BIO-01: 14-Year Changes in Plasma Neurofilament Concentrations in Middle-Aged and Older Men

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Background:
Phosphorylated neurofilament heavy (pNF-H) and neurofilament light (NF-L) concentrations represent promising biomarkers linked with neurodegenerative and neuromuscular outcomes. However, less is known about the feasibility of using long-term stored blood samples to measure neurofilament levels and about long-term changes in neurofilament levels among healthy individuals.

Objectives:
To perform a pilot study in preparation for a larger-scale study of the natural history of pNF-H and NF-L.

Methods and design:
For this pilot study, we measured pNF-H and NF-L concentrations in stored blood samples collected 14 years apart from 50 participants in the Physicians' Health Study aged ≥40 years at baseline with no history of stroke, transient ischemic attack, Parkinson's disease, multiple sclerosis, Alzheimer's disease, dementia, amyotrophic lateral sclerosis, or spinal muscular atrophy during follow-up. We also measured the coefficients of variation among 30 individuals with blinded split samples at baseline and 14-year follow-up.

Results:
To examine the role of baseline age, we a priori selected 26 men aged 40-49 years, 12 men aged 50-59 years, and 12 men aged ≥60 years at baseline. The median change in pNF-H over 14 years was 97.1 pg/mL (IQR: 5.0 pg/mL to 242.0 pg/mL), with a Spearman's correlation of 0.67 ($p < 0.01$) between baseline and 14 years. The largest 14-year changes in pNF-H concentrations were observed among men aged 60+ years (median=125.0 pg/mL; IQR: 34.5 pg/mL to 267.0 pg/mL). The median change in NF-L over 14 years was 2.12 pg/mL (IQR: -2.69 pg/mL to 3.39 pg/mL), with a Spearman's correlation of 0.86 ($p < 0.01$) between baseline and 14 years. Similar to the results for pNF-H, the largest 14-year changes in NF-L concentrations were observed among men aged 60+ years (median=8.33 pg/mL; IQR: 7.14 pg/mL to 11.24 pg/mL) at baseline. The overall coefficient of variation was 13.2 for pNF-H and 7.02 for NF-L, indicating adequate assay reproducibility.

Discussion/conclusions:
In this pilot study, we demonstrated the feasibility of measuring pNF-H and NF-L concentrations in stored blood samples and found suggestions that changes in neurofilament concentrations increase with age. Future research is needed to understand factors which may predict increases in pNF-H and NF-L concentrations, and to understand how the natural history of neurofilament levels affects the future risk of neuromuscular outcomes.

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BIO-03: Alterations in Leptin, CCL16 and sTNF-RII as a distinctive plasma immune profile in patients with fast progressing ALS

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Background:
Amyotrophic lateral sclerosis (ALS) is clinically highly heterogeneous disease with survival rate ranging from months to decades following initial diagnosis. Approximately 10-20% of patients develop a rapidly progressive disease and may die within the first year following initial diagnosis [1]. Therefore, there is an increasing need for an early detection of unique molecular signatures associated with more aggressive forms of disease as it may help identify distinct disease-specific therapeutic targets. Growing evidence suggests that chronic deregulation of immune response may represent one of the key elements in the pathobiology of ALS.

Objectives and methods:
To examine whether distinct immune profiles are associated with more aggressive forms of disease, we measured 62 immune markers in plasma of sporadic ALS patients (sALS) (raybiotech cytokines array). We recruited 45 sALS patients and 35 age-matched healthy controls. The immune profiles were then compared between normal (37) vs fast progressing ALS (8). We also measured the immune markers in SOD1G93A mice plasma across disease evolution.

Results:
To our surprise, the major pro-inflammatory markers were not significantly changed in sALS. However, we found that leptin, an important metabolic sensor, was significantly downregulated in plasma of sALS patients ($p=0.0005$) and more importantly in fast progressing disease ($p=0.0477$), while immune markers such as CCL16 ($p=0.0260$) and sTNF-RII ($p=0.0022$) were significantly increased in rapidly progressing disease as compared to normal ALS. We also found that leptin was significantly downregulated in plasma of SOD1G93A mice from pre-onset to late disease stage. The downregulation in leptin was caused by an increased in levels of phospho-AMPK in mice adipocytes, a metabolic sensor which control leptin secretion. In addition, our results revealed that cultured human adipocytes, when exposed to plasma of the fast sALS vs normal sALS expressed reduced levels of leptin and increased levels of phospho-AMPK. This was reversed by the inhibition of AMPK.

Discussion:
Therefore, we suggest that leptin may have a role in modulating the rate of disease progression by its impact on immune-metabolic homeostasis. Based on our preliminary results, we propose that the unique immune/metabolic profile in fast sALS is causing dysfunction in metabolic homeostasis by hyperactivation of AMPK pathways in adipocytes resulting in altered leptin signaling. It is our hope that the results of this study will increase our knowledge about the immune-metabolic signaling in ALS and help identify molecular signatures associated with rapidly progressing disease and possibly identify treatment for fast progressing ALS.

References:
[1] Swinnen B, Robberecht W. The phenotypic variability of amyotrophic lateral sclerosis. Nat Rev Neurol 2014. https://doi.org/10.1038/nrneurol.2014.184.

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BIO-04: An explorative analysis of the effect of CSF neurofilament on the diagnostic delay in ALS

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Background:
A diagnostic delay of approximately one year is observed in patients with ALS, resulting in a high uncertainty for the patient, a missed opportunity for proper treatment and a challenge for clinical trials to include patients at early disease stage.

Objective:
To explore if neurofilaments in cerebrospinal fluid (CSF) could shorten the diagnostic delay.

Methods:
172 patients with ALS, all diagnosed at the Neuromuscular Reference Center (NMRC) of the University Hospitals Leuven according to the revised El Escorial criteria, were included in this observational study with i) 41 patients with a neurofilament assessment by a local neurologist, before referral to the NMRC, ii) 38 patients with a neurofilament assessment at the NMRC and, iii) 93 patients without a neurofilament assessment. A control cohort of 43 patients with a suspicion of a MND, yet finally not diagnosed with ALS was included. All samples were measured for neurofilament light (NfL) and phosphorylated neurofilament heavy (pNfH) with commercially available IVD-labelled immunoassays.

