E-Methods

EXTENDED METHODS SECTION FOR THE SWEDISH BIOFINDER STUDY

Subjects

The study population was part of the prospective and longitudinal Swedish BioFINDER study (Biomarkers For Identifying Neurodegenerative Disorders Early and Reliably). BioFINDER consists of four cohorts; 1) Cognitively healthy elderly. 2) Consecutively included, non-demented patients with mild cognitive symptoms (MCS) which have been characterized as either having subjective cognitive decline (SCD) or mild cognitive impairment (MCI). 3) Patients with parkinsonian symptoms. 4) Patients with dementia.

Subjects from cohorts 1 and 2 were selected for the present study. Briefly, the cognitively healthy elderly were enrolled since 2009 from the population-based EPIC cohort. Cognitive impairment was ruled out using a cognitive test battery and by the assessment of physicians experienced in dementia disorders. At the time of the analysis, the longitudinal data had not yet been assembled and evaluated for the healthy controls. The MCS patients were between 60–80 years, had been referred to one of three participating memory clinics in Sweden due to perceived cognitive impairment, did not fulfill any dementia criteria, had an MMSE score of 24–30, spoke Swedish fluently and did not have other condition that clearly could explain the cognitive impairment. For more details about the inclusion and exclusion criteria, see www.biofinder.se. Only subjects with a complete Aβ PET scan and CSF analysis were eligible for the present study. From cohort 2, patients who had converted to AD dementia (hereafter refered to as MCI-AD) were selected for the present study. The follow-up diagnosis (AD at the dementia stage) was determined by a consensus group (KN, PJ, SP), which reviewed the follow-up diagnoses in September 2014 according to the criteria for
probable AD. The consensus group was blinded to all biomarker data. The patients were classified as having MCI based on a neuropsychological battery assessing the cognitive domains of verbal ability, visuospatial construction, episodic memory, and executive functions, as well as the clinical assessment of a senior neuropsychologist (S.V.). Subjective cognitive decline (SCD) was defined as those in the MCS cohort who did not fulfill the criteria for MCI. At baseline in the MCS cohort, 3 (9%) had SCD and 31 (91%) had MCI at baseline (48% amnestic single-domain, 39% amnestic multi-domain and 12% non-amnestic). Thus, for this analysis, 3 patients with SCD at baseline were included in the MCI-AD group.

**Aβ PET scanning and analysis**

The cerebral Aβ burden of the patients was visualized using $^{18}$F-flutemetamol PET. PET/CT scanning of the whole brain was conducted at two sites using the same type of scanner, a Philips Gemini TF 16. PET sum images from 90-110 min post injection were generated describing the average uptake of $^{18}$F-flutemetamol over this time span. MRI data were not involved since they do not improve the quantification of $^{18}$F-flutemetamol data. The images were analyzed using the software NeuroMarQ provided by GE Healthcare. A volume of interest (VOI) template was applied for the following 9 bilateral regions: prefrontal, parietal, lateral temporal, medial temporal, sensorimotor, occipital, anterior cingulate, posterior cingulate/precuneus and a global neocortical composite region. The standardized uptake value ratio (SUVR) was defined as the regional tracer uptake in a VOI, normalized for the mean uptake in the cerebellar cortex.

