Editorial

Giulia Musso and Mario Plebani

Blood biomarkers in neurology: "a call to arms" for laboratory professionals

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The Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) 2016 revealed neurological disorders to be the leading cause of disability and the second cause of death after cardiovascular diseases [1]. Neurological disorders – particularly central nervous system (CNS) disorders – have suffered from a relative lack of biomarkers for screening, diagnosis and/or follow-up. With the exception of CSF oligoclonal bands, amyloid β_{1-42} and the amyloid β_{1-42}/amyloid β_{1-40} ratio, total tau protein and phosphorylated tau_{181}, and genetic testing for inherited conditions, the role of clinical laboratories has been so far relegated to the autoimmunity section, due to the breakthrough discovery of CNS autoantibodies (i.e. AQP4, MOG, onconeural and neuronal surface antibodies).

Not a long time ago, CSF circulating neurofilaments have come under the spotlight as a biological epiphenomenon of neuroaxonal damage [2] and their light chain isoforms (NFL) have been measured in different neurological disorders, with their increase being highly specific for neurodegenerative, inflammatory, vascular and traumatic CNS disorders and related to disability outcomes [3, 4]. Further research has then been focused on correlating CSF to serum quantification of NFL, also encouraged by the pivotal finding of a functional lymphatic system in the CNS, which led to questioning the classic concept of the immune privilege of the brain [5].

The "gold rush" toward blood-based biomarkers, more easily accessible and repeatable, took a step forward when highly sensitive assay technologies, such as electrochemiluminescence (ECL) and single-molecule array (Simoa), came up beside the usual enzyme-linked immunosorbent assays (ELISA). Unsurprisingly, the work by Jens Kuhle's group who performed an analytical comparison of three available methods for quantification of NFL in blood [6] won this year's CCLM Award for the most cited article in the last 3 years [7], thus highlighting the need for robust laboratory validation of research-developed biomarkers. Since then, several studies have emphasized the potential role of serum NFL mostly in multiple sclerosis (MS) [8–10], Alzheimer disease (AD) [11] and amyotrophic lateral sclerosis (ALS) [12, 13].

Proceeding with their innovative work on biomarker validation, Kuhle and coworkers publish in this issue of the *Journal* a paper on the evaluation of the phosphorylated heavy isoform of neurofilaments (pNFH) [14], setting comparative testing between patients with ALS, frontotemporal dementia (FTD) and control subjects. Considering the current lack of optimal assessment of pNFH in CSF and serum, and of their correlation, this comprehensive work provides an accurate description of the analytical performances of three different testing methods (homebrew Simoa, commercial Simoa and ELISA, respectively), showing they all allowed ALS patients to be distinguished from controls and recognizing pNFH as a reliable test and a valid biomarker for neurodegenerative diseases. Notably, their further correlation of pNFH to NFL concentration underlined a possible different clearance dynamics between the two and suggested the need for the careful evaluation of preanalytical variables. Nevertheless, the heuristic process of finding biomarkers has not halted: in this issue of the *Journal*, another pioneering research by Karolina Minta and colleagues (also from a joint collaboration of academic institutions in Sweden, USA and UK) provides evidence on the possible use of extracellular matrix (ECM) proteins in traumatic brain injury (TBI) [15]. ECM proteins brevican, neurocan, tenascin-C and tenascin-R, highly specific for CNS as produced both by neurons and glial cells (with the exception of tenascin-C also expressed in the muscle tissue), carry the potential to predict clinical outcome in TBI. If this can be affirmed clearly for CSF brevican, tenascin-C and tenascin-R, serum correlation analysis did not show equal prognostic power, especially if compared to previously established biomarkers such as S100B and NFL. Therefore, further data regarding circulation and degradation of CNS proteins in peripheral blood should be collected.

Recently, Sid E. O'Bryant commented on JAMA Neurology that the "Holy Grail" of blood-based biomarkers has the potential to revolutionize clinical practice and clinical trials in AD [16]; this statement should be easily extended to other main neurodegenerative processes. However,

some issues still have to be fixed and, first and foremost, the need to define a clear context of use (COU) [16] for each proposed test is of primary interest. It appears indeed clearly that we are not currently deprived of possible biological hallmarks of neurological damage; rather, we need to first define how to apply them in clinical practice, to which population and with which reference ranges.

The time seems ripe for Laboratory Medicine to enter the field with extensive studies to crystallize the different COUs, define clear age-related reference ranges and decisional cut-offs, harmonize validated methods and contribute to validate the others, and, lastly, standardize pre-analytical steps. In addition, there is the need to elaborate diagnostic and prognostic algorithms pairing already consolidated tests with the novel developed ones, and to finally introduce blood-biomarkers into neurological clinical practice: we have now the right chance for involving laboratory professionals in a new exciting field of research and clinical practice.

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Corresponding author: Mario Plebani, Department of Laboratory Medicine, University-Hospital of Padova, Via Giustiniani 2, Padova 35128, Italy, Phone: +39 0498212792, Fax: +39 049663240, E-mail: mario.plebani@unipd.it. https://orcid.org/0000-0002-0270-1711
Giulia Musso: Department of Laboratory Medicine, University-Hospital of Padova, Padova, Italy