Results:
No differences were observed in age, gender or disease progression rate between the three ALS cohorts. When CSF was collected around 2 months and 10 days (range: 0.7 - 12.0 months; n = 41) before visiting the NMRC and the neurofilament levels were reported prior to referral, the diagnostic delay was significantly shorter (median: 8.00 months, range: 2.37 - 34.1 months) in comparison with patients whose neurofilaments were assessed at the NMRC (median: 12.8 months, range: 2.93 - 86.5 months; $p < 0.05$), but not when compared to the diagnostic delay in patients without neurofilament assessment (median: 9.97 months, range: 2.57 - 43.0 months; $p = 0.16$). The median pNfH and NfL concentration in CSF collected before patients visit a NMRC were 2434 pg/mL (range: 746 - 6211 pg/mL) and 5481 pg/mL (range: 1414 - 15163 pg/mL), respectively, which were higher than in the control cohort (CSF pNfH and NfL concentration of 394 pg/mL (range: 80 - 13478 pg/mL; $p < 0.0001$) and 977 pg/mL (range: 211 - 15738 pg/mL; $p < 0.0001$), respectively). The cutoff value for CSF pNfH was 1130 pg/mL with an area under the curve (AUC) of 0.922 (0.854 - 0.990), yielding a sensitivity of 92.7% (80.6 - 97.5%) and a specificity of 90.7% (78.4 - 96.3%). The cutoff value for CSF NfL was 1890 pg/mL with an AUC of 0.903 (0.828 - 0.978), yielding a sensitivity of 95.1% (83.9 - 99.1%) and a specificity of 81.4% (67.4 - 90.3%).

Discussion:
Our study provides early evidence that neurofilaments in CSF might shorten the diagnostic delay in ALS, possibly by faster referral to the NMRC, with high diagnostic accuracy. Our findings warrant a randomized controlled trial to investigate if neurofilaments can shorten the diagnostic delay in ALS.

BIO-05: Aβ1-42 and Tau as Potential Biomarkers for Diagnosis and Prognosis of Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease, but its definitive diagnosis delays around 12 months. Although the research for biomarkers for ALS diganosis is a highly active field, the absence of specific biomarkers contributes to the long delay in ALS diagnosis. Another strategy of biomarker identification based on less specific but sensitive biomolecules may be of interest in the clinical practice. For example, markers related to other neurodegenerative diseases such as Alzheimer's disease (AD) could be fully explored. Here, we compared baseline levels of amyloidβ1-42 (Aβ1-42), total Tau, and phosphorylated-Tau (phospho-Tau) protein in the cerebrospinal fluid (CSF) of ALS patients to controls and correlated it with clinical parameters of ALS progression collected over 12 months. We observed increased levels of Aβ1-42 (controls: 992.9 ± 358.3 ng/L; ALS: 1277.0 ± 296.6 ng/L; $p < 0.0001$) and increased Aβ1-42/phospho-Tau ratio and Innotest Amyloid Tau Index (IATI) (both $p < 0.0001$). IATI and the phospho-Tau/total Tau ratio correlated positively with ALSFRS-R and weight at baseline. Multivariate analysis revealed that baseline ALSFRS-R was associated with Aβ1-42 and phospho-Tau/total Tau ratio ($p = 0.0109$ and $p = 0.0013$, respectively). Total Tau and phospho-Tau levels correlated negatively with ALSFRS-R variation at months 6 and 9, respectively ($p = 0.02$ and $p = 0.04$, respectively). Phospho-Tau/total Tau ratio correlated positively with ALSFRS-R variation at month 9 ($p = 0.04$). CSF levels of Aβ1-42 could be used as a complementary tool for ALS diagnosis, and total Tau and phospho-Tau levels may help establishing the prognosis of ALS. Further studies merit exploring the pathophysiological mechanisms associated with these markers. Despite their lack of specificity, phospho-Tau/total Tau and Aβ1-42 should be combined to other biological and clinical markers in order to improve ALS management.

BIO-06: Identification of an miRNA fingerprint of ALS/MND in neural-enriched extracellular vesicles from blood plasma

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Background:
Biomarkers for amyotrophic lateral sclerosis/motor neuron disease (ALS/MND) are currently not clinically available but could improve patient outcomes by enabling earlier intervention.

Objectives:
We hypothesized that using a neural-enriched fraction of extracellular vesicles the microRNA (miRNA) fingerprint within would provide a unique ALS signature.

Methods:
Extracellular vesicles (Evs) from ALS/MND patients (Phase IIa human clinical trial, NCT03580616), and controls were extracted from blood plasma using polyethylene glycol. Antibodies (L1CAM) separated the Evs and resulted in a neural-enriched fraction (NEE, Banack et al. 2020). NEE were characterized by ZetaView NTA System and ELIZA. RNA was extracted for Next Generation Sequencing (NGS) and qPCR using ExoRNEasy Kit from Qiagen after which NGS and qPCR were conducted. The entire experiment was replicated using a second cohort of patients and controls.

Results:
NGS of NEEs returned 1704 total small RNA species of which 419 were miRNA and 101 showed differential expression levels between ALS/MND patients and controls. In order to quantitate these findings, we selected 30 miRNA of interest and conducted qPCR to measure differential expression.

Eight miRNA sequences significantly distinguished ALS/MND patients from controls in this replicated experiment of two independent cohorts.

In order to determine the specificity of our eight miRNA fingerprint, we compared these findings to plasma samples from Alzheimer's disease (AD) and additional control samples. We report no significant difference in the eight miRNA markers in AD patients compared to controls. This confirms that our eight miRNA fingerprint is not a measure of neurodegeneration alone but is likely specific to ALS.

Discussion:
Neural-enriched extracellular vesicles from blood plasma resulted in a unique set of miRNA sequences which may yield new insights ALS disease mechanisms. These eight miRNA are being further investigated to see if they can assist in early diagnosis of ALS/MND.

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Banack SA, Dunlop RA, Cox PA. 2020. An miRNA fingerprint using neural-enriched extracellular vesicles from blood plasma: toward a biomarker for ALS/MND. Royal Society Open Biology, 10: 200116. http://dx.doi.org/10.1098/rsob.200116

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BIO-07: pTDP-43 pathology in peripheral motor nerves from patients with ALS: diagnostic and pathogenic implications

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Objective:
We have previously shown that motor nerve biopsy may be used for an early diagnosis in lower motor neuron syndromes (LMNS). Here, we defined the diagnostic performance of motor neve biopsy in amyotrophic lateral sclerosis (ALS) and non-ALS mimics. Most importantly, we sought to demonstrate TAR DNA-binding protein-43 (TDP-43) deposits in motor nerves under the hypothesis that they may represent an useful pathologic biomarker for ALS.

Methods:
We retrospectively evaluated 102 LMNS patients with a diagnostic indication of motor nerve biopsy. Histopathologic criteria of motor neuron disease (MND) and motor neuropathy (MN) were applied by two independent evaluators, who were blind for clinical data. TDP-43 and phosphorylated TDP-43 (pTDP-43) were evaluated by immunohistochemistry (IHC), and results compared to final diagnosis to assess their diagnostic utility in terms of sensitivity and specificity.
Results: Histopathologic patterns for MND and MN yielded a sensitivity of 78·9% and 66·7% and a specificity of 83·9% and 100%, respectively. Higher levels of axonal degeneration were able to predict shorter survival in ALS. An axonal pTDP-43 signal was observed in 98·2% of ALS patients, while 70·2% had pTDP-43 accumulation in Schwann cell cytoplasms. Notably, we were also able to detect pTDP-43 aggregates in ALS cases displaying normal features at standard histopathological analysis.