**CSF analysis**

The procedure and analysis of the CSF followed the Alzheimer’s Association Flow Chart for CSF biomarkers. Lumbar CSF samples were collected at the three centers, stored in 1 ml
polypropylene tubes at -80°C and later analyzed at the same occasion and same center using two different enzyme-linked immunosorbent assay (ELISA) methods. CSF total tau (T-tau), Aβ40 and Aβ42 were analyzed by EUROIMMUN \(^{\text{EI}}\) ELISAs (EUROIMMUN AG, Lübeck, Germany) directly after thawing of the 1 ml CSF samples. The rest of the CSF was aliquoted into smaller CSF portions in LoBind tubes and stored at -80°C until analyzes. CSF Aβ42 and tau phosphorylated at Thr181 (P-tau) were analyzed by INNOTEST \(^{\text{IT}}\) ELISAs (Fujirebio Europe, Ghent, Belgium). The following 8 variables were derived from the CSF analyses: Aβ42\(^{\text{IT}}\), Aβ42\(^{\text{EI}}\), Aβ42\(^{\text{IT}}\)/Aβ40\(^{\text{EI}}\), Aβ42\(^{\text{IT}}\)/T-tau\(^{\text{EI}}\), Aβ42\(^{\text{IT}}\)/P-tau\(^{\text{IT}}\), Aβ42\(^{\text{EI}}\)/Aβ40\(^{\text{EI}}\), Aβ42\(^{\text{EI}}\)/P-tau\(^{\text{IT}}\), Aβ42\(^{\text{EI}}\)/T-tau\(^{\text{EI}}\).

**Statistical analysis**

Differences between the controls and MCI-AD groups were calculated with the Mann-Whitney U test (Table 1). The area under the receiving operating characteristic (ROC) curve (AUC) was used to examine the diagnostic accuracy of the continuous CSF and PET variables (Table 2). The 95% confidence interval (CI) and significance for differences between the AUCs were calculated using bootstrap techniques\(^8\), with 5000 bootstrap replicas. The AUCs of the combined CSF and PET variables were derived from logistic regression analysis. Cutoffs were established for all CSF and PET variables using Gaussian mixture modeling applied on the study population, which produces cutoffs that are completely unbiased by the underlying diagnosis.\(^9\) Sensitivity and specificity values were calculated based on the unbiased cutoffs. A Youden’s index (YI) was used for comparison of the aggregate diagnostic performance at particular cutoffs (sensitivity + specificity - 1). Sex, age, APOE genotype (presence of at least one APOE e4 allele), memory function and hippocampal volume were entered as co-variates in multivariate logistic regression analyses to examine the adjusted effects of the CSF and PET biomarkers (tested separately) as predictors of MCI-AD. The
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statistical analyses were performed with MedCalc version 14 (MedCalc Software, MariaKerke, Belgium), SPSS software, version 22.0 (SPSS Inc., Chicago, IL), MATLAB release 2014, Statistics Toolbox (MathWorks, Natick, MA) and R version 3.0.2.¹⁰

EXTENDED METHODS SECTION FOR THE ADNI COHORT

Study design

Parts of the data used in the preparation of this article were obtained from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies and non-profit organizations, as a $60 million, 5- year public-private partnership. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer’s disease (AD). Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials.

The Principal Investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California – San Francisco. ADNI is the result of efforts of many co-investigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the U.S. and Canada. The initial goal of ADNI was to recruit 800 subjects but ADNI has been followed by ADNI-GO and ADNI-2. To date these three protocols have recruited over 1500 adults, ages 55 to 90, to participate in
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the research, consisting of cognitively normal older individuals, people with early or late MCI, and people with early AD. The follow up duration of each group is specified in the protocols for ADNI-1, ADNI-2 and ADNI-GO. Subjects originally recruited for ADNI-1 and ADNI-GO had the option to be followed in ADNI-2. For up-to-date information, see www.adni-info.org.

Participants

Our study population consisted of control and MCI subjects from ADNI-2.

Inclusion/exclusion criteria are described at http://www.adni-info.org. Briefly, all subjects included in ADNI-2 were between the ages of 55 and 90 years, had completed at least 6 years of education, were fluent in Spanish or English, and were free of any significant neurologic disease other than Alzheimer’s disease. Control subjects had Mini Mental State Examination (MMSE) score ≥24, and Clinical Dementia Rating scale (CDR) score 0. MCI subjects had MMSE score ≥ 24, objective memory loss as shown on scores on delayed recall of the Wechsler Memory Scale Logical Memory II (>0.5 standard deviations below the normal mean), CDR 0.5, preserved activities of daily living, and absence of dementia.
Supplementary text

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