Markers of axonal injury in blood and tissue triggered by acute and chronic demyelination

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Abstract

Neuroaxonal injury is a major driver of irreversible disability in demyelinating conditions. Accurate assessment of the association between demyelination and axonal pathology is critical for evaluating and developing effective therapeutic approaches. Measuring neurofilament light chain (NfL) in blood could putatively allow longitudinal monitoring of neuroaxonal injury at "single protein resolution" with high pathological specificity. Here, we demonstrate a robust association between blood and tissue NfL-based assessment of neuroaxonal injury and severity of inflammatory demyelination in experimental autoimmune encephalitis (EAE).

In EAE, high levels of NfL were evident at peak of demyelination and correlated with tissue evidence of NfL loss when using antibodies that target the same NfL epitopes. In addition, we validate the longitudinal NfL dynamics in relation to de- and remyelination in an inducible genetic model of inflammatory-independent myelin loss. Through inducible knockout of myelin regulatory protein (Myrf) in proteolipid protein (PLP) expressing cells in $Myrf^{fl/fl}$ PLP1-CreERT ($Myrf^{\Delta iPLP}$) mice, serum NfL peaked at time of demyelination and were reduced following effective remyelination. In people with multiple sclerosis, the most common demyelinating condition, we confirmed the association between NfL and myelin breakdown proteins in two independent cohorts using Olink proximity extension assays, the ReBUILD clinical trial, and the MS participants in the UK-Biobank.

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Our study provides a translational framework to understand the biology behind NfL changes in the context of de- and remyelination and reveals novel aspects related to monitoring potentially reversible neuroaxonal pathology in humans and rodents.

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Introduction

Neuroaxonal injury drives permanent disability in a large number of neurological conditions despite their different underlying pathophysiology. Substantial neuroaxonal injury occurs in Alzheimer's disease, traumatic brain injury, demyelinating diseases (i.e., multiple sclerosis [MS]), motoneuron disease (i.e., amyotrophic lateral sclerosis), and small vessel ischemic disease. An accurate understanding of the timing of neuroaxonal loss and its relationship with the different factors that cause this injury in human diseases and their corresponding animal models is critical for developing effective preventative strategies.

The study of body fluid biomarkers putatively allows monitoring ongoing injury at a "single protein resolution" with high pathological specificity. The measurement of neurofilament light chain (NfL) in the blood has been proposed as a means to track structural neuroaxonal injury longitudinally.[1] Indeed, levels of NfL, a structural component of the neuronal cytoskeleton, are elevated in numerous neurological conditions such as MS, ALS, and stroke, among many others.[1,2] Moreover, NfL levels are lower following treatment that mitigates neuroaxonal insult (e.g., various disease-modifying treatments in MS and Pitisiran in peripheral amyloid neuropathy[3,4]). Recently, the Food and Drug Administration (FDA) granted NfL assays a breakthrough designation to monitor disease activity in MS.[3,5] One particular assay uses antibodies that have been recently shown to target neurodegeneration-specific epitopes in the NfL rod segment.[6]

Despite these promising indications, little is known about the biology behind NfL elevation in neurological conditions in general or specifically in demyelinating diseases like MS. The current

hypothesis postulates that axonal structural damage is followed by NfL diffusion or release in the interstitial fluid, cerebrospinal fluid, and then ultimately to blood, but this theory has not been empirically evaluated. Our recent findings showing lower NfL concentration following therapeutic remyelination challenges the assumption that loss of axonal structural integrity is required for elevated circulating NfL levels. Indeed, active treatment with clemastine, a remyelinating agent without known anti-inflammatory effects or known direct axon modulating effects,[7-10] has been associated with a reduction of NfL concentrations in MS.[11] While remyelination induced neuroprotection could potentially explain the NfL reduction, the short time frame of the ReBUILD study renders this less likely to be the sole explanation. Rather, our previous findings suggest that (1) structural damage of the neuronal cytoplasmic membrane is not a prerequisite for serum NfL elevation, and (2) changes in myelin integrity alone might be sufficient to induce changes in NfL measurements.

To better understand the complex interplay between demyelination, neuroaxonal damage, and circulating NfL levels, we evaluated NfL-based evidence of neurodegeneration in tissue and blood of different animal models of demyelination. In addition, we validated our findings using liquid biopsies from people with multiple sclerosis (pwMS) by applying proteomic approaches on patient-derived samples from two independent cohorts. We hypothesized that demyelination is sufficient to induce neuroaxonal changes that can be quantified through NfL-based measurements.

Materials and methods

Experimental autoimmune encephalomyelitis

As previously described[12] experimental autoimmune encephalomyelitis (EAE) was induced in 8-10-week-old female mice by subcutaneous (s.c.) injection of 200 µg MOG_{35-55} peptide (Genemed Synthesis, San Antonio, TX) emulsified in complete Freund's Adjuvant (CFA) containing 0.2 mg *Mycobacterium tuberculosis* H37Ra into the flanks of both hindlimbs. CFA control animals received s.c. injections without MOG_{35-55} peptide. On the day of immunization and 2 days later, all mice were also administered 300 ng of pertussis toxin (List biological laboratories, Campbell, CA) intraperitoneally. Weights and clinical scores were checked and recorded regularly (score 0.5: distal tail limpness; score 1: complete tail limpness, score 1.5: limp tail and hindlimb weakness,

score 2: mild hindlimb paresis; score 2.5: unilateral hindlimb paralysis; score 3: bilateral hindlimb paralysis; score 4: moribund; score 5: death). No sham mice developed clinical symptoms.

