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Healthy elderly in clinical trials: how to define preclinical Alzheimer's Disease for clinical trial participation

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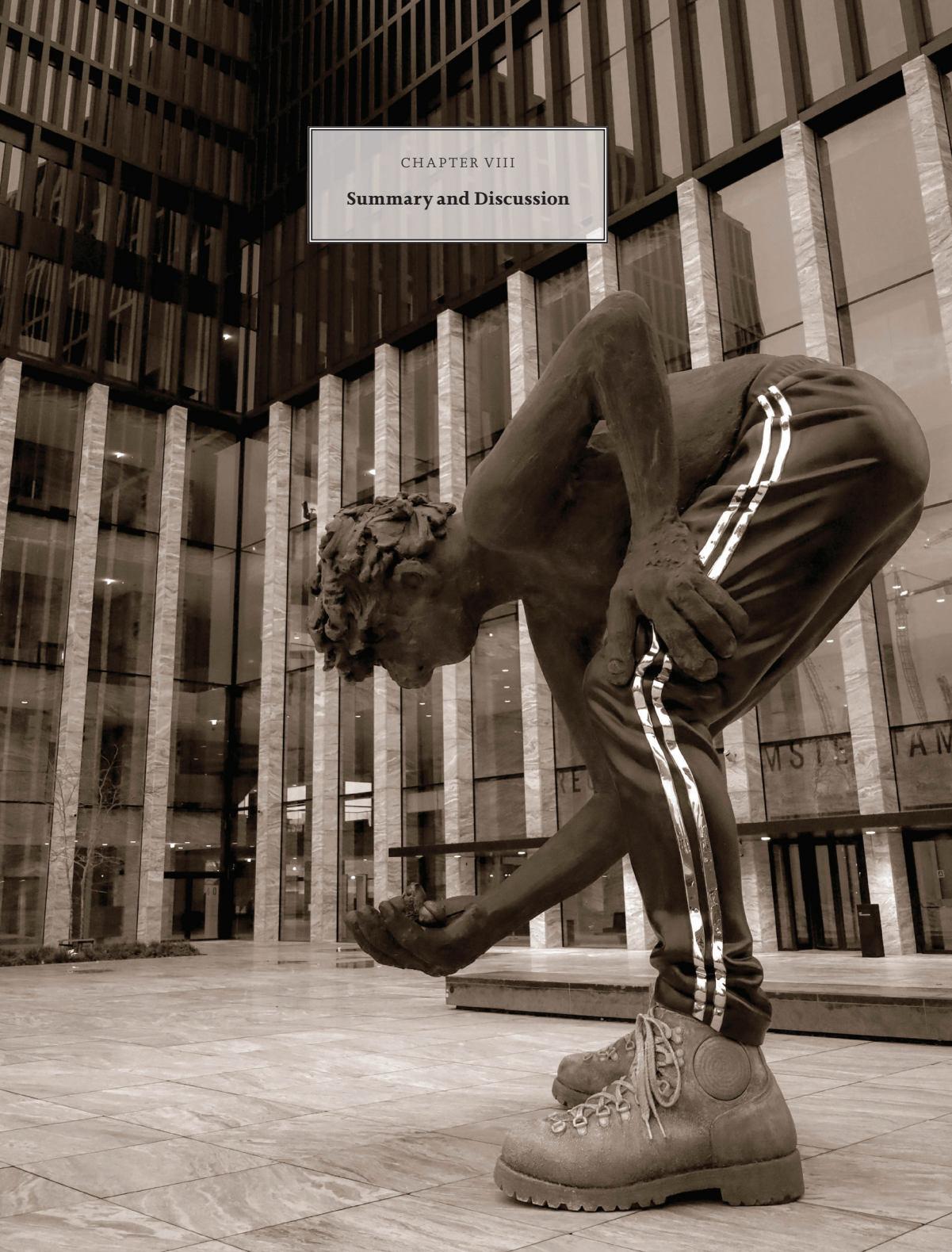
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CHAPTER VIII

Summary and Discussion



SUMMARY

Over a 100 years after Alois Alzheimer discovered amyloid plaques surrounding brain cells and neurofibrillary tangles inside the cells of a deceased patient naming it Alzheimer's Disease (AD),¹ we still have not been able to solve the mystery of this disease.

