by cross-validation and bootstrapping. While several model types achieved a steady-state AUC > 0.90, a 7-feature logistic regression function was the most parsimonious and generalizable to future datasets.

Protein	P-value	AUC	Coefficient 95% C.I.	|Importance|
CD6	1.95E-04	0.76	(0.799,1.412)	0.196
IL-1RT2	1.77E-03	0.706	(0.466, 1.186)	0.146
COL4A1	2.05E-03	0.679	(-1.258, -0.702)	0.273
LEPR	2.16E-03	0.694	(-1.548,-0.945)	0.141
AGR2	8.15E-03	0.666	(-1.393, -0.799)	0.288
BCAN	2.59E-02	0.662	(0.900, 1.472)	0.313
CSTB	3.25E-02	0.658	(0.318, 0.957)	0.194
sNfL	3.16E-02	0.611	N/A	N/A

Figure 1. The 7 proteins in the logistic regression classification model (sNfL shown for comparison). Exploratory data analysis revealed normality across each protein signal distribution, and with n > 50, this assumption was safe to apply throughout. 2-sample, 1-sided homoscedastic t-tests were used for calculation of p-values. AUC is a trapezoidal integration of TPR, FPR across all 60 samples. The 95% confidence interval for each coefficient was computed by a 50/50 bootstrap procedure (B=100,000) to determine if explanatory variables in this optimal model are significant (nonzero). Standardized coefficients were used to compute relative model importance.

RESULTS-II

A multivariate logistic regression model that included 7 biomarkers achieved performance of 0.923 ± 0.072 AUC and 0.831 ± 0.024 Accuracy for classifying High vs. Low relapse rate specimens. The model was evaluated using leave-one-out cross-validation and a 50/50 train/test split simulated B=100,000 trials to establish confidence margins. The multivariate model significantly outperformed all univariate biomarkers including sNfL (0.611 AUC, 0.583 ± 0.0177 Accuracy). Cluster of Differentiation 6 (CD6), a protein associated with T-cell activation and proliferation as well as being an identified risk gene for MS[8], was identified as the strongest separating biomarker.

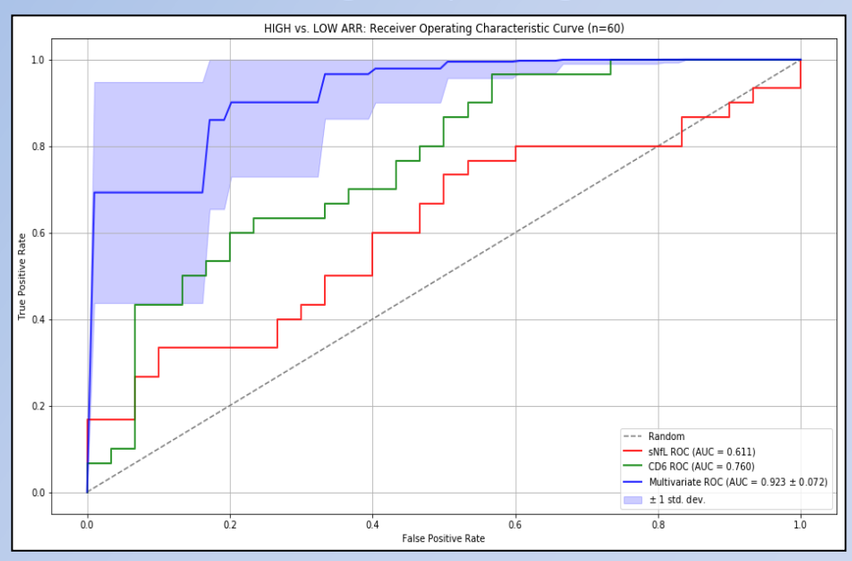


Figure 2. The Receiver Operating Characteristic visualizes the true and false positive rates of various thresholds to separate the normalized protein signals across samples. In this cohort, sNfL marginally outperformed random guessing for separating relapse rates in MS patients. CD6 was able to distinguish patients more sensitively and specifically, and the 7-feature multivariate model was able to significantly outperform all univariate markers. Correlational analysis between all features in the top-performing model (Pearson's R < 0.37 for all pairwise combinations) demonstrates the lack of collinearity in our system.

CONCLUSIONS

- Multivariate serum protein biomarker models representing several biological pathways were able to effectively classify subjects with high vs. low annualized relapse rates.
- Multivariate models outperformed all univariate approaches with a single biomarker, including CD6 and sNfL.
- These models may assist with predicting disease course and risk of disability progression to guide appropriate treatment planning and patient counseling.
- Further investigation with larger sample numbers and from additional cohorts is warranted.

REFERENCES

1)Wingerchuk, Dean M., and Jonathan L. Carter. "Multiple sclerosis: current and emerging disease-modifying therapies and treatment strategies." *Mayo Clinic Proceedings*. Vol. 89. No. 2. Elsevier, 2014. 2) Macaron, Gabrielle, and Daniel Ontaneda. "Diagnosis and Management of Progressive Multiple Sclerosis." *Biomedicines* 7.3 (2019): 56. 3) Comabella, Manuel, and Xavier Montalban. "Body fluid biomarkers in multiple sclerosis." *The Lancet Neurology* 13.1 (2014): 113-126.4) Barro, Christian, et al. "Serum neurofilament as a predictor of disease worsening and brain and spinal cord atrophy in multiple sclerosis." *Brain* 141.8 (2018): 2382-2391. 5) Martinelli, V., et al. "Multiple biomarkers improve the prediction of multiple sclerosis in clinically isolated syndromes." *Acta Neurologica Scandinavica* 136.5 (2017): 454-461. 6)Jokubaitis, Vilija G., et al. "Predictors of long-term disability accrual in relapse-onset multiple sclerosis." *Annals of neurology* 80.1 (2016): 89-100. 7) Vercellino, M., et al. "Multiple sclerosis relapses: a multivariable analysis of residual disability determinants." *Acta Neurologica Scandinavica* 119.2 (2009): 126-130. 8) Yan Li et al. "CD6 as a potential target for treating multiple sclerosis." *Proceedings of the National Academy of Sciences* Mar 2017, 114 (10) 2687-2692

DISCLOSURES

Dr. Sattarnezhad has received research support from Serono and Verily; Ms Saxena received research support from Octave, Serono and Verily. Ms. Gonzalez, Mr. Lokhande and Dr. Glanz have received research support from Serono and Verily.Mr. Qureshi, Mr. Becich and Mr. Qureshi are employees of Octave Bioscience. Dr Weiner reports grants from National Institutes of Health, grants from National Multiple Sclerosis Society, grants from Verily, grants from EMD Serono, grants from Biogen, grants from Teva Pharmaceuticals, grants from Sanofi, grants from Novartis, grants and personal fees from Genentech, Inc, grants and personal fees from Tilos Therapeutics, personal fees from Tiziana Life Sciences, personal fees from IM Therapeutics, personal fees from MedDay Pharmaceuticals, personal fees from vTv Therapeutics, outside the submitted work. Dr. Chitnis has served on advisory boards for Biogen, Novartis, and Sanofi-Genzyme; has participated in clinical trials sponsored by Sanofi-Genzyme and Novartis; has received research support from the Department of Defense, National MS Society, Guthy Jackson Charitable Foundation, Novartis, Octave, Serono and Verily-Genzyme.

SUPPORT

This study was supported by Octave Bioscience. U.S. Department of Defense (T.C). The CLIMB study has received support from EMD-Serono, the National MS Society, the Nancy Davis Center without Walls and Philanthropy.

Contact email address:

n.sattarnejad@gmail.com

tchitnis@rics.bwh.harvard.edu

disorder of the central nervous system (CNS)

- No molecular biomarkers of relapses are currently available in clinical settings
- Serum neurofilament light chain (sNfL) is a candidate biomarker which was shown to correlate with relapses in MS

OBJECTIVE

- The objective of this study is to assess sNfL as a biomarker of disease activity in MS

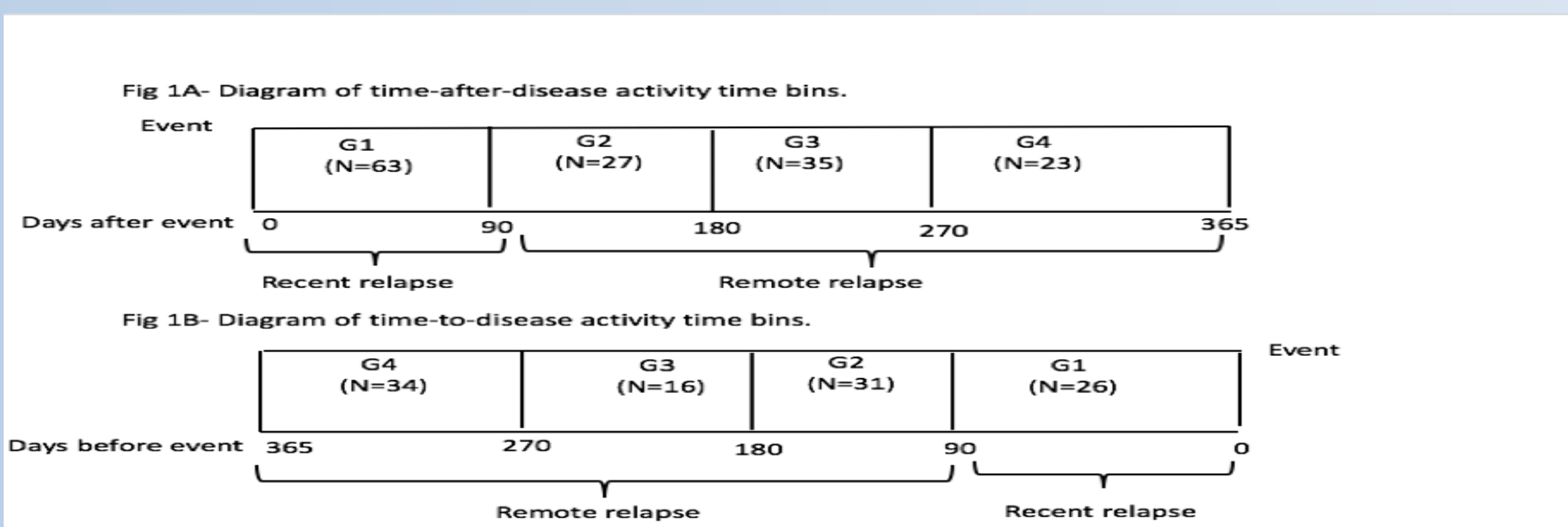
METHODS

SUBJECTS

- This study included 94 MS patients enrolled in the Comprehensive Longitudinal Investigation of MS at the Brigham and Women's Hospital (CLIMB) study
- sNfL levels were measured with a single-molecule array (SIMOA) assay annually over ten years

STATISTICAL ANALYSIS

- We used multivariable linear mixed effects models with random intercept to test the association of related to time after and before a clinical relapse and/or a new gadolinium-enhancing lesion to sNfL levels as the outcome
- The model was adjusted for age, disease duration, sex, and therapy
- Significance levels were evaluated at an alpha level of <0.05.
- SAS 9.4 software (SAS Inc., Cary, NC) and R Studio were used for statistical analysis



