PERSPECTIVE

Bioelemental patterns in the cerebrospinal fluid as potential biomarkers for neurodegenerative disorders

Neurodegenerative disorders like Parkinson's disease (PD) or atypical Parkinsonian syndromes including the different synucleinopathies and tauopathies are an important burden for patients, relatives, care providers and incur mounting costs for the health care system in our aging society. The lack of disease modifying strategies and the failure in translating promising molecules from bench to bedside is also attributable to a relatively late diagnosis: when patients become symptomatic and seek medical advice, neurodegeneration has already widely spread through the central nervous system and thus represents a major obstacle for disease-modifying and/or regenerative therapies. The detection of neurodegenerative processes at an earlier stage by biomarkers is urgently needed to define state and trait of a disease condition, which ideally would increase precision of diagnosis, allow for stratified therapeutic interventions and monitoring of treatment effects. The mechanisms causing neurodegeneration in aggregation-related disorders like PD are not completely understood but most likely are multifactorial, including factors like oxidative stress, autophagic-lysosomal dysfunction, mitochondrial dysfunction and prion-like spreading of misfolded proteins (Maiti et al., 2017). Importantly, biometals and other bioelements were shown to modify these mechanisms under multiple circumstances. For example, aggregation of alpha-synuclein, a hallmark of PD and other synucleinopathies, can be enhanced by different biometals like iron, copper, aluminium and magnesium (Uversky et al., 2001). Pathological protein aggregation triggered by bioelements has not only been shown for PD but also for other neurodegenerative disorders like Amyotrophic lateral sclerosis (ALS) and Alzheimer's disease (Aizenman and Mastroberardino, 2015), suggesting a common disease mechanism. In addition to effects on protein aggregation, biometals can be involved in neurodegenerative pathogenesis by catalysis of oxidative reactions. One example is the contribution of iron to the Haber-Weiss- and Fenton-reactions, which results in increased oxidative stress in dopaminergic neurons, their dysfunction and consequent demise (Carboni and Lingor, 2015). Because disease pathology is unlikely to be influenced by one single element alone, the characterization of bioelemental patterns may be more promising for the identification of a biomarker. Advanced bioinformatics methods extending beyond descriptive statistics are helpful to detect changes in such multivariable patterns. A number of computational machine learning algorithms allowing for predictive pattern recognition are available and can be used for analysis of complex data sets in biomedical research applications. Recently, our group used machine learning techniques to analyse patterns in the elemental composition of cerebrospinal fluid (CSF) samples of PD patients and age-matched healthy controls. CSF has a close spatial relation to the affected brain regions in neurodegenerative pathology and thus has the potential to reflect pathophysiological changes of the disease processes. Although the acquisition of CSF is more invasive in comparison to blood samples, CSF may represent a more faithful biomarker source when it comes to neurodegenerative disorders like PD. Elemental serum levels may be variable for example in correlation to specific food and supplement intake while CSF levels are more tightly regulated and thus may be more meaningful in regard to their diagnostic value. In our discovery trial (Maass et al., 2018), inductively-coupled plasma mass spectrometry (ICP-OES and ICP-sf-MS) was used for element determination and a total of 28 elements were quantified in CSF samples of 36 PD patients and 42 age-matched controls, including some elements in trace concentrations down to nanogram levels. Nineteen out of those initial 28 elements were stably detectable and used for further analysis. In the single element comparison, arsenic, magnesium and selenium showed significantly higher mean CSF levels in the PD group compared with the control group after multiple adjustments. However, previous trials showed contradictory results on the levels of single elements, which may be due to their high variability and limited sample size (Jiménez-Jiménez et al., 2014). These limitations also applied to our trial and we therefore aimed at the identification of patterns instead of individual elemental levels. In order to establish differences in the bioelemental pattern, machine-learning was applied to this dataset, showing the best performance for the discrimination of PD patients and controls for the gradient tree boosting algorithm, yielding a good area under the receiver-operated curve (AUROC) of 0.83. The term "boosting" belongs to an ensemble method in the field of machine learning, which creates a strong classifier from multiple weak classifiers, in this case based on different decision trees (**Figure 1**). The classification decision tree itself is a predictive model, based on a binary tree-like system with nodes