As mentioned in **Chapter 1**, the growing elderly population worldwide creates a great burden on the health care systems. The WHO estimates 1 in 6 people in the world to be over the age of 60 by 2030.² As more people generally have access to (better) health care throughout life, people get older. With increasing age, the chance to develop a form of dementia also increases. In 2020, the prevalence of dementia was approximately 50 million people worldwide. The most common form of dementia is Alzheimer's disease which accounts for approximately 70% of the dementia cases.³ Biomarker research has yielded many new insights in AD over the past decades. Biomarker evidence of AD pathology has shown to be measurable up to 20 years before clinical symptoms appear.⁴ Apart from measuring amyloid beta and tau in brains of deceased patients, these proteins can now also be measured using cerebrospinal fluid (CSF), positron emission tomography (PET) using tracers and even in blood. New biomarkers associated with AD have also been identified related to inflammatory processes in the brain, astroglial activation and neuronal damage. The large numbers of patients emphasize the need for a disease modifying treatment. Clinical trials have been improved making use of randomized (placebo) controlled trials reducing bias in trial results. At present time (2022), 119 disease modifying compounds are in development for the treatment of AD.⁵ Currently, only symptomatic treatment is available for AD patients. In June 2021, the first DMT for the treatment of AD was approved by the FDA in the United States of America.⁶ Aducanumab promises to remove amyloid plaques from the brain that have accumulated due to AD disease progression. Inconclusive results from the preceding clinical trials led to this acceptance and therefore the EMA did not approve the drug in the European Union. The label of aducanumab has been adjusted since approval by the FDA. Initially the FDA approved aducanumab for all patients with AD, but they now adjusted the approval by restricting the label to patients with mild cognitive impairment or mild AD, in whom the drug was also tested in the phase 3 clinical trials. This stresses that subject selection is of great importance in AD research. Performing clinical trials in early phase of AD or even preclinical AD might prevent further disease progression as there is less disease pathology in the brain. When a healthy subject with no cognitive complaints has a lowered CSF protein A β 42 level, comparable

with AD, this subject is considered to have preclinical AD according to the NIA-AA standards from 2011.⁷ This shift in subject selection is noticeable in current clinical trials with 14 DMT trials including subjects with preclinical AD.⁵

Cognitive performance is important to take into account when looking at the clinical manifestation of AD. **Chapter II** described age related decline in cognitive performance measured by the NeuroCart. The NeuroCart is a neuropsychological and neurophysiological test battery that is used to detect pharmacodynamic effects of drugs in the context of (early phase) drug development.⁸ Over the years it has been used in hundreds of studies in healthy subjects and patient populations. This retrospective study encompassed 93 studies, performed at CHDR between 2005 and 2020 that included NeuroCart measurements, which resulted in 2729 subjects with data from at least one of five NeuroCart measurements. The five NeuroCart tests included in the study were: Eye Movements - Smooth and Saccadic Eye Movements, Body movement- Body sway, Attention and Eye-Hand Coordination- Adaptive Tracking, Memory Consolidation-Visual Verbal Learning Task, Delayed Recognition, Working Memory-N-Back. Results show that the NeuroCart can detect age-related decreases in performance in healthy subjects, which were not affected by sex. The NeuroCart was able to detect significant differences in performance between healthy volunteers and patients with AD, Parkinson's Disease, Huntington's Disease and Vascular dementia at the mean age of the disease group. Because disease durations were unknown, this cross-sectional study was not able to show age-related decline after disease onset. Therefore, the speed of deterioration as a consequence of neurodegenerative disease could not be quantified reliably. The healthy elderly participating in this study, declined in performance on all NeuroCart measurements on a yearly basis. After clinical onset of the studied neurodegenerative diseases, this decline increases significantly.

In **Chapter III** a broad overview of biomarkers found in human AD and a comparison to biomarkers in animal studies is described. The number of currently existing and emerging pathophysiological hypotheses, mechanisms, theories, and processes related to AD is high and is still increasing. Currently, we lack sufficient information and understanding of processes in the onset and early stage of the disease. This contributes to the fact that we cannot yet diagnose or initiate treatment in the earliest phase of AD. This highlights the need to find adequate, preferably body-fluid-based biomarkers of AD. Currently, the biomarkers that are mostly measured in human studies are A β , P-TAU, T-TAU, neurogranin, SNAP-25, GFAP, YKL-40, and NFL.⁹ Additionally, there is a high volume of animal research, in which the emphasis has mostly been on A β . Animal studies can be

smartly designed to provide mechanistic information on the interrelationships between the different AD processes in a longitudinal fashion and may also include the combinations of different conditions that may reflect comorbidities in human AD, according to the Mastermind Research approach.¹⁰ The Mastermind Research approach is for strategic and systematic CNS drug research using advanced preclinical experimental designs and mathematical modeling and is able to model data extracted from animal research to predict CNS drug distribution in humans without the need of animal experiments.