Interpretation:
Motor nerve biopsy is a useful adjunct for the diagnosis and management of LMNS. We demonstrated that a specific pTDP-43 signature is present in the peripheral nervous system of ALS patients, and might be exploited a specific, accessible tissue biomarker. The demonstration of pTDP-43 aggregates within motor nerves of ALS patients in the earliest stages of disease suggest that this is an early event, that might contribute to ALS pathogenesis, and thus may represent a novel therapeutic target for the new strategies aimed at preventing ALS-linked neurodegeneration.

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BIO-08: Raman Spectroscopy and Mass Spectrometry lipidomics reveal biochemical differences in plasma derived extracellular vesicles from sporadic amyotrophic lateral sclerosis patients

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Background:
There is no validated blood-based biomarker for sporadic Amyotrophic Lateral Sclerosis (ALS). Extracellular vesicles (EVs) have the potential to solve this unmet clinical need; in fact they can be involved in the pathogenesis and/or progression of neurodegenerative diseases (Selmaj et al., 2017). Lipids are essential molecular components of extracellular vesicles, but at the moment the knowledge about their distribution and function in EVs is limited.

Objective:
The aim of this work was to find biomarkers of ALS by investigating biochemical composition of plasma-derived EVs with Raman Spectroscopy (RS) and HPLC-MS (High Performance Liquid Chromatography-Mass Spectrometry).

Methods:
We isolated small and large extracellular vesicles (sEVs and lEVs), from blood plasma of 20 sporadic ALS patients and a matched group of healthy controls, by differential centrifugation/ultracentrifugation.
We characterized sEVs, lEVs and blood plasma firstly by RS and subsequently by HPLC-MS targeting a panel of around 200 lipids.
Statistical analysis included univariate and multivariate analysis techniques such as PCA (Principal Component Analysis) and PLS-DA (Partial Least Squares-Determinant Analysis).

Results:
Raman spectroscopy highlighted lEVs as a particularly promising biomarker for ALS. Raman spectra showed in fact that sporadic ALS patients have a different lipid content and less intense bands relative to the aromatic amino acid phenylalanine.
HPLC-MS revealed some lipid species discriminating between ALS and healthy subjects. They were mainly phospholipids, belonging to the subclasses of phosphatidylcholines (PC), phosphatidylethanolamines (PE) and phosphatidylinositols (PI), and sphingolipids, belonging to the subclasses of ceramides (Cer), mono-hexosyl-ceramides (MHC) and di-hexosylceramides. In particular the increase of PC(34:1), MHC(24:1) and Cer(24:1) was observed in either plasma, lEVs and sEVs from ALS patients. Some phosphatidylinositols exhibited a higher concentration in ALS EVs lipidome, with the species PI(36:3) upregulated in both large and small vesicles.

Discussion:
Interestingly, some species significantly altered in our analysis of plasma lipidome, overlap with the ones highlighted by Blasco et al. in their work on cerebrospinal fluid (CSF) (Blasco et al. 2017), namely the phosphatidylcholine PC(38:2) the mono-hexosyl-ceramides MHC(24 :1) and the plasmalogen PCO(34 :1). This supports the idea of plasma and plasma-derived EVs as easily available source of robust biomarkers. Among the other results, the perturbed sphingolipids are particularly relevant as they are involved in key pathways for ALS patogenesis, such as autophagy, energy metabolism and neuroinflammation.

References:
Selmaj I. et al., J Neuroimmunol., 2017; 306:1–10
Blasco H. et al., Scientific Report, 2017; 7(1):17652

BIO-09: Gamma fibrinogen as a predictor of survival in Amyotrophic Lateral Sclerosis

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Background:
Amyotrophic lateral sclerosis (ALS) is an aggressive neurodegenerative disorder related to neuroinflammation that is associated with increased risk of thrombosis.

Objectives:
We aimed to evaluate γ' fibrinogen plasma level (an in vivo variant of fibrinogen) as a biomarker in ALS, and to test its role as a predictor of disease progression and survival.

Methods:
We studied 67 consecutive ALS patients followed in our center. In the same period and using the same methodology we studied 82 healthy controls. Plasma γ' fibrinogen levels were measured in plasma by ELISA. We performed Cox regressions firstly by grouping ALS patients by γ' fibrinogen levels.

Results@
γ' fibrinogen levels in plasma were significantly higher in the ALS patients (51.58 ± 24.50 mg/dL) than in controls (38.66 ± 16.65 mg/dL), $p < 0.001$. The survival analysis (via Cox proportional hazard regressions) provided strong evidence towards the existence of an association between higher γ' fibrinogen levels and longer survival in ALS patients.

Discussion:
We disclosed positive associations between γ' fibrinogen levels and both motor function (assessed by ALSFRS-R global scores) . We found, for the first time to our knowledge, a link between γ' fibrinogen level and survival: patients with higher γ' fibrinogen plasma levels survived longer.
Remarkably, we found that increased levels of γ' fibrinogen can have a neuroprotective role increasing survival of ALS patients, which is against a possible action promoting neuro-inflammation and causing neuronal degeneration.
Our findings regarding the association between γ' fibrinogen and survival suggests that this new avenue should be further investigated in ALS.

BIO-10: Cross-site validation of the diagnostic accuracy of a ncRNA biomarker panel in ALS

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Objective biomarkers for the clinically heterogeneous adult-onset neurodegenerative disorder amyotrophic lateral sclerosis (ALS) are crucial to facilitate assessing emerging therapeutics, and improve the diagnostic pathway. We have studied non-coding RNA (ncRNA) transcripts, including microRNA (miRNA), piwi-RNA (piRNA), and transfer RNA (tRNA) present in human biofluids.

Serum samples from our Oxford-based discovery cohort of ALS patients, disease mimics, and age- and sex-matched healthy controls (n=81) were profiled for ncRNA expression using RNA-seq, which showed a wide range of ncRNA to be dysregulated. We confirmed significant alterations with RT-qPCR in the expression of hsa-miR-16-5p, hsa-miR-21-5p, hsa-miR-92a-3p, hsa-piR-33151, TRV-AAC4-1.1, and TRA-AGC6-1.1. Furthermore, hsa-miR-206, previously associated with ALS, showed a binary-like pattern of expression in our samples. Using the expression of these ncRNA, we were able to discriminate ALS samples from healthy controls in our discovery cohort using a random forest analysis with 94% classification accuracy with promise in predicting progression rate.

