DEMTEST

Biomarker based diagnosis of rapid progressive dementias - optimisation of diagnostic protocols

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Rapidly progressive dementia (RPD), in contrast to the more common forms of dementia, is a subset that follows an accelerated course of cognitive, behavioural and motor decline over a period of less than 2 years. A major problem in the diagnosis of RPD is that current methodologies are very heterogeneous because there are serious variations between different reference centers. A lack of knowledge or expertise results in a large number of misdiagnosed cases in several countries.

Therefore, the main objective of Joint Programming Neurodegenerative Disease (JPND) was to establish a large European and global collaboration to include national surveillance units from both within and outside the European Union. To improve the international collaboration, we initiated international scientific project meetings, workshops, student exchanges, ring trials and several dissemination activities. By this we achieved a huge progress in diagnostic assay development, protocol standardization, sample pre-handling and treatment during the last 3 years. In this period of time we established a well-characterised sample bank (consisting of blood, CSF or tissue mostly brain) from rapidly progressive dementia patients which was provided to the consortium.

Within the consortium we shared and developed common protocols for biomaterial collection and storage. Progress was achieved regarding the standardization of pre-analytic conditions influencing sample stability and marker degradation. Moreover, we established, validated and implemented a novel ELISA-assays for 14-3-3 and alpha synuclein (also validate other biomarkers such as, tau, p-tau etc.) detection in CSF in prion disease diagnostic. Both marker proteins are specifically up-regulated in sporadic Creutzfeldt-Jakob disease (sCJD) and revealed high sensitivities and specificities (>90%). They are now easy measurable and the ELISA assay of 14-3-3 is less time-consuming than Western blotting. Within the framework of a European Community we further initiated a longitudinal multicentre study, where we analysed the spectrum of rapid progressive dementia diagnoses, their potential influence on 14-3-3 specificity as well as results of other dementia markers (tau, phosphorylated tau and amyloid-β(1-42)) and evaluated the specificity of 14-3-3 in Creutzfeldt-Jakob disease diagnosis for the years 1998-2008. A total of 29 022 cerebrospinal fluid samples were analysed for 14-3-3 protein and other cerebrospinal fluid dementia markers in patients with rapid dementia and suspected Creutzfeldt-Jakob disease in the participating centres. We observed a total specificity of 90-92%.

Substantial progress was achieved in the development, harmonization and standardization of the Real-Time-Quaking induced-conversion (RT-QuIC) assay, which is an in-vitro aggregation assay for misfolded proteins. We implemented this assay in our routine diagnostic since March 2014. It exhibits a specificity of 99.9% and a specificity of approximately 85% for prion diseases. The robustness against short- and long term storage conditions of the CSF samples (up to 1 week at room temperature and up to 10 years at -80°C) and the good reproducibility in different laboratories, indicated by ring trial studies between 13 international partner-laboratories makes this novel test valuable for diagnostic and makes autopsies less necessary. In addition to diagnostics, this assay exhibits tremendous potential in research and therapy, in particular in studying novel compounds which may influence the conversion and aggregation of misfolded proteins in vitro. In addition to diagnostics, this assay exhibits hugh potential in studying the aggregation behaviour of different protein types or strains in vitro. Altogether, the JPND project supported the establishment of a global network of different international laboratories involved in RPD diagnostics and it contributed to a substantial improvement of RPD-diagnostics.