Chapter IV combined plasma-based biomarkers for AD with cognitive biomarkers measured with the NeuroCart to predict CSF amyloid beta status of healthy elderly. The study aimed to develop an algorithm based on less-invasive (plasma) biomarkers for AD pathology, to be used for pre-selection of subjects who are suspected of lowered, abnormal, CSF A β levels ('A β positive subjects') consistent with the presence of AD pathology. The algorithm that resulted from the study includes sex, 7 cognitive tests measured with the NeuroCart (MMT, VVLT, finger tapping, N-Back, SART, Face and EEG) and one plasma biomarker (YKL-40) and was successful in predicting CSF A β + in healthy elderly with a sensitivity of 70.82% and specificity of 89.25%. When using this algorithm, 70% fewer lumbar punctures will have to be performed to enroll subjects based on lowered A β CSF. The overall subject burden and costs of trials will reduce as fewer lumbar punctures will need to be performed. This may also increase subject's willingness to participate in drug studies.¹¹

Verberk et al.¹² showed that plasma A β ₄₂/A β ₄₀ ratio has the potential to identify Alzheimer pathological changes in subjects with subjective memory decline. Further, the inclusion of age and ApoE ϵ 4 carriership in their multivariate model improved the likelihood of identification. Based on these results, Verberk and colleagues postulated that plasma A β ₄₂/A β ₄₀ ratio could be a potential prescreener to identify the earliest AD pathological changes in individuals with subjective memory decline. Using plasma-based biomarkers in identifying and characterizing the preclinical AD state is a breakthrough in clinical research as taking a blood sample is less invasive than taking a CSF sample which decreases the burden for healthy subjects and patients. However, results are still preliminary and should be reviewed with caution. Results could, however, not be reproduced in a (slightly) different subject group as discussed in **Chapter V** of this dissertation. We aimed to extend the findings of Verberk et al, using the same statistical methods, but in a different population, namely healthy elderly subjects without memory complaints (n=189). The sensitivity and specificity of the plasma A β ₄₂/A β ₄₀ ratio in our study were 30.8% and 71% respectively, compared to 76% and

75% in Verberk et al. The results of our logistic regression and receiver operating characteristic (ROC) analyses showed that the plasma $A\beta_{42}/A\beta_{40}$ ratio did not significantly affect ROC curves discriminating between cerebrospinal fluid (CSF) amyloid abnormal and amyloid normal individuals, in a multivariate model including age and ApoE ϵ_4 carriership. Not cross validating a model can lead to overfitting of the sampled data. Also, different populations were used in comparing the results. Stating that plasma amyloid is a prescreener for the earliest signs of AD pathology is, in our opinion, a premature statement.¹³

What Alois Alzheimer did not know in 1906, but what we have learned since then is that AD is not simply caused by amyloid plaques and neurofibrillary tangles. As discussed in **Chapter vi**, inflammation also plays a major role. This exploratory study investigated plasma biomarkers related to neuroinflammation associated with AD in a cohort of subjects with preclinical AD, and compared them to healthy elderly, defined by $A\beta_{1-42}$ CSF status. Four inflammatory plasma biomarkers were investigated. YKL-40 (also known as chitinase-3-like protein-1 [CHI3L1]) is a glycoprotein, which is mainly expressed in astrocytes. Patients with AD have significantly higher YKL-40 levels in the CSF compared to healthy controls however it is not a specific biomarker for AD, because it merely reflects the inflammatory progress.¹⁴ Glial fibrillary acidic protein (GFAP) is a marker for astrogliosis and was reported to be increased postmortem in brains of patients with AD and in CSF of patients with AD.¹⁵ Two chemokines (monocyte chemoattractant protein-1 [MCP-1] and eotaxin-1) have previously been reported to be correlated with greater memory impairment in MCI and AD.¹⁶ Of the four inflammatory plasma biomarkers investigated in the study, only GFAP was significantly higher in subjects with preclinical AD compared to healthy elderly. When post hoc defining preclinical AD based on the PTAU181/ $A\beta_{1-42}$ ratio, GFAP and YKL-40 were significantly different between groups. This could indicate that GFAP and YKL-40 are more sensitive markers of the incipient inflammatory process that occurs in response to the beta amyloid misfolding and aggregation that is ongoing as indicated by the lowered $A\beta_{1-42}$ protein levels in the CSF.¹⁷