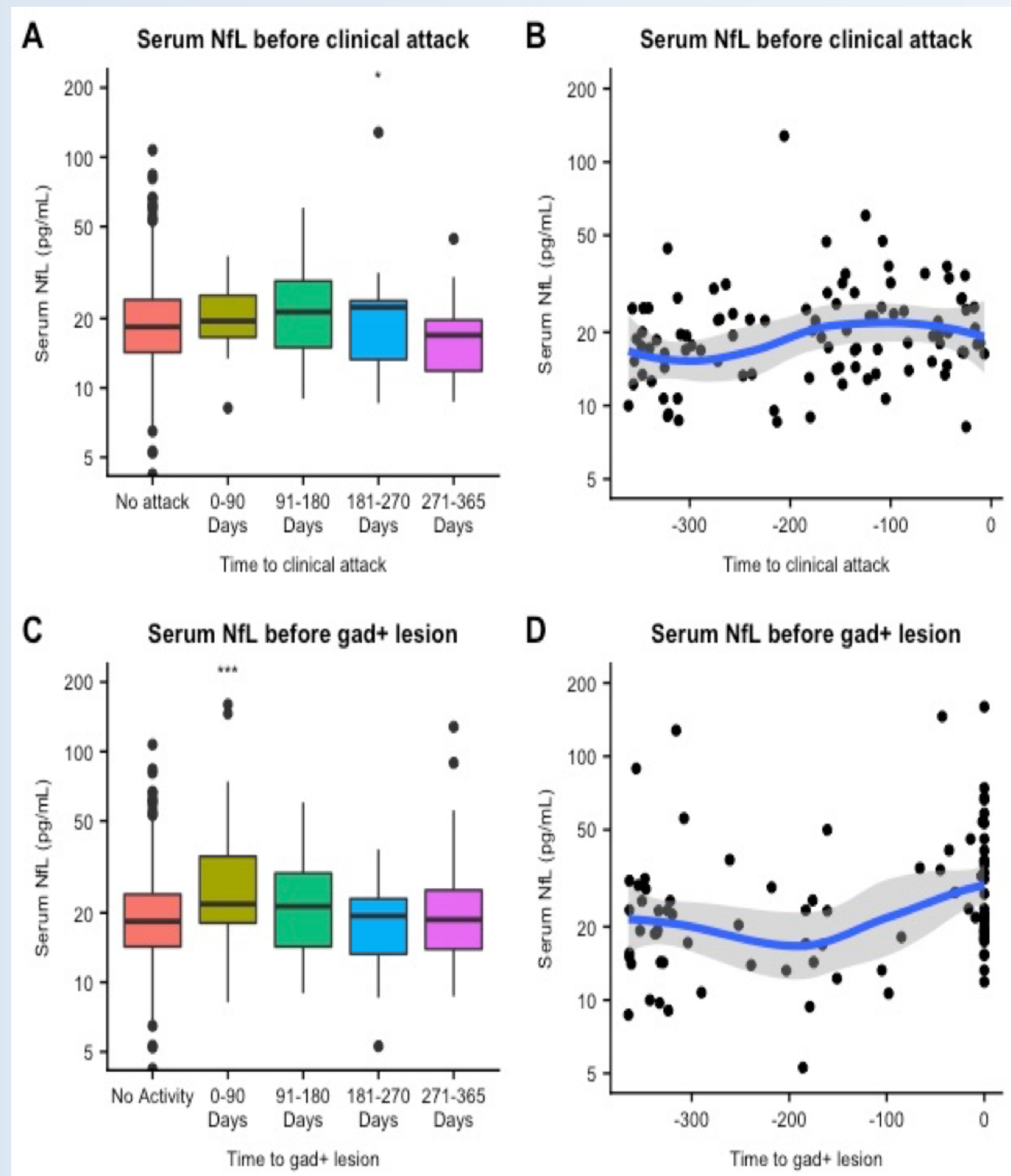
neurofilament light chain in the presence of gadolinium-enhancing lesions

- In the three months after an MRI scan, we observed an average 35% elevation in serum neurofilament light (p<0.0001)
- An average 32.3% elevation in serum neurofilament light was seen in the three months prior to MRI (p=0.002)
- When we classified clinical attacks according to attack location, spinal cord attacks were associated with an average 23.4% elevation in serum neurofilament light (p=0.03)

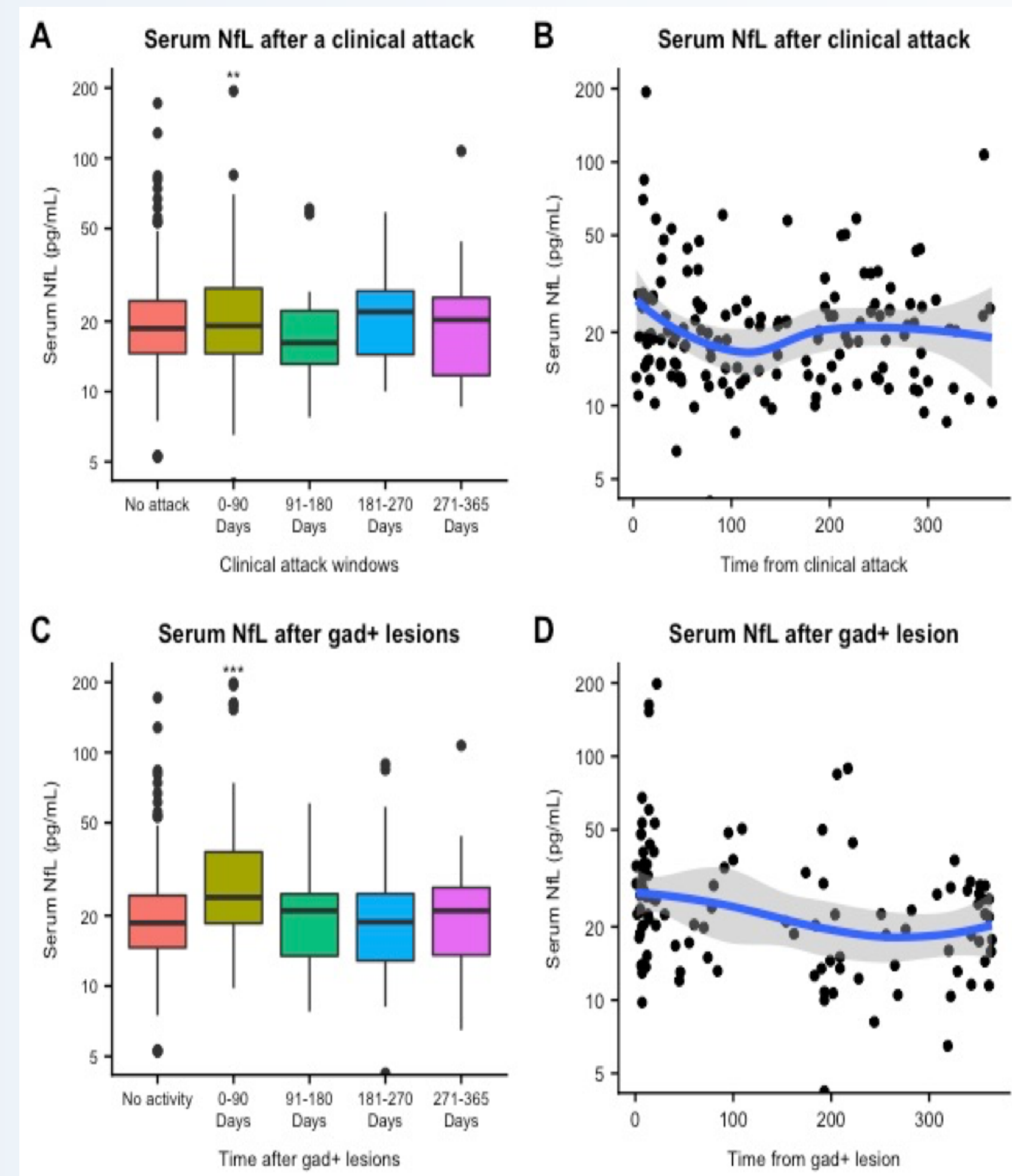
Characteristics		MS patients (n=94)
Sex, n (%)	Female	69 (73%)
	Male	25 (27%)
Race, n (%)	White	90 (96%)
	African American	1 (1%)
	Unknown	2 (2%)
	More than one race	1 (1%)
Age, years at baseline (mean, SD)		37.4 ± 8.9
Disease duration, years at baseline (mean, SD)		2.3 ± 1.4
EDSS at baseline (median, interquartile range)		1.0 (0 – 2.0)

Legend:
EDSS= expanded disability status scale; MS= multiple sclerosis; n= patient count; SD= standard deviation

Serum NfL before Disease Activity



Serum NfL after Disease Activity



Multivariate Protein Biomarker Models More Accurately Predict Multiple Sclerosis MRI Disease Activity Compared to Serum Levels of Neurofilament Light Chain Alone

Tanuja, Chitnis[1] Hajime Yano[1], Shrishti Saxena[1], Hrishikesh Lokhande[1], Neda Sattarnezhad[1], Anu Paul[1], Fermisk Saleh[1], Mikaela Collins[1], Bonnie Glanz[1], Charles Guttmann[1], Rob Bakshi[1], Ferhan Qureshi[2], Michael Becich[2], Remus Osan[2], Victor Gehman[2], Howard Weiner[1]

[1]Partners MS Center, Ann Romney Center for Neurologic Diseases, Department of Neurology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [2] Octave Bioscience, Menlo Park, CA, USA

INTRODUCTION

BACKGROUND:

•Multiple sclerosis(MS) is a chronic inflammatory demyelinating disease of the central nervous system with various phenotypes and heterogenous disease course. [1]

•While exact pathophysiology of MS remains elusive, both inflammatory and degenerative processes are believed to play a role in the disease mechanism and disability progression. [1,2]

•Identifying disease-specific biomarkers may assist with predicting the diverse disease course and classifying patients to high risk versus low risk for disease activity and progression. [3,4]

•Use of multivariate models reflecting multiple biological pathways that are involved in the complex pathophysiology of MS will most likely increase predictive accuracy of these biomarkers. [4]

•Serum levels of neurofilament light chain (sNfL) are associated with neurodegeneration in Multiple Sclerosis (MS) and correlate with measurements of disease activity (DA), including the presence of gadolinium enhancing (GAD+) lesions.

•The inclusion of additional inflammatory and neurodegenerative protein biomarkers, can provide deeper insights and reveal stronger correlations to radiographic DA than sNfL individually.

OBJECTIVES:

To compare the performance of multivariate protein biomarker models with sNfL individually to classify samples from subjects with and without GAD+ lesions from the Comprehensive Longitudinal Investigation of Multiple Sclerosis at Brigham and Women's Hospital (CLIMB) study.

METHODS and MATERIALS

SUBJECTS: A total of 326 serum samples drawn within close-proximity (median interval 1 day) to a contrast-enhanced MRI were measured for 1196 proteins including sNfL using Proximity Extension Assays (PEA) from Olink and 215 proteins using Luminex based immunoassays from Rules Based Medicine (RBM). Samples represented both 113 longitudinal pairs (n=226) and non-paired specimens (n=100) that were categorized by the number of GAD+ lesions per Table 1. 58 samples had been measured previously for 1104 proteins using the Olink platform as a proof of concept study.

Table 1: GAD lesion count distribution for paired and unpaired samples

Sample Group (# of GAD+ lesions)	Sample Pairs	Individual Samples	0 lesions	1 lesion	2 lesions	≥3 lesions
A (0 and ≥1)	98	196	98	66	19	13
B (1 and ≥2)	15	30	0	15	7	8
C (≥ 2)	0	100	0	0	77	23
Totals	113	326	98	81	103	44

STATISTICAL ANALYSIS: Univariate and multivariate machine learning-driven biostatistical techniques were used to classify samples with and without GAD+ lesions. Analysis was performed both on the entire cohort (n=326) and restricted to longitudinal pairs which strictly included a sample with 0 GAD+ lesions. Five-fold cross-validation and regularization (L2) were used in tandem with sequential feature selection to minimize overfitting and ensure generalizability for predicting DA of new samples. Area Under the Curve (AUC) and Accuracy were selected as the key metrics for comparison.

RESULTS-I

FEATURE SELECTION: Exploratory data analysis was conducted to filter noise, reduce dimensionality & avoid collinearity. Univariate significance was combined with multivariate importance from simulated models as shown in Table 2.