Cross-validation of this novel signature was undertaken using a geographically distinct cohort of samples containing ALS, disease mimics, neurological controls, and healthy control samples from the Queen Mary University of London (n=141; classification accuracy 75%) and from the University of Ulm (n=130; classification accuracy 43%). Compared with the very consistent cross-site experience with other ALS biomarkers e.g. neurofilaments and chitinases, ncRNA may be susceptible to more subtle variations in sample acquisition, processing and storage. Multi-centre, collaborative 'round robin' analyses are needed to understand these.

BIO-11: CSF t-Tau levels are predictive of a shorter survival in patients with Amyotrophic Lateral Sclerosis

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Aims:
Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder, characterized by a rapid progression and a relatively short survival. Several studies have been performed to identify biomarkers useful for diagnostic and prognostic purposes. Over the years, much debate has occurred about the role of total Tau (tTau) and Tau phosphorylated at threonine 181 (pTau) as potential biomarker in ALS and how changes in their CSF levels could be interpreted (1, 2).

In the present study, we assayed pTau and tTau levels and calculated pTau/tTau (Tau ratio) in CSF of ALS patients and disease controls (i.e., patients affected by non-neurodegenerative diseases)(CTRL), and evaluated their relationship with different clinical variables, the rate of progression and survival.

Patients and Methods:
A total of 197 ALS patients (Bulbar: 35.1%; Spinal: 64.9%; M/F:1.21) and 91 CTRL were recruited. Demographic and clinical variables were recorded, and ΔFS was used to rate the disease progression. For each patient and control, CSF was taken at the time of the diagnostic work-up and stored following the published guidelines. CSF tTau and pTau were assayed with commercially available chemiluminescence enzyme immunoassay (CLEIA) kits on a fully automated platform, according to the manufacturer.

Results:
Significant differences between ALS and CTRL were found in tTau levels [ALS: 246pg/ml (IQR:168-335) and CTRL: 146pg/ml (IQR:87-251); $p<0.001$] and pTau/tTau ratio [ALS: 0.12 (0.09-0.14) and CTRL 0.18 (0.14-0.26); $p<0.001$), but no in pTau levels [ALS: 27.3(21-36) and CTRL: 23.9 (17-34); $p=0.073$]. ROC analyses showed a discrete specificity for tTau [58% C.I. (47-69)] and for pTau/tTau [66% C.I. (49-80)] in discriminating ALS patients from CTRL, in relation with an higher sensitivity [tTau: 76% C.I. (69-81); pTau/tTau ratio: 83% C.I.(77-87)].
CSF pTau and tTau levels were then studied in ALS according to different demographic and clinical variables. CSF Tau proteins correlated positively with age at onset (pTau: $r=0.358$, $p<0.001$; tTau: $r=0.358$, $p<0.001$) and rate of progression (pTau: $r=0.157$, $p=0.033$; tTau: $r=0.162$, $p=0.027$), and negatively with survival (pTau: $r= - 0.194$ $p=0.040$; tTau: $r=- 0.282$, $p=0.002$). Kaplan– Meier survival curves of ALS patients showed a significantly shorter survival of patients with higher CSF tTau ($p= 0.005$; Log- Rank test), but no in patients with higher CSF pTau ($p=0.61$).

Conclusions:
Higher CSF tTau levels at diagnosis time are indicative of a shorter survival in ALS patients, making it a potential prognostic biomarker useful for stratification and management of ALS patients.

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BIO-12: Decoding distinctive features of plasma extracellular vesicles in amyotrophic lateral sclerosis

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Background:
Amyotrophic lateral sclerosis (ALS) is a multifactorial, multisystem motor neuron disease for which currently there is no effective treatment. There is an urgent need to identify biomarkers to tackle the disease's complexity and help in early diagnosis, prognosis, and therapy. Extracellular vesicles (EVs) are nanostructures released by any cell type into body fluids. Their biophysical and biochemical characteristics vary with the parent cell's physiological and pathological state and make them an attractive source of multidimensional data for patient classification and stratification.
Objectives:
To define whether plasma EVs are potential prognostic and diagnostic biomarkers in ALS.

Methods:
We analyzed plasma-derived EVs of ALS patients (n= 60) and controls (n= 56), and SOD1G93A and TDP-43Q331K mouse models of ALS. We purified plasma EVs by innovative nickel-based isolation, characterized their EV size distribution and morphology respectively by nanotracking analysis and transmission electron microscopy, and analyzed EV markers and protein cargos by Western blot and proteomics. We used machine learning techniques to predict diagnosis and prognosis.
Results:
Our procedure resulted in high-yield isolation of intact and polydisperse plasma EVs, with minimal lipoprotein contamination. There were more particles in the plasma of ALS patients and the two mouse models of ALS while their average diameter was smaller. HSP90 and phosphorylated TDP-43 were differentially represented in ALS patients and mice compared to the controls. In terms of disease progression, the levels of phosphorylated TDP-43 and cyclophilin A, with the EV size distribution, distinguished fast and slow disease progressors, suggesting a new means for patient stratification.
Discussion:
Our analysis unmasked features in plasma EVs of ALS patients with potential straightforward clinical application. We conceived an innovative mathematical model based on machine learning which, by integrating EV size distribution data with protein cargoes, gave very high prediction rates for disease diagnosis and prognosis.
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Acknowledgements: This project received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 752470 (to M.B.); grants from the Italian Ministry of Health (GR-2016-02361552 to M.B.), from Intesa San Paolo S.p.A., project no. B/2018/0061 (to V.B.).

BIO-13: Do measures of neurofilament light chain reflect upper vs. lower motor neuron dysfunction?

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Background:
Elevated levels of neurofilament proteins have been reported in several neurodegenerative disorders and are considered to be a biomarker of axonal breakdown. Levels of serum neurofilament light chain (NfL) have been noted to be higher in ALS than in other disorders. HYPOTHESIS: Serum NfL levels reflect a greater contribution from the breakdown of motor axons in the periphery than central neuronal degeneration.

Methods:
We examined whether clinical signs of upper motor neuron (UMN) or lower motor neuron (LMN) were correlated with serum or CSF NfL levels in a cohort of 50 carriers of C9orf72 expansion mutations. Clinically the cohort was heterogeneous with a range of ALS-FTD phenotypes and has been previously described (Floeter et al Neurology 2017). CSF and/or serum were obtained from 1 to 4 evaluations in each patient over an 18-30 month period. Serum NfL levels were available for 121 visits; CSF NfL levels were available for 86 visits. To quantify severity of LMN involvement, points were given for clinic exam signs of atrophy, fasciculations, and weakness in cranial and limb muscles (maximum score =57). To quantify severity of UMN involvement, points were given for clinical exam signs of spastic tone, hyperactive DTRs, pathological reflexes, presence of any frontal release sign (maximum score = 18). Levels of CSF NfL were measured using quanterix Simoa assay in multiple batches. Levels of serum NfL were measured using quanterix Simoa assay, as a batch using a single kit.