The neurofibrillary tangles discovered by Alois Alzheimer have been studied profoundly in the past decades. **Chapter vii** described specific isotopes of tau, namely phosphorylated types and comparing results found in CSF to plasma. The study investigated P-TAU at threonine 181, 217 and 231 in CSF and P-TAU181 and P-TAU231 in plasma in subjects with preclinical AD and healthy elderly defined by $A\beta_{1-42}$ CSF status, to investigate whether phosphor-tau CSF and plasma biomarkers offer a good alternative to distinct healthy elderly from preclinical AD subjects. CSF PTAU217 was significantly higher in subjects with preclinical

AD compared to healthy elderly. CSF PTAU181 and CSF PTAU231 were increased at higher age but there was no group difference between the two studied groups. All PTAU isoforms in CSF and plasma show high correlations. As PTAU seems to emerge in the preclinical phase of AD as a response to upcoming A β misfolding in the brain, this could be the earliest possible intervention window for treatment before neurofibrillary tangles arise. Measuring PTAU in plasma can be used for the measurement of target engagement of specific anti-tau DMT and early phase removal or lowering of ptau might lead to less subjects progressing from pre-clinical AD to AD. As this study does not confirm the discriminating power of PTAU in preclinical AD, more (longitudinal) research is needed to provide more insight into the usefulness of plasma PTAU biomarkers for distinction between preclinical AD and healthy subjects.

FUTURE PERSPECTIVE OF THE USE OF BIOMARKERS IN HEALTHY SUBJECTS IN THE PRECLINICAL PHASE OF ALZHEIMER'S DISEASE

In this dissertation the focus has been on preclinical AD. How we define a subject to be in the preclinical phase of AD had been a topic of discussion in the past decade. Subjects with preclinical AD included in the studies mentioned in this thesis were characterized based on the NIA-AA standards from 2011, which state that if an otherwise healthy subject without cognitive complaints has evidence of A β pathology in CSF, this subject is classified as being in the preclinical phase of AD.¹⁸ Having A β pathology is not a guarantee that a subject will actually develop AD later in life although the odds are greatly increased. Current research states that approximately 40-60% of subjects with subjective cognitive complaints will develop AD from the preclinical phase.^{19,20} New suggestions about the definition of preclinical AD have been proposed, including the use of PET to determine amyloidosis in the brain and measuring tau pathology in CSF (2014). The most recent recommendation about the classification of preclinical AD is evidence of both A β and tau pathology measured by either PET and/or CSF.⁷ This standard is, however, still only applied in some research facilities and are not part of standard clinical care. Also, including both PET and CSF for the classification of an otherwise healthy individual is costly and invasive, which influences the willingness of a subject to undergo these procedures but also the availability of these diagnostic tools is far from common. A β measured with PET is concordant with measurements in CSF, which makes performing both assessments unnecessary.²¹

The development of blood-based biomarkers in the detection of (early) AD is very promising and might improve the ever-challenging field of AD research as it is a less invasive procedure. When biomarkers that are well established in CSF,

such as A β and specific tau isotopes, can be validated properly in blood or plasma samples, this would make early diagnosis more accessible, less invasive, and far less costly. Unfortunately, we are not there yet. New high-sensitive blood-based assays have emerged with promising results on consistency between different cohorts and agreement when comparing these results with CSF and PET.²²⁻²⁴ More (long term) research is needed to determine the validity of these blood-based biomarkers before these can be implemented as standard (research) practice.

And what about the cognitive aspect of preclinical AD? As described in this thesis, combining a blood-based YKL-40 test with cognitive tests using the NeuroCart can predict CSF A β outcome. These findings are especially useful for clinical research as, per definition, subjects in the preclinical phase of AD do not have cognitive complaints and overall do not perform worse on cognitive tests. Asking trial subjects to perform cognitive tests and a blood draw may increase willingness to participate in clinical trials and may lower costs of clinical (due to fewer PET and/or CSF measurements).