Myrf conditional knockout mice

To determine how genetic demyelination impacts axonal health, we use a genetically modified mouse line that inducibly deletes myelin regulatory factor (*Myrf*) in oligodendrocytes. Conditional knockout of the *Myrf* gene in oligodendrocytes was induced through 5 consecutive days of tamoxifen dosing at 100mg/kg i.p. in corn oil in mice (8-week-old) with homozygous 'floxed' Myrf alleles (JAX 010607), positive or negative for the PLP1-CreERT transgene20 (JAX 005975). $Myrf^{fl/fl}$ PLP1-CreERT ($Myrf^{\Delta iPLP}$)[13] mice allow for the inducible deletion of Myrf in mature oligodendrocytes, resulting in CNS-wide oligodendrocyte loss and demyelination by 10 weeks post-tamoxifen. This is followed by widespread OPC-mediated remyelination by 20 weeks post-tamoxifen.

Rodent blood processing and NfL measurement

Serum was collected through two different approaches. Longitudinal blood in EAE and sham mice was collected from the submandibular veins. In $Myrf^{fl/fl}$ PLP1-CreERT mice, blood was collected through terminal cardiac puncture. Blood was centrifuged for 15 min at 1500 G and serum was collected and stored in -80 till day of analysis. NfL concentration was measured on the Simoa platform using the NF-light advantage kit V2. To encounter the higher concentration in mice, which might go beyond the highest point of the calibrator, serum was bench-diluted to 1:4 or 1:8 (depending on available sample volume). On Simoa, another 1:4 online dilution followed as part of the standard assay procedure, and the final concentration was corrected for the applied dilution factor. Samples were measured by an experienced lab technician blinded to the experimental group. All included samples were included in the analysis.

Immunohistochemistry of EAE

Mice were deeply anesthetized with Avertin and perfused transcardially with 4% paraformaldehyde in 1X PBS. Spinal cord was isolated and postfixed overnight at 4°C in the same solution. Samples were cryoprotected with 30% sucrose in PBS and cryosectioned (20 μm) on a cryostat. Free-floating sections were permeabilized and blocked with 0.2% Triton X-100 and 10%

normal goat serum in 1X PBS for 1hr at room temperature. Sections were incubated with primary antibodies in blocking solution at 4°C overnight. Primary antibodies used were anti-MBP 1:200 (rat MAB386; Millipore) and anti-NfL DegenoTag 1:500 (mouse MCA-1D44; EnCor Biotechnology); Sections were incubated with the secondary antibodies Alexa Fluor 594 and 647 human and mouse respectively (1:1000; all raised in goat; Jackson ImmunoResearch) for 2h at RT. Cell nuclei were labeled with DAPI (Vector Laboratories). Tiled Z-stacks (with 2 µm steps) spanning 20 µm sections of spinal cord, were taken with a Zeiss Axio Imager Z1 with ApoTome attachment and Axiovision software, using a 10X objective.

Immunohistochemistry of Myrf$^{\Delta iPLP}$

10um cryosections of the spinal cord were cut. Sections were blocked in 10% FCS with 0.2% Triton X-100 and stained overnight in the following antibodies: mouse anti-NfL DegenoTag (MCA-1D44; EnCor Biotechnology) at 1:500. Sections were then incubated with the appropriate fluorescent conjugated antibodies (Alexa Fluor; Life Technologies). For all images, Z-stacks (every 2 microns) were acquired. Exposure times and any post-capture adjustments in brightness or contrast were kept constant for all images to be directly compared. For analysis, the Z-stacks were compressed into a single maximum-intensity projection image.

ReBUILD cohort

The ReBUILD trial (NCT02040298)[7]; a double-blind, randomized, placebo-controlled, within-groups comparison trial was conducted to evaluate the remyelinating potentials of clemastine fumarate.[9] 50 PwMS were randomized into two groups; the first group (G1) received daily clemastine fumarate for the first 90 days, followed by a placebo for 60 days. In group 2 (G2), patients were initially treated with a placebo for 90 days, followed by the active substance for 60 days. Visual evoked potentials (VEP) were conducted at each visit, including the screening visit. The primary outcome parameter was the improvement of P100 latency following the initiation of clemastine. The study design included evaluation of relapse history, DMT treatment, EDSS assessment, and focal MRI inflammatory metrics (FLAIR lesions) at each visit.

Serum samples were collected from a subset of participants, who additionally consented to longitudinal blood sample collection at each study visit (baseline, month 1, month 3, month 5). Processed serum was stored at the local biobank at -80°C and all available samples were included

in this analysis. Unbiased blood proteomics analysis was conducted using the proximity extension assay (PEA) with the Olink explorer kit 3072 (Olink®, Uppsala, Sweden) at Olink analytical facility (Boston, USA). In summary, the Olink Explorer kit utilizes compatible antibody pairs to measure 2947 unique proteins over eight 384-plex panels. For each protein, relative protein concentration was reported as normalized protein expression (NPX) on a log-2 scale. Quality control (QC) was conducted for each sample and assay. Sample QC was assessed for each sample using the using internal controls (incubation and Amplification controls) and count per sample. Internal controls NPX beyond ± 0.3 NPX from the plate median or count per sample may not be less than 500 counts, resulting in a sample warning. Assays measuring median NPX of three triplicates of negative control > 5 SD from the predefined values set for each assay received assay warning. Datapoints receiving QC or assay warnings were not included in the final dataset used for the analysis. In addition, we performed a PCA analysis to identify samples with mean NPX > 5 SD of average total samples NPX were also excluded. Here, we conducted a hypothesis-driven analysis focusing on myelin proteins; Myelin oligodendrocyte glycoprotein (MOG), and oligodendrocyte-myelin glycoprotein (OMgp) as well as NfL.

