2D BioPAD

Supple Graphene Bio-Platform for point-of-care early detection and monitoring of Alzheimer's Disease

D1.1 MCI to AD Biomarker Deep Dive Analysis for Early Diagnosis

Q-PLAN

24/04/2023

Funded by the European Union

Document Information

Issued by:	Q-PLAN
Issue date:	24/04/2023
Due date:	31/03/2023
Work package leader:	ICN2
Dissemination level:	PU

Document History

Version	Date	Modifications made by
0.1	30/01/2024	Table of Contents provided by Q-PLAN
0.1	12/02/2024	First draft with input from partners circulated
0.2	21/03/2024	Updated version for refinements on desk research by partners
0.2	25/03/2024	Feedback collected from clinical and technical partners & interview results integrated by Q-PLAN
0.3	05/04/2024	Integration of ECR and online survey results by EVNIA
0.3	17/04/2024	Final draft circulated for peer review
0.4	23/04/2024	Peer-review delivered by ICN2 and UEF
1.0	24/04/2024	Final version available for submission.

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Executive Summary

This report was developed within the context of the 2D-BioPAD project, funded by the European Union's Horizon Europe Framework Programme for Research and Innovation 2021-2027, to shed light on (i) biomarkers for early detection of MCI to AD progression, (ii) clinical needs and challenges; (iii) available or forthcoming technological solutions such as 2D-material-based point-of-care (PoC) diagnostic devices; (iv) key actors; and (v) socioeconomic perspectives for clinics and health systems.

To investigate the aforementioned aspects, a Desk Research was conducted, which was later validated and fine-tuned through 26 Semi-Structured Interviews. These interviews involved technology providers, healthcare professionals (HCPs), patients, caregivers, and decision makers, from the 2D-BioPAD consortium, the Scientific and Industrial Advisory Board (SIAB), as well as external key stakeholders with emphasis on the 2D-BioPAD clinical centers.

To expand the collected knowledge, a broader Online Survey was conducted, which yielded in total 90 responses, out of 197 enrolled participants. The 2D-BioPAD Online Survey was meticulously crafted to capture insightful information from a diverse European audience, with a specific focus on gaining a deeper understanding of their needs, concerns, and barriers to acceptance regarding AD and PoC IVD tools. The targeted participant groups for the Online Survey comprised Primary and Specialized Healthcare Professionals (HCPs), Patients, Caregivers, Decision Makers, and Biomarker Experts.

Developed based on the Semi-Structured Interviews, the Online Survey integrated insights gleaned from the Interviews already performed at the time and the results of the Desk Research. This comprehensive approach ensured that the Online Survey covered pertinent topics and addressed key areas of interest aligned with the project's objectives. By incorporating insights from both qualitative and quantitative sources, the questionnaire aimed to gather comprehensive data that would advance the project's goals and offer valuable insights into the subject matter.

The analysis of the responses further shed light in the user-driven dimensions of PoC IVDs for early diagnosis and progression monitoring of AD. As a result of these efforts, several insights for the design and implementation of the 2D-BioPAD system were identified:

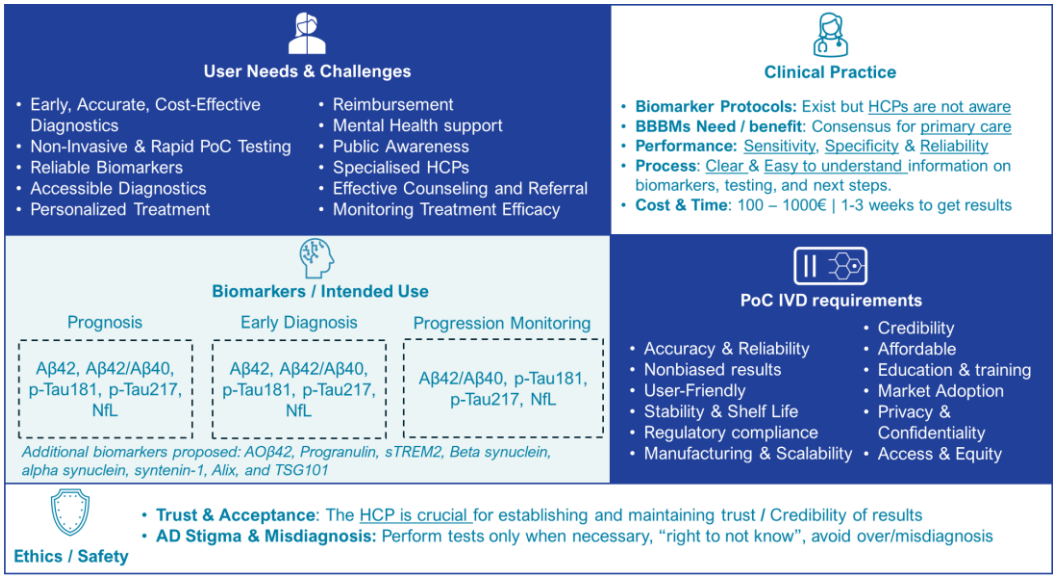

Figure 1. Summary of main user needs, challenges and PoC IVDs requirements for AD.

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Table 1: Terms and Definitions

Abbreviation	Definition	Abbreviation	Definition
Aβ	Amyloid Beta	IPR	Intellectual Property Rights
AD	Alzheimer's Disease	IVD	In-Vitro Diagnostics
ADNI	Alzheimer's Disease Neuroimaging Initiative	IVDR	In-Vitro Diagnostics Regulation
AI	Artificial Intelligence	KER	Key Exploitable Result
APOE	Apolipoprotein E gene	LFA	Lateral-flow biosensor assays
"ATN"	Research framework which covers amyloid abnormalities ('A'), tau protein changes ('T'), and evidence of neurodegeneration ('N'), irrespective of clinical phenotypes	LMICs	Low-and middle-income countries
BBBM	Blood-based Biomarker	LOD	Limit of Detection
BRU	Brain Research Unit at UEF	MCI	Mild Cognitive Impairment
CIS	Clinical Information System	MDR	Medical Device Regulation
CSF	Cerebrospinal fluid	MNPs	Magnetic Nanoparticles
D	Deliverable	MRI	Magnetic Resonance Imaging
DFT	Density-functional theory	NACC	National Alzheimer's Coordinating Center
DMP	Data Management Plan	NFL	Neurofilament Light
DNA	Deoxyribonucleic acid	NIA-AA	National Institute on Aging and Alzheimer's Association
DNS	Digital Neuro Signature	NPs	Nanoparticles
EC	European Commission	NTA-tau	N-terminal containing tau fragments
ECR	Ethical Consideration Roadmap	PCR	Polymerase chain reaction
EDC	Electronic Data Capture	PDB	Protein Data Bank
ELISA	Enzyme-linked immunosorbent assay	PET	Positron emission tomography
ePADs	Electrochemical paper-based analytical devices	PhD	Philosophy Doctorate
ESC	Ethics Steering Committee	PoC	Point-of-Care
EU	European Union	Post-Doc	Post Doctoral
FAIR	Findable, Accessible, Interoperable and Re-usable	PPIE	Patient and Public Involvement and Engagement
FDG	Fluorodeoxyglucose	RNA	Ribonucleic acid
FG	Fluorographene	QA	Quality Assurance
GAAIN	Global Alzheimer's Association Interactive Network	QC	Quality Control
GCP	Good Clinical Practice	QoL	Quality of Life
GDPR	General Data Protection Regulation	RWE	Real World Evidence
GFAP	Glial Fibrillary Acidic Protein	SELEX	Systematic Evolution of Ligands by Exponential Enrichment
GFET	Graphene field effect transistor	SCI	Subjective Cognitive Impairment
GGC	Greenlight Guru Clinical	SOP	Standard operating procedure
GMP	Good Manufacturing Practice	sTREM2	Soluble triggering receptor expressed on myeloid cells 2
GP	General practitioner	tau	Tau protein
HCPs	Healthcare Professionals/Practitioners	TDP-43	TAR DNA-binding protein 43
HICs	High-income countries	WMA	World Medical Association
hPSCreg	Human Pluripotent Stem Cell Registry	WP	Work Package

1. Introduction

Alzheimer's Disease (AD) is the most prevalent form of dementia[1]. With more than 1 in 9 people aged 65 and older having AD, the disease is one of the most severe factors driving brain dysfunction in elderly people. It is expected to affect roughly 18.8 million people by 2050 in Europe alone[2], with enormous financial burden for healthcare, long-term care, and hospice (over $355b just in the US in 2021, without including additional ~$257b in unpaid caregiving) at a global scale (Figure 2).

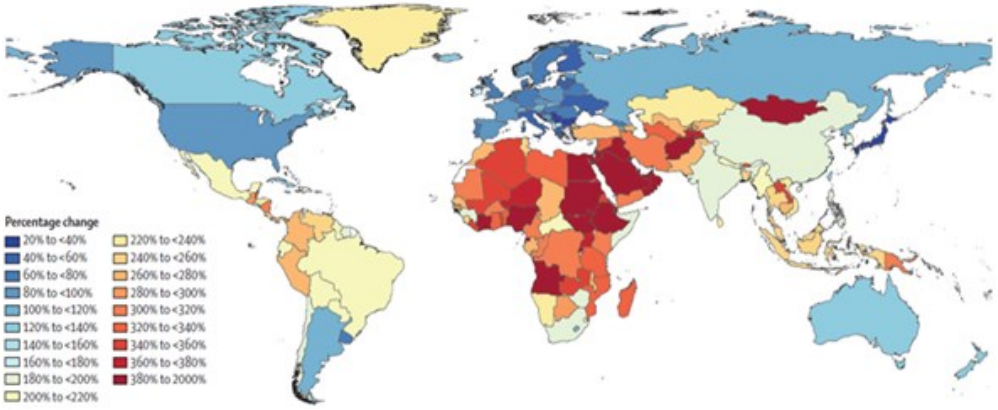

Figure 2. 2019-2050 Percentage change in global prevalence in all-age number of individuals with dementia

For caregivers, AD poses additional emotional and psychological burden, often leading to substantial levels of stress and higher likelihood to experience depression, anxiety, and other symptoms. Perhaps more importantly than all, the incidence and mortality due to AD keep rising, with an increase of 145.2% from 2000 to 2019, while heart diseases have decreased by 14%. This situation will worsen due to population aging; life expectancy in Europe is expected to increase by ~10% in 2065 reaching 92.8 years for women and 90.5 years for men[3].

Recognizing the need for early diagnosis of AD in order for patients to be able to have all the information needed and to alter their lifestyle early on, to avoid the rapid escalation of AD, 2D-BioPAD aims to create a cost-effective, non-invasive point of care/self-testing tool for the early and accurate prognosis (assistive diagnosis) of AD, with special focus on earlier stages such as Subjective or Mild Cognitive Impairment (SCI/MCI).

In the context of 2D-BioPAD, Work Package 1 "WP1: Requirements & System Architecture" holds a pivotal role in identifying and mapping the needs and challenges for early PoC diagnostics for AD, analysing the needs, challenges and available solutions, creating design guidelines and co-designing the 2D-BioPAD system's requirements and architecture. In this regard, the Work Package was divided in 3 tasks (Figure 3), out of which the results of T1.1 are elaborated in this report.

[1] https://www.alz.org/media/documents/alzheimers-facts-and-figures.pdf
[2] https://www.alzheimer-europe.org/dementia/prevalence-dementia-europe
[3] Janssen, F., et al. Future life expectancy in Europe taking into account the impact of smoking, obesity, and alcohol. Elife, 10, e66590, 2021.

Figure 3: The outline of 2D-BioPAD Work Package 1

The document at hand is Deliverable (D)1.1 "MCI to AD Biomarker Deep Dive Analysis for Early Diagnosis", elaborated in the context of Task 1.1: "Point-of-care AD diagnostics' User-centred Requirements, Needs and Challenges". The report focuses on creating a detailed mapping of end-users' needs, disease challenges, and requirements.