Taking the information from this thesis into account, questions arise what the perfect biomarker combination would be in a clinical trial and which trial subjects should be enrolled to improve clinical trials in preclinical AD. Based on the research performed in this thesis and recent literature, the suggested biomarkers to incorporate in a clinical trial would be a combination of CSF, blood-based- and cognitive biomarkers. Preselecting healthy subjects in an age range with higher prevalence of AD pathology results in including subjects from the age of 65 years old as approximately 20% will have A β pathology measured in CSF.²⁵ Submitting these subjects to a variety of cognitive tests (e.g., memory consolidation, verbal learning, sustained attention, motor movement and EEG) and blood-based biomarkers (GFAP, YKL-40 and ptau217) will increase the chance of finding subjects with preclinical AD likely to develop AD in the future. Taking the cognitive- and biomarker results into account, a selection of these subjects would be asked to undergo CSF sampling or a PET scan to confirm preclinical AD status based on A β ₁₋₄₂ and ptau217. Improving the selection criteria for clinical trials to be performed in preclinical AD can be expected to lead to a less heterogenic patient population, lower primary outcome variability and greater effect size of the intervention and thereby a better powered RCT with a larger chance of a positive outcome.

It is important that subjects with preclinical AD are well characterized. Evidence of AD pathology needs to be established in order to enroll these subjects in clinical trials with DMTs aimed at prevention of progression of developing AD pathology. Recruiting patients with Alzheimer's disease in clinical trials can be a challenge due to various reasons, e.g., study burden, cognitive burden,

progression of disease and study compliance. Focusing more on enrolling subjects with preclinical AD will save time and money as trials will be completed at a faster pace due to higher compliance and lower burden for healthy subjects compared to patients with cognitive decline. On the other hand, trials may have to last longer before change on a biomarker level can be observed.²⁶ Finding the optimal therapeutic window for DMTs in AD will have to include subjects with preclinical AD to find the earliest window for modification. Currently ongoing longitudinal studies aimed at elucidating biomarker evolution over time will shed more light on the feasibility of inclusion of subjects with preclinical AD. Examples of these large trials are the European Prevention of Alzheimer's Disease Consortium (EPAD) and PResymptomatic Evaluation of Experimental or Novel Treatments for AD (PREVENT-AD), which collect (biomarker) data of healthy elderly over several years in CSF and blood but also PET imaging when available, genetics and cognitive information.^{27,28}

FUTURE CONSIDERATIONS FOR CLINICAL TRIALS IN ALZHEIMER'S DISEASE WITH DISEASE MODIFYING TREATMENTS

After selecting the ideal trial subject characterized to be in the preclinical AD phase, what would be the best design for a clinical trial with a disease modifying compound? As mentioned by Hariton and Locascio (2018), the gold standard for effectiveness research is the randomized controlled trial (RCT) design.²⁹ First step is to carefully select the studied population, as mentioned above. Also, the interventions that will be compared and the outcomes of interest should be determined prior to the start of the trial. A power calculation to predefine the number of subjects needed to obtain reliable results should be done beforehand. Trials should be registered to avoid selective reporting of trial outcomes. When subjects are recruited, preferably a computerized system randomizes the subjects into different trial arms to prevent selection bias. Using double-blinded conditions, meaning the trial subjects, physicians and researchers do not know which subject is in which treatment arm, further minimalizes bias. Results should be based on intention-to-treat analyses opposed to only including subjects who have completed treatment in the analyses. A problem with RCTs can be that subjects do not represent the patients for whom the results of the trial will be used in the future. Maximizing the treatment response by selecting a more limited homogeneous study population helps with demonstrating treatment effect but becomes less representative for the patient population. Including biomarker data and including subjects in the preclinical phase of a disease should minimize this generalization issue. RCT are usually more costly as more conditions are added to

a trial resulting in more data, however, this should be compared to performing trials in a less optimized way resulting in having to perform more trials with debatable outcomes which will cost more in the end. Reproducibility of a trial is important. As shown in **chapter v** of this dissertation one single study does not represent certainty and multiple comparable studies should be performed before any definite conclusions can be drawn. Accordingly, RCTs in subjects with preclinical AD, phase 3 studies in patients with AD should follow the same guidelines. Patients with AD should be well characterized on biomarker level to include patients with similar pathology as to what the DMT is targeting.