Results:
CSF and serum NfL levels were strongly correlated ($R=0.76$, $p < 0.001$) for the 85 visits where both were available. LMN scores were correlated with both serum NfL and CSF NfL to a similar extent (serum NfL: $R=0.58$; CSF NfL $R=0.62$). UMN scores correlated with serum NfL ($R=0.70$) and CSF NfL ($R=0.56$). All correlations were significant ($P<0.001$).

Conclusion:
Measures of serum and CSF NfL are correlated with disease severity, but do not distinguish between the severity of upper and lower motor neuron findings on clinical examination.

Acknowledgements:
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BIO-14: Increased natural killer lymphocytes in blood of patients with amyotrophic lateral sclerosis and their role in the disease progression

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Aims:
Neuroinflammation is involved in the degeneration and progression of Amyotrophic Lateral Sclerosis (ALS) (1). Natural Killer (NK) lymphocytes may play a role in the pathophysiology of the disease (2-4). Here, we studied the role of the circulating lymphocytes in ALS. In this preliminary report we focused on the relationship between blood lymphocytes changes, ALS clinical subtype and disease severity.

Subjects and Methods:
Blood samples were collected from 42 patients with sporadic ALS, 8 patients with other MND and 14 patients affected by primary progressive multiple sclerosis (PPMS) with inactive plaques. Circulating lymphocytes were analyzed by flow cytometry on a FACSC-anto II Flow Cytometer, upon staining cells with the following antibodies: antiCD3-FITC, antiCD16, antiCD56-PE, antiCD45-PerCpCy5.5, antiCD19-PeCy7, antiCD8-APC and antiCD4-APCH7. For each sample, values were expressed as both percent and absolute number (n/µl) of viable lymphocytes. Reference values for the adult healthy population were taken from Comans-Bitter et al,1997 (5). Both percent and absolute values of lymphomonocyte subpopulations (T lymphocyte CD3+, CD3+/CD4+T, CD3+/CD8+T, CD19+ B cells and CD3-/CD56+ NK cells) in ALS were compared with those of other MND and PPMS. Correlations with site of onset, changes in ALSFRS-R and disease progression rate (calculated as ΔFS score) were made.

Results:
Age at onset was 68y(63-73) in ALS (spinal 71%; bulbar, 29%), 68y(60-74) in MNDs and 50y(40-59) PPMS. Absolute blood levels of the lymphocytes in the different cohorts were within normal range (5). Furthermore, while levels of lymphocytes T and B were not different between disease groups, NK cells (n/µl) were increased in the ALS cohort (ALS=210[142-339] vs MND=138[108-196] and PPMS=149[112-211], p=0.038). In ALS, NK cells correlated with the disease duration (r=0.38,p=0.011) and blood levels were lower in patients with a rapid disease progression, though the differences between groups did not reach significance (Slow=249[147-351] vs intermediate=179[143-321] and rapid=157[124-250],p=0.36). Site of onset did not affect the levels of the different lymphocytes' populations.

Conclusions:
In this preliminary report, on relatively small ALS and disease control cohorts, we show that blood NK cells are selectively increased in ALS and that their level appear lower in the rapid progressing patients. Site of onset does not affect the NK levels. Other variables are being studied. NK cells may infiltrate degenerating motorneurons, especially in rapidly progressing patients (6), which would explain the reduced levels in this group. This reinforces the suggested role of NK cells in modulating the disease progression in ALS.

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BIO-15: Monocyte-derived Macrophages contribute to Chitinolytic Dysregulation in Amyotrophic Lateral Sclerosis: A Pilot Study

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Background:
Neuroinflammation significantly contributes to ALS pathology. Reports of elevated chitinase levels in ALS are interesting as they are surrogate markers of a chronic inflammatory response. Although post-mortem studies have reported glial expression of CHIT1 and CHI3L1, the cellular origins of these targets remain to be fully understood.
Objectives: This pilot study aimed to examine whether the peripheral immune system also contributes to chitinolytic dysregulation in ALS. Therefore, we examined the temporal expression of CHIT1, CHI3L1 and, CHI3L2 in non-polarized monocyte-derived macrophages (MoMas) in patients with ALS and healthy controls (HCs).

Methods:
MoMas were cultured over 9 days from a) patients with confirmed ALS (n = 8), b) and HCs (n = 8). Cell lysates and supernatants were collected at Days 1, 3, 6, and 9. CHIT1, CHI3L1, and CHI3L2 expression were evaluated at the transcriptomic and secreted protein level. The ALSFRS-R and the novel D50 progression model were used to quantify disease aggressiveness. Two-way mixed ANOVAs were used to assess between-group differences. Statistical significance was set at $p < 0.05$.

Results:
Age was included as a covariate in all analyses as ALS patients were significantly older than HCs. CHIT1 and CHI3L1 displayed similar temporal expression patterns in both groups; relative gene and protein expression for both targets were minimal at earlier timepoints and increased over time. However, profound between-group differences were apparent for the later timepoints i.e. when monocytes were fully differentiated. Relative CHIT1 expression was significantly higher in ALS MoMas on Days 6 and 9 ($p < 0.01$); this was recapitulated at the protein level, wherein ALS MoMas secreted significantly higher CHIT1 amounts on Day 9. This group-effect persisted despite the inclusion of age as a covariate. An analogous upregulation was noted for CHI3L1: expression at both the transcriptomic and protein level was significantly higher for the ALS group at later timepoints ($p = 0.01$). However, this effect did not retain significance after accounting for age. Finally, neither a time- or group-driven regulation was noted for CHI3L2.

Discussion:
Here, we demonstrate that non-polarized MoMas contribute to chitinolytic dysregulation in ALS. CHIT1 and CHI3L1 expression are influenced by disease state and time, while CHI3L2 isn't, indicating that individual chitinases display distinct regulatory profiles. These data are particularly interesting given evidence from other conditions that chitinase secretion is a feature of pro-inflammatory "M1-like" macrophages and that monocytes from ALS patients are more readily differentiated towards an M1 phenotype. Studies with larger age-matched cohorts are required to 1) distinguish between the effects of age and disease and 2) examine the implications for disease activity.

Acknowledgements:
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BIO-16: Network analysis of the CSF proteome supports TDP-43 dysfunction as a core element of ALS pathogenesis

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Background:
Loss of protein homeostasis is a theme common to neurodegenerative disorders, and cytoplasmic accumulation of TDP-43 aggregates is the pathological hallmark of nearly all cases of ALS. Disruption of the cellular functions of TDP-43 have been implicated as the core pathogenic event, but arising from a broad range of upstream perturbations. While proteomic analysis of CSF has identified many individual protein biomarkers, analysis of the proteome at a network level allows the identification of potentially convergent pathways in pathogenesis.

Objectives:
To characterise the CSF proteomic correlation network in healthy controls in comparison with ALS and Parkinson's disease.