The remaining document consists of the following sections:

- **Section 2** articulates the overall methodological approach followed in Task 1.1 (e.g., desk research, interviews etc.).
- **Section 3** provides information on the clinical needs different key actors have regarding point of care AD diagnostics, as well as the challenges and barriers identified.
- **Section 4** offers information on the plasma biomarkers that qualify for early detection of AD, as well as for progression monitoring.
- **Section 5** lists the in-vitro biosensing technologies for AD biomarkers, offering details on aptamers, graphene based biosensing technologies, magnetic nanoparticles used for sample purification and the use of AI in the design and operation of a PoC IVD device.
- **Section 6** provides information on Ethical requirements and principals across Europe and the globe, detailing the procedures, responsibilities and principles applied in the framework of 2D-BioPAD.
- **Section 7** provides an integrated summary of the results of the 26 interviews contacted with expert stakeholders in order to fine-tune the results of the desk research, including the opinions of (i) Patients & Caregivers; (ii) HCPs & Biomarker experts; (iii) Decision Makers related healthcare systems; (iv) Technology Providers.
- **Section 8** elaborates on the feedback gathered through the Online Survey targeted to (i) Patients; (ii) Caregivers; (iii) Primary and Specialised HCPs; (iv) Biomarker Experts (Researchers/Scientists); (v) Decision Makers (Policy Makers and Advocacy Groups), designed to capture higher-level information on their needs, concerns, and acceptance barriers for AD.
- **Section 9** provides some concluding remarks and guides the project's next steps.

The Annexes include (I) more details on promising biomarkers that have been identified as candidates for the 2D-BioPAD system; (II) the 2D-BioPAD Ethics Management Milestone Overview; (III) the ECR – Self Assessment template; (IV) the Interview Guide; (V) the Online survey; and (VI) the Demographic Characteristics per participant profile.

2. Methodological approach

2.1 Desk research

As a first step for our analysis, we identified the current state-of-play regarding (i) biomarkers for early detection of MCI to AD progression, (ii) clinical needs and challenges; (iii) available or forthcoming technological solutions such as 2D material-based point-of-care diagnostic devices; (iv) key actors (industry, policy and research arena); and (v) socioeconomic perspectives for clinics and health systems, based on relevant online literature as well as existing knowledge and expertise from the consortium experts.

The consortium actively collaborated to provide input, refine and enhance our research to include the latest advancements in the intersection of PoC IVD and biomarkers for early detection and progression monitoring of AD, resulting in a more comprehensive and up-to-date version.

This exercise was a key component of our research as it provided preliminary key insights for the implementation of the 2D-BioPAD system, guiding our next steps and allowing us to move forward in a more informed and efficient manner.

As research on biomarkers for AD is constantly evolving, the desk research, focusing mainly on new literature findings, continued in parallel and until the submission of this report.

2.2 Semi-structured interviews

To complement and validate previous findings, further refine them and effectively identify any unanticipated dimensions, towards defining the design inputs and expected outcomes for the 2D-BioPAD systems, semi-structured interviews were designed, planned and organised, including key stakeholders from within the consortium, the Scientific and Industrial Advisory Board, as well as from other external to the consortium stakeholders, such as HCPs, patients and caregivers, following a Patient and Public Involvement and Engagement (PPIE) approach.

The interviews were designed to engage with 4 distinct target groups:

- Patients & Caregivers: to gauge the overall awareness, perceptions, concerns, needs, and attitudes towards AD, from a wider citizen perspective;
- HCPs & Biomarker experts: to explore the current state of research, knowledge gaps, and priorities in AD research from the perspective of experts in the field, while understanding challenges, needs, and experiences of individuals who provide care for Alzheimer's patients, including their concerns and barriers to accessing support and resources; and
- Decision Makers related healthcare systems: to understand the policy landscape, funding priorities, and advocacy efforts related to AD at the national and European levels.
- Technology Providers: to collect technology-oriented feedback on the challenges and enablers of PoC IVD systems, towards expanding our understanding of the relevant domain and anticipating limitations and enabling factors.

2.2.1 Data Collection

To split the associated effort and increase the interviews' efficiency, the interviews were distributed among the consortium members, to cover the four target groups mentioned above. Partners were provided with Interview Guides in English (see Annex IV) to facilitate the activity, and the flexibility to conduct them in the interviewees' mother tongue. The Guides comprised a summary of the 2D-BioPAD project, an overview of the scope and objectives of the interview, as well as notes/tips to ensure a clear understanding of each question's expected outcome.

The interview guide was also a template for partners to "transcript" the interviews, which allowed for a more structured data collection and analysis.

Finally, an informed consent form was also provided to all partners, who then used them for informing the interviewees and receiving their consent for performing the interviews.

Emphasis was given to the three clinical centers, via which, 3 out 4 target groups were engaged.

2.2.2 Semi-structured Interviews' Timeline

The Semi-structured Interviews were performed deployed over a period of approximately 1 month, from early February until early March 2024. Preparatory steps ensured that all partners performing the interviews had all the required material available (guidelines, informed consent forms, etc.), whereas a subsequent step focused on the analysis and aggregation of the results. Key findings were extracted early along the way to feed into the design of the Online Survey.

Figure 4. Semi-structured interviews' timeline.

2.2.3 Data Analysis

The data analysis of the results from the Semi-structured interviews was performed following a stepwise processing. A first preprocessing and cleaning of the results was performed by the interviewer, who transferred the interview transcripts into clear notes for further analysis. The second analysis step was performed during the aggregation of results per stakeholder category, followed by the final qualitative analysis of the aggregated results. During the aggregation (step 2 of the analysis), key aspects were extracted to support the design of the Online Survey.

It is important to note that the structure of the Semi-Structured interviews was slightly different than the Online Survey, as the latter was a more structured and less open-ended improvement of the former. Hence, some of the questions are not fully aligned between the two instruments.

The results of the semi-structured interviews performed are presented in Section 7.

2.3 Online Survey

The 2D-BioPAD online survey was meticulously designed to capture higher-level information from a wider European audience, focusing on a better understanding of their needs, concerns, and acceptance barriers for AD.

The online survey was developed by integrating insights gained from project interview guidance and extensive desk research. This comprehensive approach ensured that the survey covered relevant topics and addressed key areas of interest related to the project's objectives. By drawing upon both qualitative and quantitative sources, the survey aimed to gather comprehensive data that would contribute to the project's goals and provide valuable insights into the subject matter.

The questionnaire aimed to focus on the following target groups:

- Patients: To gauge the overall awareness, perceptions, concerns, needs, and attitudes towards AD among the broader European populace;
- Caregivers: To understand the challenges, needs, and experiences of individuals who provide care for Alzheimer's patients, including their concerns and barriers to accessing support and resources;
- HCPs: To gather varied insights from healthcare providers in different healthcare settings (e.g., primary and/or specialized care) regarding their perspectives on diagnosing, treating, and managing AD, as well as their experiences with patients (if any);
- Biomarker Experts (Researchers/Scientists): To explore the current state of research, knowledge gaps, and priorities in AD research from the perspective of experts in the field; and
- Decision Makers (Policy Makers and Advocacy Groups): To understand the policy landscape, funding priorities, and advocacy efforts related to AD at the national and European levels.

2.3.1 Data Collection

To safeguard the voluntary participation and the anonymity/confidentiality of the targeted audience a public registration approach was chosen as a data collection method. The Online Survey was promoted through the 2D-BioPAD's social media accounts (e.g., LinkedIn) and was also directed to key clinical centers and network stakeholders related to AD.

The Online Survey was created by EVNIA through the GGC EDC platform. Forms which included all relevant questions for all targeted participant groups were produced alongside with processes (including process and validation rules) which facilitated a smooth transition from the paper format of the Survey questionnaire to an active electronic version.

The Survey used and aimed to collect two forms of data:

- Qualitative: Qualitative data including interviewee/expert comments and information derived from the previously conducted interviews and desk research to form the Survey questionnaire that was used for active data collection. Additionally, open-ended Survey questions were used to capture the supplementary perspectives of the targeted audience;
- Quantitative: Quantitative data including Survey questions aimed to extract category/rating/frequency/preference ranking metrics to evaluate different scientific- and socioeconomic aspects of AD.

2.3.2 Online Survey Timeline

The Online Survey was deployed over a period of approximately 2 weeks in total. The most important dates in the preparation, deployment, and analysis of results, are depicted in the below figure. The database was locked on 06/03/2024[4].

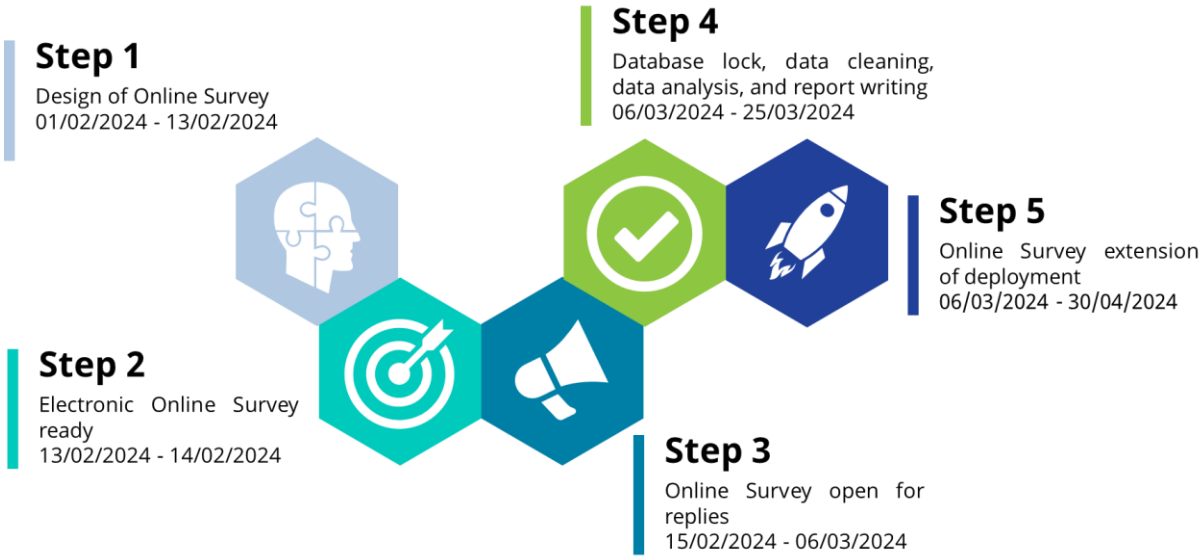

Figure 5. Online Survey's timeline

2.3.3 Data Analysis

Preceding to the formal data analysis and following database lock data cleaning and preparation were conducted as follows:

a. The dataset was checked for any incomplete or erroneous responses;
b. The dataset was cleared from characters not included in the English language (i.e., grammatical characters included in the Greek language) that were present in some of the participant responses to the open/ended questions. Then appropriate translation of excluded characters was conducted to ensure data credibility and reliability without any other data interference;
c. The dataset was checked for any additional outliers or unusual values that may have skewed the analysis;
d. The dataset was re-coded and/or categorized all included variables as needed to perform the analysis.

The analysis of the Online Survey results utilised descriptive statistics and graphical methods. For categorical data, frequency and percentage calculations were conducted, accompanied by graphical representations such as pie charts and bar charts.

[4] Note that the database was locked in a dataset of 90 completed Survey participants. The Online Survey will remain open upon submission of the current Survey report until 30/04/2024 to facilitate larger participant engagement. Results will be documented accordingly through scientific publications.

During the data analysis, as some of the provided answers seemed incomplete or inaccurately worded, additional verification with respondents was undertaken to assess response accuracy and participant credibility. This step was crucial to uphold the integrity and reliability of the collected data and to address any potential discrepancies or uncertainties in the responses.

For the raw data of the Online Survey, which has been extracted from the GGC EDC platform, please refer to the 2D-BioPAD Zenodo Community hosting the respective dataset.

The results of the Online Survey are presented in Section 8.

3. The need for Point of Care AD diagnostics

3.1 The shift to earlier stages and accurate screening and monitoring

With no widely available and effective disease-modifying drugs, and current main therapeutic approaches targeting the symptoms rather than the cause[5], the need to diagnose AD as early as possible and get a better insight on its progression is of utmost importance, especially at earlier stages such as Subjective and Mild Cognitive Impairment (SCI/MCI). An early and accurate AD diagnosis can offer significant benefits such as (i) a better chance of benefiting from treatment, (ii) lessening emotional and social burden, (iii) allowing more time and better quality of life, and (iv) saving trillions in terms of overall costs[6]. This is particularly important in the context of emerging promising treatments through novel disease-modifying drugs. However, such drugs would require extensive screening to identify the people in early AD stages, while also aiming to limit adverse effects, by frequent and accurate monitoring[7].

In fact, currently available diagnostic techniques contain magnetic resonance imaging (brain MRI), lumbar puncture (biomarkers in cerebrospinal fluid), amyloid and tau positron emission tomography (PET), fluorodeoxyglucose (FDG)-PET, and neuropsychological assessment. The most reliable methods are either expensive or invasive and they are commonly used to confirm the AD diagnosis only after the onset of significant symptoms; although it is possible to detect AD-related pathologies in the preclinical stage.