ETHICAL CONSIDERATIONS IN PRECLINICAL ALZHEIMER'S RESEARCH

This dissertation focused on research in healthy elderly and subjects in the pre-clinical phase of AD, in whom no cognitive symptoms are (yet) measurable but in the presence of biomarkers (in this case CSF A β 42) that are consistent with AD pathology. Ethical considerations should be taken into account when performing research in otherwise healthy elderly subjects. Since 2018, the General Data Protection Regulations (GDPR) are in place protecting all personal data of EU citizens. At the time of data collection for the studies mentioned in this thesis (**chapter IV, v, VI and VII**) the GDPR was not yet fully applicable and therefore informed consent forms were less specific about handling of personal information and the possibility of requesting personal results collected during study participation. Currently, clinical trial participants are more aware of the (personal) data collected during trial participation and requests for detailed information can be more common.

In The Netherlands, a license is needed when performing research that involves screening the population on severe diseases or abnormalities for which no treatment or prevention is available (Wet op bevolkingsonderzoek [WBO]).³⁰ Most biomarker research in preclinical AD related to trial participation is of course not population-based research but does investigate severe diseases or abnormalities with no treatment or prevention. Question is if large population-based studies, which would provide us with valuable information about the development of AD, would even be allowed by the Dutch government. Current so called secondary prevention trials that screen large groups of healthy elderly for presence of AD related biomarkers and genetic information in order to select subjects for trials have been approved by ethical committees, also in the Netherlands.^{27,28} These trials are not by definition population-based trials as not all people above a certain age are invited but do aim to include a large number of otherwise healthy elderly.

As an example, EPAD registered over half a million people across Europe. Also, sharing information based on biomarker data indicating the possible presence of an untreatable disease to an otherwise healthy elderly will have great consequences. No disease modifying treatment is yet available (in The Netherlands) for AD and the presence of biomarkers consistent with AD is not 100% predictive of developing AD later on in life. Also, biomarkers consistent with AD can be present up to 20 years before actual disease onset, so actively diagnosing a preclinical state may lead to a long period of unnecessary worry. Enrolling subjects with preclinical AD means screening healthy subjects looking for specific AD pathology, which leads to many subjects that will have to be screened which is both time consuming and costly. Also, the treatment period for subjects with preclinical AD might have to be longer as the effect of treatment will take more time with less profound pathological damage.²⁶ Exposing preclinical subjects to treatment for a longer period of time must be safe and benefits of the trial results must justify the burden.

The question if the preclinical biomarker results should be shared with otherwise healthy trials subject with no cognitive complaints remains unanswered. Research shows that there might be benefits to an early diagnosis. Subjects implemented specific health behavioral changes to everyday life when learning about being an ApoEE4 carrier, according to Chao et al., (2008),³¹ even when knowing that these lifestyle changes were not proven to prevent AD. Disclosing genetic information could affect trial outcome as shown by Lineweaver et al., (2014) who concluded that subjects who were familiar with their genetic disposition for AD performed worse on cognitive tests.³² Input from the patient community and better understanding the concept of biomarkers by the general population might help researchers to understand what degree of risk is found to be acceptable in clinical trials. Knowing ones' AD biomarker status also influences the willingness to participate in clinical trials due to altruistic reasons but also to reduce personal risk of developing AD.³³ As many biomarkers in AD research are not specific for AD, extra caution is needed for the possibility of misdiagnosing subjects. The diagnostic accuracy of CSF biomarkers for AD in the MCI stage is high, with sensitivity and specificity up to 85%-90%.^{34,35} These are high accuracy numbers, but still lead to many misdiagnosed subjects.^{35,36} For example, even with a specificity of 90%, assuming a prevalence of preclinical AD of approximately 20% among healthy elderly above the age of 65 years, the positive predictive value of a positive test (as in a CSF profile consistent with AD) will be as low as 50%, leading to a large number of misdiagnosed subjects.

Being diagnosed with a disease that influences cognitive performance can be of great influence on certain legal rights. Caution about the consequences of having a preclinical 'diagnosis' on these rights should be taken into account. Subjects with AD can lose the right to hold a driver's license and, in the USA, lose the right to hold a gun (which might not be such a bad thing). As for legal arrangements, early diagnoses do force subjects to think about their future and for instance draw up their wills before reaching the incapacitated phase. If knowledge about biomarker status becomes common, this could also influence the health care system and in particular could influence health care insurance policies.