P-value	AUC	Protein
6.06E-15	0.746	sNfL
0.015	0.585	CNTN5
0.046	0.552	E-Cad
0.006	0.575	TNC
0.030	0.577	SORT1
0.070	0.574	PDGFC
0.009	0.573	CTSF
0.006	0.582	TNFRSF10A
0.023	0.568	VWA1
0.014	0.569	IL18
2.55E-04	0.62	CCL20
1.39E-04	0.622	CDCP1
2.50E-04	0.627	CXCL9
0.019	0.572	CCL3
0.004	0.588	PODXL2
0.004	0.582	EZR
0.028	0.568	LIF-R
0.082	0.567	GDF-15
0.040	0.556	MFGE8
0.012	0.569	CXCL6
0.020	0.566	YKL-40

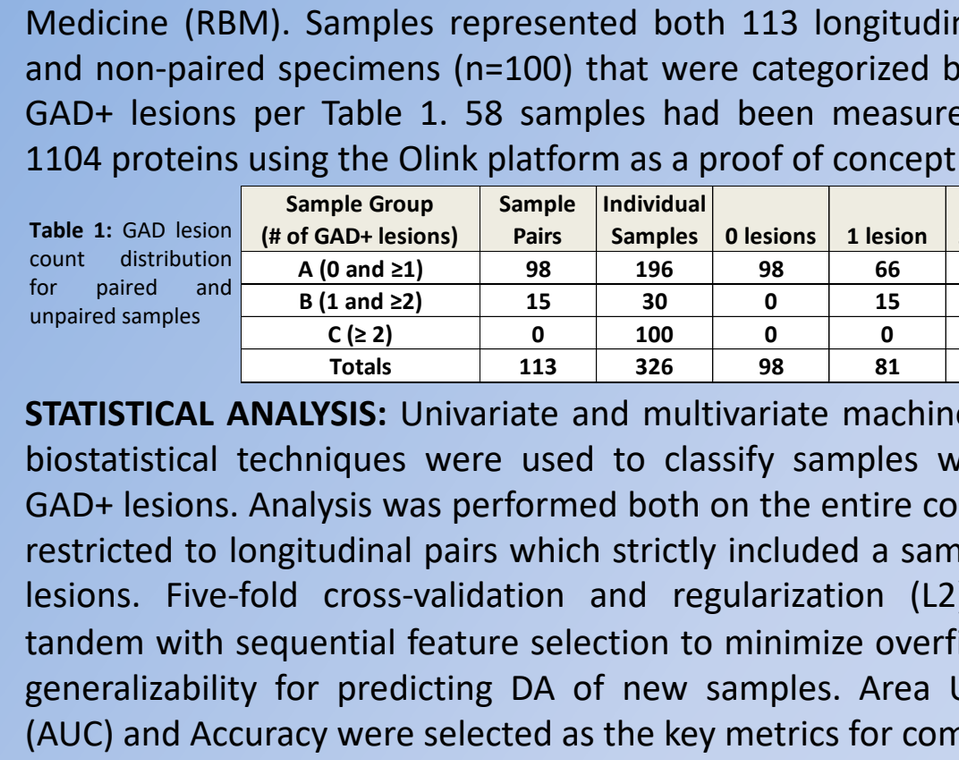


Table 2: Top 21 features ranked by feature importance (across LR, SVM, LDA, and QDA models), shown by accompanying p-value (2 sample, 1-sided homoscedastic t-test) and univariate AUC (trapezoidal integration of TPR, FPR across all 326 samples). sNfL passed multiple hypothesis correction filters (Bonferroni) for paired and unpaired samples while the remaining markers contribute orthogonal signal that were deemed significant explanatory variables (nonzero) through 95% confidence intervals after 100,000 bootstrap iterations. A similar procedure was conducted for the 196 paired samples to identify the strongest shifts (not shown).

RESULTS-II

MODEL-BUILDING: Forward selection, combined with grid search hyperparameter-tuning, as measured by 5-fold stratified cross-validation, achieved strong separation potential across supervised classification models: AUC_{LR} = 0.836 ± 0.066, AUC_{SVM} = 0.834 ± 0.039, AUC_{QDA} = 0.827 ± 0.055, AUC_{LDA} = 0.822 ± 0.065. The highest-performing parsimonious model (a 7-feature logistic regression model) was then validated using 100,000 iterations of repeated 50/50 cross-validation to produce the ROC curves in Fig. 1.

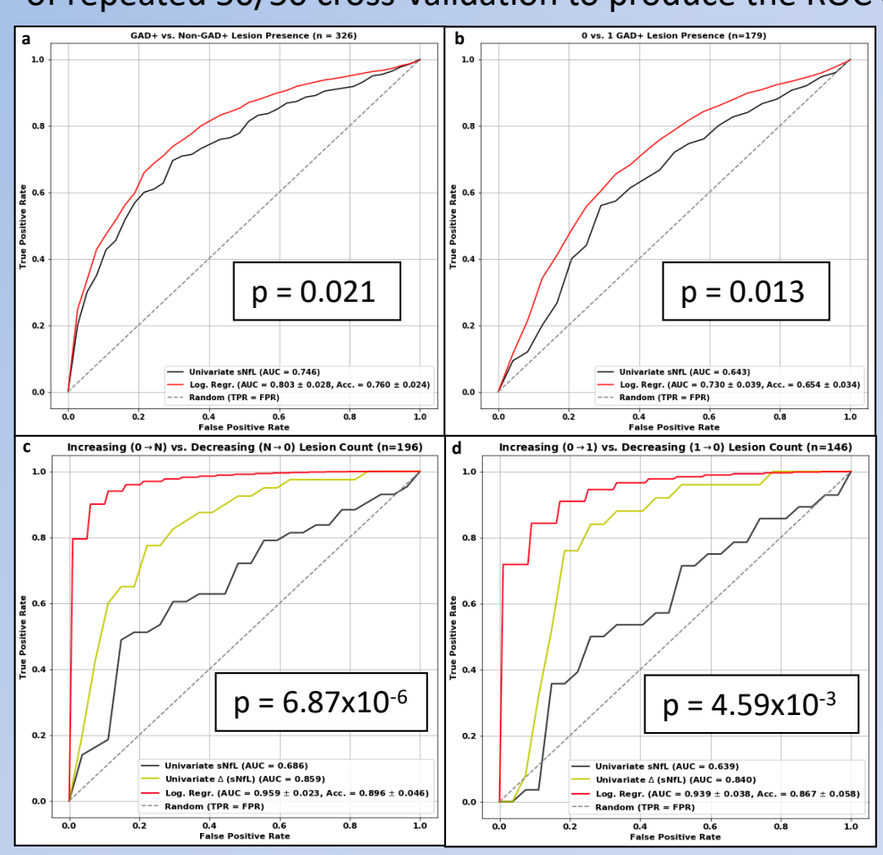


Figure 1. The Receiver Operating Characteristic (ROC) curve visualizes the true and false positive rates of various thresholds to separate the protein levels across samples. The p-value represents the statistical significance of the multivariate model's AUC being significantly greater than the AUC of sNfL (or Δ sNfL). ROC plots b and d reflect the power of the model to discriminate 0 vs. 1 lesions (thereby representing subtle disease activity). Different features were pulled in for the longitudinal analysis; however, in all 4 breakdowns of the study (a-d), logistic regression models showed significantly ($p < 0.05$) improved sensitivity and specificity (as measured by AUC).

Biomarkers that were selected as important features in the multivariate classifier were investigated for relevance and interactions using biological network models. In addition to neurodegeneration, proteins related to inflammatory and immune pathways were identified.

CONCLUSIONS

- Multivariate protein biomarker models representing several biological pathways predicted radiographic DA with greater statistical significance than sNfL alone.
- A multivariate model based on shifts in patient protein levels (between 2 samples, which better controls for age/sex/BMI) was able to strongly predict directionality of lesion activity (AUC=0.96). This not only outperforms sNfL alone, but also improves upon the multivariate model's ability to predict predict lesion presence from an individual MS patient's blood sample (AUC=0.80).
- Further investigation with larger sample numbers and from additional cohorts is warranted.

REFERENCES

1)Wingerchuk, Dean M., and Jonathan L. Carter. "Multiple sclerosis: current and emerging disease-modifying therapies and treatment strategies." *Mayo Clinic Proceedings*. Vol. 89. No. 2. Elsevier, 2014. 2) Macaron, Gabrielle, and Daniel Ontaneda. "Diagnosis and Management of Progressive Multiple Sclerosis." *Biomedicines* 7.3 (2019): 56. 3) Comabella, Manuel, and Xavier Montalban. "Body fluid biomarkers in multiple sclerosis." *The Lancet Neurology* 13.1 (2014): 113-126.4) Barro, Christian, et al. "Serum neurofilament as a predictor of disease worsening and brain and spinal cord atrophy in multiple sclerosis." *Brain* 141.8 (2018): 2382-2391.

DISCLOSURES

Tanuja Chitnis has served on advisory boards for Biogen, Novartis, and Sanofi-Genzyme; has participated in clinical trials sponsored by Sanofi-Genzyme and Novartis; has received research support from the Department of Defense, National MS Society, Guthy Jackson Charitable Foundation, Novartis, Octave, Serono and Verily Hajime Yano has received research support has received the research grant from Yoshida Scholarship Foundation, Japan Shrishti Saxena has received research support from Octave, Serono and Verily Hrishikesh Lokhande has received research support from Serono and Verily Neda Sattarnezhad has received research support from Serono and Verily Maria Claudia Manieri has no financial conflicts of interest to disclose Anu Paul has no financial conflicts of interest to disclose Fermisk Saleh has no financial conflicts of interest to disclose Mikaela Collins has no financial conflicts of interest to disclose. Bonnie Glanz has received research support from Serono and Verily Charles Guttmann has no financial conflicts of interest to disclose Rohit Bakshi has received consulting fees from Bayer, Biogen, Celgene, EMD Serono, Genentech, Guerbet, Sanofi-Genzyme, and Shire and research support from EMD Serono and Sanofi-Genzyme Ferhan Qureshi, Michael Becich, Remus Osan, and Victor Gehman are employees of Octave Bioscience. Howard Weiner reports grants from National Institutes of Health, grants from National Multiple Sclerosis Society, grants from Verily, grants from EMD Serono, grants from Biogen, grants from Teva Pharmaceuticals, grants from Sanofi, grants from Novartis, grants and personal fees from Genentech, Inc, grants and personal fees from Tilos Therapeutics, personal fees from Tiziana Life Sciences, personal fees from IM Therapeutics, personal fees from MedDay Pharmaceuticals, personal fees from vTv Therapeutics, outside the submitted work.

SUPPORT

This study was supported by Octave Bioscience. U.S. Department of Defense (T.C). The CLIMB study has received support from EMD-Serono, the National MS Society, the Nancy Davis Center without Walls and Philanthropy.

