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Biochemical markers in dementia

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Summary

The objectives of this thesis were:

1. To produce and characterize mAbs against A β .
2. To validate measurement systems for AD biomarkers in CSF.
3. To study the implementation of AD biomarker measurement systems in the clinic.

In this chapter, the main findings of the studies presented in this thesis, will be summarized. Additionally the conclusions will be discussed and future directions will be recommended.

Chapter 2

Production and characterization of monoclonal antibodies against A β

2.1

Since biochemical changes in the brain are thought to be reflected in cerebrospinal fluid (CSF), several peptides and proteins (i.e. Tau, P-Tau, SAP, inflammation factors, cytokines and vitamins) have been investigated and measured extensively in CSF of Alzheimer's Disease (AD) patients (with an ELISA system). To do this in a reliable manner a robust ELISA system is needed, and this can only be achieved when mAbs are well characterized and found to detect the target antigen with high affinity and specificity.

In AD, the current paradigm assumes that the accumulation of amyloid-beta1-42 (A β 42), amyloid-beta1-40 (A β 40), and truncated forms of these peptides in the brain result in a complex cascade of biochemical and cellular changes that culminates in memory and cognitive impairment. As a result, the A β peptide in CSF is one of the most common proteins that have been investigated and measured by ELISAs. Results obtained with ELISAs built by various laboratories to measure A β are difficult to compare, since different mAbs, analytical strategies and standards are being used. Theoretically this problem can be overcome by using the same ELISA, however, when doing so, high inter-assay variations and different reference values are still found by different labs.

Probably these problems are caused by ELISA batch-to-batch differences or problems in the standardization/calibration of the ELISA system. With these measurements problems in mind, we tried to build an ELISA system that can measure A β 40 in CSF in a reliable manner, with the goal to use this system as a diagnostic tool. To this aim we developed and characterized a monoclonal antibody (mAb), VU- α -A β 40. This mAb recognizes with high affinity only the C-terminus of A β 40, making it a unique mAb. Consequently, this mAb was built in an ELISA system, which was extensively validated, standardized and calibrated to measure A β 40 in human CSF. This validation (including the determination of a detection limit, recovery, stability, precision and parallelism) showed that this system was robust and that it could indeed be used for clinical purposes.

2.2

One of the neuropathological hallmarks of AD is progressive accumulation of A β plaques and, as a result, the neuropathological diagnosis of AD is partly based on the detection of these A β deposits. Most of the stainings for A β rely on pre-treatment with formic acid (FA), which is a relatively harsh chemical method. This pre-treatment results in conformational changes of proteins, making double stainings impossible. Double stainings can be used to investigate the co-localization of different A β forms and A β associated factors in various stages of plaque development in AD. Therefore it would be advantageous to omit FA in pre-treatment. In chapter 2.2, we described the production and characterization of two newly developed mAbs. VU- α -A β 1-17 and IC16 were used for several immunohistochemical experiments, showing that these mAbs could indeed be used for double stainings without FA pre-treatment. The omission of FA opens new possibilities to investigate the co-localization of various A β forms and A β associated factors in different stages of plaque development in AD.

Chapter 3

CSF measurements in AD: Clinical chemical properties

3.1

In the current study, we evaluated the quality by investigating the changes in CSF levels of Tau and P-Tau with time in the same cohort. These patients underwent two spinal taps and the baseline samples were assayed twice

in a sandwich ELISA: once after the first spinal tap and once, in a separately stored aliquot simultaneous with the follow-up sample. We assessed the influence of assay variation on the results of Tau and P-Tau measurement and we found higher variability of baseline and follow-up Tau and P-Tau levels when determined in different assays compared to assessment in the same assay. Probably, this higher variability is caused by batch-to-batch ELISA differences. We concluded that in case of repeated spinal taps, determination of these markers should be performed in the same assay.