On the other hand, while the neuropsychological tests can detect mild cognitive changes even in the prodromal stage, they are inadequate to distinguish the different neuropathological changes underlying progression to dementia, and thus, an early treatment regimen is impossible. Additional challenges[8] have also been identified, due to diagnostic uncertainty and associated risks for the patient, accompanied by significant delays and costs for both the patients and their families, but also for the health systems across the globe[9]. Nowadays, the situation has further worsened, as recent findings on post-COVID-19 research indicate an increased risk of cognitive impairment, in even younger age[10]. Attention should also be given though to the fact that screening increases the risks of unnecessary anxiety and overtreatment compared with regular healthcare[7].

It is also interesting to highlight that for the neuropathologic diagnosis of AD using biomarkers, the National Institute on Aging and Alzheimer's Association (NIA-AA) "ATN" research framework can be used[11], which covers amyloid abnormalities ('A'), tau protein changes ('T'), and evidence of neurodegeneration ('N'), irrespective of clinical phenotypes and even in the absence of cognitive symptoms. Although these guidelines can support the detection of AD pathology in the brain, they

[5] A Kumar, et al., A review on Alzheimer's disease pathophysiology and its management: an update. Pharmacological reports 67.2 (2015): 195-203
[6] https://www.alz.org/alzheimers-dementia/diagnosis/why-get-checked
[7] Gustavsson, E., et al. Novel drug candidates targeting AD: ethical challenges with identifying the relevant patient population. J. of Medical Ethics, 47(9), 608-614, 2021.
[8] AP Porsteinsson, et al. Diagnosis of Early Alzheimer's Disease: Clinical Practice in 2021. Journal of Prevention of Alzheimer's Disease (2021): 1-16
[9] Nichols, E., & Vos, T. The estimation of the global prevalence of dementia from 1990-2019 and forecasted prevalence through 2050: An analysis for the Global Burden of Disease (GBD) study 2019. Alzheimer's & Dementia, 17, e051496, 2021.
[10] MN Gordon, et al. Impact of COVID-19 on the Onset and Progression of Alzheimer's Disease and Related Dementias: A Roadmap for Future Research. Alzheimer's & Dementia, 18(5), 1038-1046, 2022.
[11] Jack Jr, C. R., Bennett, D. A., Blennow, K., Carrillo, M. C., Dunn, B., Haeberlein, S. B., ... & Silverberg, N. (2018). NIA-AA research framework: toward a biological definition of Alzheimer's disease. Alzheimer's & Dementia, 14(4), 535-562.

cannot support the diagnosis of the clinicopathologic syndrome recognized as AD, and as such, there is a lot of debate for their use in clinical practice. Recognising these limitations, the discussion on these guidelines is ongoing, with suggestions on their evolution to expand with additional biomarkers (e.g., such as the AT(X)N, where 'X' represents novel candidate biomarkers for additional pathophysiological mechanisms such as neuroimmune dysregulation, synaptic dysfunction and blood–brain barrier alterations[12]).

There has been tremendous research to identify new methods and tools for AD diagnostics, with a focus not only to early or pre-clinical stages, but also closer to the patient (i.e., PoC) and/or at primary healthcare settings (e.g., general practitioners or family doctors). Recently, biosensing technologies have been gaining attention for their potential to offer affordable, easy-to use, fast and reliable *in-vitro* diagnostics (IVD) for early detection and monitoring of the pathophysiological changes and cognitive impairment caused by AD. Nevertheless, even though their potential is promising, these biosensors are still under intense research and their deployment to clinical practise is still questionable.

3.1.1 Clinical Needs

The situation in Europe is highly diverse in terms of clinical needs and processes. Focusing more on the 2D-BioPAD clinical centres, an overview of the current clinical practice in terms of AD care in Finland, Germany and Greece is presented, followed by the viewpoint of key actors involved.

AD care in Finland, Germany and Greece

Finland

People aged 70+ are usually referred to Geriatrics clinics (primary care equivalent) for assessment of a suspected dementia-related disease. This is most commonly done as part of public healthcare. The entire evaluation process from first primary care contact to diagnosis can take up to 1 year.

People of working age with cognitive complaints are usually referred by occupational healthcare to specialized Neurology clinics (secondary/tertiary care "memory clinics"). The entire evaluation process from first contact with occupational healthcare to memory clinic diagnosis can take about 6 months. Occupational healthcare is usually provided by private (not public) healthcare providers, which vary depending on the employers' choices. Specialized Neurology clinics are part of public healthcare (usually university hospitals). Following diagnosis and initiation of the appropriate care plan by Neurology, the main responsibility for longer-term management is transferred back to occupational or primary care.

People aged 70+ with atypical symptoms/unclear diagnosis can be referred by Geriatrics clinics to specialized Neurology clinics. If needed, the Neurology clinic can take over the entire diagnostic process, but it is more common that patients are only referred for e.g., CSF, amyloid-PET or other specialized investigations that Geriatrics clinics cannot do, and the results are transferred back to Geriatrics who keeps main responsibility for diagnosis and management.

Cut-off for "working age" is expected to change as retirement age increases and more people choose to work longer. With the recent Finnish healthcare reform, there may be changes at regional and/or national level regarding public healthcare and existing agreements with private occupational healthcare providers.

[12] Hampel, H., Cummings, J., Blennow, K., Gao, P., Jack Jr, C. R., & Vergallo, A. (2021). Developing the ATX (N) classification for use across the Alzheimer disease continuum. Nature Reviews Neurology, 17(9), 580-589.

Germany

Germany has a well-resourced healthcare system compared to countries of similar size and economic wealth, with a large number of practicing physicians. Elderly people usually turn to their primary care physician (PCP) for a first evaluation of cognitive symptoms. 30% of all elderly subjects (age 60+), are assumed to undergo a brief cognitive screening in primary care either because of a subjective memory complaint or as part of a routine assessment. At this case-finding step, 2/3 of these patients turn out to be in the mild-moderate stages of dementia, only 1/3 are at the stage of MCI.

Approx. 50-60% of patients with detectable cognitive deficits are referred by their PCP to Neurologists/Psychiatrists (secondary care) for further assessment of a suspected dementia-related disease, while the remaining 40% are treated in primary care settings. The work-up usually includes short psychometric testing and CT/MRI investigations. Geriatricians and geriatric clinics are few and usually not involved in the work-up of milder cognitive deficits. 10-20% of patients may by-pass the PCP and go directly to secondary care.

Approximately 10% of patients with cognitive complaints or deficits arrive at specialist memory clinics (tertiary care) for a full work-up including comprehensive neuropsychological testing, MRI and biomarker-based diagnosis. Only 1-2% of patients in secondary care and 20-30% of patients in tertiary care receive a biomarker-based diagnosis, although this is the state-of-the-art procedure for mild cognitive impairment or mild dementia according to the most recent German medical specialist guideline. Biomarker testing mainly consists of CSF analysis in 80% of patients, whereas the remaining cases may receive a PET scan. Reimbursement of biomarker diagnostics is inadequate and usually cross-financed by research investigations. Under the current conditions, wait times would be around 50 months, if all patients were referred to specialists based on a brief cognitive assessment, and all were to receive a biomarker-based diagnosis. Adding a blood test (or a similarly easy-to-handle test) for Alzheimer's pathology as additional triage step would reduce wait times to below 24 months.

The evaluation process from first primary care contact to a formal diagnosis can take up to 1-1.5 years. Current antidementia drugs can be prescribed by all physicians as result of their diagnostic work-up. Due to limitations in competence and setting requirements, this will not apply for future monoclonal antibody infusions. All steps of the diagnostic work-up and prescription of antidementia drugs are fully reimbursed by the health insurances. Social services and occupational healthcare are widely available and their costs are partly reimbursed by the long-term care insurance or health insurances, respectively. Benefits and payment levels are determined based on an individual's dependency status as determined by a medical review board. After initiation of the appropriate care plan by Neurologists/Psychiatrists, the responsibility for longer-term management is shared between PCP's and specialists. Memory clinic specialists are usually not involved in longer-term management.

Greece

In the majority of cases in Greece, when relatives start to notice certain cognitive or behavioural issues, usually late, they directly visit a specialised Neurologist either private or working at Memory Clinics at Public or Private Hospitals or Public Day Centers. From there, the diagnostic procedure is carried out with neuropsychological examination, blood tests and imaging. Rarely (<10%) lumbar puncture and/or genetic testing for APOE alleles is also employed to further complement the diagnostic procedure. Yet these examinations are not paid for by the Government. Usually, the family doctor (a General Practitioner) will prescribe the drugs that the neurologists suggest but is not

involved in the diagnosis. In half of the cases, the examined individuals are at the stage of MCI and the other half are already in the moderate or severe stages, when they first visit an HCP to seek advice. The entire evaluation process from first HCP contact to diagnosis can take from 1 month to several months (depending on the age of the subject, the tests that need to be performed, etc.).

Occupational doctors (where available) are not trained to assess cognitive issues, and therefore they are not in a position to evaluate relevant symptoms. In most cases, they will either send the subject to a specialised doctor (i.e., neurologist or psychiatrist) or to the hospital. There are memory clinics in public universities and non-university hospitals, but they have a long waiting list that can take up to 6 months to be reassessed.

People with atypical symptoms/unclear diagnosis are mainly investigated with the means available in university hospitals, but then the treating neurologist is responsible, possibly on a private basis, rather than geriatricians, who are not yet well established in Greece.

Viewpoint of key actors

Decisions Makers/Executives

Nowadays, decision-making (e.g., acquisition of lab equipment, which biomarkers are covered by public healthcare, etc.) are mainly handled at the hospital/clinic level. As EU Member states adopt national strategies for dementia and AD, the decision-making may move from hospital/clinic level to regional or even national level. This is also expected to be affected by the introduction of the new AD medicines (pending EMA approval), and it is still unclear who would or should be eligible for the new medicines, how often should they be administered, what will the reimbursement conditions be, etc.

Decision makers and health system executives (particularly for public health systems) will require a number of additional data (e.g., cost-benefit analysis, health technology assessment, socio-economic trade-offs, etc.) beyond clinical performance and clinical safety for adopting a new diagnostic system in routine healthcare[13]. This is also linked with national and regional policies and strategies, which may result to substantial delays for widespread adoption.

Healthcare Professionals

Until now it has been possible for almost all public healthcare doctors to choose which tests and which biomarker measurements to order for patients. However, it is unclear if this possibility will remain, for how long, and which biomarkers (in bodily fluids or digital ones) will be affected by changes at regional/national level or by the introduction of new medicines for AD.

However, this flexibility offers diagnostic capacity up to a certain degree at primary healthcare settings, as HCPs do not have the knowledge and tools to tap into the potential offered by state-of-the-art biomarker research for early diagnosis and effective monitoring. This often leads to a misdiagnosis for 50% to 70% of patients with AD-related symptoms.

On the other hand, even though specialised HCPs are much better equipped, knowledge and tools wise, the respective percentage is still important, with 25% to 30% of patients with a clinical diagnosis of AD dementia are misdiagnosed[14].

[13] Jönsson, L., Wimo, A., Handels, R., Johansson, G., Boada, M., Engelborghs, S., ... & Winblad, B. (2023). The affordability of lecanemab, an amyloid-targeting therapy for Alzheimer's disease: an EADC-EC viewpoint. The Lancet Regional Health–Europe, 29.

[14] Hansson, O., Blennow, K., Zetterberg, H., & Dage, J. (2023). Blood biomarkers for Alzheimer's disease in clinical practice and trials. Nature Aging, 3(5), 506-519.

On the other hand, HCPs are nowadays required to adopt a person-centred approach, to offer a more personalised and precise experience to the patient and their caregivers, while enabling a warm familiar environment that can address worries and concerns both during the pre- and post-diagnosis stages. However, to be able to do so, they should be able to confidently and in a short timeframe support the diagnosis of the disease and the drafting of an advanced care pathway.

With the introduction of telemedicine and the digitalisation of the healthcare system, the provision of healthcare services has become even more challenging, with the COVID-19 pandemic as a critical example of an increased need for remote clinical assessment and disclosure of the diagnosis of dementia.

Therefore, more elaborate information on how to use digitalised devices and services for biomarker quantification is most needed at the level responsible for diagnostic and treatment purposes (e.g. geriatrics clinics or specialized neurology clinics). This also extends to clear and detailed guidelines on interpretation of results including a discussion of inconclusive data.