CLIMB PATHWAYS TO CURING MS

octave BIOSCIENCE

ECTRIMS 2019

Contact email address:
tchitnis@rics.bwh.harvard.edu

ANNALS of Clinical and Translational Neurology
Open Access
ANA American Neurological Association since 1875

RESEARCH ARTICLE

Neurofilament light chain serum levels correlate with 10-year MRI outcomes in multiple sclerosis

Tanuja Chitnis[1,2,3], Cindy Gonzalez[2,3], Brian C. Healy[2,3,4], Shrishti Saxena[2,3], Mattia Rosso[2,3], Christian Barro[5], Zuzanna Michalak[5], Anu Paul[2,3], Pia Kivisakk[2,3], Camilo Diaz-Cruz[2,3], Neda Sattarnezhad[2,3], Isabelle V. Pierre[2,3], Bonnie I. Glanz[1,2,3], Davorka Tomic[6], Harald Kropshofer[6], Dieter Häring[6], David Leppert[6], Ludwig Kappos[5], Rohit Bakshi[1,2,3], Howard L. Weiner[1,2,3] & Jens Kuhle[5]

[1]Department of Neurology, Partners Multiple Sclerosis Center, Brigham and Women's Hospital, Boston, Massachusetts
[2]Harvard Medical School, Boston, Massachusetts 02115
[3]Ann Romney Center for Neurologic Disease, Harvard Medical School, Boston, Massachusetts 02115
[4]Massachusetts General Hospital Biostatistics Center, Boston, Massachusetts
[5]Departments of Medicine, Biomedicine and Clinical Research, Neurologic Clinic and Policlinic, University Hospital Basel, University of Basel, Basel, Switzerland
[6]Novartis, Neuroscience Development Unit, Basel, Switzerland

Correspondence
Tanuja Chitnis, Partners Multiple Sclerosis Center, Brigham and Women's Hospital, 60 Fenwood Road, 9002K, Boston, MA. Tel: 617 525 6550; Fax: 617 732 6466; E-mail: tchitnis@rics.bwh.harvard.edu

Funding
This study was funded in part by Novartis, the Swiss National Research Foundation (320030_160221) and the U.S. Department of Defense (MS170140). The National MS Society has provided funding for the CLIMB and SUMMIT cohort studies. We thank Merck Serono and the Nancy Davis Center Without Walls for their support of the CLIMB study.

Received: 21 May 2018; Revised: 31 July 2018; Accepted: 6 August 2018

doi: 10.1002/acn3.638

Abstract

Objective: To assess the value of annual serum neurofilament light (NfL) measures in predicting 10-year clinical and MRI outcomes in multiple sclerosis (MS). **Methods**: We identified patients in our center's Comprehensive Longitudinal Investigations in MS at Brigham and Women's Hospital (CLIMB) study enrolled within 5 years of disease onset, and with annual blood samples up to 10 years (n = 122). Serum NfL was measured using a single molecule array (SIMOA) assay. An automated pipeline quantified brain T2 hyperintense lesion volume (T2LV) and brain parenchymal fraction (BPF) from year 10 high-resolution 3T MRI scans. Correlations between averaged annual NfL and 10-year clinical/MRI outcomes were assessed using Spearman's correlation, univariate, and multivariate linear regression models. **Results**: Averaged annual NfL values were negatively associated with year 10 BPF, which included averaged year 1–5 NfL values (unadjusted $P < 0.01$; adjusted analysis $P < 0.01$), and averaged values through year 10. Linear regression analyses of averaged annual NfL values showed multiple associations with T2LV, specifically averaged year 1–5 NfL (unadjusted $P < 0.01$; adjusted analysis $P < 0.01$). Approximately 15–20% of the BPF variance and T2LV could be predicted from early averaged annual NfL levels. Also, averaged annual NfL levels with fatigue score worsening between years 1 and 10 showed statistically significant associations. However, averaged NfL measurements were not associated with year 10 EDSS, SDMT or T25FW in this cohort. **Interpretation**: Serum NfL measured during the first few years after the clinical onset of MS contributed to the prediction of 10-year MRI brain lesion load and atrophy.

Introduction

Multiple sclerosis (MS) is a demyelinating and degenerative disease with a heterogeneous disease course.[1] Patients experience periodic relapses and varying ranges of disability accrual over their lifetime.

Neurofilament light chain (NfL) is a major component of the neuronal cytoskeleton and is important for axonal growth, stability, and intracellular transport.[2,3] NfL are released upon axonal or neuronal damage or degeneration, and can be found as a consequence, in the CSF and blood. Prior studies have shown that NfL concentrations in cerebrospinal fluid (CSF) are associated with the occurrence of MRI lesions, relapses, neurological disability, and treatment status in MS.[4–7] Additional studies have demonstrated predictive value of CSF neurofilament light or heavy chain

levels with clinical outcomes,[8–10] and MRI measures.[11] More recently, single molecule array (SIMOA) based assays, which offer improved sensitivity for detection of molecules, have been used to measure NfL in serum samples. SIMOA-based assays of serum NfL have demonstrated high correlation with CSF values[7,12,13] and potentially provide a more accessible means to monitor MS patients. Serum NfL measurements by SIMOA correlate with disease state as well as short-term outcomes in MS,[12–18] however, the associations of serum NfL levels in predicting longer term outcomes, have not been explored.

In this study, we assessed serum NfL levels collected annually for 10 years in a cohort of MS patients with first sample within the first 5 years of disease onset. We assessed correlation with clinical, cognitive, and MRI outcomes at 10 years.

Methods

Subjects

The MS subjects included in this study were patients enrolled in the Comprehensive Longitudinal Investigation of MS at the Brigham and Women's Hospital (CLIMB, www.climbstudy.org).[19] This study has enrolled over 2100 patients since 2000, and patients are followed longitudinally with biannual standardized clinical exams, annualized MRI scans, and stored blood samples. Subjects in this analysis met additional specific inclusion criteria: (1) enrolled in the quality of life (QOL) subgroup of the CLIMB study; (2) met the diagnostic criteria of MS by the 2010 McDonald criteria at last visit[20]; (3) first blood drawn within 5 years of first symptom onset; (4) at least 8/10 annual blood draws from first collection to year 10; (4) provided consent for sample sharing. EDSS and T2FW are collected in all CLIMB subjects annually. Subjects in the QOL subgroup of CLIMB annually completed several patient reported outcomes (PROs) and this analysis included a fatigue measurement (modified fatigue impact scale, MFIS)[21] and cognition (symbol digit modalities test, SDMT).[22]

Standard protocol approvals, registrations, and patient consents

Institutional Review Board approval was granted by the Partners Human Research Committee, and participants provided written informed consent for participation.

NfL measurements

Serum samples were collected at annual CLIMB visits and were stored at −80°C following standardized procedures. The NfL serum samples were shipped on dry ice from Boston to Basel in a temperature controlled container and were measured by SIMOA assay as previously described.[13] Inter-assay coefficients of variation (CV) for three native serum samples were 10.8%, 8.3%, and 5.7% for control samples with mean concentrations of 9.2 pg/mL, 24.4 pg/mL, and 101.4 pg/mL, respectively. The mean intra-assay CV of duplicated determinations for concentration was 5.1%. Repeat measurements were performed for few samples with intra-assay CV above 20%. 4 samples showed an NfL value below 1.3 pg/mL (i.e., the lower limit of quantification), these were extrapolated from the standard curve and 12 values were measured as zero.

Untransformed NfL levels were used in all analyses. Several subjects were missing NfL measurements at some timepoints, and these subjects were removed from analyses related to that specific timepoint. In some analyses, NfL values were averaged across multiple time points (e.g., averaged yearly 1–2 NfL was calculated by the sum of the year 1 and year 2 NfL, then were divided by (2). If subjects were missing one or more of the values for the interval, the average was calculated using the available measurements. In additional analyses not presented in this paper, log-transformed NfL levels were also analyzed, and we converted all 0's to 1 prior to log transformation.

Clinical outcomes

The primary clinical outcome for our analyses was disability measured by the Expanded Disability Status Scale (EDSS) at year 10. Secondary outcomes at year 10 were SDMT, MFIS, and Timed 25-Foot Walk (T25FW). The SDMT tested executive function and processing speed which was a sensitive early marker of longitudinal cognitive changes in MS. The MFIS was a commonly used measure of fatigue for MS patients, and has three subscale scores (physical, mental, and psychosocial) as well as a total fatigue score. For the T25FW, there were 16 (1.62%) individuals who had high values for T25FW or were unable to complete the walk. For these patients, a score of 25 was assigned to limit the impact of those extreme observations on the analysis. The SDMT, MFIS, and T25FW measurements closest to the 10-year sample were used for analysis. Also, a calculation in the difference between year 10 and year 1 SDMT, T25FW, and MFIS were performed.

MRI acquisition and processing

Brain MRI acquisition protocol was performed on a 3T unit (Siemens Skyra) which used a 20-channel head coil, comprised of 3 sagittal sequences, and covered the whole

head with 1 mm^3 isotropic voxel sizes. This included a 3D T1-weighted gradient echo (TE/TR = 2.96/2300 msec, TI = 900 msec, flip angle = 9 deg), 3D T2 spin echo (TE/TR = 300/2500 msec, echo train length = 160), and 3D T2-FLAIR (TE/TR = 389/5000 msec, TI = 1800 msec, echo train length = 248). The sequences were optimized in contrast for depicting brain-cerebrospinal fluid (CSF) interfaces and white matter lesions. The main steps of the fully automated quantitative analysis pipeline were outlined in Meier et al.[23] Key steps were co-registration of the three MR sequences, anatomical parcellation with heuristic misclassification correction, and an expectation-maximization algorithm. The output provided brain T2 hyperintense lesion volume (T2LV) and brain parenchymal fraction (BPF), a surrogate of whole brain atrophy. This pipeline showed high accuracy and reliability.[23] Intraclass correlation coefficients of 0.95, 0.91, and 0.86 were obtained for T2LV, CSF, and BPF accuracy. A scan-rescan reliability experiment showed coefficients of variation (COVs) of 8%, 2%, and 0.4% for T2LV, CSF volume, and BPF. For this study, BPF values were multiplied by 100 to yield interpretable estimates for our analyses, and T2LV was log transformed due to skewness.

Statistical analysis

MS patients: To assess the potential long-term association between NfL and clinical/MRI outcomes, correlations between each NfL sample year and the 10-year clinical/MRI outcomes were assessed using Spearman's correlation and linear regression. Beyond the individual NfL measurements, the association between averaged yearly NfL values from specific intervals, the 10-year clinical/MRI outcomes, and the year 1 and year 10 differences were also assessed using linear regression models. In addition, multiple linear regression models adjusted for sex, age, and disease duration at baseline. Additionally, in order to quantify the additional variance explained by adding NfL levels to the multiple regression model, we reported the R-squared from reduced (the absence of the averaged yearly NfL) and full models. To further investigate the relationship between NfL and clinical disability, logistic regression was used to compare the relationship of NfL values with year 10 EDSS measurement ($\pm$1.5 years). We additionally performed all analyses using log NfL values and the results were generally similar compared to the untransformed NfL values presented in this paper. Also, given we completed 21 comparisons for each outcome, the Bonferroni corrected alpha level was 0.0024. *P*-values for all analyses will be compared to 0.05 as well as 0.0024 to account for multiple comparisons. All analyses were performed using the Statistical Analysis System (SAS) 9.4 (Cary, NC).

Results

Patients and NFL characteristics

The baseline demographic and clinical characteristics of our MS patient cohort are shown in Table 1. 66% of patients were treated with a DMT at year 1 NfL measurement and the proportion of treated patients increased in year 2 to 85%. The arithmetic mean of NfL values per year show the highest levels at years 1 and 5 (Fig. 1A), and a spaghetti plot of individual MS patient trajectories showed variability (Fig. 1B).

Association of NfL levels with MS clinical outcomes

In the MS cohort, the median EDSS at year 10 was 1.5, and approximately 11% of patients had an EDSS of 3 or higher. We assessed the correlation of each yearly NfL measurement with year 10 EDSS, and only year 2 NfL showed an association ($r_s = 0.21$, $P = 0.04$). When we assessed the association between yearly NfL measurements and averaged yearly measurements with univariate and multiple linear regression models, no statistically significant associations were observed (Table 2 for averaged

Table 1. Participant demographics.