Statistical analysis

Comparison between the NfL levels in EAE and sham mice and different Myrf models was conducted with student t-test, and ANOVA, respectively. The association between longitudinal NPX of myelin-proteins (dependent variables) and NfL was evaluated using mixed linear effect models, accounting for repeated measures per subject. In the multivariant models, we corrected for age at screening, sex, body mass index (BMI), and for potential effect of active treatment with clemastine. The analyses were performed using IBM® SPSS® and R (version 4.2.2).

UK Biobank cohort

Detailed description of UK Biobank Olink measurement and related QC procedures are reported elsewhere.[14] For this analysis, proteomic datasets from 53014 participants were available. We selected participants with the diagnosis of MS (ICD-10 code G35) in the diagnosis data fields (s_41202_0_0 to s_41205_0_11). For those participants, we selected NPX levels for NfL, MOG, and OMgp. The association between MOG, OMgp, and NfL (dependent variable) was assessed

using uni- and multivariant linear regression models (correcting for age, BMI, and sex). The analysis was conducted using Python (version 3.6.8).

Results

Demyelination is associated with evidence of neuroaxonal pathology in experimental autoimmune encephalitis

First, we evaluated blood evidence and corresponding tissue markers of cytoskeleton pathology in an animal model of inflammatory demyelination (EAE). To that end, longitudinal levels of NfL were measured in the same EAE and sham mice at the peak of clinical symptoms in EAE (21 days post-immunization) as well as post-peak (day 35) as well as in the chronic stage (day 68). In addition, we performed immunohistochemistry with myelin markers and NfL DegenoTag™ antibody to define the tissue extent of axonal injury. DegenoTag™ antibody targets the same specific pathological epitopes of NfL that are targeted with one of the antibodies in the highly sensitive single molecule array (Simoa) assay. These epitopes on the NfL rod segment are accessible only following protease-induced neuroaxonal pathology.[6]

In mice that underwent EAE, we observed significant serum NfL elevation at day 21 post-immunization (*n*=4, median [IQR]: 3,844 pg/ml [2,160 - 8,520]) compared to age-matched sham mice (*n*=7, 230 [115 - 326], *P*=0.006) (**Figure 1a, 1b)**. In line with the findings in serum, we found a greater proportion of DegenoTag-positive axons of EAE mice (**Figure 1c)** compared to sham mice. DegenoTag-positive axons colocalized with tissue markers of inflammatory cell infiltration and myelin loss (**Figure 1c)**. These data indicate that serum NfL levels correlate with axonal changes induced by inflammatory-associated demyelination. To further explore the longer-term impact of inflammatory-induced demyelination on neuroaxonal injury, we evaluated NfL concentrations at two additional time points (day 35 and day 68 post-immunization). Indeed, NfL concentrations remained elevated at both time points in EAE compared to sham mice (1,642.0 [1,112.4 - 1,710.0] vs 138.8 [80.8 - 180.0] and 656.0 [436.0 - 748.0] vs 105.8 [51.2 - 136.0], *P*=0.010 and 0.024, respectively) (**Figure 1b)**. Those results support the short and longer-term impact of demyelination on neuroaxonal health in EAE.

However, prominent inflammation in EAE makes it impossible to distinguish the impact of demyelination from the immune-mediated injury on axons. Indeed, inflammatory cascades can damage both myelin and axons coincidentally.[12] To disentangle inflammation-mediated axonal damage from demyelination-induced axonal damage, we evaluated NfL dynamics in a transgenic animal model of demyelination and remyelination that is not driven by immune-mediated attacks on the central nervous system. In $Myrf^{fl/fl}$ PLP CreERT ($Myrf^{\Delta iPLP}$) mice, tamoxifen administration leads to inducible deletion of *Myrf* in mature oligodendrocytes but not oligodendrocyte progenitor cells. Therefore, treated mice will be unable to maintain myelin and suffer from widespread demyelination (peak at week 10), followed by considerable remyelination at week 20.[13-15] In line with findings in EAE, we found NfL elevation at the peak of demyelination (326.6 pg/ml [180.4 – 403.0]), week 10, n=10) compared to the remyelination stage (113.8 pg/ml [108.1 – 165.9], week 20, n=10, P=0.001) and $Myrf^{fl/fl}$ mice (n=23, P<0.001) (**Figure 2a, 2b**). Similar to EAE, the blood NfL peak follows the dynamics of DegenoTag staining in the white matter of the spinal cord section (**Figure 2c-f**). Spontaneous remyelination in $Myrf^{\Delta iPLP}$ mice at week 20 (remyelination) was associated with much lower NfL concentrations compared to samples collected at the peak of demyelination (Week 10) (**Figure 2b)** and a lower number of NfL DegenoTag+ axons (**Figure 2d)**.