Disclosing results of biomarker and genetic testing is a complex task and should only be done by trained specialists. The decision to learn about one's biomarker or genetic status should be made by the trial subject him- or herself. However, because of the importance of finding a cure for AD research related to biomarkers and genetics in the field of AD should continue. In our opinion, specific trial data (biomarker and genetic results) should only be disclosed at an individual's explicit request, after thorough (psychological) education about the possible consequences. Future research should take the ethical considerations into account, especially with longitudinal studies characterizing otherwise healthy human beings and study how biomarker disclosure impacts an otherwise healthy subject. Once DMTs are available for the preclinical stage, the ethical considerations will change drastically and will need to be reevaluated. At this point, clinical research in subjects with preclinical AD including biomarker information has a solid scientific basis and needs to be able to move forward in order to ultimately find a cure for AD.

REFERENCES

- 1 Alzheimer, A., *Über eine eigenartige Erkrankung der Hirnrinde.* 1907(64): p. 146-8.
- 2 WHO. <https://www.who.int/news-room/fact-sheets/detail/dementia>. <https://www.who.int/news-room/fact-sheets/detail/dementia> 2021 02 September 2021 [cited 2021 07Oct2021].
- 3 2021 Alzheimer's disease facts and figures. *Alzheimers Dement*, 2021. 17(3): p. 327-406.
- 4 Jack, C.R., et al., Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *The Lancet Neurology*, 2010. 9(1): p. 119-128.
- 5 Cummings, J., et al., Alzheimer's disease drug development pipeline: 2022. *Alzheimers Dement (NY)*, 2022. 8(1): p. e12295.
- 6 FDA. <https://www.fda.gov/news-events/press-announcements/fda-grants-accelerated-approval-alzheimers-drug>. <https://www.fda.gov/news-events/press-announcements/fda-grants-accelerated-approval-alzheimers-drug> 2021 content current as of 06Jul2021 [cited 2021 23Nov2021].
- 7 Dubois, B., et al., Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria. *Alzheimer's & dementia: the journal of the Alzheimer's Association*, 2016. 12(3): p. 292-323.
- 8 Groeneveld, G.J., J.L. Hay, and J.M. Van Gerven, Measuring blood-brain barrier penetration using the NeuroCart, a CNS test battery. *Drug Discov Today Technol*, 2016. 20: p. 27-34.
- 9 Qin, T., et al., Utility of Animal Models to Understand Human Alzheimer's Disease, Using the Mastermind Research Approach to Avoid Unnecessary Further Sacrifices of Animals. *Int J Mol Sci*, 2020. 21(9).
- 10 de Lange, E.C., The mastermind approach to CNS drug therapy: translational prediction of human brain distribution, target site kinetics, and therapeutic effects. *Fluids Barriers CNS*, 2013. 10(1): p. 12.
- 11 Prins, S., et al., A cross-sectional study in healthy elderly subjects aimed at development of an algorithm to increase identification of Alzheimer pathology for the purpose of clinical trial participation. *Alzheimers Res Ther*, 2021. 13(1): p. 132.
- 12 Verberk, I.M.W., et al., Plasma Amyloid as Prescreener for the Earliest Alzheimer Pathological Changes. *Ann Neurol*, 2018. 84(5): p. 648-658.
- 13 Prins, S., A. Zhuparris, and G.J. Groeneveld, Usefulness of Plasma Amyloid as a Prescreener for the Earliest Alzheimer Pathological Changes Depends on the Study Population. *Ann Neurol*, 2020. 87(1): p. 154-155.
- 14 Haas, D., Chapter 15 - Biomarker for Alzheimer's Disease, in *Precision Medicine*, H.-P. Deigner and M. Kohl, Editors. 2018, Academic Press. p. 333-349.
- 15 Jesse, S., et al., Glial fibrillary acidic protein and protein S-100B: different concentration pattern of glial proteins in cerebrospinal fluid of patients with Alzheimer's disease and Creutzfeldt-Jakob disease. *J Alzheimers Dis*, 2009. 17(3): p. 541-51.
- 16 Bettcher, B.M., et al., MCP-1 and eotaxin-1 selectively and negatively associate with memory in MCI and Alzheimer's disease dementia phenotypes. *Alzheimers Dement (Amst)*, 2016. 3: p. 91-7.
- 17 Prins, S., et al., Inflammatory plasma biomarkers in subjects with preclinical Alzheimer's disease. *Alzheimer's Research & Therapy*, 2022. 14(1): p. 106.
- 18 Jack, C.R., Jr., et al., Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*, 2011. 7(3): p. 257-62.
- 19 DeCarli, C., Mild cognitive impairment: prevalence, prognosis, aetiology, and treatment. *Lancet Neurol*, 2003. 2(1): p. 15-21.
- 20 Hansson, O., et al., Prediction of Alzheimer's Disease Using the CSF A β 42/A β 40 Ratio in Patients with Mild Cognitive Impairment. *Dementia and Geriatric Cognitive Disorders*, 2007. 23(5): p. 316-320.
- 21 Spallazzi, M., et al., CSF biomarkers and amyloid PET: concordance and diagnostic accuracy in a MCI cohort. *Acta Neurol Belg*, 2019. 119(3): p. 445-452.
- 22 Teunissen, C.E., et al., Blood-based biomarkers for Alzheimer's disease: towards clinical implementation. *The Lancet Neurology*, 2022. 21(1): p. 66-77.
- 23 Zetterberg, H., Blood-based biomarkers for Alzheimer's disease - An update. *Journal of Neuroscience Methods*, 2019. 319: p. 2-6.
- 24 Chong, J.R., et al., Blood-based high sensitivity measurements of beta-amyloid and phosphorylated tau as biomarkers of Alzheimer's disease: a focused review on recent advances. *J Neurol Neurosurg Psychiatry*, 2021. 92(11): p. 1231-1241.