Patients & Caregivers

Patients & caregivers are primarily concerned with long waiting times in the public healthcare system. There are different approaches across different countries. For example, Finland does not assign specific doctors, nurses etc. to patients (not even in primary care). The assignment is based on who is available at a specific time, i.e. patients & caregivers can meet different doctors, nurses etc. at different appointments. This can be particularly difficult for older patients with multimorbidity. On the other hand, Greece recently adopted the “family doctor” framework, which is a GP assigned to each family and offers public primary healthcare services. However, for demanding diseases, such as cognitive decline, patients most often skip this consultation and visit either private HCPs or dedicated public institutions (such as GAADRD) for more detailed examinations and testing.

All lab results from public & private healthcare end up in electronic medical records systems, which can be accessed by different stakeholders depending on the health system (e.g., in Finland Electronic Medical Record systems can be the same within a single wellbeing services region, but differ between regions; however, in Greece, hospitals in the same city do not share clinical data). Where online digital services are available (e.g. Kanta services in Finland), citizens can view their own health records at any time. Results can be shown online with some delay, but it often happens that patients can see test results before they meet the doctor to discuss them. The test result is usually shown together with the “normal range” provided by the lab, i.e. patients can see if something is within/outside the normal range, but without any further information provided. This is also the case for non-digitalised processes, through which patients visit an external lab for e.g., blood testing, and then receive (after some time) their results and they are responsible for getting them back to their doctor.

Both patients and their caregivers are in dire need of faster, accurate, and easy-to understand information about their need to be tested, the results of these tests, as well as the way forward in terms of diagnosis/prognosis and beyond.

Clinical Information System/IT support

The healthcare digitalisation of both primary and specialised care follows completely different pathways across Europe, with Electronic Patient Records becoming a necessity for future clinical and research endeavours. There are numerous clinical information systems, that do not necessarily follow the same health information standards (e.g., information models, ontologies, etc.). Even within the same country, different regions or even organisations can have different systems, and systems can change within one region depending on budgets, etc. There are also some national-wide systems (e.g.,

the Greek prescription system known as e-Government center for social security), however information is not largely shared with citizens and not in a user-friendly approach. As such, any new digitalised medical device would require a highly customisable electronic interface for connection to clinical information systems.

A promising development at European level is the introduction of the European Health Data Space[15]; a health specific ecosystem comprised of rules, common standards and practices, infrastructures and a governance framework that aims at (i) empowering individuals through increased digital access to and control of their electronic personal health data, and support to their free movement, as well as fostering a genuine single market for electronic health record systems, relevant medical devices and high risk AI systems (primary use of data), and (ii) providing a consistent, trustworthy and efficient set-up for the use of health data for research, innovation, policy-making and regulatory activities (secondary use of data). Upon adoption, the integration of new digitalised medical devices will be significantly accelerated without requiring resource-intensive activities from HCPs and IT personnel for, most often manually, transferring data across analogue and digital systems.

3.1.2 Challenges and barriers

There are several challenges and barriers identified in several reports in the literature. Most of them are also captured by the World Alzheimer's Reports[16] by the Alzheimer's Disease International.

Lack of awareness/knowledge

A significant roadblock to obtaining a diagnosis is a lack of knowledge and awareness about the disease by the public. Although there has been an effort to increase promotion and media attention over the past decade, even with books and movies, there are limited cases, at national level, that public awareness campaigns that provide information about the signs and symptoms of the condition. As a result, progressive cognitive decline and/or changes in behaviour are often thought to be associated with normal ageing or depression or mistaken for other mental illnesses. This is also coupled with the confusion created about the HCP's expertise that is needed to be advised for reaching to an accurate diagnosis.

This barrier also extends after diagnosis, as patients and caregivers are often left without adequate knowledge on what are the next steps. Yet again, this has two perspectives. On the one hand, HCPs (especially in primary healthcare settings) lack the training, and therefore the knowledge and tools, to handle properly such cases. Thus, this can lead from misdiagnosis to inadequate medication, and of course absence of appropriate guidance and follow-ups. On the other hand, patients and caregivers have limited understanding of their diagnosed conditions, which doesn't allow them to take the proper preventive steps to address its symptoms, better monitor their condition, and follow a preventive strategy to delay progression by drafting an advanced care plan.

Finally, from the broader citizens' perspective (also considering their right "not to know"), there is a significant lack of awareness regarding current examinations and specifically lab tests. People rarely know what it is that they are needed to be tested about and why. In fact, it is often that people get the results before their HCP, having to deal with the anxiety of not understanding what they mean, which is more confusing if a value is marked outside the lower or upper "healthy" thresholds.

[15] https://health.ec.europa.eu/ehealth-digital-health-and-care/european-health-data-space_en
[16] https://www.alzint.org/reports-resources/

Therefore, transparency on this process is essential, including information about the "full package" that needs to be examined, and how a specific "package" fits best with the relevant budget and care pathways.

Stigma

Stigma[17] in general, is a complex concept and may occur at the individual, interpersonal, family, societal, and institutional level. This is still a major barrier to diagnosis, not only for patients and caregivers, but also for HCPs, since approximately one third of them believe that nothing can be done.

Rosin et al.[18], delved deeper into the AD stigma, exploring how the status of "non-normal" ageing, that is related to the lack of ability to care for oneself or function independently (among many other characteristics), affects the subject's decision-making for managing their own health, increasing their quality of life, actively participate in drafting their own care pathway, and in general their sense of autonomy and self-worth. In some cases, stigma can lead to even more adverse effects such as enduring psychological and physiological harms, avoiding diagnosis and treatment, losing relationships and jobs, and even facing a shortened life expectancy[19].

Complexity of diagnosis and prognosis – the risk of misdiagnosis

With the shift in diagnosis to earlier stages, i.e., pre-clinical and prodromal AD including Subjective and/or Mild Cognitive Impairment (SCI/MCI), accurate diagnosis becomes even more challenging, especially considering prognosis and the assessment of the risk to progress (and how fast) to AD. At the same time, in most cases, dementia in general remains undiagnosed in primary healthcare[20].

According to Howard and Scott[21], there is a high frequency of 40% in cases diagnosed with MCI, out of which 50% are expected to not show any decline in the subsequent 5 years. The use of disease-specific biomarkers as a screening mechanism is extensively explored as the answer to this challenge, mainly to identify the ones that are most likely to progress to dementia or AD. However, to day, screening instruments for fluid biomarkers alone have insufficient specificity to establish a valid diagnosis.

On the other hand, false-positive screening results increase the probability of misdiagnosis, could result in unnecessary examinations and treatments, and might cause anxiety and depression in the affected individuals. Even in later stages, some studies have found that the AD phenotype is not always indicative of AD pathology in the brain. Finally, several treatable brain disorders are commonly diagnosed as dementia, which also add up to the complexity of an early and accurate diagnosis followed by an advanced prevention care plan.

Cost of existing diagnostics

Currently, the clinical diagnosis of dementia is reached in the primary healthcare setting, following a rather standardised procedure (although not followed as widely as expected) that covers, medical history, symptoms, physical examinations, as well as blood testing and imaging to rule out other

[17] An attribute that is deeply discrediting which leaves the bearer "tainted" and "discounted".
[18] Rosin, E. R., et al., (2020). A narrative review of Alzheimer's disease stigma. *Journal of Alzheimer's disease*, *78*(2), 515-528.
[19] Best, R. K., & Arseniev-Koehler, A. (2023). The stigma of diseases: unequal burden, uneven decline. American Sociological Review, 88(5), 938-969.
[20] Boustani, M., et al., (2005). Implementing a screening and diagnosis program for dementia in primary care. Journal of general internal medicine, 20(7), 572-577.
[21] Howard, R., & Schott, J. (2021). When dementia is misdiagnosed. International Journal of Geriatric Psychiatry, 36(6), 799-801.

(treatable) causes of cognitive decline. The costs up to this point are not excessive, however results are most often not conclusive or inaccurate.

From there onwards, and for cases that are demonstrating atypical dementia symptoms, early-onset or rapidly progressive dementias, such as AD, patients are referred to specialised care units (e.g., geriatrics, memory clinics, etc.). Diagnosis is reached then following a combination of examinations, that span from cognitive tests to medical imaging and lumbar puncture. The cost of these examinations varies significantly across Europe, with CSF or blood testing ranging from €100 (Bulgaria) to €500 (Italy & Finland) for 3-5 biomarkers together, MRI from €300 (Bulgaria) to €1200[22] (Italy), and a PET scan from ~€600 (Ukraine) to ~€3000 (UK). Altogether, the overall cost per patient exceeds several hundreds of euros, even in the lower limits, making it highly inaccessible in countries where these costs are not covered by a public health system and a tremendous financial burden for public health systems that do so.

This high cost is a significant barrier for the diagnosis of AD, especially at early asymptomatic stages in which their benefit is unclear, but also for the new disease-modifying medicines, which will not only require a thorough screening of everyone to allow their prescription, but also frequent follow-up to monitor progression and avoid adverse effects.

Recent studies suggest that the use of plasma biomarkers (i.e., p-Tau217) could avoid ~57% of PET scans needed for selecting the appropriate treatment option[23].

Time-intensive Process

Clinical diagnosis in primary healthcare settings takes time. It is generally finalised at the second visit, usually within six months after the initial assessment, but can also take up to a year, depending on the circumstances. This is also related to the various tests that need to be performed and are not usually available in primary healthcare settings, and thus require collaboration with external laboratories. It is quite often that it takes weeks (for several reasons) for the HCP to get the full “picture” from all the diagnostic tests that are needed.

Monitoring on the other hand is more streamlined, but also requires a lot of time. It occurs every year for most cases or every 6-months for high-risk and/or critical cases (biomarker in “grey zone”, quick progression, manifestation of comorbidities, etc.). Yet again, if tests are performed in primary healthcare settings, several weeks are needed for the results to reach the HCP.

The “time” element for both aspects will be even more of a challenge when new medicines are approved. Initial testing may be needed at 3- or 6-months to e.g., confirm if the medicine is working for a specific patient and/or to justify the need to continue treatment.

Clinical Heterogeneity

There are widely accepted examinations and tests among clinical centers in Europe for the diagnosis of AD. However, there are also several of them that are not commonly accepted, used or aligned (e.g., due to language differences). This is even more the case for biomarker results, where different equipment (or even different reagents in the same equipment) can lead to different cut-offs.

[22] Depends greatly on the equipment, e.g., 1T vs 3T MRI, etc.

[23] Mattsson-Carlgren, N., Collij, L. E., Stomrud, E., Binette, A. P., Ossenkoppele, R., Smith, R., ... & Hansson, O. (2024). Plasma biomarker strategy for selecting patients with Alzheimer disease for antiamyloid Immunotherapies. JAMA neurology, 81(1), 69-78.

At the same time, when evaluating biomarkers' accuracy performance, a range of measures/metrics can be found in the literature, without following a standardized approach.

These "data" disparities create significant bottlenecks in dementia research, since they lead to non-replicable and non-comparable outcomes. Hence, there is a clear need for technical consistency over an extended period of time and with high analytical precision across different cohorts, in various regions and clinical settings in Europe.

Lack of digital Interoperability

On the other hand, even in the cases where clinical data are comparable, their digitalisation in clinical information systems, does not follow a commonly agreed standard or ontology (even though there are widely accepted frameworks, such as the OMOP CDM, HL7 FHIR, and OpenEHR), making it extremely difficult to be shared across different clinical settings at regional, national, or European levels.

According to the Alzheimer's Association, using electronic health records is an important step in treating patients with AD. There are of course security and ethical concerns, but data interoperability and EHRs are beneficial because they allow for increased communication and sharing between HCPs. This collaboration is beneficial to patients living with AD (or at-risk of AD), but also for researchers who are trying to find the way to treat the disease.

Sex, gender and cultural bias

Research unambiguously shows that sex, gender and cultural biases are found in clinical practice, subjects, samples, and data. Extracted evidence suggests that minority groups and women are at higher risk of underdiagnosis or getting diagnosed at a later stage and receiving a less comprehensive diagnostic evaluation. This is quite important considering for example that two-thirds of diagnosed patients are women, and the same percentage also reflects caregivers. These diagnosis disparities have severe consequences to patients as they are not in a position to receive the benefits of early and accurate diagnosis.

There is insufficient awareness of how sex, gender and cultural factors influence the diagnostic journey, as such groups are greatly underrepresented in both research and early AD testing. This challenge is more complicated than the lack of awareness, as there are enough data to support a more effective and culturally optimal diagnosis and care.

A great recent example, of what would be the consequences of such biases, is the overestimation of arterial oxygen saturation levels by pulse oximetry occurs in patients of racial and ethnic minority groups with COVID-19, which was found to contribute to unrecognized or delayed recognition of eligibility to receive COVID-19 therapies[24].