Demyelination is associated with neuroaxonal pathology in pwMS

To validate results from the EAE and $Myrf^{\Delta iPLP}$ models in people with MS (pwMS), we evaluated the impact of acute and chronic demyelination on neuroaxonal health. Various physiological and imaging markers have been previously proposed to assess the severity of demyelination in pwMS, but they suffer from numerous shortcomings. While using visually evoked potentials (VEPs) as a measure of de- and remyelination is biologically validated,[12]it is pathway-specific and incapable of distinguishing recent from temporally remote myelin damage. Advanced magnetic resonance imaging (MRI) has recently shown promising results in this direction but lacks cellular specificity.[15,16] Recent approaches, such as PEA, allow for proteome-wide unbiased biomarker discovery with high specificity and sensitivity using next-generation sequencing readout methods. The PEA approach used here through Olink® Explore kit 3072 can measure up to 2947 unique proteins in blood. Of those proteins, MOG levels can reflect myelin injury and correlate with clinical severity markers, cognitive deficits, and structural MRI damage.[17] Another protein of

interest is OMgp, a structural protein of oligodendrocytes but not a structural component of myelin. To assess the relationship between markers of myelin damage and NfL in pwMS, we conducted a proteome-wide analysis of available samples from participants in the ReBUILD trial (**Table 1)**.

ReBUILD is a prospective, case-crossover study that demonstrated the remyelinating potential of clemastine in pwMS without any evidence of acute inflammatory activity, particularly without recent optic neuritis. In this targeted analysis, we found that MOG (a structural myelin protein) and OMgp (structural protein of oligodendrocyte [OL] but not compact myelin) normalized protein expression (NPX) were associated with higher blood levels of NfL (estimate and 95% confidence interval [95% CI]: 0.62 [0.36 – 0.89] and 0.19 [0.08 – 0.30], *P*=<0.001 for both) (**Table 2, Figure 3a-d)**. In addition, we found a similar association between VEP latency and NfL abundance (0.02 [0.01 – 0.03], *P*=0.003). Those associations remained significant after correcting for age, BMI, sex, and any potential impact of treatment with clemastine. Of note, the association between NfL and OMgp was no longer significant when correcting for MOG levels (*P*=0.904).

To further validate the association between MOG and NfL reported in ReBUILD, we leveraged the data from MS participants in a large population study, The Proteomics Project of the UK Biobank. Here, plasma profile was characterized in a subset of the UK Biobank participants.[14] We explored the association between NfL-, MOG-, and OMgp-NPX in UK Biobank MS participants (*n*=407, mean age 54.9 years [±7.8], BMI 26.8 [±5.0], 70.8% females). Only samples and assays passing the QC criteria were included in this analysis. We found a positive association between MOG-NPX and NfL-NPX (*n*=386, univariant coefficient 0.59 [0.42 – 0.75], *P*<0.001, multivariant coefficient 0.45 [0.28 – 0.62], *P*<0.001) (**Figure 4a**). There was no association between OMG-NPX and NfL-NPX (*n*=381, univariant coefficient -0.03 [-0.06 – 0.12], *P*=0.518, multivariant coefficient -0.03 [-0.06 – 0.12], *P*=0.552) (**Figure 4b**).

Discussion

Our study provides a translational framework for understanding the biology behind changes in circulating NfL levels in the context of de- and remyelination. Our findings can be summarized in the following: 1) Blood NfL increases specifically following demyelination and correlates with the severity of myelin injury; 2) These blood NfL changes correlate with the degree of axonal

pathology in the CNS; 3) The association between demyelination and axonal injury is evident in both during the acute and chronic stages of demyelination; 4) Spontaneous remyelination is associated with a reduction of tissue and blood NfL-based markers of neuroaxonal injury. Those results expand our previous findings showing lower NfL levels following therapeutic remyelination with clemastine ReBUILD participants.[11]

We demonstrate high NfL levels during acute demyelination in EAE, an animal model of inflammatory demyelination. This NfL elevation aligns with the large body of evidence showing NfL elevation following clinical and imaging-based inflammatory disease activity in pwMS.[1,3] In EAE, NfL elevation could be attributed to the significant neuroaxonal structural damage following inflammatory cell influx from blood and direct immune-mediated damage of the axons. This assumption is supported by our results showing co-localization of cellular infiltration and DegenoTag+ axons in EAE spinal cords and emerging results from EAE models. There is a growing body of evidence regarding NfL measurements in the context of EAE. In all studies, NfL levels were substantially elevated at peak.[18-21] and at late stages of EAE.[18,19,21] In some of those studies, NfL concentration correlated as well with cellular infiltrates in lesion[18]. Interestingly, most of studies conducted with Simoa revealed concentrations of NfL in a similar range to what was observed in our study. Our findings regarding inflammatory demyelination-associated NfL increase, alongside the other reports of NfL elevation in EAE strongly support the utility of NfL in EAE.

However, the NfL elevation detected in Myrf$^{\Delta iPLP}$, a transgenic animal model of demyelination, is crucial. These data support a robust temporal association between demyelination and NfL elevation without acute immune-mediated demyelination. After the peak of demyelination (Week 10), NfL levels decreased substantially with increasing spontaneous remyelination (Week 20). It is important to recognize that demyelination in the Myrf$^{\Delta iPLP}$ was not associated with reduced axonal count as reported recently.[22] This observation suggests that NfL elevation at Week 10 is not associated with irreversible axonal loss, but likely with reversible axonopathy. Another important observation is the substantially higher levels of serum NfL in EAE mice at peak of demyelination compared to Myrf$^{\Delta iPLP}$. Indeed, inflammatory-induced demyelination and axonal loss is expected to be the main driver of NfL elevation in inflammatory demyelination conditions (such as MS) considering the magnitude and severity of acute structural axonal injury. Of note,

remyelination in Myrf$^{\Delta iPLP}$ is incomplete, which might explain the residually higher NfL concentrations at week 20 compared to Myrf$^{fl/fl}$.