Methods:
Analysis of CSF proteomic data from patients with apparently sporadic ALS (n=43), idiopathic Parkinson's disease (n=20) and healthy age-similar control participants (n=20) was performed. Weighted correlation network analysis identified modules within the CSF protein network with gene ontology enrichment analysis to identify functional annotation of module proteins. Differential correlation analysis of the CSF protein network was used to compare ALS and Parkinson's disease groups with healthy controls. Longitudinal analysis of the ALS CSF proteome was also performed.

Results:
Weighted correlation network analysis identified 10 modules, including modules enriched for terms involved in gene expression (module 1), immune system function (module 2), membrane proteins (module 3) and axonal fasciculation (module 4). The 19 altered protein correlations in ALS were enriched for module 1 (OR 3.01, p = 0.036) and module 3 (OR 3.62, p = 0.017) proteins, including intramodular hub proteins involved in transcription, translation, cytoskeleton, stress granules and carbohydrate metabolism, including several proteins identified as TDP-43 interactors. Proteins decreasing longitudinally in ALS were enriched in module 4. Proteins with altered correlation in Parkinson's disease showed no module enrichment.

Discussion and conclusions:
Analysis of the CSF protein co-correlation network provides indirect in vivo support for the hypothesis that dysfunction of TDP-43 is a key driver of ALS pathogenesis.

BIO-17: Assessment of microRNAs as biomarkers of disease progression in Amyotrophic Lateral Sclerosis

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Background:
Despite major advances in understanding the molecular biology of ALS, molecular biomarkers of disease are scarce and unspecific. Thus, there is an urgent need for molecular biomarkers that could provide reliable information on the onset and progression of ALS in clinical practice. Several putative biomarkers of ALS have been evaluated in cerebrospinal fluid (CSF); however, these are invasive, and not useful for routine screening. Therefore, a minimally invasive blood-based biomarker of disease and progression would be essential to clinicians, and could guide diagnosis, therapy, and follow-up. Recently, increasing evidence has indicated that deregulation of small non-coding RNAs, like microRNAs (miRNA), is involved in ALS pathogenesis. miRNAs are short RNA molecules that play an important role as endogenous regulators of gene expression. Importantly, miRNAs are exceptionally stable in plasma and serum. Indeed, some miRNA have been identified as potential ALS biomarkers.

Objectives:
Hence, our aim is to assess a panel of microRNAs in plasma samples and determine a signature of biomarkers of ALS diagnosis and progression.

Methods:
To achieve our aim, we collected plasma samples from 68 ALS patients and 17 healthy controls. Total RNA from each sample was isolated using an available commercial kit, and 16 biological samples of each group were selected to create a pool for the quantification of 1008 miRNAs using 96-well plate format from miScript miRNA PCR Arrays from Qiagen. This sample pooling allowed to perform an initial exploratory study.

Results:
We obtained a total of 325 differently expressed between ALS patients and healthy controls. From which, 95 were downregulated and 230 upregulated in ALS patients. From these, 5 were significantly (p-value ≤ 0.05) differentially expressed between ALS patients and healthy controls (upregulated: hsa-miR-150-5p, hsa-miR-576-5p, hsa-miR-337-3p, hsa-miR-223-5p; downregulated: hsa-miR-140-3p).

Discussion:
Using DIANA-miRPath v3.0 web server we identified 6 KEGG pathways regulated by this set of miRNAs, being one ErbB signalling pathway, a known pathway involved in neurodegenerative diseases. Within this pathway the genes putatively regulated are CBL, PAK2, CDKN1A, CDKN1B and ABL1.
In conclusion, we identified a general upregulation of miRNA in the ALS group, and a signature of dysregulated miRNAs that may be a promising plasma biomarker of ALS disease. From this point forward, we will assess the expression levels of the selected miRNA individually in each ALS patient and control.

BIO-18: Characterising the role of SARM1 in ALS axon degeneration

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The mechanisms underlying the loss of axon integrity in upper and lower motor neurons in amyotrophic lateral sclerosis (ALS) are unclear. Recently the Sarm1 protein was identified as a key mediator of Wallerian degeneration, a well characterized degeneration process triggered after an axon severing event. Sarm1 has also been identified as a genetic risk variant in sporadic ALS. This project will investigate the potential role of Sarm1 in axon degeneration associated with ALS. We hypothesize that removing Sarm1 will prevent axonal pathology in a mouse model of ALS and slow functional decline.
We knocked out Sarm1 in the mSOD1G93ATg mouse model of ALS and examined the resulting effect on motor behaviour phenotype and axon pathology across male littermates. Animals were weighed and monitored weekly for ALS disease severity. Motor function was assessed at 16 weeks of age through a battery of motor tests for balance, strength, and coordination. Animals were perfused at end point (20 weeks), with tissue harvested for immunohistochemistry and blood collected for protein analysis. Neurofilament light (NFL) protein concentration, as an indicator of axon degeneration, was measured from serum using single molecule array (SiMoA) technology.
At 16 weeks mSOD1G93ATg animals, showed a stereotypical reduction in weight, however the onset and progression of neuromotor decline in mSOD1G93ATg was not attenuated by deletion of Sarm1. The loss of Sarm1 failed to improve impaired motor skills, functional strength, or coordination during the late clinical stages of ALS in mSOD1G93ATg mice, and failed to protect motor neurons from degeneration and cell death. However, loss of functional Sarm1, protected excitatory VGlut1 immunopositive puncta in mSOD1G93ATg mice. mSOD1G93ATg animals had significantly increased NFL protein serum levels compared to control littermates ($p < 0.0001$), where loss of Sarm1 resulted in a decrease in NFL concentration in mSOD1G93ATg animals (Sarm1KO; 2115 ± 423 pg/ml; Sarm1WT 3812 ± 1400 pg/ml). This likely indicates a protective effect of Sarm1KO, in mSOD1G93ATg animals. This preliminary analysis highlights the potential role of Sarm1 in axon degeneration in an ALS model. These results will be extended by future immunohistochemical analyses of axonal and motor circuitry alterations.

BIO-19: Differential expression of microRNAs from serum exosomes in patients with amyotrophic lateral sclerosis.

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Background:
There are no clinically established biomarkers yet in amyotrophic lateral sclerosis. Exosomes can reflect the physiologic changes of the various cells, including proteins and nucleic acids, which have potential as biomarkers. In parallel with exosomes, miRNAs have been identified as potential biomarkers in many diseases. Since small RNAs including miRNAs are enriched in exosomes and the lipid layer of exosomes protect miRNA from degradation by enzymes, miRNAs from exosomes are good candidate of potential biomarkers. So, we compared the expression level of miRNAs derived from serum exosomes between ALS patients and healthy controls.