Characteristics	MS (*N* = 122)
Race, *n*(%)	
Black or African American	2 (1.64%)
Missing	1 (0.82%)
More than one race	1 (0.82%)
Unknown or not reported	1 (0.82%)
White	117 (95.90%)
Sex, *n*(%)	
Female	89 (72.95%)
Male	33 (27.05%)
Age at first sample years (mean $\pm$ SD)	37.95 $\pm$ 9.09
Age at first symptom, years (mean $\pm$ SD)	36.35 $\pm$ 9.01
Disease duration at first visit, years (mean $\pm$ SD)	1.61 $\pm$ 1.08
EDSS at year 10, *N* = 117 (mean $\pm$ SD)	1.61 $\pm$ 1.36
T25FW at year 10, *N* = 117 (mean $\pm$ SD)	4.87 $\pm$ 2.80
SDMT at year 10, *N* = 99 (mean $\pm$ SD)	59.16 $\pm$ 13.33
MFIS at year 10, *N* = 79 (mean $\pm$ SD)	21.68 $\pm$ 13.87
3T BPFx100, *N* = 91 (mean $\pm$ SD)	78.32 $\pm$ 3.77
3T Log T2Lesion volume, *N* = 91 (mean $\pm$ SD)	0.79 $\pm$ 1.31
SDMT at year 1, *N* = 27 (mean $\pm$ SD)	53.67 $\pm$ 10.71
SDMT at year 10, *N* = 27 (mean $\pm$ SD)	59.19 $\pm$ 14.40
MFIS at year 1, *N* = 31 (mean $\pm$ SD)	24.45 $\pm$ 17.04
MFIS at year 10, *N* = 31 (mean $\pm$ SD)	20.48 $\pm$ 14.99
T25FW at year 1, *N* = 92 (mean $\pm$ SD)	4.74 $\pm$ 1.09
T25FW at year 10, *N* = 92 (mean $\pm$ SD)	5.02 $\pm$ 3.13

EDSS, expanded disability status scale; T25FW, timed 25-foot walk; SDMT, symbol digit modalities test; MFIS, modified fatigue impact scale.

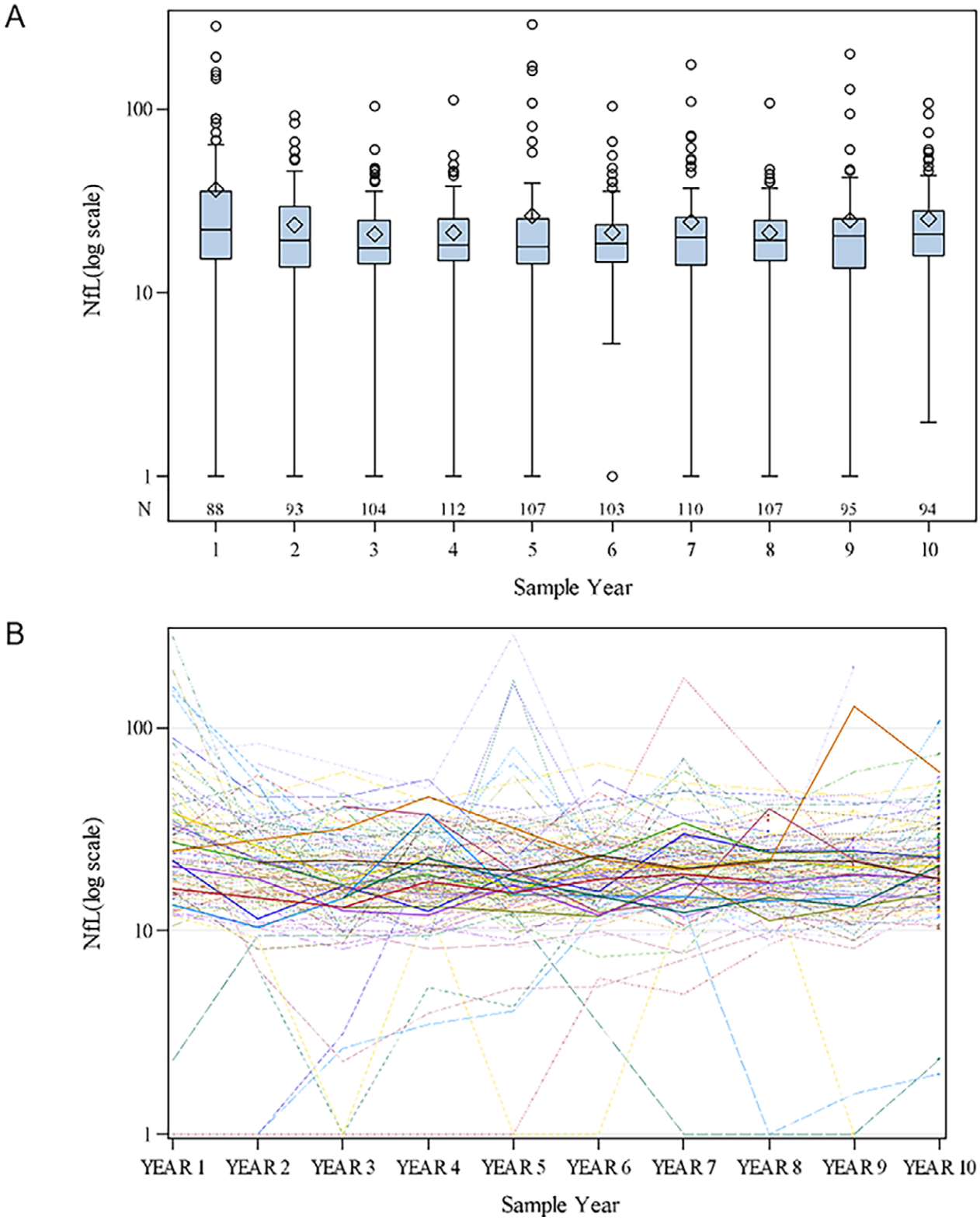


Figure 1. Boxplot of NfL distribution for each year. (A) Boxplot distributions of NfL during each sample year (*N* subjects=122), using the log scale. (B) Spaghetti plot of the arithmetic mean observed trajectories per each subject

Table 2. Linear regression (univariate & multivariate) models show associations of averaged yearly NfL with Year 10 EDSS.

	Univariate					Multivariate						
Variable	*N*	Estimate	95% CI	*P*-value	Correction[1]	*N*	Estimate	95% CI	*P*-value	Correction[1]	R^2 Full	R^2 Reduced
Average year 1–2 NfL	104	0.075	−0.002,0.017	0.1374	1.0000	104	0.076	−0.002,0.018	0.1373	1.0000	0.074	0.053
Average year 1–3 NfL	114	0.103	−0.004,0.024	0.1497	1.0000	114	0.101	−0.004,0.024	0.1642	1.0000	0.073	0.056
Average year 1–4 NfL	117	0.121	−0.005,0.029	0.1716	1.0000	117	0.117	−0.006,0.029	0.1866	1.0000	0.074	0.060
Average year 1–5 NfL	117	0.078	−0.008,0.024	0.3382	1.0000	117	0.066	−0.010,0.023	0.4259	1.0000	0.065	0.060
Average year 1–6 NfL	117	0.096	−0.009,0.028	0.2977	1.0000	117	0.076	−0.011,0.026	0.4180	1.0000	0.065	0.060
Average year 1–7 NfL	117	0.103	−0.010,0.031	0.3151	1.0000	117	0.083	−0.012,0.029	0.4292	1.0000	0.065	0.060
Average year 1–8 NfL	117	0.103	−0.012,0.032	0.3574	1.0000	117	0.074	−0.015,0.030	0.5096	1.0000	0.063	0.060
Average year 1–9 NfL	117	0.087	−0.012,0.030	0.4149	1.0000	117	0.059	−0.015,0.027	0.5838	1.0000	0.062	0.060
Average year 1–10 NfL	117	0.092	−0.012,0.030	0.3941	1.0000	117	0.062	−0.015,0.028	0.5702	1.0000	0.062	0.060
Average year 2–10 NfL	117	0.037	−0.019,0.026	0.7447	1.0000	117	−0.001	−0.022,0.022	0.9950	1.0000	0.060	0.060
Average year 3–10 NfL	117	0.023	−0.020,0.024	0.8357	1.0000	117	−0.015	−0.023,0.020	0.8908	1.0000	0.060	0.060
Average year 4–10 NfL	117	0.029	−0.019,0.024	0.7900	1.0000	117	−0.009	−0.022,0.021	0.9328	1.0000	0.060	0.060
Average year 5–10 NfL	117	0.020	−0.018,0.021	0.8409	1.0000	117	−0.018	−0.021,0.018	0.8527	1.0000	0.060	0.060
Average year 6–10 NfL	117	0.047	−0.017,0.027	0.6733	1.0000	117	0.008	−0.021,0.023	0.9412	1.0000	0.060	0.060
Average year 7–10 NfL	117	0.029	−0.016,0.022	0.7633	1.0000	117	0.001	−0.019,0.019	0.9942	1.0000	0.060	0.060
Average year 8–10 NfL	117	0.064	−0.013,0.026	0.5164	1.0000	117	0.021	−0.017,0.022	0.8277	1.0000	0.060	0.060
Average year 9–10 NfL	110	0.037	−0.008,0.016	0.5398	1.0000	110	0.024	−0.010,0.014	0.6982	1.0000	0.052	0.050
	116	−0.001	−0.015,0.014	0.9839	1.0000	116	−0.030	−0.018,0.012	0.6911	1.0000	0.066	0.065

(*Continued*)

Table 2. Continued.

	Univariate					Multivariate						
Variable	*N*	Estimate	95% CI	*P*-value	Correction[1]	*N*	Estimate	95% CI	*P*-value	Correction[1]	R^2 Full	R^2 Reduced
Average year 5–6 NfL												
Average year 5–7 NfL	117	0.001	−0.018,0.018	0.9954	1.0000	117	−0.028	−0.021,0.015	0.7561	1.0000	0.061	0.060
Average year 5–8 NfL	117	−0.014	−0.022,0.019	0.8928	1.0000	117	−0.055	−0.026,0.015	0.5921	1.0000	0.062	0.060
Average year 5–9 NfL	117	0.001	−0.020,0.020	0.9950	1.0000	117	−0.039	−0.024,0.016	0.6984	1.0000	0.061	0.060

The estimate corresponds to the change in the mean of the outcome for a 10 pg/mL increase in NfL.
[1]Adjusted for multiple comparisons using Bonferroni correction.

yearly NfL and Table S1 for yearly NfL measurements). Further, we did not find any associations of yearly or averaged yearly NfL values with the status of benign (EDSS≤2) or nonbenign (EDSS>2) at year 10 (data not shown).

We next examined the association of NfL with other clinical measures. Averaged NfL levels during years 1–3 were associated with the increase between baseline (year 1) and year 10 fatigue score measured by the MFIS[21] (R^2 full = 0.207, Puncorrected = 0.04, PBonferroni = 0.87, n = 31) (Table 3). When we assessed the association between yearly NfL measurements and averaged yearly measurements with univariate and multiple linear regression models, no statistically significant associations were observed in the changes in year 1 and year 10 for SDMT (Table 4) or T25FW with averaged NfL levels (Table 5). We found no significant associations of either annual or averaged yearly NfL with year 10 SDMT, T25FW, and MFIS measures (data not shown) in univariate or multiple linear regression models.

Association of NfL levels with MRI outcomes and variance

When the associations between NfL levels and year 10 BPF were assessed, we found a negative correlation between year 5 NfL levels with year 10 BPF (r_s=−0.22, P = 0.0479). Linear regression analysis of yearly NfL values and averaged yearly NfL values were provided in Table S2 and Table 6, respectively. Several averaged yearly NfL values had a statistically significant association with year 10 BPF. In the univariate analysis present in Table 6, a 10 pg/mL increase in the average yearly 1–5 NfL was associated with a mean reduction of 0.849% in the BPF (Puncorrected < 0.01, PBonferroni = 0.0035, n = 91). In the multivariate analysis, adjusted for sex, baseline age and disease duration, a 10 pg/mL increase in the averaged yearly 1–5 NfL was associated with a mean reduction of 0.920% in the BPF (Puncorrected < 0.01, PBonferroni = 0.0027, n = 91) (Table 6). Overall, 5% of the variance in BPF was predicted from the variables sex, baseline age and disease duration. While 20% of the BPF variance was predicted from the variables averaged yearly 1–5 NfL, sex, baseline age, and disease duration, 15% variance is accounted for by averaged yearly 1–5 NfL.