Privacy and confidentiality

With the digitalisation of the healthcare system in most countries, access to electronic health records/data has become quite a controversial topic of discussion. In fact, the topic of who owns health data and who should control the secondary use of health data (for research) is both complex

[24] Fawzy, A., Wu, T. D., Wang, K., Robinson, M. L., Farha, J., Bradke, A., ... & Garibaldi, B. T. (2022). Racial and ethnic discrepancy in pulse oximetry and delayed identification of treatment eligibility among patients with COVID-19. JAMA internal medicine, 182(7), 730-738.

and subject to the laws under which the data was collected, and the citizenship of the individual on whom the data was collected[25].

Even though there are processes for pseudo anonymisation or anonymization, the development of sophisticated artificial intelligence (AI) algorithms that can process collections of such anonymized data has made it possible to re-identify individuals. As a result, there are increasing concerns about the use of health data, leading to the lack of trust and the propagation of misconception phenomena.

Ageing Population

By 2030, 1 in 6 people in the world will be aged 60 years or over[26]. High-income countries (HICs) are projected to experience an approximately 56% rise in older adult populations by 2050, a percentage that is expected to go beyond 150% in low-and middle-income countries (LMICs).

This is expected to be translated into an important increase of demand for dementia diagnostics at primary healthcare settings. This is attributed to the fact that the prevalence of dementia cases, including AD, will also reach a higher rate. At the same time, as awareness improves, citizens will seek earlier to be tested and receive guidance. Combined, it becomes quite evident that healthcare systems need to be better prepared and equipped to address this increase in demand.

Primary healthcare preparedness

With all the above in mind, the diagnostic infrastructure of existing healthcare systems, particularly in a primary healthcare setting, is not prepared or properly equipped to address the current demand or its projected increase in the near future.

This is even more critical in cases where specialised care is not easily accessible (e.g., rural areas).

Deployment of new PoC IVDs

There are significant scientific breakthroughs in biosensing technologies for PoC IVDs for early detection and monitoring of AD. However, they are not clinically validated and subsequently not regulatory compliant (i.e., based on the MDR and IVDR) to be used in clinical practice. And even if there are approved medical devices that showcase suitable performance, capable of supporting HCPs in their decision-making, clinical use will still be dependent on higher decision-making, which will hinder deployment in primary healthcare settings.

Deployment can be currently done within a research framework (not routine healthcare), e.g. via research units closely connected to memory clinics, such as the Brain Research Unit (BRU) at UEF, and the relevant units in GAADRD and ZI. To have the best chance of changing the clinical standard of care, the test should be easy to perform and provide a readily understandable result that can be integrated to electronic health records for longitudinal monitoring.

[25] Kahn, S. D., & Terry, S. F. (2024). Who owns (or controls) health data? Scientific Data, 11(1), 156.
[26] https://www.who.int/news-room/fact-sheets/detail/ageing-and-health

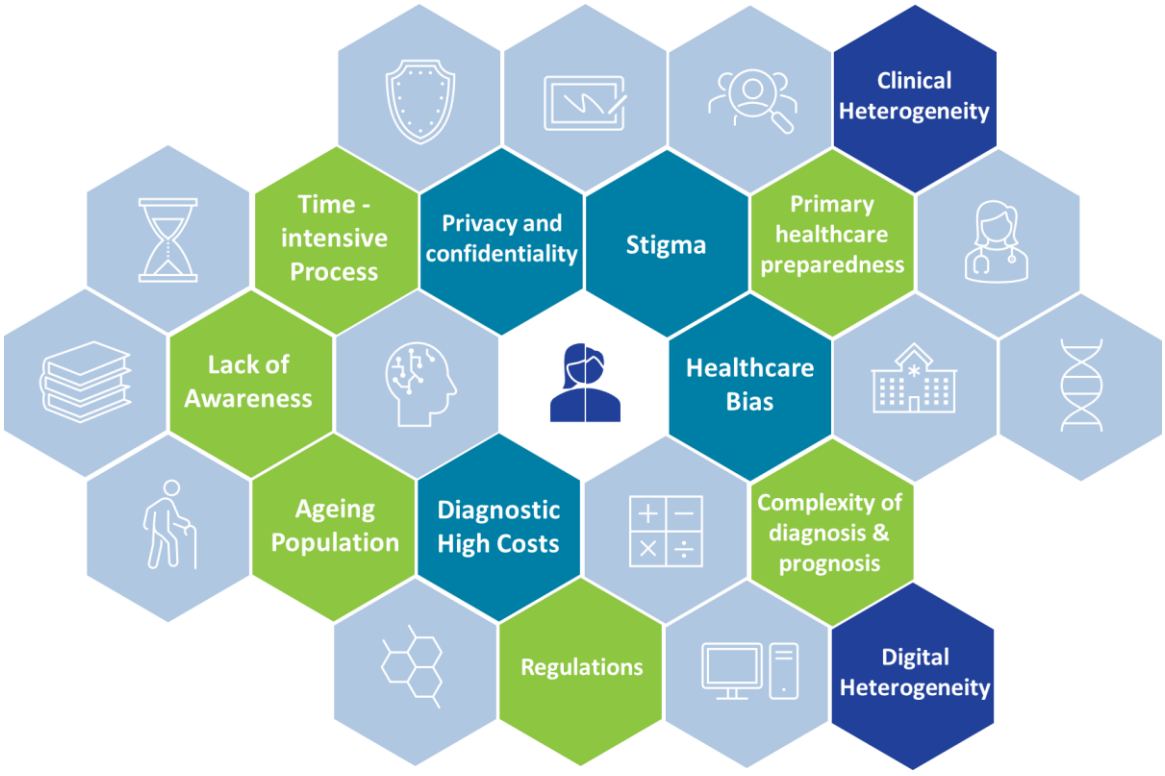

Figure 6. Main challenges related to early detection and progression monitoring of AD

4. Plasma biomarkers for early detection and progression monitoring

In 2014, Kiddle et al.[27], reviewed 163 candidate blood biomarkers of AD onset and progression. They managed to replicate 94 of them, out of which only 9 were found to be associated with AD phenotypes. This is just an example of the extensive research devoted to AD (blood) biomarker research for over a decade. However, up to today there aren't any validated and clinically-approved blood biomarkers that can be widely used in clinical practice.

Since 2018[28], validated biomarkers of AD pathology have been introduced to clinical practice, including Aβ and p-Tau PET; the CSF concentration of Aβ42 and the Aβ42/Aβ40 ratio; the CSF concentrations of total tau (t-tau) and p-Tau181. Additionally, over the years, MRI has increasingly been used to support differential diagnosis. Hence, from the very beginning of using them and until today, biomarkers alone, are not (yet) sufficient to confidently diagnose AD or predict disease progression and should be supplementary to a more complete clinical assessment to help inform the diagnosis of AD[29].

PET and CSF biomarkers are widely used in clinical research. However, AD biomarkers are not routinely incorporated into clinical care for most patients presenting with cognitive decline symptoms. In fact, knowing of amyloid PET status changed patient management in approximately 60% of patients[30]. However, PET and CSF biomarkers' evaluations have several limitations, including high cost, insufficient accessibility, and invasiveness. In search of a viable and reliable alternative, blood-based biomarkers are considered a convenient, cost-effective, and less invasive screening tool[31], with the scientific community converging to a more specific list of blood-based biomarkers as also shown in Figure 7.

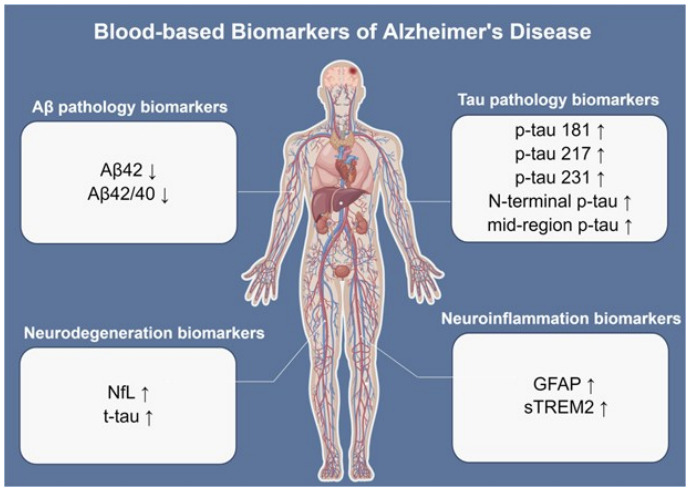

Figure 7. Overview of promising blood-based AD biomarkers[32].

[27] Kiddle, S. J., Sattlecker, M., Proitsi, P., Simmons, A., Westman, E., Bazenet, C., ... & Dobson, R. J. (2014). Candidate blood proteome markers of Alzheimer's disease onset and progression: a systematic review and replication study. Journal of Alzheimer's Disease, 38(3), 515-531.
[28] Jack Jr, C. R., Bennett, D. A., Blennow, K., Carrillo, M. C., Dunn, B., Haeberlein, S. B., ... & Silverberg, N. (2018). NIA-AA research framework: toward a biological definition of Alzheimer's disease. Alzheimer's & Dementia, 14(4), 535-562.
[29] Dubois, B., von Arnim, C. A., Burnie, N., Bozeat, S., & Cummings, J. (2023). Biomarkers in Alzheimer's disease: role in early and differential diagnosis and recognition of atypical variants. *Alzheimer's Research & Therapy*, *15*(1), 175.
[30] Hampel, H., Hu, Y., Cummings, J., Mattke, S., Iwatsubo, T., Nakamura, A., ... & Schindler, S. E. (2023). Blood-based biomarkers for Alzheimer's disease: Current state and future use in a transformed global healthcare landscape. Neuron.
[31] Teunissen, C. E., Verberk, I. M., Thijssen, E. H., Vermunt, L., Hansson, O., Zetterberg, H., ... & Del Campo, M. (2022). Blood-based biomarkers for Alzheimer's disease: towards clinical implementation. The Lancet Neurology, 21(1), 66-77.
[32] Tao, Q. Q., Lin, R. R., & Wu, Z. Y. (2023). Early Diagnosis of Alzheimer's Disease: Moving Toward a Blood-Based Biomarkers Era. Clinical Interventions in Aging, 353-358.

Summarising, Hampel et al. in their recent review[30], introduced a clinical care pathway (

Figure **8**) on how blood biomarkers are and could be used to address the needs, challenges and barriers mentioned above. These intended uses are:

- Early detection of "*at-risk*" healthy individuals at primary healthcare.
- Early detection of AD onset (i.e., SCI or MCI) at primary healthcare.
- Differential diagnosis and treatment selection at specialised care.
- Treatment response and/or disease progression monitoring at specialised care.

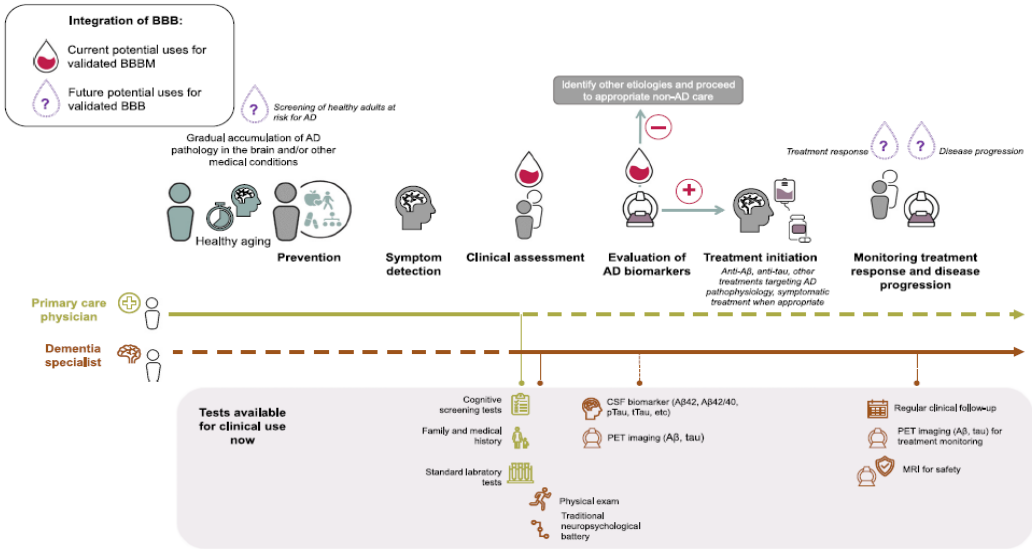

Figure 8. An overarching illustration of integration of Blood-based Biomarkers into patient journey[30].