- 25 Jansen, W.J., et al., Prevalence of Cerebral Amyloid Pathology in Persons Without Dementia A Meta-analysis. *Jama-Journal of the American Medical Association*, 2015. 313(19): p. 1924-1938.
- 26 Molinuevo, J.L., et al., Ethical challenges in preclinical Alzheimer's disease observational studies and trials: Results of the Barcelona summit. *Alzheimers Dement*, 2016. 12(5): p. 614-22.
- 27 Tremblay-Mercier, J., et al., Open science datasets from PREVENT-AD, a longitudinal cohort of pre-symptomatic Alzheimer's disease. *NeuroImage: Clinical*, 2021. 31: p. 102733.
- 28 Ritchie, C.W., et al., Development of interventions for the secondary prevention of Alzheimer's dementia: the European Prevention of Alzheimer's Dementia (EPAD) project. *Lancet Psychiatry*, 2016. 3(2): p. 179-86.
- 29 Hariton, E. and J.J. Locascio, Randomised controlled trials - the gold standard for effectiveness research: Study design: randomised controlled trials. *BJOG: an international journal of obstetrics and gynaecology*, 2018. 125(13): p. 1716-1716.
- 30 CCMO. <https://www.ccmo.nl/onderzoekers/wet-en-regelgeving-voor-medisch-wetenschappelijk-onderzoek/wetten/wet-op-het-bevolkingsonderzoek-wbo>. <https://www.ccmo.nl/onderzoekers/wet-en-regelgeving-voor-medisch-wetenschappelijk-onderzoek/wetten/wet-op-het-bevolkingsonderzoek-wbo> 2022 [cited 2022 17Aug2022].
- 31 Chao, S., et al., Health behavior changes after genetic risk assessment for Alzheimer disease: The REVEAL Study. *Alzheimer Dis Assoc Disord*, 2008. 22(1): p. 94-7.
- 32 Lineweaver, T.T., et al., Effect of knowledge of ApoE genotype on subjective and objective memory performance in healthy older adults. *Am J Psychiatry*, 2014. 171(2): p. 201-8.
- 33 Grill, J.D., et al., Risk disclosure and preclinical Alzheimer's disease clinical trial enrollment. *Alzheimer's & Dementia*, 2013. 9(3): p. 356-359.e1.
- 34 Hansson, O., et al., Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. *Lancet Neurol*, 2006. 5(3): p. 228-34.
- 35 Mattsson, N., et al., CSF Biomarkers and Incipient Alzheimer Disease in Patients With Mild Cognitive Impairment. *JAMA*, 2009. 302(4): p. 385-393.
- 36 Mattsson, N., D. Brax, and H. Zetterberg, To Know or Not to Know: Ethical Issues Related to Early Diagnosis of Alzheimer's Disease. *International Journal of Alzheimer's Disease*, 2010. 2010: p. 841941