Methods:
We obtained serum samples from 11 ALS patients and 12 healthy subjects at Seoul National University and Boramae medical center, who wrote informed consents. Serum exosomes were isolated using exoEasy kit (Qiagen, US). The exosomal RNAs were extracted using exoRNeasy serum/plasma maxi kit (Qiagen, US). The 10 ng of RNA isolated from each samples was used to construct sequencing libraries with the SMARTer smRNA-Seq Kit for Illumina. We used three different algorithms, DESeq2, edgeR, and quantile normalization, to identify differentially expressed miRNAs. Lastly, we used TAM 2.0 as functional enrichment analysis tool.

Results:
We identified 266 miRNAs in exosome-enriched fractions of serum samples. Of 266 miRNAs, 20 miRNAs were differentially expressed between ALS patients and healthy controls (absolute fold change>2, p-value<0.05). Of 20 miRNAs, five miRNAs were differentially expressed in all three algorithms. Among these five miRNAs, two miRNAs (miR-23c and miR-324-3p) were up-regulated and three miRNAs (miR-192-5p, miR-32-5p, and miR-378a-5p) were down-regulated. In the validation study, miR-192-5p and miR-378a-5p were differentially expressed by droplet digital PCR in an independent cohort. Pathway enrichment analysis showed that the differentially expressed miRNAs were related to several functions including adipocyte differentiation, apoptosis, inflammation, lipid metabolism, and immune response.

Discussion:
We identified five miRNAs derived from serum exosome extractions that significantly differentiate ALS patients from healthy subjects. By validation study, we confirmed that two miRNAs, miR-192-5p and miR-378a-5p, were downregulated in ALS patients. MiR-192 had been shown to be involved in TGF-β/Smad3 pathway in renal fibrosis. Si Y et al reported TGF-β and Smad expression were increased in muscle samples from ALS patients. Herein, we postulated miR-192-5p might be related to TGF-β/Smad signaling in ALS patients. Krist B et al. reported that miR-378 was involved in vascularization of skeletal muscle and miR-378 might be involved in muscle regeneration process in ALS. We expect these miRNAs might be playing a role as biomarkers.

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BIO-20: Macquarie University Neurodegenerative Disease Biobank

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Macquarie University's Centre for MND research and Macquarie Health Neurology clinic joined forces to establish the Neurodegenerative Disease (ND) Biobank in 2013.
MND patients, family members and controls are invited to participate in the biobank by donating blood, urine, hair and skin biopsies. Extensive demographic, clinical and lifestyle data is collected from each participant.

This resource has grown rapidly with more than 800 participants: 65 familial patients, 320 sporadic patients, 429 controls and 17 asymptomatic participants. The biobank also contains multi-generational participants from large Australian MND families. Biological samples and data are collected longitudinally in order study disease progression. Nearly 2,500 collections of DNA, RNA, plasma, serum, urine and hair have been obtained, thus far. In addition, there is a bank of more than 20 fibroblast cell lines developed from participant skin biopsies.

Since 2013, more than 20 research projects have availed of this resource. Most of these are large complex, national and international collaborative projects. Multiple biomarker projects study longitudinal collections of plasma, serum, urine and hair, to identify biomarkers. Fibroblasts are used to investigate protein degradation and generate iPSCs. DNA samples are used in numerous studies including genomics and gene discovery. RNA samples have been used in studies of gene expression. Environment projects search for evidence of neurotoxin and pollutant exposure in plasma samples from sporadic patients living within geographical clusters of MND.

The quality of the biobank is supported by NSW Health biobank certification received in 2019. Access to samples and data is obtained via an access committee and a cost recovery fee applies.

As one of the largest MND biobanks in the world, with a collection of well-characterized samples and extensive clinical and lifestyle data, it is an extremely valuable resource to investigate MND pathogenesis and treatment strategies.

https://www.mq.edu.au/mnd

BIO-21: Oxidative stress biomarkers in ALS and FTD patients: a longitudinal study in lymphocytes

Bch Gabriel García-Salamero[1], PhD Daniel Borrego-Hernández[1], Professor Ana Cristina Calvo-Royo[2], Bch María del Carmen Herrero-Manso[3], Bch Pilar Cordero-Vázquez[1], PhD Alberto Villarejo-Galende[4], PhD Sara Llamas-Velasco[4], PhD Marta González-Sánchez[4], PhD Miguel Ángel Martín-Casanueva[5], PhD Jesús Esteban-Pérez[1], Professor Rosario Osta-Pinzolas[2], Professor Alberto García-Redondo[1]

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Background:
Previous studies performed on muscle biopsies from mice SOD1G93A suggested that this animal model presents an alteration in the expression of several genes (MEF2C, GSR, Col19A1, CALM1, SOD1 and SNX10). In this point, two of the previous genes (GSR and SOD1) are involved as an important part of defense against that stress cellular metabolism in ALS as in other neurodegenerative diseases.
Therefore, with these previous results, the expression of those genes could behave as potential prognostic biomarkers of longevity as well as diagnostic biomarkers.

Methods:
cDNA and protein extract serial samples from lymphocytes of 45 patients with ALS (27 males and 18 females) and 58 patients of FTD (33 males and 25 females) were subjected to qPCR methods in order to study expression levels of GSR and SOD1.
The levels found in every sample were related to the main clinical parameters like days since symptoms onset, clinical variant, and others.
Statistical analyses were made through SPSS Statistics 24 and diagrams and graphics were made with GraphPad Prism software support.

Results:
Significant differences in gene expression levels were found in GSR in the ALS cohort compared to the control group in the cross-sectional study. Furthermore, the ALS cohort could be divided into two populations based on gene expression levels of GSR and its descriptive analysis, cause dividing that cohort, the standard deviation of the outcomes was normalized. In this way, one of the subpopulations, GSR1, presented a significant low expression compared with control group. Besides, Spearman correlation analyses led to a negative relationship between GSR expression and survival. Respect to subpopulation, logistic regression indicates that GSR1 correlated with sex in the ALS group.
With respect to SOD1, a significant difference between FTD's patients and controls were found in the cross-sectional study. Spearman correlation analyses led to a positive relationship with survival and the logistic regression indicated that SOD1 correlated with sex in FTD's patients.
Cox regression indicates that SOD1 correlated with ALSFRS-r slope and with survival in the FTD group, indicating its possible importance as a risk factor associated with survival in the FTD group.

Discussion:
Expression levels of GSR and SOD1 and their corresponding rates seem as good diagnostic biomarkers of ALS. Unfortunately, the progression study does not yield concordant data based on the prognostic capacity of these genes as biomarkers.
It is necessary to evaluate biomarkers' potential with other cohorts in multicenter assays to validate their utility and diagnosis capacity.

On the other hand, significant alteration of those biomarkers in ALS and FTD groups on respect to the control group indicates a possible treatment target for deepening in the pathogeny of both diseases.

BIO-22: Plasma hyaluronan: a biomarker for change in functional capacity in patients with Motor Neuron Disease.