Biomarker analysis from ReBUILD samples and the UK Biobank demonstrated an association between various markers of demyelination and NfL levels. The association in the absence of disease activity in ReBUILD confirms the human biological relevance of the findings from Myrf$^{\Delta iPLP}$ mice. Reversible axonopathy has been previously suggested in the context of lesions for which neuropathology was available in MS.[23] Our results support the use of circulating NfL as an indicator of reversible axonal microstructural changes and not only of irreversible axonal loss. Another interesting observation was the lack of association between OMgp (OL injury) with NfL after correction for MOG (myelin injury). This suggests, *statistically*, that the association between OL injury and axonal pathology is mediated by myelin injury. OL play an important role in supporting axonal health[24]. For example, OL metabolically support axons through proteins such as monocarboxylate transporter 1, which colocalize with myelin proteins (e.g., myelin basic protein and CNPase).[25] Therefore, it could be postulated that myelin loss could result in loss of the main means, through which OL provide such metabolic support.

The findings reported here, and in our previous work,[11] have numerous clinical and translational implications. First, blood NfL measurement can be a powerful tool for monitoring axonal stress due to myelin injury in animal models and people with neurological conditions. NfL elevation should not be considered a definite sign of axonotmesis (i.e., irreversible axonal injury) but potentially a marker of reversible and irreversible axonal pathology and a sign of axonal vulnerability. This is particularly advantageous in contrast to immunohistochemistry used in animal models as it allows in-vivo monitoring of axonal health. Circulating NfL can be measured longitudinally in mice, and standardized measurement enables comparison between experiments conducted under different settings. Furthermore, circulating NfL can serve as a rapidly responsive marker in remyelination trials. NfL measurement is accessible and reproducible. In addition, NfL is remarkably resilient to pre-analytical conditions. [26-28] Thus, remote blood collection to measure NfL could improve accessibility, allow a larger sample size, and enable a short study duration. From the clinical point of view, a more precise understanding of the underlying biological causes for changes in circulating NfL levels will enable clinicians to understand the timing of neurodegeneration in pwMS, validate some of the key pathologic concepts, and hone in on the best window for administration of remyelinating/neuroprotective treatments.

Data availability

Experimental data will be made available upon request to the corresponding authors. Individual-level proteomic data described in this study are available to bona fide researchers as per the UK Biobank data-access protocol (https://www.ukbiobank.ac.uk/enable-your-research/apply-for-access). Further details and instructions about registration for access to the data are available at http://www.ukbiobank.ac.uk/ register-apply/. UK Biobank project number 2112 for this study.

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Competing interests

The authors report no competing interests.

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Figure legends

Figure 1 Blood and tissue evidence of neuroaxonal injury in EAE. (**A**) Mice were immunized with myelin oligodendrocyte glycoprotein (MOG)35-55, and blood and spinal cord were collected at and after the peak of the disease. Created in BioRender. Abdelhak, A. (2025) https://BioRender.com/jba0i1w. (**B**) Serum neurofilament light chain (NfL) concentration was

higher at the peak of experimental autoimmune encephalomyelitis (EAE) compared to sham mice and persistent elevation was seen post-peak (days 35 and 68). (**C**) EAE spinal cord tissue showed a strong increase in the number of DegenoTag+ NfL axons which colocalized with inflammatory cell infiltration and myelin loss.

Figure 2 Blood and tissue markers of neuroaxonal injury in non-inflammatory, inducible models of demyelination. In Myrf$^{fl/fl}$ PLP CreERT (Myrf$^{\Delta iPLP}$) mice, tamoxifen (TAM) injection leads to widespread death of mature oligodendrocytes (Ols) and demyelination, which is largely reversible through differentiation of the endogenous oligodendrocyte progenitor cells (OPCs). **(A)** Blood and tissue samples were collected at week 10 (peak of demyelination) and week 20 (peak of remyelination). Created in BioRender. Abdelhak, A. (2025) https://BioRender.com/py7zb18. **(B)** NfL concentration followed the temporal dynamic of de- and remyelination with high concentrations at week 10 and significant reduction at week 20. **(C)** Similarly, the intensity of DegenoTag+ NfL axons was higher at week 10 compared to week 20 in Myrf$^{\Delta iPLP}$ mice.

Figure 3 Association between blood biomarkers of demyelination, oligodendrocyte injury and neuroaxonal injury in people with MS in ReBUILD study. In the ReBUILD study, **(A)** higher myelin oligodendrocyte glycoprotein (MOG) normalized protein expression (NPX) was associated with higher neurofilament light chain (NfL) NPX in **(B)** both uni- and multivariant mixed linear models. **(C)** There was a similar association between oligodendrocyte injury (oligodendrocyte myelin glycoprotein[OMgp] and NfL NPX in **(D, Multivariant 1)** both uni- and Multivariant mixed models. **(D, Multivariant model 2)** Nevertheless, this association did not remain significant after correction for MOG levels. Beyond markers of acute myelin injury, increased VEP P100 latency as a marker of more distant, chronic demyelination was associated with higher NfL NPX. **Multivariant Model 1**: correcting for age at screening, body mass index, sex, and active treatment with clemastine. **Multivariant Model 2**: Model 1 + correcting for MOG-NPX. Cohen f^2 is a measure of strength of association in mixed models; Cohen's $f^2 \geq 0.02$, ≥ 0.15, and ≥ 0.35 represent small, medium, and large effect sizes, respectively.

