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Controversies in MS

Exosomal profiling should be used to monitor disease activity in MS patients: No

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Research in the field of exosome profiling is becoming increasingly popular, as evidenced by the growing number of publications related to the subject. Exosomes are a subpopulation of extracellular vesicles (EV) which are membrane-bound vesicles secreted by almost all cell types. Initially considered as platelet dust, EV are now considered as mediators of intercellular communication.[1] Their surface markers or their protein-, lipid-, or RNA-containing cargo can vary depending on the parent cell and various physiological or pathological conditions. In the setting of multiple sclerosis (MS), microRNAs, proteins, and lipids associated with EV have already been identified as potential biomarkers of the disease in serum, plasma, peripheral blood mononuclear cells, or cerebrospinal fluid.[2–5] The ability of EV to cross the blood–brain barrier also makes them a promising non-invasive biomarker that may be used to reflect the inflammatory state of the central nervous system.[6] However, these potential biomarkers still have a long way to go from bench to bedside, due to several challenges and limitations that still need to be addressed, before exosomal profiling can become a reliable and cost-effective tool for improving patient care.

Indeed, several steps are required to implement a biomarker identified from pre-clinical explorations in clinical routine.[7] This initial discovery phase usually involves a low-throughput method for EV isolation and detection of the EV-associated target. This step is followed by an "assay development phase" involving analytical validation of the biomarker in retrospective studies with larger cohorts, and the development of a robust high-throughput assay. Prospective studies are then required to demonstrate clinical utility, such as the diagnostic accuracy or prognostic potential of the candidate marker. These results will determine whether the candidate will gain regulatory approval and advance into clinical practice or not.

First, a marker that exhibits good sensitivity and specificity in the condition of interest and shows measurement robustness and accuracy needs to be identified. For instance, the differentially expressed serum exosome-associated miRNA found dysregulated between healthy controls (HC) and relapsing-remitting MS patients by small RNA sequencing in the studies by Ebrahimkhani et al.[2] and Selmaj et al.[3] show very little overlap. Besides the variability between cohorts, methodological heterogeneity may also explain the lack of reproducibility. For example, EV isolation (size exclusion chromatography vs using a polymer formulation method) differed between the two studies, potentially affecting the ratio of EV-specific to non-specific miRNA.[8] Therefore, the primary obstacle in adopting exosomal profiling as biomarker remains the lack of standardization between studies and the lack of consensus regarding the methods which should be adopted, thus making comparison difficult. Even preanalytical variables such as the blood collection tube and processing time may be sufficient to affect sample quality and lead to confounding effects.[9]

Next, the specificity of the marker of interest has to be confirmed in studies involving large cohorts of patients. Small size pre-clinical studies often involve carefully selected patients with few or no comorbidities. But the release of EV and the composition of their cargo can be influenced by many physiological factors, comorbidities, and concomitant medications.[10] All these confounding factors will need to be identified to enable a cautious interpretation of the test results and to define appropriate reference intervals.

The study of these targets in larger cohorts also calls for the development of a methodology that can enable high-throughput analysis. For EV isolation, the use of commercial size exclusion chromatography columns or commercial precipitation kits could find their way to a clinical laboratory as they are not time consuming, easy to use and show little lot-to-lot variation. The method for assaying the chosen EV cargo(s) must

be accessible in most clinical laboratories, not be a specific technique devoted to basic research as is often the case and have a sufficient dynamic range and resolution. The cost-effectiveness of the test will also need to be determined.

Several studies regarding exosomal profiling in the field of oncology, preeclampsia or stroke are registered in ClinicalTrials.gov but none for MS. Most of the studies related to EV profiling in MS are small-scale pre-clinical studies comparing MS patients versus healthy controls, indicating that we are only at the beginning of the development process leading to implementation of this potential biomarker.

While exosomal profiling in general, but also in the field of MS, holds promise as a non-invasive, liquid biopsy-based approach for disease diagnosis, prognostication, and monitoring, there still is a lack of standardization of isolation and profiling technique protocols. The most appropriate matrix (cerebrospinal fluid or blood) is not yet determined. Whether whole exosomes or cell-specific exosomes should be analyzed should be studied. Apart from the technical challenges, exosome analysis remains costly and labor-intensive, constituting a barrier to its implementation in the clinic. Heterogeneity of the exosomal cargo further adds to the technical and analytical complexity of their potential value as biomarker. What are the most performant exosomal markers (microRNAs, lipids, or proteins) to measure, either alone or in combination should be investigated. Clinical validation is thus still in its exploratory stage. Finally, regulatory concerns must also be addressed.

In conclusion, the challenges outlined hereabove need first to be overcome before implementing the monitoring of MS patients in clinical practice through exosomal profiling. Nevertheless, beyond their strong potential as biomarker, research related to EV/exosomes also remains an excellent opportunity to unravel new pathways and targets involved in the complex pathophysiology of MS.

Declaration of Conflicting Interests

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