When the association between NfL values and year 10 T2LV were assessed, there were positive correlations between years 1 through 4 with T2LV (year 1 r_s = 0.39, P < 0.01; year 2 r_s = 0.38, P < 0.01; year 3 r_s = 0.24, P = 0.04; year 4 r_s = 0.32, P < 0.01), which suggested that higher NfL levels were associated with higher brain lesion load. Table S3 and Table 7 showed the linear regression analysis of yearly NfL values and averaged yearly NfL values. In the univariate analysis, a 10 pg/mL increase in the average yearly 1–5 NfL was associated with a mean log-transformed T2LV increase of 0.307 (Puncorrected < 0.01, PBonferroni = 0.0017, n = 91) (Table 7). When adjusted for sex, baseline age and disease duration, there was a mean log-transformed T2LV increase of 0.335 (Puncorrected < 0.01, PBonferroni = 0.0014, n = 91) (Table 7). Overall, 2% of the variance in log-transformed T2LV was predicted from the variables sex, baseline age, and disease duration, whereas 18% of the variance in log T2LV was predicted from these variables, and including averaged yearly 1–5 NfL.

Table 3. Linear regression (univariate & multivariate) models show associations of averaged yearly NfL with year 1 and year 10 MFIS difference.

	Univariate					Multivariate						
Variable	*N*	Estimate	95% CI	*P*-value	Correction[1]	*N*	Estimate	95% CI	*P*-value	Correction[1]	R^2 Full	R^2 Reduced
Average year 1–2 NfL	31	1.360	−0.032,0.304	0.1083	1.0000	31	1.514	−0.024,0.327	0.0878	1.0000	0.167	0.067
Average year 1–3 NfL	31	2.177	−0.014,0.449	0.0640	1.0000	31	2.506	0.011,0.490	0.0413	0.8668	0.207	0.067
Average year 1–4 NfL	31	3.078	0.024,0.592	0.0346	0.7257	31	3.438	0.052,0.635	0.0227	0.4763	0.238	0.067
Average year 1–5 NfL	31	3.616	0.030,0.693	0.0334	0.7019	31	4.056	0.064,0.747	0.0219	0.4593	0.240	0.067
Average year 1–6 NfL	31	4.019	0.028,0.776	0.0361	0.7589	31	4.662	0.079,0.853	0.0201	0.4223	0.245	0.067
Average year 1–7 NfL	31	4.587	0.025,0.892	0.0389	0.8162	31	5.477	0.102,0.994	0.0180	0.3787	0.250	0.067
Average year 1–8 NfL	31	5.302	0.052,1.008	0.0308	0.6475	31	6.304	0.134,1.127	0.0149	0.3119	0.260	0.067
Average year 1–9 NfL	31	5.231	0.038,1.008	0.0355	0.7462	31	5.827	0.083,1.083	0.0241	0.5068	0.235	0.067
Average year 1–10 NfL	31	5.788	0.082,1.076	0.0240	0.5033	31	6.348	0.121,1.148	0.0174	0.3647	0.252	0.067
Average year 2–10 NfL	31	6.320	0.037,1.227	0.0380	0.7989	31	7.113	0.102,1.321	0.0239	0.5013	0.236	0.067
Average year 3–10 NfL	31	6.123	−0.016,1.240	0.0556	1.0000	31	6.765	0.029,1.324	0.0411	0.8636	0.207	0.067
Average year 4–10 NfL	31	5.511	−0.059,1.162	0.0750	1.0000	31	5.665	−0.065,1.198	0.0766	1.0000	0.174	0.067
Average year 5–10 NfL	31	4.534	−0.140,1.047	0.1293	1.0000	31	4.571	−0.160,1.074	0.1397	1.0000	0.143	0.067
Average year 6–10 NfL	31	4.428	−0.154,1.040	0.1400	1.0000	31	4.336	−0.186,1.053	0.1619	1.0000	0.135	0.067
Average year 7–10 NfL	31	3.674	−0.154,0.889	0.1603	1.0000	31	3.344	−0.215,0.884	0.2218	1.0000	0.120	0.067
Average year 8–10 NfL	31	3.138	−0.110,0.737	0.1406	1.0000	31	2.846	−0.162,0.731	0.2016	1.0000	0.124	0.067
Average year 9–10 NfL	31	1.536	−0.160,0.467	0.3245	1.0000	31	1.304	−0.195,0.456	0.4179	1.0000	0.090	0.067
Average year 5–6 NfL	31	3.168	−0.288,0.921	0.2927	1.0000	31	4.388	−0.224,1.101	0.1850	1.0000	0.129	0.067
Average year 5–7 NfL	31	2.995	−0.399,0.998	0.3879	1.0000	31	4.495	−0.284,1.183	0.2192	1.0000	0.120	0.067
Average year 5–8 NfL	31	5.119	−0.273,1.297	0.1927	1.0000	31	6.519	−0.189,1.493	0.1231	1.0000	0.150	0.067
Average year 5–9 NfL	31	3.840	−0.227,0.995	0.2089	1.0000	31	3.971	−0.233,1.027	0.2067	1.0000	0.123	0.067

The estimate corresponds to the change in the mean of the outcome for a 10 pg/mL increase in NfL.
[1]Adjusted for multiple comparisons using Bonferroni correction.

Discussion

In this study, we found that averaged annual serum NfL levels correlated with 10-year MRI derived brain lesions (T2LV) and whole brain atrophy (BPF) in MS patients. We found an association with increased averaged annual NfL levels with fatigue score worsening between years 1 and 10. However, we did not find significant correlations with clinical measures including EDSS, benign status, SDMT, or T25FW.

Serum NfL levels have emerged as an important measurable biomarker for several neurological diseases which included MS,[12–17] Alzheimer's,[24–26] ALS, and head and spinal cord trauma.[24,27–29] Serum NfL levels have shown

Table 4. Linear regression (univariate & multivariate) models show associations of averaged yearly NfL with year 1 and year 10 SDMT difference.

	Univariate					Multivariate						
Variable	*N*	Estimate	95% CI	*P*-value	Correction[1]	*N*	Estimate	95% CI	*P*-value	Correction[1]	R^2 Full	R^2 Reduced
Average year 1–2 NfL	27	0.163	−0.147,0.179	0.8382	1.0000	27	0.109	−0.164,0.186	0.8985	1.0000	0.056	0.055
Average year 1–3 NfL	27	0.252	−0.264,0.314	0.8590	1.0000	27	0.189	−0.287,0.325	0.8994	1.0000	0.056	0.055
Average year 1–4 NfL	27	0.735	−0.322,0.469	0.7051	1.0000	27	0.656	−0.351,0.482	0.7474	1.0000	0.060	0.055
Average year 1–5 NfL	27	0.152	−0.448,0.478	0.9465	1.0000	27	0.057	−0.481,0.492	0.9807	1.0000	0.055	0.055
Average year 1–6 NfL	27	0.047	−0.528,0.537	0.9856	1.0000	27	−0.017	−0.561,0.558	0.9952	1.0000	0.055	0.055
Average year 1–7 NfL	27	0.108	−0.507,0.529	0.9661	1.0000	27	−0.075	−0.549,0.534	0.9773	1.0000	0.055	0.055
Average year 1–8 NfL	27	0.097	−0.516,0.536	0.9699	1.0000	27	−0.157	−0.567,0.536	0.9534	1.0000	0.056	0.055
Average year 1–9 NfL	27	−0.492	−0.615,0.517	0.8594	1.0000	27	−0.819	−0.676,0.512	0.7776	1.0000	0.059	0.055
Average year 1–10 NfL	27	0.490	−0.547,0.645	0.8669	1.0000	27	0.114	−0.618,0.640	0.9703	1.0000	0.056	0.055
Average year 2–10 NfL	27	0.158	−0.588,0.620	0.9575	1.0000	27	−0.306	−0.677,0.616	0.9226	1.0000	0.056	0.055
Average year 3–10 NfL	27	0.034	−0.604,0.611	0.9910	1.0000	27	−0.447	−0.696,0.606	0.8881	1.0000	0.056	0.055
Average year 4–10 NfL	27	0.296	−0.531,0.590	0.9143	1.0000	27	−0.123	−0.611,0.586	0.9663	1.0000	0.056	0.055
Average year 5–10 NfL	27	−0.127	−0.495,0.470	0.9574	1.0000	27	−0.449	−0.557,0.467	0.8574	1.0000	0.057	0.055
Average year 6–10 NfL	27	0.394	−0.352,0.431	0.8377	1.0000	27	0.152	−0.399,0.429	0.9399	1.0000	0.056	0.055
Average year 7–10 NfL	27	0.562	−0.235,0.347	0.6944	1.0000	27	0.373	−0.271,0.345	0.8041	1.0000	0.058	0.055
Average year 8–10 NfL	27	1.126	−0.505,0.730	0.7103	1.0000	27	0.855	−0.617,0.788	0.8031	1.0000	0.058	0.055
Average year 9–10 NfL	27	0.934	−0.521,0.708	0.7569	1.0000	27	0.600	−0.620,0.740	0.8565	1.0000	0.057	0.055
Average year 5–6 NfL	27	−4.267	−1.066,0.213	0.1815	1.0000	27	−4.421	−1.129,0.245	0.1956	1.0000	0.126	0.055
Average year 5–7 NfL	27	−0.948	−0.474,0.285	0.6114	1.0000	27	−1.093	−0.507,0.288	0.5742	1.0000	0.069	0.055
Average year 5–8 NfL	27	−0.591	−0.445,0.327	0.7552	1.0000	27	−0.781	−0.483,0.327	0.6932	1.0000	0.062	0.055
Average year 5–9 NfL	27	−1.418	−0.614,0.331	0.5420	1.0000	27	−1.727	−0.669,0.324	0.4785	1.0000	0.077	0.055

The estimate corresponds to the change in the mean of the outcome for a 10 pg/mL increase in NfL.
[1]Adjusted for multiple comparisons using Bonferroni correction.

associations with short-term outcomes in MS, however, no published studies have explored long-term outcomes. We examined the value of averaged annual NfL levels on disease course, since annual or periodic measurements may reflect what occurred in clinical practice.

Short-term studies have found that in patients with high baseline NfL, brain volume decreased more rapidly ($P = 0.05$ at 12 months and $P = 0.008$ at 24 months).[15] Our BPF measurements of brain atrophy at 10 years consistently correlated with averaged yearly NfL levels, however, limited strength of association was gained by measurements beyond years 1–5. In fact, point estimates for the averaged values in years 1–2 and 1–3 were similar to the years 1–5 associations, suggesting that early axonal

Table 5. Linear regression (univariate & multivariate) models show associations of averaged yearly NfL with year 1 and year 10 T25FW difference.