Figure 4 Association between blood biomarkers of demyelination, oligodendrocyte injury and neuroaxonal injury in people with MS in UK Biobank. (A) In UK Biobank participants with MS, higher myelin oligodendrocyte glycoprotein (MOG) normalized protein expression (NPX) was associated with higher neurofilament light chain (NfL) NPX in both uni- and multivariant mixed linear models. **(B)** There was no statistically significant association between oligodendrocyte injury (oligodendrocyte myelin glycoprotein [OMgp]) and NfL NPX. Lines and shaded areas represent linear regression estimates and 95% confidence intervals, respectively.

Table 1 Clinical characteristics in the included ReBUILD participants

Clinical criteria of ReBUILD participants (*n* = 47)	
Baseline age in years (mean, SD)	39.36 (10.27)
Sex (Female: Male)	31:16
Baseline BMI (mean, SD)	27.25 (7.54)
Baseline DMT (count, percent)	
Platform Treatments	25 (53.2%)
High Potency Treatments	18 (38.3%)
Treatment-naive	4 (8.5%)
Baseline EDSS (mean, SD)	2.17 (1.07)
Baseline Disease Duration in years (mean, SD)	4.43 (3.60)

BMI = Body mass index; DMT = Disease-modifying treatment; EDSS = Expand disability status scale

Table 2 Association between NfL and evaluated myelin/ oligodendrocyte markers in ReBUILD

NPX (*n* of samples)	**Model**	**Estimate [95% CI]**	***P*-value**	**Cohen's f^2**
MOG (153)	Univariant model	0.62 [0.36 to 0.89]	<0.001	0.21
	Multivariant model 1	0.55 [0.27 to 0.83]	<0.001	0.21
OMgp (149)	Univariant model	0.19 [0.08 to 0.30]	<0.001	0.11
	Multivariant model 1	0.17 [0.05 to 0.28]	0.004	0.12
	Multivariant model 2	0.01 [−0.16 to 0.18]	0.904	0.12

Estimates and 95% confidence interval from mixed linear models assessing the association between NfL NPX (dependent variable) and NPX of MOG/OMgp. Multivariant Model 1: correcting for age at screening, body mass index, sex, and active treatment with clemastine. Multivariant Model 2: Model 1 + correcting for MOG-NPX. Cohen f^2 measures the strength of association; Cohen's f^2 ≥0.02, ≥0.15, and ≥0.35 represent small, medium, and large effect sizes, respectively. NPX = Normalized protein expression; MOG = Myelin oligodendrocyte glycoprotein; OMgp = Oligodendrocyte myelin glycoprotein.

