Arranging the achievement of a new revolution in the assessment of CSF orexin-A levels: ELISA analysis!

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Dear Editor,

We read the recent paper published in Your *Psychiatry and Clinical Neurosciences Journal* by Dr. Ono and co-Authors showing the possibility to measure CSF orexin-A levels by using the enzyme-linked immunosorbent assay (ELISA) instead of radioimmunoassay (RIA).[1] Quantification of CSF orexin-A levels is essential for the diagnosis of narcolepsy type 1, expecially in challenging cases, as documented in the International Classification of Sleep Disorders (ICSD-3) 3rd Edition.[2] Although RIA is a widely used method worldwide (but few centers perform this assay), concerns have been expressed about the reliability and the reproducibility of the results. Moreover, RIA represents an expensive and potentially dangerous method to measure CSF orexin-A levels;

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moreover, this technique can undergo cross-reactivates with matrix components causing intrusions and resulting in high variability between assays in a low accuracy ways[3].

In the past years, alternative methods have been tested to measure CSF orexin-A levels: fluoroimmunoassay, mass spectrometry, and ELISA test.[3-6] In particular, ELISA test has been already used in studies to quantify CSF orexin-A levels in narcoleptic patients compared to controls, with significant results also using a different laboratory kit based on ELISA to quantify CSF orexin-A concentrations.[5-6] It appeared evident by comparing the study by Ono and co-Authors to both studies previously pubblished by using ELISA method that CSF orexin-A levels obtained with this latter method were about 4 times lower than the RIA results. This finding can be explained by the sensitivity differences in the orexin-A epitopes detects by the antibody. If orexin-A undergoes a proteolytic change after sampling the RIA method, it can recognize and bound several non-specific peptide fragments instead of the specific length of the orexin-A peptide measured by ELISA antibodies sandwiches. Indeed, it appeared evident that the previously validated cut-off of 110 pg/mL cannot be used when CSF orexin-A concentrations were determined by ELISA test. Consistently, the ICSD-3 specify that also CSF orexin-A levels lower than 1/3 of controls can be considered diagnostic for brain orexin neurons depletion.[2] In keeping with this indication, in the past years a different cut-off for pathological CSF orexin-A levels has been supposed, and in particular for ELISA test is has been suggested the cut-off of approximately 50 pg/mL.[5,6]

Hence, considering the need of developing a simpler and less expensive measurement method for CSF orexin-A levels, we further support the work by Dr. Ono and co-Authors and claim for a new controlled method for assessing of CSF orexin-A concentrations.

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