Finally, the Alzheimer's Association Workgroup (2023-2024) has recently released a draft of the Revised Criteria for Diagnosis and Staging of Alzheimer's Disease, with supplementary material for relevant clinical criteria, which provides information about the ongoing classification of biomarkers, intended uses and biological staging.

4.1 Beta amyloid (Aβ)

Under normal conditions, Aβ is a normal, soluble product of neuronal metabolism that regulates synaptic function beginning early in life[33]. In fact, monomeric Aβ40 and Aβ42 are the predominant forms required for synaptic plasticity and neuronal survival. However, in pathological conditions, Aβ forms aggregates, from soluble oligomers to extended fibers to the amyloid or senile plaques, which are found outside and in between neurons[34]. This pathological behaviour has been associated with an imbalance between Aβ neuronal production and its extracellular clearance of Aβ, which allows for the aggregation of the Aβ fibrils into plaques. This is especially in the case of Aβ42 which is considered more "toxic" since is more likely to aggregate.

[33] Parihar, M. S., & Brewer, G. J. (2010). Amyloid-β as a modulator of synaptic plasticity. Journal of Alzheimer's Disease, 22(3), 741-763.
[34] Hampel, H., et al. (2021). The amyloid-β pathway in Alzheimer's disease. Molecular psychiatry, 26(10), 5481-5503.

This extracellular accumulation/excess of Aβ has several implications from oligomers directly affecting the health of neurons to plaques occupying space, thus preventing the formation of neural networks either in terms of communication among existing ones or the activation of new ones.

Furthermore, it is apparent that if Aβ remains in the brain (which is not the case under physiological conditions), then the concentrations of Aβ in CSF, blood, or other bodily fluids is reduced. This "reduction" has been largely observed both in CSF and blood – with the latter being significantly lower (e.g., in Klafki et al.[35], the concentration of Aβ40 in CSF was measured in the scale of nanograms per ml compared to hundreds of picograms per ml in blood, whereas in the case of Aβ42 from hundreds of pg/ml in CSF to a few dozen pg/ml in blood) – making Aβ one of the core biomarkers for AD.

Plasma Aβ40, Aβ42, and their ratio (Aβ42/Aβ40) have been evaluated extensively in the literature[30,36], with most studies showcasing lower concentration levels of Aβ in patients with AD compared with cognitively unimpaired individuals. Notably, the most recent consensus is that the Aβ42/Aβ40 ratio has a stronger correlation with burden and better diagnostic and prediction accuracy than either Aβ42 or Aβ40 alone.

A less researched, but interesting "shorter" species of Aβ that is gaining attention is Aβ38. Although mainly focused on CSF, it has been observed that a higher concentration of Aβ38 is associated with less or slower cognitive decline (measures with MMSE score) and with lower to no risk of conversion to AD dementia[37]. The concentration of Aβ38 in plasma appears to be of the same magnitude as that of Aβ42[38].

4.2 Tau protein (tau)

Tau protein is predominantly associated with axonal microtubules and is also present at a lower level in dendrites where it is engaged in signalling functions[39]. Under abnormal conditions, tau becomes excessively phosphorylated (and aggregates into neurofibrillary tangles – NFTs) which causes its dysfunction and leads to microtubule disintegration, tau filaments formation and intraneuronal signalling disorder and, consequently, to cell death[40].

Besides examining the total tau (t-tau), the protein has multiple phosphorylation sites, and currently available research, as well as commercial assays, are mainly focused on three different cases, namely the p-Tau181, p-Tau217, and p-Tau231[31]. There are also other phosphorylation sites, such as threonine 199, 202, 205, 235, and 236[41], however they are less researched. These isoforms are quite important as they have been associated not only with diverse diagnostic benefits but also with temporal knowledge on the progression pathway. In particular, literature findings suggest that tau is phosphorylated at different sites over the course of AD[42], starting with p-Tau231, then moving onto p-Tau217, followed by p-Tau181 and later p-Tau205. This "sequence" has been observed both in CSF

[35] Klafki, H. W., et al., (2022). Is plasma amyloid-β 1–42/1–40 a better biomarker for AD than AβX–42/X–40? Fluids and Barriers of the CNS, 19(1), 96.
[36] Pais, M. V., Forlenza, O. V., & Diniz, B. S. (2023). Plasma biomarkers of Alzheimer's disease: a review of available assays, recent developments, and implications for clinical practice. Journal of Alzheimer's Disease Reports, (Preprint), 1-26.
[37] Cullen, N., et al., (2022). Association of CSF Aβ38 levels with risk of Alzheimer disease–related decline. Neurology, 98(9), e958-e967.
[38] Ovod, V., et al., (2017). Amyloid β concentrations and stable isotope labeling kinetics of human plasma specific to central nervous system amyloidosis. Alzheimer's & Dementia, 13(8), 841-849.
[39] Grundke-Iqbal, I., et al., (1986). Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. Proceedings of the National Academy of Sciences, 83(13), 4913-4917.
[40] Mietelska-Porowska, A., et al., (2014). Tau protein modifications and interactions: their role in function and dysfunction. International journal of molecular sciences, 15(3), 4671-4713.
[41] Karikari, T. K., et al., (2022). Blood p-Tau in AD: analysis, interpretation, and clinical utility. Nature Reviews Neurology, 18(7), 400-418.
[42] Barthélemy, N. R., Horie, K., Sato, C., & Bateman, R. J. (2020). Blood plasma phosphorylated-tau isoforms track CNS change in Alzheimer's disease. Journal of Experimental Medicine, 217(11), e20200861.

and plasma. Finally, most recent research[43] focuses on N-terminal containing tau fragments (NTA-tau), that appears to increase across the AD continuum, especially during mid-to-late stages of the disease (even though concentration in plasma is reported to be below the pg/ml scale).

Plasma levels of t-tau have shown large inter-group overlaps that limit its AD diagnostic potential, even though it has been identified to reliably reflect neurodegeneration in AD when measured in CSF. t-tau concentration in plasma can be also related to its peripheral production. However, it can be used for prognostic purposes, as high concentration in plasma has been associated with faster cognitive decline and neurodegeneration[44].

In regard to, the phosphorylated versions, plasma p-Tau181, p-Tau217 and p-Tau231 have all been found to have a similar pattern with t-tau, with elevated concentration in AD patients and a strong link with Aβ and tau pathologies. They are considered good predictors of AD and cognitive decline in MCI[45]. Out of the three, p-Tau217 has the largest fold-change between AD and non-AD disorders and is more related to AD progression[46]. Janelidze et al.[47] assessed 10 different plasma p-Tau assays and identified that p-Tau217, and in particular mass spectrometry-based measures, perform best when identifying mild cognitive impairment patients with abnormal brain Aβ or those who will subsequently progress to Alzheimer's dementia. Recent reviews converge to the outcome that p-Tau217 is superior across different reference standards and different assay platforms[30].

Finally, NTA-tau in plasma has recently been found to increase across the AD continuum, especially in the mid-to-late AD stages[43], complementing the other tau-based biomarkers which hold the most value in earlier stages.

4.3 Neurofilament Light and Neurodegeneration

The neurofilament light (NfL) polypeptide or neurofilament light chain is a component of the neural cytoskeleton, and it is a well-established marker of neuroaxonal injury and neurodegeneration. Under normal physiological conditions, NfL after being released into the interstitial fluid from where it reaches the CSF (~1000-3000 pg/ml) as well as plasma (~10-50 pg/ml). However, in related neurodegenerative pathologies (e.g., upon neuronal injury and loss), the amount that reaches these fluids is increased[48].

Plasma NfL corresponds well to CSF measures, for both MCI and AD, making it a good blood-based biomarker, however not specific to AD[49], as several other reasons lead to that type of neurodegeneration, including other dementia types, such as frontotemporal, vascular and HIV-associated dementias[50]. At the same time, like the phosphorylated tau isoforms, patients with neurodegenerative diseases who have higher levels of NfL are associated with faster disease

[43] Lantero-Rodriguez, J., et al., (2024). Plasma N-terminal containing tau fragments (NTA-tau): a biomarker of tau deposition in Alzheimer's Disease. Molecular Neurodegeneration, 19(1), 1-22.
[44] Arslan, B., Zetterberg, H., & Ashton, N. J. (2024). Blood-based biomarkers in Alzheimer's disease–moving towards a new era of diagnostics. Clinical Chemistry and Laboratory Medicine (CCLM), (0).
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[46] Ashton, N. J., et al., (2023). Plasma and CSF biomarkers in a memory clinic: head-to-head comparison of phosphorylated tau immunoassays. Alzheimer's & Dementia, 19(5), 1913-1924.
[47] Janelidze, S., et al., (2023). Head-to-head comparison of 10 plasma phospho-tau assays in prodromal AD. Brain, 146(4), 1592-1601.
[48] Yuan, A., Rao, M. V., & Nixon, R. A. (2017). Neurofilaments and neurofilament proteins in health and disease. Cold Spring Harbor perspectives in biology, 9(4), a018309.
[49] Gaetani, L., et al., (2019). NfL chain as a biomarker in neurological disorders. Journal of Neurology, Neurosurgery & Psychiatry, 90(8), 870-881.
[50] Bridel, C., et al., (2019). Diagnostic value of cerebrospinal fluid neurofilament light protein in neurology: a systematic review and meta-analysis. JAMA neurology, 76(9), 1035-1048.

progression[51], which could be associated with faster damage to the neurons. Moreover, NfL increase in people older than 70 years of age is observed even in non-dementia cases[52].

4.4 Neuroimmflamation

Brain pathologies such as the ones related to the Aβ aggregation (i.e., plaque deposition) or the hyperphosphorylation of tau (i.e., accumulation of neurofibrillary tangles) induce microglia and astrocyte activation, two cell types involved in important physiological roles, such as synaptogenesis, synaptic plasticity, and neuronal support. Although this response in physiological conditions is anti-inflammatory, if excessive, it induces a pro-inflammatory glial phenotype, thus fostering AD progression[53].

Glial fibrillary acidic protein (GFAP) is one of the most studied glial markers (over 50 reported in the literature[54], which is likely released as a quick response to Aβ pathology in the context of AD. In particular, GFAP is found in astrocytes, which if damaged, release GFAP into cerebrospinal fluid and blood.

Plasma GFAP (~50-400 pg/ml) has the potential to predict cognitive decline to AD dementia in patients with MCI[55] as well as to support differential diagnosis among dementia diseases. It is also interesting that plasma GFAP outperforms its CSF counterpart, with a higher magnitude of change for plasma GFAP and more accurate discrimination of individuals with or without Aβ pathology[56].

The soluble triggering receptor expressed on myeloid cells 2 (sTREM2) has also been researched as an associated neuroinflammation biomarker for AD. Nevertheless, the majority of interesting results are focus on identification in CSF, whereas plasma related studies are limited and ambiguous[32].

Expanding research on GFAP, Prins et al.[57] explored additional neuroinflammatory biomarkers in plasma, namely YKL-40, MCP-1, and eotaxin-1. They confirmed that GFAP was significantly higher in subjects with preclinical AD compared to healthy elderly, however their findings on YKL-40 (which is also believed to be linked to cardiovascular diseases and diabetes[32]) are not conclusive, whereas no results on MCP-1 and eotaxin-1 were introduced.

In a similar recent approach, Foley et al.[58] evaluated six AD-related (Aβ40, Aβ42, Aβ42/40, p-Tau181, t-tau, and NfL) and five inflammatory biomarkers (TNFα, IL6, IL8, IL10, and GFAP), demonstrating significant positive correlations between pro- and anti- inflammatory markers, which is believed to suggest (1) overactivation of multiple immune system regulators, (2) multiple insults occurring at once creating opposing inflammatory effects, (3) dysregulation of the communication between inflammatory signals, and (4) multiple temporal inflammatory signals occurring simultaneously. These

[51] Preische O, et al., Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease. Nat Med. 2019;25(2):277–283.
[52] Nyberg, L., et al., (2020). Elevated plasma neurofilament light in aging reflects brain white-matter alterations but does not predict cognitive decline or Alzheimer's disease. Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring, 12(1), e12050.
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[55] Cicognola C., et al., Plasma glial fibrillary acidic protein detects Alzheimer pathology and predicts future conversion to Alzheimer dementia in patients with mild cognitive impairment. Alzheimer's Res Ther. 2021;13:68.
[56] Zimmer, E. R., Benedet, A. L., Suárez-Calvet, M., & Blennow, K. (2021). Differences between plasma and cerebrospinal fluid glial fibrillary acidic protein levels across the Alzheimer Disease continuum. JAMA neurology. Chicago. Vol. 78, no. 12 (Dec. 2021), p. 1471-1483.
[57] Prins, S., de Kam, M. L., Teunissen, C. E., & Groeneveld, G. J. (2022). Inflammatory plasma biomarkers in subjects with preclinical Alzheimer's disease. Alzheimer's Research & Therapy, 14(1), 106.
[58] Foley, K. E., et al., (2024). Alzheimer's disease and inflammatory biomarkers positively correlate in plasma in the UK-ADRC cohort. Alzheimer's & Dementia, 20(2), 1374-1386.

findings could justify the extensive list of brain inflammatory biomarkers believed to be of value for AD, and thus require substantial more research, not only individually but also combined.