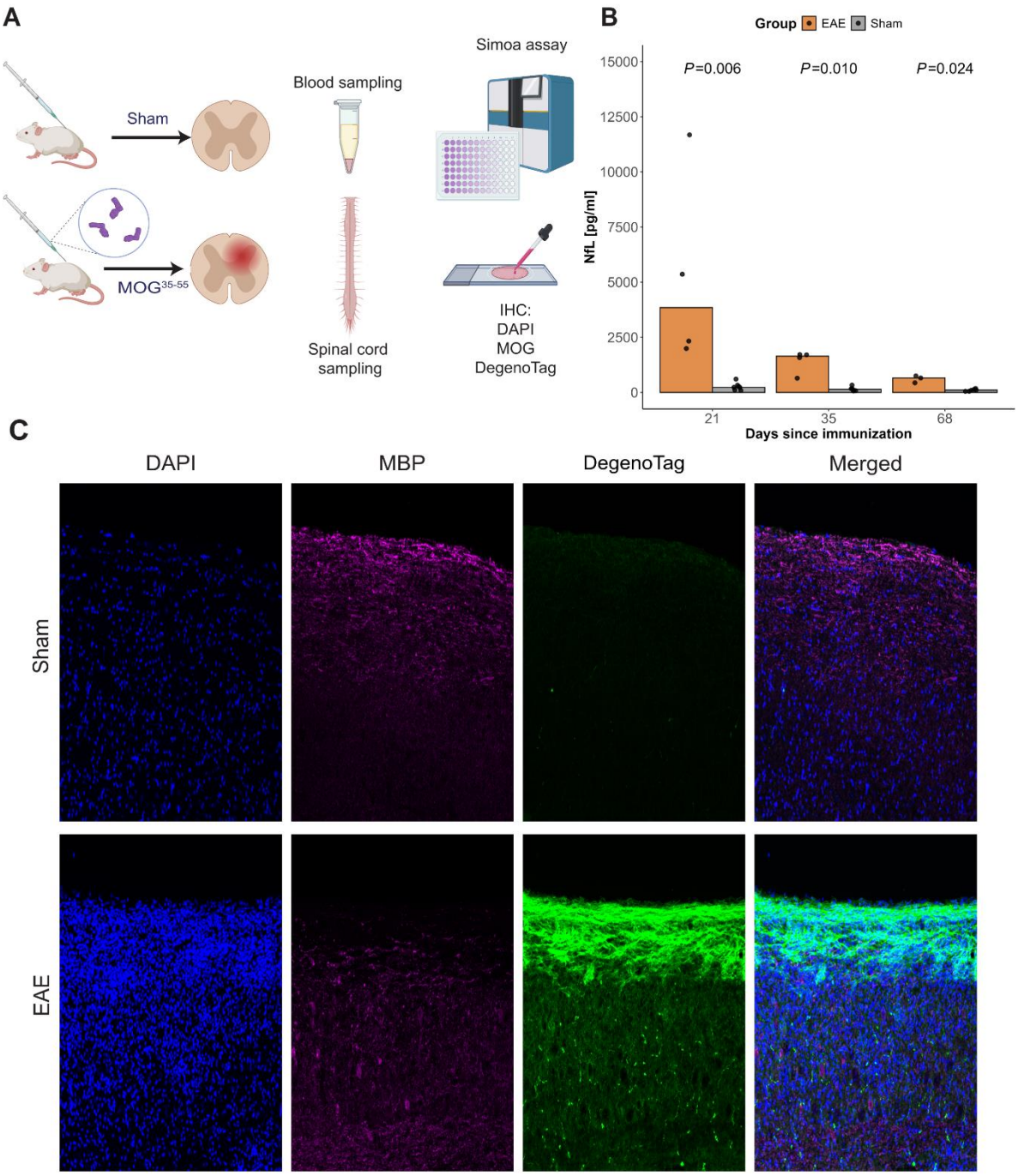

Figure 1
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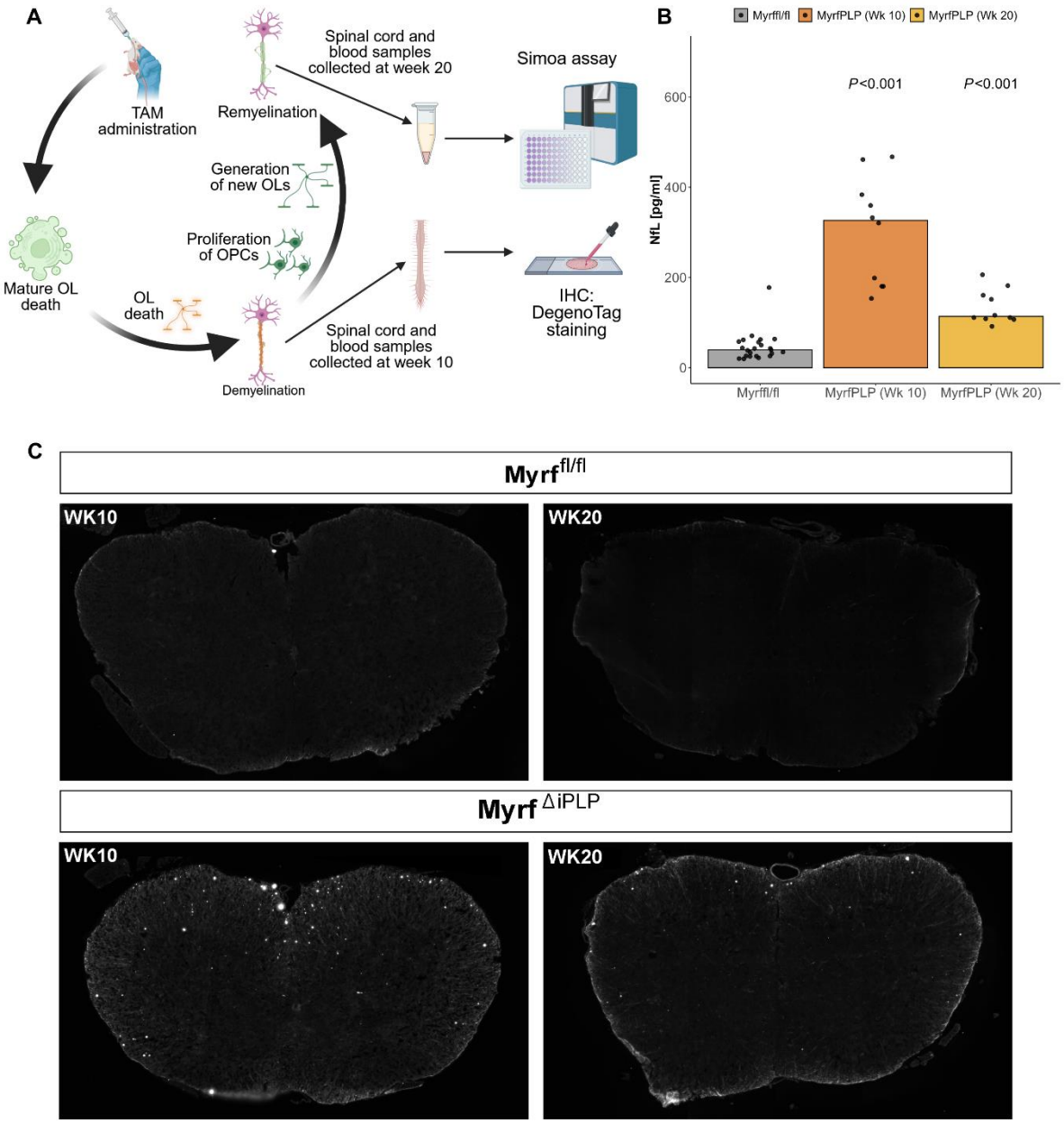