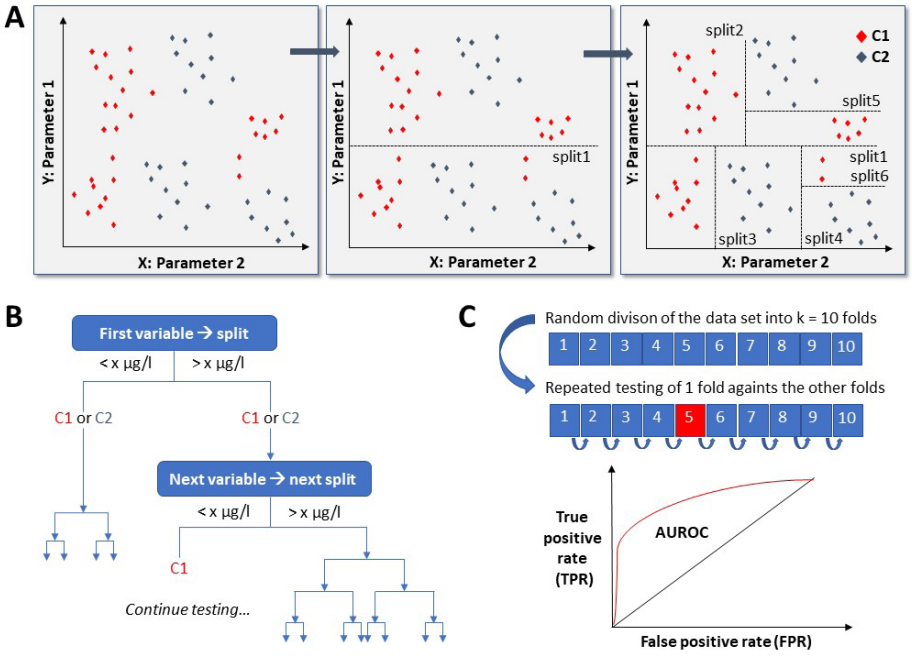

Figure 1 Scheme showing a classification task by "decision tree" machine learning.
(A) Two different conditions (C1 and C2, *e.g.*, patients and controls) can be separated with a non-linear approach using different variables (decision tree expression levels) and multiple splits trough the algorithm. (B) Structure of a classification tree showing the principle of binary splitting, taking the levels of multiple variables into account. (C) N-times, k-fold cross-validation divides the data set into multiple subsets, allowing to train and test the model repeatedly. The performance of the algorithm can be described using the area under the receiver operating characteristic curve (AUROC).

representing the input variable (abundance of bioelements) used for prediction, branches representing the test outcome and leaves representing the output class label (PD *vs*. control). Since the analysis of many elements is not economical, we aimed to reduce the number of required elements to a minimum using the AUC results from the 10 times repeated 10-fold cross validations as a feature selection criterion to determine the optimal set of elements for a good classifier. This resulted in the identification of a cluster of six single elements (Se, Fe, As, Ni, Mg, Sr), which most importantly contributed to the sample discrimination. Based on the six remaining elements from the feature selection analysis, the feature importance for each of these elements was quantified *via* 10 times 10-fold cross validation of a classifier trained on these elements only. It can be suspected that the elemental pattern identified in this analysis is not a mere epiphenomenon but reflects the role of these elements in disease pathogenesis. Changes in endogenous elemental pattern may represent a disease specific dysregulation, which could be influenced by external (*e.g*., environmental) factors. Across the contiguous 48 states of the USA, PD mortality rates have a significant positive correlation with soil strontium concentrations, and an inverse correlation with soil selenium concentrations (Sun, 2018). Further investigation in other industrial areas is needed to validate this potential relationship, *e.g*., in Scandinavian countries, where epidemiological registers of high quality are available. While the role of iron in the pathogenesis of PD has been widely described, our analysis argues for an important contribution of selenium, which had the highest impact in this model. There is growing evidence for the importance of selenium and selenoproteins in neurodegenerative disorders like PD but there is also evidence for an involvement in Alzheimer's disease, Multiple sclerosis, ALS and Huntington's disease (Cardoso et al., 2015). In PD, one function of selenoproteins seems to be the protection of dopaminergic neurons against oxidative stress and cell death. A more detailed characterization of selenoproteins could have the potential to enhance the sensitivity of the bioelemental fingerprint, likely yielding an even better sensitivity. Our analysis with a proof-of-concept approach showed that changes in elemental signatures in the CSF detected by machine learning techniques may have the potential to be used as a biomarker signature for the diagnosis of neurodegenerative diseases like PD. This approach using the CSF composition of elements as a biomarker for neurodegeneration seems also to be promising in ALS. Alterations in heavy metals, such as lead, cadmium and mercury, have been described in this motoneuron disease (Vinceti et al., 2017a). Changes in elemental levels were also recently detected in serum, urine and hair samples of ALS patients, again showing dysregulated selenium levels as a marker for neurodegeneration (Oggiano et al., 2018). In Alzheimer's disease, higher levels of inorganic selenium in the CSF may predict the conversion from mild cognitive impairment (Vinceti et al., 2017b). In the field of Parkinsonian syndromes, such bioelemental patterns may also be useful for an earlier and precise differential diagnosis between idiopathic PD and atypical Parkinsonian syndromes (*e.g*., Multisystem atrophy, Dementia with Lewy bodies, Progressive supranuclear palsy or Corticobasal degeneration), which is a more frequent challenge in the clinical setting than the mere diagnosis of PD. Biomarker patterns could also facilitate the design of disease-modifying trials, particularly when it comes to the identification of subgroups with different progression rates.

In a currently ongoing analysis, we are now validating the elemental pattern identified by the gradient tree boosting algorithm in a prospective multicentre validation cohort including more PD patients and controls as well as patients from different centres to exclude a center bias. Increasing the number of patients may also yield a sufficient power to detect correlations of disease severity (assessed by the Hoehn & Yahr stage or the Unified PD rating scale, UPDRS) and the bioelemental pattern, which was not possible in the original discovery cohort. Since our patient cohort is followed-up for at least one year, we will also be able to include longitudinal data on individual patients that will permit to correlate bioelemental patterns with disease progression, which could reflect disease progression more faithfully than commonly used clinical scales.

We expect that the analysis of elemental profile patterns, including biometals, as well as associated metalloproteins will contribute to a better accuracy in the diagnosis of PD. Ongoing analyses including patients with other neurodegenerative disorders will show whether this also holds true for phenotypic mimics. A better differentiation of patients particularly in the early disease stages will permit to choose the most appropriate therapy, to better inform patients and caregivers about the individual prognosis and to stratify patients for clinical trials. Data from trials analyzing single elements in other neurodegenerative disorders suggests that the identification of bioelemental patterns has also the potential to be translated to other disease entities.

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Fabian Maass, Paul Lingor*
Department of Neurology, University Medical Center Goettingen, Germany (Maass F, Lingor P)
Cluster of Excellence Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB), Goettingen, Germany (Lingor P)
***Correspondence to:** Paul Lingor, M.D., plingor@gwdg.de.*
***orcid:** 0000-0001-9362-7096 (Paul Lingor)*
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