4.5 Synaptic Degeneration

Synaptic degeneration has been identified as a prominent feature of AD, with evidence suggesting that synaptic degeneration is the best neuropathological correlate of cognitive decline in AD[59].

According to Mohaupt et al.[60], β-synuclein is the only detectable blood biomarker for synaptic degeneration in AD. Their findings support significantly higher levels of β-synuclein in the blood of AD patients, including also higher values in pre-clinical and prodromal AD, even earlier than p-Tau181.

Another recent study[61] has also identified higher levels of β-synuclein in preclinical AD and even higher in MCI and AD dementia. Their findings also support that synaptic degeneration occurs early in the AD continuum, and even before tau pathology.

4.6 Apolipoprotein E

The E4 allele of the apolipoprotein E gene (APOE), which translates to the APOE4 isoform, is the strongest risk factor for Alzheimer's disease (AD)[62] and is also considered quite important in the context of new anti-amyloid drugs[63], i.e. APOE4 carriers (particularly homozygotes) have higher risk of adverse events such as ARIA and related symptoms.

However, limited information is currently available on APOE4 and the pathological role of plasma APOE4 remains unclear. Existing information however is quite contradictory with some findings[64] suggesting important associations between low plasma apoE levels, Aβ pathology, and progression from aMCI to a clinical ADD diagnosis. In general, results are not replicable, and thus the value of this protein remains ambiguous.

4.7 TAR DNA-binding protein 43 (TDP-43)

TAR DNA-binding protein 43 (TDP-43) is a highly conserved nuclear RNA/DNA-binding protein involved in the regulation of RNA processing. The accumulation of TDP-43 aggregates in the central nervous system is a common feature of many neurodegenerative diseases, including AD[65]. Under pathological conditions, cleavage, hyperphosphorylation and ubiquitination of TDP-43 can occur, leading to cytoplasmic accumulation and aggregation of TDP-43, which subsequently leads to cognitive disorders.

TDP-43 pathology is observed in up to 50% of AD patients in general and in 75% of patients with severe AD. However, the contribution of TDP-43 in AD is not yet clear, in contrast to Frontotemporal

[59] Tzioras, M., McGeachan, R. I., Durrant, C. S., & Spires-Jones, T. L. (2023). Synaptic degeneration in Alzheimer disease. Nature Reviews Neurology, 19(1), 19-38.
[60] Mohaupt, P., Pons, M. L., Vialaret, J., Delaby, C., Hirtz, C., & Lehmann, S. (2022). β-Synuclein as a candidate blood biomarker for synaptic degeneration in Alzheimer's disease. Alzheimer's Research & Therapy, 14(1), 179.
[61] Oeckl, P., Janelidze, S., Halbgebauer, S., Stomrud, E., Palmqvist, S., Otto, M., & Hansson, O. (2023). Higher plasma β-synuclein indicates early synaptic degeneration in Alzheimer's disease. Alzheimer's & Dementia, 19(11), 5095-5102.
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Dementia (FD), where it appears to be dominant[66]. Hence, it could be considered a valuable biomarker for differential diagnosis.

However, in a recent review by Grigoli et al.[67], it was highlighted that only AD patients (and not FTLD) showed neurofibrillary tangles and neural cytoplasmic inclusion in addition to TDP-43 phosphorylated at serines 409/410, contrary to FD, which has TDP-43 phosphorylated at serines 403/404. Hence, a lot more research is required to evaluate the potential of this protein.

More detailed information about these biomarkers is presented in Annex I.

[66] Chiu, P. Y., Yang, F. C., Chiu, M. J., Lin, W. C., Lu, C. H., & Yang, S. Y. (2022). Relevance of plasma biomarkers to pathologies in Alzheimer's disease, Parkinson's disease and frontotemporal dementia. Scientific Reports, 12(1), 17919.
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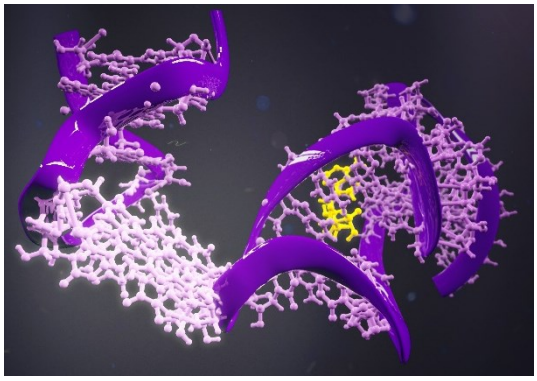
5. *In-vitro* biosensing technologies for AD biomarkers

There is a variety of *in-vitro* biosensing commercial products in our daily life, from the glucometer and the pregnancy test, to the most recently known *rapid test* for COVID-19. There is also an extensive list of research endeavours for biosensing technologies for AD, both *in-vitro* and *in-vivo*[68]. There is also an increasing number of lab-oriented assays that measure CSF and blood-based biomarkers at all stages of the AD continuum, with the most recent example being the FDA Breakthrough Device Designation for Blood-Based p-Tau 217 Test in AD from Quanterix[69].

Focusing on the 2D-BioPAD technologies, key concepts and recent literature findings are presented in the following sections.

5.1 Aptamers as binding agents



Aptamers are short single-stranded sequences of DNA or RNA) that can form unique 3D structures like proteins. The aptamers were primarily designed to mimic antibodies and hence often referred as 'chemical antibodies'. As an alternative recognition element to antibodies, have comparable or higher affinities, are less expensive, do not require *in vivo* production, are easier to reproduce, have smaller molecular size[70], are biocompatible, non-toxic. The aptamers have excellent specificities and can also distinguish between different isoforms of the same target/protein, while being more stable under harsh conditions.

These advantages make aptamers ideal for targeting a range of analytes. They have become a potent rival of antibodies in therapeutics and bio-analysis. The remarkable binding affinities of the aptamers combined with facile synthesis and easier modifications have led to the use of aptamers in both immobilization-based and easy immobilization-free assays such as graphene oxide-based. The versatility of aptamers has allowed the incorporation of aptamers into various nanomaterials such as metallic nanoparticles, carbon materials, and functional nanospheres. The so developed aptasensors have improved the analytical performance and commercial application of aptamers[71]. Such biosensing devices hold immense value for low-cost solutions, especially for PoC IVD systems.

Aptamers are identified through an *in vitro* experimental approach from the 90s named *Systematic Evolution of Ligands by Exponential Enrichment* (SELEX)[72,73], which is still used with several variations but with certain barriers[74,75]: (1) it requires from weeks to months to acquire aptamer candidates; (2)

[68] Murti, B. T., Putri, A. D., Huang, Y. J., Wei, S. M., Peng, C. W., & Yang, P. K. (2021). Clinically oriented Alzheimer's biosensors: expanding the horizons towards point-of-care diagnostics and beyond. RSC advances, 11(33), 20403-20422.
[69] https://www.quanterix.com/press-releases/quanterix-granted-breakthrough-device-designation-from-u-s-fda-for-blood-based-p-Tau-217-test-for-alzheimers-disease/ (announced on the 4th of March 2024).
[70] Shui, B., et al., (2018). Biosensors for Alzheimer's disease biomarker detection: a review. Biochimie, 147, 13-24.
[71] Kim, Y. S., Raston, N. H. A., & Gu, M. B. (2016). Aptamer-based nanobiosensors. Biosensors and Bioelectronics, 76, 2-19.
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[74] Chen, Z., et al., (2021). Artificial intelligence in aptamer–target binding prediction. International journal of molecular sciences, 22(7), 3605.
[75] Mikuła, E., & Malecka-Baturo, K. (2023). An Overview of the Latest Developments in the Electrochemical Aptasensing of Neurodegenerative Diseases. Coatings, 13(2), 235.

the success rate is still low; (3) not all aptamer candidates can be synthesized for affinity characterization; (4) one SELEX protocol cannot be used to select aptamers for all targets. However, the advances in aptamer discovery with improved selection methods can deliver aptamers that can recognize various target molecules with high specificity and affinity, including proteins.

The first aptamers targeting Aβ peptides were reported in 2002 by Ylera et al.[76] Since then, a lot of studies have used aptamers to target biomarker proteins for dementia and AD[77]. In general, electrochemical aptasensors have been gaining a lot of attention, including those targeting neurodegenerative diseases[75]. Interestingly enough, the authors of this review state that *"…there has only been one aptasensor developed for the simultaneous detection of two biomarkers"*, referring to the core challenge of delivering an aptasensor that can target more than two analytes simultaneously.

Focusing on AD, there are quite a few examples in the literature mainly targeting Aβ40[78], Aβ42[79] (or both[80]) as well as tau protein isoforms (such as p-Tau231[81]), all claiming to have superior performance over the conventional ELISA methods.

A more detailed exploration of the literature as well as benchmarking related to the 2D-BioPAD aptamers will be presented under WP2 activities.

5.2 Graphene-based biosensing technologies

In simple terms, Graphene is a one-atom-thick layer of carbon atoms arranged in a hexagonal lattice. Its' discovery[82] and its subsequent groundbreaking experiments regarding this 2D material led Andre Geim and Kostya Novoselov to be awarded the 2010 Nobel Prize in Physics[83]. As introduced by the Graphene Flagship Initiative the following properties make graphene unique.

Graphene is …:

- … the world's thinnest material, only one atom thick, which also makes it extremely light. It is one million times thinner than a human hair whereas less than 1 g of graphene layer can cover a football/soccer field.
- … very strong, stronger than steel and diamond, offering outstanding stiffness and durability
- … very flexible, ideal for wearable devices and foldable electronics.
- … transparent.
- … a great conductor of electricity and heat, allowing the creation of conductive materials, such as inks for electronic circuits and gels that dissipate heat.
- … selectively permeable, allowing (or not) selective passage of atoms.

[76] Ylera, F., Lurz, R., Erdmann, V. A., & Fürste, J. P. (2002). Selection of RNA aptamers to the Alzheimer's disease amyloid peptide. Biochemical and biophysical research communications, 290(5), 1583-1588.
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[78] Khang, A., Idegwu, N., & Lee, J. H. (2023). A cost-effective aptasensor capable of early diagnosis and monitoring of Alzheimer's disease with the rapid analysis of beta-amyloid peptide 1–40. Sensors & Diagnostics, 2(2), 409-417.
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[82] Novoselov, K. S., et al., (2004). Electric field effect in atomically thin carbon films. Science, 306(5696), 666-669.
[83] https://www.nobelprize.org/uploads/2018/06/press-9.pdf

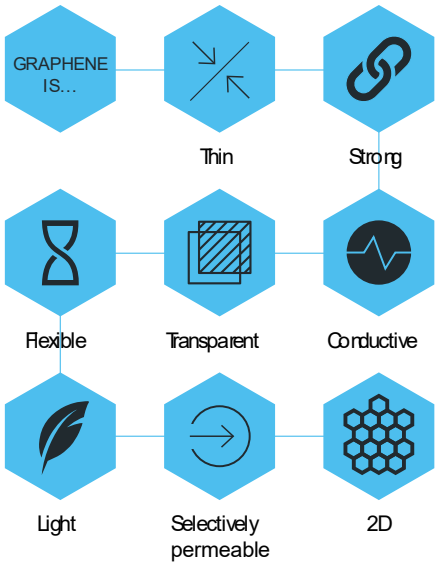

Figure 9. Graphene properties [Source: Graphene Flagship Initiative]

Due to its unique physical structure, as well as its chemical and electrical properties, graphene has become a cornerstone material in life sciences and other fields, as it offers a simple, low-cost, highly stable and modular platform for biosensing applications, towards real-time assessment of miscellaneous biomolecular analytes, important for health monitoring. Graphene combines together, among many other properties, an excellent conductivity and a large surface area with ultimate thinnest which de facto confines the electrical currents on the surface thus creating a strong interaction between conducting electrons and chemical adsorbates on its surface. Furthermore, its capacity to immobilize different molecules (either bioreceptors/probes or analytes)[84], which makes them excellent transducers (i.e., the part of the sensor, which converts chemical information into a measurable signal).