	Univariate					Multivariate						
Variable	*N*	Estimate	95% CI	*P*-value	Correction[1]	*N*	Estimate	95% CI	*P*-value	Correction[1]	R^2 Full	R^2 Reduced
Average year 1–2 NfL	80	−0.077	−0.034,0.019	0.5691	1.0000	80	−0.115	−0.039,0.016	0.4123	1.0000	0.053	0.044
Average year 1–3 NfL	89	−0.135	−0.051,0.024	0.4734	1.0000	89	−0.194	−0.058,0.019	0.3221	1.0000	0.051	0.039
Average year 1–4 NfL	92	−0.188	−0.064,0.026	0.4061	1.0000	92	−0.258	−0.072,0.021	0.2749	1.0000	0.052	0.039
Average year 1–5 NfL	92	−0.165	−0.055,0.022	0.3979	1.0000	92	−0.260	−0.067,0.015	0.2085	1.0000	0.056	0.039
Average year 1–6 NfL	92	−0.169	−0.061,0.027	0.4455	1.0000	92	−0.283	−0.075,0.018	0.2267	1.0000	0.055	0.039
Average year 1–7 NfL	92	−0.208	−0.069,0.028	0.3974	1.0000	92	−0.322	−0.083,0.019	0.2113	1.0000	0.056	0.039
Average year 1–8 NfL	92	−0.219	−0.075,0.031	0.4112	1.0000	92	−0.336	−0.088,0.021	0.2251	1.0000	0.055	0.039
Average year 1–9 NfL	92	−0.186	−0.068,0.031	0.4597	1.0000	92	−0.291	−0.081,0.022	0.2647	1.0000	0.052	0.039
Average year 1–10 NfL	92	−0.172	−0.067,0.033	0.4954	1.0000	92	−0.277	−0.079,0.024	0.2875	1.0000	0.051	0.039
Average year 2–10 NfL	92	−0.161	−0.067,0.035	0.5327	1.0000	92	−0.253	−0.077,0.027	0.3371	1.0000	0.049	0.039
Average year 3–10 NfL	92	−0.145	−0.064,0.035	0.5647	1.0000	92	−0.234	−0.074,0.028	0.3629	1.0000	0.048	0.039
Average year 4–10 NfL	92	−0.145	−0.064,0.035	0.5586	1.0000	92	−0.234	−0.074,0.027	0.3563	1.0000	0.048	0.039
Average year 5–10 NfL	92	−0.108	−0.055,0.034	0.6312	1.0000	92	−0.193	−0.065,0.026	0.4017	1.0000	0.046	0.039
Average year 6–10 NfL	92	−0.092	−0.060,0.042	0.7204	1.0000	92	−0.154	−0.067,0.036	0.5541	1.0000	0.042	0.039
Average year 7–10 NfL	92	−0.107	−0.055,0.034	0.6343	1.0000	92	−0.147	−0.059,0.030	0.5141	1.0000	0.043	0.039
Average year 8–10 NfL	92	−0.071	−0.052,0.038	0.7531	1.0000	92	−0.151	−0.061,0.030	0.5114	1.0000	0.043	0.039
Average year 9–10 NfL	85	−0.036	−0.024,0.017	0.7313	1.0000	85	−0.049	−0.026,0.016	0.6466	1.0000	0.008	0.006
Average year 5–6 NfL	92	−0.072	−0.040,0.026	0.6638	1.0000	92	−0.158	−0.050,0.019	0.3656	1.0000	0.048	0.039
Average year 5–7 NfL	92	−0.134	−0.055,0.028	0.5202	1.0000	92	−0.218	−0.064,0.021	0.3102	1.0000	0.050	0.039
Average year 5–8 NfL	92	−0.149	−0.062,0.032	0.5285	1.0000	92	−0.241	−0.072,0.024	0.3187	1.0000	0.050	0.039
Average year 5–9 NfL	92	−0.126	−0.058,0.033	0.5801	1.0000	92	−0.212	−0.067,0.025	0.3619	1.0000	0.048	0.039

The estimate corresponds to the change in the mean of the outcome for a 10 pg/mL increase in NfL.
[1]Adjusted for multiple comparisons using Bonferroni correction.

damage has the greatest impact on 10-year BPF. Further studies to understand the mechanisms and impact of early damage are needed.

T2LV can be considered a cumulative measure of total lesion formation, although it may not reflect the accumulation of all new Gd+ lesions along the disease course, since new lesions may undergo spontaneous regression/repair without leaving a permanent MRI change[30]. Shorter term studies have found correlations between new gadolinium-enhancing lesions and serum NfL values.[13,15,31] Patients with either brain, spinal, or both brain and spinal gadolinium-enhancing lesions had higher serum NfL than those without.[13] Our study found associations between averaged NfL values up to year 9 with

Table 6. Linear regression (univariate & multivariate) models show associations of averaged yearly NfL with BPF.

	Univariate					Multivariate						
Variable	*N*	Estimate	95% CI	*P*-value	Correction[1]	*N*	Estimate	95% CI	*P*-value	Correction[1]	R^2 Full	R^2 Reduced
Average year 1–2 NfL	81	−0.422	−0.068,−0.017	0.0014	0.0291	81	−0.440	−0.070,−0.018	0.0013	0.0280	0.164	0.041
Average year 1–3 NfL	89	−0.645	−0.103,−0.026	0.0012	0.0262	89	−0.683	−0.108,−0.028	0.0011	0.0227	0.156	0.041
Average year 1–4 NfL	91	−0.776	−0.126,−0.029	0.0021	0.0447	91	−0.823	−0.133,−0.032	0.0017	0.0355	0.150	0.046
Average year 1–5 NfL	91	−0.849	−0.128,−0.042	0.0002	0.0035	91	−0.920	−0.137,−0.046	0.0001	0.0027	0.197	0.046
Average year 1–6 NfL	91	−0.922	−0.142,−0.043	0.0004	0.0081	91	−0.985	−0.151,−0.046	0.0004	0.0079	0.177	0.046
Average year 1–7 NfL	91	−0.985	−0.155,−0.041	0.0009	0.0192	91	−1.058	−0.167,−0.045	0.0009	0.0179	0.162	0.046
Average year 1–8 NfL	91	−1.019	−0.165,−0.039	0.0019	0.0402	91	−1.064	−0.174,−0.039	0.0023	0.0478	0.144	0.046
Average year 1–9 NfL	91	−0.972	−0.158,−0.037	0.0020	0.0413	91	−1.004	−0.165,−0.036	0.0026	0.0540	0.142	0.046
Average year 1–10 NfL	91	−0.907	−0.152,−0.029	0.0045	0.0938	91	−0.911	−0.156,−0.026	0.0069	0.1446	0.124	0.046
Average year 2–10 NfL	91	−0.777	−0.145,−0.011	0.0234	0.4911	91	−0.738	−0.144,−0.003	0.0399	0.8378	0.092	0.046
Average year 3–10 NfL	91	−0.673	−0.133,−0.002	0.0442	0.9275	91	−0.624	−0.131,0.006	0.0740	1.0000	0.081	0.046
Average year 4–10 NfL	91	−0.627	−0.128,0.003	0.0611	1.0000	91	−0.560	−0.124,0.012	0.1075	1.0000	0.074	0.046
Average year 5–10 NfL	91	−0.568	−0.116,0.003	0.0605	1.0000	91	−0.496	−0.112,0.012	0.1153	1.0000	0.073	0.046
Average year 6–10 NfL	91	−0.183	−0.095,0.059	0.6368	1.0000	91	−0.054	−0.084,0.073	0.8910	1.0000	0.046	0.046
Average year 7–10 NfL	91	−0.183	−0.090,0.054	0.6151	1.0000	91	−0.060	−0.079,0.067	0.8707	1.0000	0.046	0.046
Average year 8–10 NfL	91	−0.331	−0.093,0.027	0.2775	1.0000	91	−0.213	−0.083,0.040	0.4949	1.0000	0.051	0.046
Average year 9–10 NfL	86	−0.350	−0.072,0.002	0.0659	1.0000	86	−0.303	−0.069,0.008	0.1196	1.0000	0.074	0.046
	90	−0.564	−0.096,−0.017	0.0057	0.1188	90	−0.561	−0.098,−0.014	0.0093	0.1952	0.120	0.047

(*Continued*)

Table 6. Continued.

	Univariate					Multivariate						
Variable	*N*	Estimate	95% CI	*P*-value	Correction[1]	*N*	Estimate	95% CI	*P*-value	Correction[1]	R^2 Full	R^2 Reduced
Average year 5–6 NfL												
Average year 5–7 NfL	91	−0.614	−0.115,−0.008	0.0253	0.5323	91	−0.613	−0.118,−0.004	0.0354	0.7442	0.094	0.046
Average year 5–8 NfL	91	−0.630	−0.127,0.001	0.0524	1.0000	91	−0.582	−0.125,0.009	0.0886	1.0000	0.078	0.046
Average year 5–9 NfL	91	−0.651	−0.125,−0.005	0.0327	0.6865	91	−0.600	−0.123,0.003	0.0607	1.0000	0.084	0.046

The estimate corresponds to the change in the mean of the outcome for a 10 pg/mL increase in NfL.
[1]Adjusted for multiple comparisons using Bonferroni correction.

T2LV measured at or close to year 10, however, the strongest associations was with averaged values from years 1 through 5 with little gain beyond that timepoint. The strongest associations of NfL with both BPF and T2LV were found with the inclusion of year 1 measures, which suggests that early axonal damage is an important contributor toward long-term MRI outcomes.

Our results showed an association with increased average annual NfL levels with increased fatigue scores between year 1–10, measured by the MFIS scale. Fatigue was known as a debilitating symptom, which was frequently reported among MS patients.[32,33] Little is understood about the mechanisms and determinants of fatigue.[34] Axonal damage as measured by N-acetylaspartate-creatinine ratio on proton magnetic resonance spectroscopy scanning have been associated with fatigue in MS patients.[35] Our results supported these findings, and may provide serum NfL as a potential biomarker and predictor of fatigue, which may be utilized in clinical monitoring or for clinical trials.

Our study examined the associations of serum NfL in predicting longer term physical disability measured with EDSS. Year 10 EDSS was not associated with either individual year, or averaged serum NfL levels. We note that in our sample, only a minority of patients (11%) had an EDSS of 3 or higher, and <5% had an EDSS of 6 or greater, which may account for the challenges in distinguishing between patients with relatively low levels of disability. Several studies have shown short-term correlations of serum NfL values and EDSS over periods of 2–3 years, which included more debilitated patients, than in our cohort. In a cohort of 42 MS patients followed in a trial of riluzole over 24 months which included MS patients with high disability scores, serum NfL levels were correlated with EDSS change ($P = 0.009$)[15]. In a study following subjects for a mean of 3.1 years, serum NfL levels were associated with EDSS assessments (beta = 1.105, $P < 0.001$).[13] As discussed above, NfL increases are closely correlated with new T2 lesions, and thus the short-term correlations may reflect the effect of relapses and new lesion formation, which has limited impact on longer term outcomes.

SDMT is a measure of processing speed, commonly used in MS. In our study, 10-year SDMT or change from year 1 through 10 SDMT showed no significant associations with annual or averaged serum NfL values, which suggests that mechanisms other than axonal shedding could contribute to those clinical outcomes. We note that in our sample, SDMT baseline scores were relatively high, did not change significantly over time, and actually improved at year 10, likely reflecting the known practice effect of this test as well as the fact that the majority of patients in the cohort were on treatment. It has been recently shown that in a large SPMS cohort, with much lower SDMT scores at baseline and relatively higher NfL levels than in this study, SDMT scores were significantly correlated with NfL levels at baseline, indicating that higher degree of neuronal damage and more severe cognitive impairment may be needed for establishing a relationship between these measures (Kuhle et al., AAN 2018 S8.006).

The strengths of this study were the longitudinal study design, with annual serum NfL measured in a well characterized cohort, with year 10 clinical and MRI outcomes. The limitations of this study were that not all subjects had MRI scans meeting criteria for analysis, and only one

Table 7. Linear regression (univariate & multivariate) models show associations of averaged yearly NfL with Log T2LV.