3.2

We addressed the quality of A β 42, Tau, and P-Tau measurements by investigating the results of these biomarkers over six years. These three biomarkers were measured by commercial ELISAs in CSF of AD patients and patients with subjective memory complaints. Sensitivity and specificity were assessed and trends over time were analyzed. This showed a lack of stability in the first half of our study period, however, results appeared to reach stability in the second half. Probably, next to the need of a robust ELISA system, a certain degree of experience is required when performing these measurements. Further, we concluded that CSF A β 42 and CSF Tau can be used to discriminate AD patients from controls. P-Tau did not have additional value over these two markers in our study.

3.3

The external quality was tested by organising the first world-wide quality control for CSF biomarkers in AD. Three CSF-pool samples were distributed to 13 laboratories in 2004 and the same samples were again distributed to 18 laboratories in 2008, with the instruction to measure A β 42, Tau, and P-Tau and compare the results. The results were reported to us and we calculated the intra-centre and the inter-centre variability. The highest variability was found for A β 42, the variability for Tau and P-Tau were lower. In addition, the centres that participated in both years showed high intra-centre variability, comparable to their inter-centre variability. This indicates that there is not only a high variation between, but also within centres. We concluded that a uniform standardization of (pre)analytical procedures and agreement on type of assay to use, is needed to decrease the variations. Once the inter-centre variability is reduced to acceptable levels, results from the different centres can be compared and an inter-centre reference range determined.

Chapter 4

Biomarkers in the clinic

4.1

In this chapter we studied the additional value of CSF A β 40 next to A β 42, Tau and P-Tau to distinguish patients with Frontotemporal lobar degeneration (FTLD), AD, and controls. Statistical analysis showed that CSF A β 40 levels added to the conventional CSF biomarkers increased the potential to discriminate subjects with dementia from controls, however it unfortunately does not discriminate FTLD from AD patients. Our findings favour the implementation of CSF A β 40 CSF in differential diagnosis between FTLD, AD and controls.

4.2

Serum-Amyloid-P-component (SAP) is an associated factor that is present in A β plaques. This plaque associated factor may protect A β -deposits against proteolysis, thereby promoting plaque formation. With this in mind, we first aimed to investigate if CSF and serum SAP levels could be used to discriminate (cross-sectionally) controls, AD and mild cognitive impaired (MCI) patients. Secondly, we were curious if SAP (in serum and/or CSF) could identify incipient AD among MCI patients. Early identification of MCI patients that progress to dementia is important, however, diagnosing incipient or prodromal AD in MCI patients remains a difficult issue. To reveal these questions, CSF and serum SAP levels were determined in the three groups, and the MCI group was clinically followed. Cross-sectionally no differences were found in SAP CSF and serum levels between groups, however, lower CSF SAP levels were found in MCI patients that progressed to dementia. These data suggest that measurement of CSF SAP levels can aid in the identification of incipient AD among MCI patients.

4.3

Numerous studies have shown a marked decrease of A β in CSF of patients with AD, however, studies on A β in plasma are contradictory. In this study we have investigated, in a preliminary cross-sectional setting, the differentiation of controls from patients with AD by determining A β 40 and A β 42 plasma levels. We found that the clinical relevance of the plasma tests is limited. The reason for this is the limited reproducibility and accuracy of the

A β measurements in plasma. Until now, available assays and technologies yield insufficient analytic sensitivity and suffer from matrix-related effects. Hopefully these problems will be resolved in future.

Chapter 5

Biomarkers in future

In this chapter, the newly proposed clinical criteria for AD were compared to the presently widely used clinical criteria (according the NINCDS-ADRDA) in AD patients versus non-demented controls and versus other dementias. Dichotomized medial temporal lobe atrophy (MTA) score on MRI and dichotomized CSF profiles (based on A β 42, Tau, P-Tau181) were used in combination with an episodic memory test to assess sensitivity, specificity and likelihood ratios of the newly proposed criteria and their components separately. This analysis showed a good specificity (controls versus demented subjects). Additionally we could conclude that when dementia is clinically doubted, at least two supportive features should be considered (i.e. abnormal MTA score and CSF profile; mentioned in the newly proposed clinical criteria).

Conclusion

Well characterized antibodies are essential to build a valid and reliable measurement system. In turn a well validated and reliable measurement system is essential for clinical applications.