Figure 2
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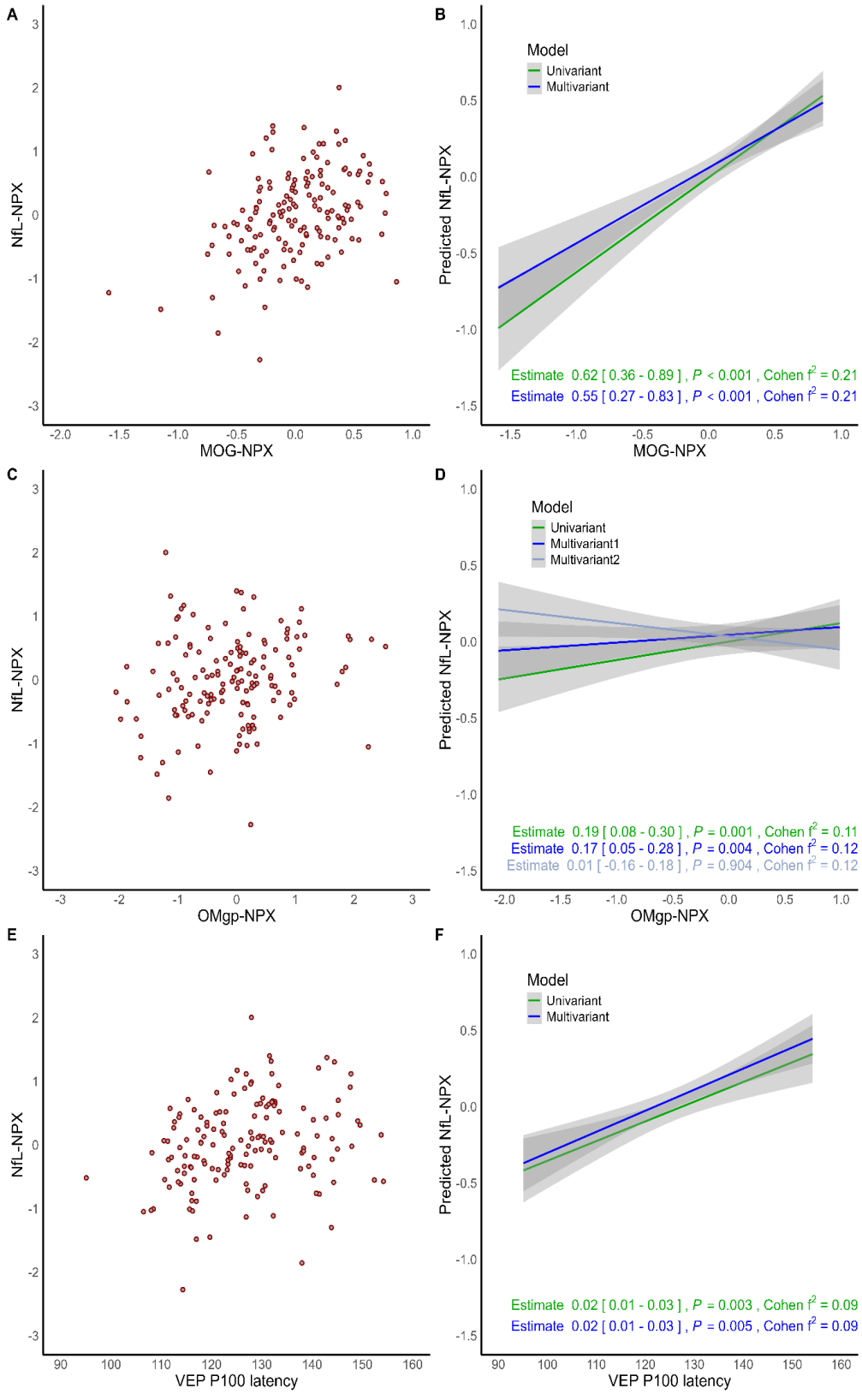

Figure 3
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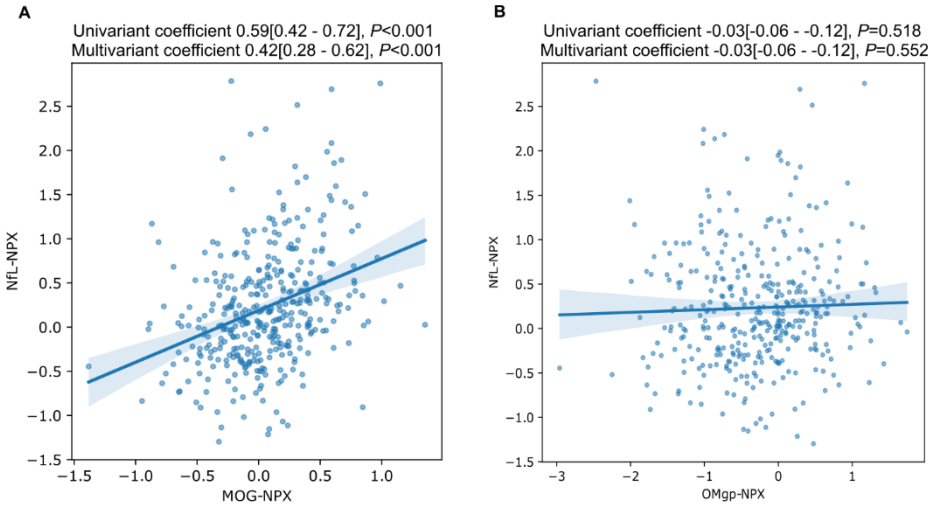

Figure 4
125x67 mm (x DPI)