	Univariate					Multivariate						
Variable	*N*	Estimate	95% CI	*P*-value	Correction[1]	*N*	Estimate	95% CI	*P*-value	Correction[1]	R^2 Full	R^2 Reduced
Average year 1–2 NfL	81	0.148	0.006,0.024	0.0019	0.0393	81	0.153	0.006,0.025	0.0022	0.0455	0.135	0.020
Average year 1–3 NfL	89	0.247	0.012,0.038	0.0002	0.0051	89	0.261	0.012,0.040	0.0003	0.0056	0.160	0.016
Average year 1–4 NfL	91	0.326	0.016,0.049	0.0002	0.0033	91	0.339	0.017,0.051	0.0002	0.0041	0.165	0.017
Average year 1–5 NfL	91	0.307	0.016,0.045	<.0001	0.0017	91	0.335	0.018,0.049	<.0001	0.0014	0.184	0.017
Average year 1–6 NfL	91	0.340	0.017,0.051	0.0001	0.0030	91	0.372	0.019,0.056	0.0001	0.0025	0.174	0.017
Average year 1–7 NfL	91	0.405	0.021,0.060	<.0001	0.0014	91	0.442	0.023,0.065	<.0001	0.0012	0.187	0.017
Average year 1–8 NfL	91	0.438	0.023,0.065	<.0001	0.0020	91	0.473	0.024,0.070	<.0001	0.0019	0.179	0.017
Average year 1–9 NfL	91	0.396	0.019,0.060	0.0002	0.0049	91	0.425	0.020,0.065	0.0002	0.0050	0.161	0.017
Average year 1–10 NfL	91	0.390	0.018,0.060	0.0004	0.0074	91	0.416	0.019,0.064	0.0004	0.0077	0.153	0.017
Average year 2–10 NfL	91	0.355	0.013,0.058	0.0025	0.0531	91	0.365	0.012,0.061	0.0034	0.0724	0.111	0.017
Average year 3–10 NfL	91	0.320	0.010,0.054	0.0053	0.1116	91	0.326	0.009,0.056	0.0072	0.1518	0.097	0.017
Average year 4–10 NfL	91	0.299	0.008,0.052	0.0093	0.1954	91	0.303	0.007,0.054	0.0122	0.2557	0.087	0.017
Average year 5–10 NfL	91	0.250	0.005,0.045	0.0165	0.3461	91	0.257	0.004,0.047	0.0193	0.4051	0.078	0.017
Average year 6–10 NfL	91	0.220	−0.004,0.048	0.1002	1.0000	91	0.218	−0.005,0.049	0.1136	1.0000	0.046	0.017
Average year 7–10 NfL	91	0.199	−0.005,0.045	0.1110	1.0000	91	0.202	−0.005,0.045	0.1165	1.0000	0.045	0.017
Average year 8–10 NfL	91	0.149	−0.006,0.036	0.1571	1.0000	91	0.156	−0.006,0.037	0.1538	1.0000	0.041	0.017
Average year 9–10 NfL	86	0.100	−0.003,0.023	0.1237	1.0000	86	0.100	−0.003,0.023	0.1388	1.0000	0.038	0.012
Average year 5–6 NfL	90	0.184	0.005,0.032	0.0092	0.1931	90	0.192	0.004,0.034	0.0113	0.2379	0.092	0.020
Average year 5–7 NfL	91	0.273	0.009,0.046	0.0037	0.0786	91	0.284	0.009,0.048	0.0051	0.1071	0.104	0.017
Average year 5–8 NfL	91	0.302	0.009,0.052	0.0068	0.1436	91	0.313	0.008,0.054	0.0083	0.1750	0.094	0.017
Average year 5–9 NfL	91	0.260	0.005,0.046	0.0135	0.2840	91	0.269	0.005,0.049	0.0162	0.3393	0.082	0.017

The estimate corresponds to the change in the mean of the outcome for a 10 pg/mL increase in NfL
[1]Adjusted for multiple comparisons using Bonferroni correction.

MRI time point was used which did not allow for calculating change over time. Also, serum samples and/or clinical outcomes were not available for all subjects for each time point throughout the 10-year period which resulted in lower participant counts for some analyses. This is a highly treated cohort of patients, with limited variability in 10-year EDSS, which potentially limits the ability to detect effects of NfL on EDSS. The variability in NfL values was lower after the second year, especially after the initiation of treatment, thereby potentially limiting predictive ability. DMT can impact NfL levels, however, secondary analysis of our results including only patients on DMT yielded similar results (data not shown). We did not have concurrent CSF samples with our serum

samples, and therefore cannot comment on additional associations of CSF NfL levels in our cohort.

Our study found a correlation of early annual and averaged yearly serum NfL levels with 10-year MRI outcomes, and worsening fatigue measures. The association of early NfL levels with long-term outcomes informs the development of predictive models, potentially identifying patients at risk for more severe disease, and more aggressive treatments. Further analyses will explore effects of specific treatments on NfL levels.[12,36,37] Future studies should validate our findings and explore the additional predictors of long-term disease course and MRI outcomes in multivariate and machine learning models[38].

Acknowledgments

Dr. Tanuja Chitnis had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. The authors thank the following colleagues at the Brigham and Women's Hospital: Mariann Polgar- Turcsanyi, MS and Mark Anderson, MS for their role in managing the Partners MS Center research database, as well as Taylor Saraceno, BA for her assistance in manuscript preparation.

Conflicts of Interest

None declared.

References

1. Weiner HL. The challenge of multiple sclerosis: how do we cure a chronic heterogeneous disease? Ann Neurol 2009;65:239–248.
2. Yabe JT, Chylinski T, Wang FS, et al. Neurofilaments consist of distinct populations that can be distinguished by C-terminal phosphorylation, bundling, and axonal transport rate in growing axonal neurites. J Neurosci 2001;21:2195–2205.
3. Bacioglu M, Maia LF, Preische O, et al. Neurofilament light chain in blood and csf as marker of disease progression in mouse models and in neurodegenerative diseases. Neuron 2016;91:56–66.
4. Lycke JN, Karlsson JE, Andersen O, Rosengren LE. Neurofilament protein in cerebrospinal fluid: a potential marker of activity in multiple sclerosis. J Neurol Neurosurg Psychiatry 1998;64:402–404.
5. Hakansson I, Tisell A, Cassel P, et al. Neurofilament light chain in cerebrospinal fluid and prediction of disease activity in clinically isolated syndrome and relapsing-remitting multiple sclerosis. Eur J Neurol 2017;24:703–712.
6. Teunissen CE, Iacobaeus E, Khademi M, et al. Combination of CSF N-acetylaspartate and neurofilaments in multiple sclerosis. Neurology 2009;72:1322–1329.
7. Novakova L, Axelsson M, Khademi M, et al. Cerebrospinal fluid biomarkers of inflammation and degeneration as measures of fingolimod efficacy in multiple sclerosis. Mult Scler 2016;23:62–71.
8. Soelberg Sorensen P, Sellebjerg F. Neurofilament in CSF-A biomarker of disease activity and long-term prognosis in multiple sclerosis. Mult Scler 2016;22:1112–1113.
9. Salzer J, Svenningsson A, Sundstrom P. Neurofilament light as a prognostic marker in multiple sclerosis. Mult Scler 2010;16:287–292.
10. Martinez MA, Olsson B, Bau L, et al. Glial and neuronal markers in cerebrospinal fluid predict progression in multiple sclerosis. Mult Scler 2015;21:550–561.
11. Petzold A. The prognostic value of CSF neurofilaments in multiple sclerosis at 15-year follow-up. J Neurol Neurosurg Psychiatry 2015;86:1388–1390.
12. Piehl F, Kockum I, Khademi M, et al. Plasma neurofilament light chain levels in patients with MS switching from injectable therapies to fingolimod. Mult Scler. 2018;24:1046–1054.
13. Disanto G, Barro C, Benkert P, et al. Serum Neurofilament light: A biomarker of neuronal damage in multiple sclerosis. Ann Neurol 2017;81:857–870.
14. Disanto G, Adiutori R, Dobson R, et al. Serum neurofilament light chain levels are increased in patients with a clinically isolated syndrome. J Neurol Neurosurg Psychiatry 2016;87:126–129.
15. Kuhle J, Nourbakhsh B, Grant D, et al. Serum neurofilament is associated with progression of brain atrophy and disability in early MS. Neurology 2017;88:826–831.
16. Amor S, van der Star BJ, Bosca I, et al. Neurofilament light antibodies in serum reflect response to natalizumab treatment in multiple sclerosis. Mult Scler 2014;20:1355–1362.
17. Novakova L, Zetterberg H, Sundström P, et al. Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. Neurology 2017;89:2230–2237.
18. Kuhle J, Barro C, Andreasson U, et al. Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. Clin Chem Lab Med 2016;54:1655–1661.
19. Gauthier SA, Glanz BI, Mandel M, Weiner HL. A model for the comprehensive investigation of a chronic autoimmune disease: the multiple sclerosis CLIMB study. Autoimmun Rev 2006;5:532–536.
20. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol 2011;69:292–302.
21. Larson RD. Psychometric properties of the modified fatigue impact scale. Int J MS Care 2013;15:15–20.
22. Benedict RH, DeLuca J, Phillips G, et al. Validity of the symbol digit modalities test as a cognition performance

outcome measure for multiple sclerosis. Mult Scler. 2017;23:721–733.
23. Meier DS, Guttmann CRG, Tummala S, et al. Dual-sensitivity multiple sclerosis lesion and CSF segmentation for multichannel 3T brain MRI. J Neuroimaging 2017;28:36–37.
24. Gaiottino J, Norgren N, Dobson R, et al. Increased neurofilament light chain blood levels in neurodegenerative neurological diseases. PLoS ONE 2013;8:e75091.
25. Weston PSJ, Poole T, Ryan NS, et al. Serum neurofilament light in familial Alzheimer disease: a marker of early neurodegeneration. Neurology 2017;89:2167–2175.
26. Mattsson N, Andreasson U, Zetterberg H, Blennow K. Alzheimer's Disease neuroimaging I. association of plasma neurofilament light with neurodegeneration in patients with Alzheimer Disease. JAMA Neurol 2017;74:557–566.
27. Julien JP. A role for neurofilaments in the pathogenesis of amyotrophic lateral sclerosis. Biochem Cell Biol 1995;73:593–597.
28. Weydt P, Oeckl P, Huss A, et al. Neurofilament levels as biomarkers in asymptomatic and symptomatic familial amyotrophic lateral sclerosis. Ann Neurol 2016;79:152–158.
29. Kuhle J, Gaiottino J, Leppert D, et al. Serum neurofilament light chain is a biomarker of human spinal cord injury severity and outcome. J Neurol Neurosurg Psychiatry 2015;86:273–279.
30. Oommen VV, Tauhid S, Healy BC, et al. the effect of fingolimod on conversion of acute gadolinium-enhancing lesions to chronic T1 hypointensities in multiple sclerosis. J Neuroimaging 2016;26:184–187.
31. Varhaug KN, Barro C, Bjørnevik K, et al. Neurofilament light chain predicts disease activity in relapsing-remitting MS. Neurol Neuroimmunol Neuroinflamm 2018;5:e422.
32. Krupp LB, Elkins LE. Fatigue and declines in cognitive functioning in multiple sclerosis. Neurology 2000;55:934–939.
33. Krupp L. Fatigue is intrinsic to multiple sclerosis (MS) and is the most commonly reported symptom of the disease. Mult Scler. 2006;12:367–368.
34. Newland P, Starkweather A, Sorenson M. Central fatigue in multiple sclerosis: a review of the literature. J Spinal Cord Med 2016;39:386–399.
35. Tartaglia MC, Narayanan S, Francis SJ, et al. The relationship between diffuse axonal damage and fatigue in multiple sclerosis. Arch Neurol 2004;61:201–207.
36. Kuhle J, Disanto G, Lorscheider J, et al. Fingolimod and CSF neurofilament light chain levels in relapsing-remitting multiple sclerosis. Neurology 2015;84:1639–1643.
37. Gunnarsson M, Malmestrom C, Axelsson M, et al. Axonal damage in relapsing multiple sclerosis is markedly reduced by natalizumab. Ann Neurol 2011;69:83–89.
38. Zhao Y, Healy BC, Rotstein D, et al. Exploration of machine learning techniques in predicting multiple sclerosis disease course. PLoS ONE 2017;12: e0174866.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Distribution of clinical outcomes at year 10.
Table S1. Linear regression (univariate & multivariate) models show associations of yearly NfL with Year 10 EDSS.
Table S2. Linear regression (univariate & multivariate) models show associations of yearly NfL with BPF at year 10.
Table S3. Linear regression (univariate & multivariate) models show associations of yearly NfL with T2LV at year 10.