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Background:
Changes in extracellular matrix factors are a consistently reported feature of disease in MND. Further exploration into these processes may provide improved avenues for development of potential biomarkers and therapeutics. Hyaluronan, a glycosaminoglycan that forms a major constituent of the extracellular matrix, has previously been shown to be increased in serum of patients with MND[1].

Objectives:
To determine the utility of measures of plasma hyaluronan as biomarker for disease progression in patients with MND.

Methods:
Seventy-two patients with probable or definite MND, and fifty-nine non-neurodegenerative disease (NND) healthy controls were enrolled for comparison of measures of plasma hyaluronan. Hyaluronan was quantified using a commercial sandwich ELISA kit (R&D #DHYAL0). Levels of hyaluronan were compared with clinical and anthropometric outcomes (including the ALS functional rating scale, King's staging, respiratory capacity, and change in body weight and fat-free mass) and risk of death.

Results:
Hyaluronan was decreased in patients with MND (60.88 ng/mL vs 75.70 ng/mL, p = 0.043). Hyaluronan was correlated with Δ FRS (i.e., patients with faster progressing disease had decreased hyaluronan, Spearman's rho = 0.363, p = 0.004), and lower limb ALSFRS-R subscores (Spearman's rho = 0.237, p = 0.045). In a Cox-regression analysis, hyaluronan was predictive for survival (HR: 0.977 [0.958, 0.996]), alongside age (HR: 1.050 [1.007, 1.095] and Δ FRS (HR: 0.230 [0.068, 0.770]), with an interaction term for hyaluronan and Δ FRS (HR: 0.951 [0.928, 0.975]).

Discussion:
Contrasting previous findings showing an increase in hyaluronan in serum and skin of patients with MND[1], we found a decrease in plasma levels of hyaluronan in MND. This observation mirrors the documented decrease of other extracellular matrix components in patients with MND[2]. We also found that measures of hyaluronan in patients with MND were proportional to ΔFRS, and predictive for survival. Our data suggest that hyaluronan may be a useful prognostic biomarker of physical function, and risk for earlier death in patients with MND.

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BIO-23: Synaptic and Tripartite Synaptic Nanostructure in the Spinal Cord in Mouse Models of Amyotrophic Lateral Sclerosis.

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Synapses are the communication junctions between neurons in the nervous system. They consist of thousands of proteins organised into nanoscale trans-synaptic signalling domains that facilitate high fidelity signal transmission. Astrocytes make specialised contacts with synapses, termed tripartite synapses, where they are capable of detecting and manipulating synaptic activity. Both synapses and astrocytes are thought to show altered functionality in pathological conditions such as Amyotrophic Lateral Sclerosis (ALS). This study aims to investigate synaptic and tripartite synaptic nanostructure in the spinal cord in healthy and ALS mice.

To visualise tripartite synapses, mice expressing PSD95-eGFP were used to visualise the excitatory postsynaptic density (PSD), along with immunolabelling of other synaptic and astrocytic structures. To investigate ALS, PSD95-eGFP mice were crossed with G93a-SOD1 mice, WT-SOD1 mice and C9orf72 BAC mouse. Over 1 million synapses and tripartite synapses have been visualised and analysed using high-resolution and super-resolution microscopy (g-STED) to produce a robust map of tripartite synaptic changes in ALS.

In the healthy adult mouse spinal cord, there is anatomical diversity in PSD nanostructure – whereby ventral horn synapses are larger and display a greater number of distinct PSD95 nanoclusters (NCs). Our preliminary data suggests that early-symptomatic 16-week-old G93a-SOD1 mice show changes in PSD size and nanostructure in intermediate and ventral laminae, including the lateral motor pools (U=14889, p≤0.00001). No such synaptic changes were observed in WT-SOD1 mice or in C9orf72 mutant mice, neither of which display motor phenotypes of ALS. Tripartite synapses, as determined by their association with presynaptic VGLUT2 and astrocytic EAAT2 or pEzrin, show a greater nanostructural complexity and enriched expression of PSD95. In symptomatic G93a-SOD1 mice, both the fraction of synapses which are tripartite (F=6.0, p≤0.05) and the size of tripartite synapses (F=7.7, p≤0.05) are reduced compared to controls.

These findings demonstrate that tripartite synapse nanostructure is altered ALS, which may lead to changes in neuronal excitability which could correspond to motor deficits. Fundamentally, this work also demonstrates the importance of nanoscale analysis of synaptic and astrocytic structure for understanding neural function in health and disease.

BIO-24: Urinary Neopterin as a Candidate Biomarker for Motor Neurone Disease

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Background:
It is well established that objective markers of disease progression and prognosis have relevance in assessing the effectiveness of treatments in motor neuron disease (MND) clinical trials. Our laboratory was the first to demonstrate urinary p75ECD as a candidate biochemical biomarker that measurably changes as disease progresses[1] and can be used to determine if treatments are working in clinical trials[2]. Urinary p75ECD is thus a putative pharmacodynamic biomarker. The addition of validated urinary biomarkers to the pharmacodynamic urinary p75ECD will help in subgrouping patients in clinical trials and increasing the ability to detect efficacy. Immune dysfunction occurs in MND, and a marker of cell mediated immune response, namely Neopterin[3] is secreted in urine.

Objectives:
A. Measure human urinary Neopterin levels in MND patients at baseline and compare to healthy controls
B. Analyse Neopterin as a prognostic and progression marker for MND
C. Compare baseline levels of Neopterin to human urinary p75ECD in MND patients

Methods:
Our study involved 28 healthy controls and 44 people with MND. Urine was sampled from MND patients at baseline (first visit to neurologist) and for a subset of 20 sampled 2-6 times over a 2 year-period. Urinary Neopterin and p75ECD were measured using enzyme-linked-immunoassays. Longitudinal changes in urinary Neopterin was examined by a linear mixed model analysis, and the prognostic value of baseline Neopterin explored by survival analysis. We also compared urinary Neopterin to p75ECD measured at baseline.

Results and Discussion:
Results to date, indicate urinary Neopterin is higher (162.9 ± 12.9 µmol Neopterin/mol creatinine, n=37) in samples from MND patients at baseline compared to controls (85.3 ± 8.9 µmol Neopterin/mol creatinine, n=18, by unpaired t-test (p=0.0003). The association between Neopterin and ALSFRS-r, age, gender is being analysed as are the prognostic value of Neopterin. Analysis of Neopterin over disease progression is also ongoing with the results to be included in final presentation. Interestingly, there was a significant correlation (r=0.52, p=0.0009) between urinary Neopterin and p75ECD measured in the same individuals at baseline (n=37). Conclusion/ Discussion: Neopterin is another candidate urinary biomarker of MND to add to p75ECD, and may be useful as an immune response biomarker. Ongoing studies will shed light on the prognostic value and natural history of urinary Neopterin over disease progression for MND, and it's value for clinical trials.

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