On the contrary, conventional sensing methods (e.g., lateral flow immunoassay, fluorescent microarray and electrochemical methods, PCR-based methods, ELISA, etc.) require highly trained personnel, expensive reagents, high-precision instruments, and quantification methods to achieve highly sensitive detection.

Thus, graphene-based biosensors, with their high sensitivity and specificity, can be particularly useful in life sciences and medicine as they can significantly enhance patient care, early diagnosis of diseases and pathogen detection.

5.2.1 Graphene-based electrochemical biosensors

Nowadays, paper-based lateral-flow biosensor assays (LFA) have become a very valuable and popular tool for PoC IVD[85,86], and have been adopted as an alternative technology to PCR[87]. Such biosensors have also been found to be excellent analytical tools for the detection of AD biomarkers as they are

[84]Peña-Bahamonde, J., et al., (2018). Recent advances in graphene-based biosensor technology with applications in life sciences. Journal of nanobiotechnology, 16(1), 75.
[85] Parolo, C. et al., (2020). Tutorial: design and fabrication of nanoparticle-based lateral-flow immunoassays. Nature Protocols 15(12), 3788-3816.
[86] Sena-Torralba, A., et al. Toward Next Generation Lateral Flow Assays: Integration of Nanomaterials. Chemical Reviews, 122(18): 14881–14910, 2022
[87] Merkoçi, A., et al. COVID-19 biosensing technologies. Biosensors and Bioelectronics, 178: 113046, 2021

easy to use, portable, and provide real-time analysis in a single-step[88] and, above all, their manufacturing process is simple and inexpensive while they show good shelf life.

However, there are also several limitations, such as low sensitivity, binary results (positive/negative), difficulty in handling complex matrices such as blood or serum, or the relatively low number of biomarkers to be detected at the same time. These limitations hinder the adoption of such techniques for pathologies like AD.

Meanwhile, the field of electrochemical paper-based analytical devices (ePADs), which were first introduced by Dungchai et al. in 2009[89], has shown the rise of electrical readout devices such as glucometers or the most recent digital pregnancy tests, which generate an electrochemical signal directly related to the amount of analyte present in the sample, further increasing the capability to act as a PoC sensor. In principle, ePADs are sensitive, portable, disposable and cost-effective (considerably due to the paper substrate) over conventional systems[90].

Electrochemical biosensors operate by converting a biochemical signal into an electrical signal, such as current (amperometric), potential (potentiometric), conductance, or impedance. This conversion process involves the generation or consumption of electrons as part of a redox reaction. The generated signal is then measured by the transducer, quantifying the biosensing result (i.e., different amplitude of the signal).

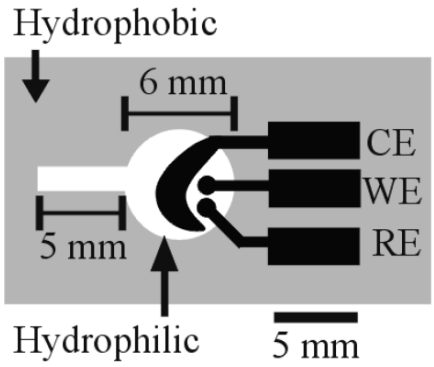

Figure 10. Basic design of the electrochemical detection cell for paper-based microfluidic devices. WE, working electrode; RE, reference electrode; CE, counter electrode[89]

A lot of research has followed, to optimise the functionality of such sensors, with emphasis on the technology of the electrodes. These include laser-induced graphene formation directly on paper[91], print/stamp graphene-based electrode development on paper-like substrates[92] (developed by ICN2), and more, to achieve higher electrical signal and one-step functionalization compared to classic carbon screen-printed electrodes.

[88] Miku, E. (2021). Recent advancements in electrochemical biosensors for Alzheimer's disease biomarkers detection. Current Medicinal Chemistry, 28(20), 4049-4073.
[89] Dungchai, W., Chailapakul, O., & Henry, C. S. (2009). Electrochemical detection for paper-based microfluidics. Analytical chemistry, 81(14), 5821-5826.
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[91] Bhattacharya, G., et al., (2022). Disposable paper-based biosensors: Optimizing the electrochemical properties of laser-induced graphene. ACS Applied Materials & Interfaces, 14(27), 31109-31120.
[92] Giacomelli, C., et al. Selective stamping of laser scribed rGO nanofilms: From sensing to multiple applications. 2D Materials, 7(2), 024006, 2020.

Such biosensors have been applied on several occasions in the detection of biomarkers for neurodegenerative diseases, including AD[93],[94]. Most of the findings in recent literature target Aβ42[95,96], however, mainly focusing on lab set-ups and with a limited number of samples.

5.2.2 Graphene Field Effect Transistors

A graphene field effect transistor (GFET) is composed of a graphene channel between two electrodes with a gate contact to modulate the electronic response of the channel (Figure 11). The graphene is exposed to enable functionalization of the channel surface and binding of receptor molecules (e.g., antibodies, aptamers, etc.) of interest to the channel surface.

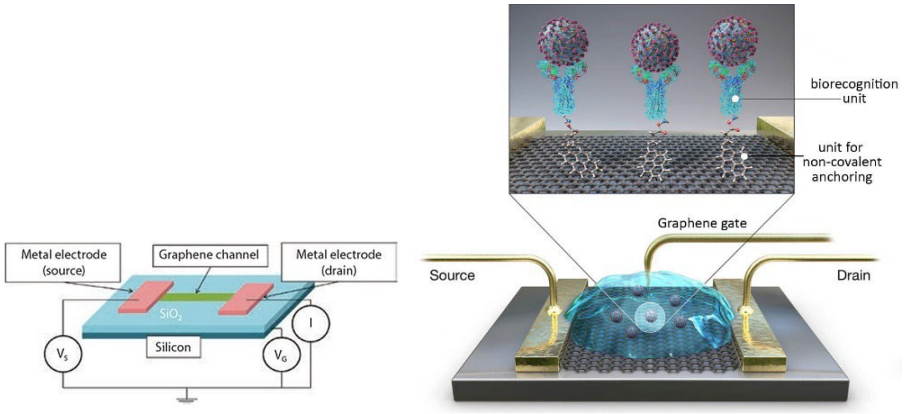

Figure 11. Structure of a graphene field effect transistor [Source: Merck (left) and Seo et al.[97] (right)]

When a target analyte is "captured" by the bioreceptor/biorecognition unit bound to the graphene surface, the redistribution of electronic charge generates a change in the electric field across the FET channel region, which, in turn, changes the electronic conductivity in the channel and the overall device response. This response (i.e., signal) can be accurately measured allowing not only the binary detection of the analyte (positive/negative) but also a quantitative representation of the concentration found.

Hence GFET-based biosensors are promising candidates for reliable and low-cost PoC IVDs. This is also the case for AD, with recent research leveraging their unique properties to deliver screening tests for AD hallmarks. Indicatively, there have been studies using GFET technologies targeting Aβ42 in serum[98], Aβ42 and t-tau in CSF and Plasma[99], or GFAP in plasma[100]. In most findings though, even though there are highly promising results, like 100% accuracy in differential diagnosis[98], distinctive output signal for

[93] Valkova, P., & Pohanka, M. (2021). Novel trends in electrochemical biosensors for early diagnosis of Alzheimer's disease. International Journal of Analytical Chemistry, 2021.
[94] Rouhi, N., et al., (2023). Recent progress in the graphene-based biosensing approaches for the detection of Alzheimer's biomarkers. Journal of Pharmaceutical and Biomedical Analysis, 222, 115084.
[95] Sethi, J., Van Bulck, M., Suhail, A., Safarzadeh, M., Perez-Castillo, A., & Pan, G. (2020). A label-free biosensor based on graphene and reduced graphene oxide dual-layer for electrochemical determination of beta-amyloid biomarkers. Microchimica Acta, 187, 1-10.
[96] Abbasi, H. Y., et al., (2021). Graphene based electrochemical immunosensor for the ultra-sensitive label free detection of Alzheimer's beta amyloid peptides Aβ (1–42). Nanoscale Advances, 3(8), 2295-2304.
[97] Seo, G., et al., (2020). Rapid detection of COVID-19 causative virus (SARS-CoV-2) in human nasopharyngeal swab specimens using field-effect transistor-based biosensor. ACS nano, 14(4), 5135-5142.
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each biomarker[99], or even competitive advantages over current state-of-the-art techniques (i.e., ELISA or SIMOA)[100], their sample size of subjects in the studies is quite limited or non-existent. Hence, it is evident that this technology (or its application in AD) is currently in its "validation" phase with much more clinical research required to further support preliminary findings.

5.2.3 Graphene materials in electrochemical and GFET biosensors

Graphene derivatives are very promising materials as key components for chemical and biochemical sensors for both electrochemical[101,102] and FET devices[103,104]. They can be used either as a functionalized gate electrode in an electrochemical sensor but, when used as single layer, graphene acts as a zero-band gap semiconductor exhibiting high electronic mobility and allows the implementation of a FET channel with excellent transconductance.

Furthermore, their high transconductance, stability, mechanical flexibility, biocompatibility and chemical inertness[10,105] offer additional features for high-quality biosensors. Thus, of densely and selectively functionalized conductive graphene transducers [106,107,108] can retain the initial semiconducting and electrochemical behaviour of the sensor surface even under the most demanding environments such as whole blood.[104]

The essential ingredient for a state-of-art advanced graphene biosensor (both in electrochemical and FET set-ups) is the tailored and reproducible chemical functionalization of graphene's surface, because it is indispensable for the effective and selective recognition of the target analytes (ions,[103] nutrients,[101] proteins,[104] genes, or viruses[109] in the samples), which in turn defines signal generation selectivity and, in some cases, ultra-high sensitivity.

This can be achieved via the chemistry of fluorographene (FG, pioneered by UP-CATRIN in 2010[110]), affording tuneable graphene derivatives (Figure 12)[111] with a functionalization degree that reaches up to 15%.

As the conjugation handles are grafted directly to the graphene backbone, avoiding long linkers, the electron transfer between the site of the biorecognition event to the electrodes is enhanced. As a result, this functionalisation approach supports a more specific binding with bioreceptors/biorecognition units and it enhances the electrochemical activity[112], improving the

[101] Wang, M, et al. A Wearable Electrochemical Biosensor for the Monitoring of Metabolites and Nutrients. Nat. Biomed. Eng 2022, 1–11.
[102] Lee, H., et al. A Graphene-Based Electrochemical Device with Thermoresponsive Microneedles for Diabetes Monitoring and Therapy. Nature Nanotech 2016, 11 (6), 566–572.
[103] Xue, M., et al. Integrated Biosensor Platform Based on Graphene Transistor Arrays for Real-Time High-Accuracy Ion Sensing. Nat Comm 2022, 13 (1), 5064.
[104] Goldsmith, B. R., et al. Digital Biosensing by Foundry-Fabricated Graphene Sensors. Sci Rep 2019, 9 (1), 434.
[105] Georgakilas, V., et al. Noncovalent Functionalization of Graphene and Graphene Oxide for Energy Materials, Biosensing, Catalytic, and Biomedical Applications. Chem. Rev. 2016, 116 (9), 5464–5519.
[106] Šedajová, V., et al. Nitrogen Doped Graphene with Diamond-like Bonds Achieves Unprecedented Energy Density at High Power in a Symmetric Sustainable Supercapacitor. Energy Environ. Sci. 2022.
[107] Jayaramulu, K., et al. Covalent Graphene-MOF Hybrids for High-Performance Asymmetric Supercapacitors. Advanced Materials, 33 (4), 2004560, 2021.
[108] Bakandritsos, A., et al. High-Performance Supercapacitors Based on a Zwitterionic Network of Covalently Functionalized Graphene with Iron Tetraaminophthalocyanine. Adv. Funct. Mater, 28 (29), 1801111, 2018.
[109] Kevadiya, B. D., et al. Diagnostics for SARS-CoV-2 Infections. Nat. Mater. 2021, 20 (5), 593–605.
[110] Zbořil, R., et al. Graphene Fluoride: A Stable Stoichiometric Graphene Derivative and Its Chemical Conversion to Graphene. Small 2010, 6 (24), 2885–2891.
[111] Bakandritsos, A., et al. Cyanographene and Graphene Acid: Emerging Derivatives Enabling High-Yield and Selective Functionalization of Graphene. ACS Nano, 11 (3), 2982–2991, 2017.
[112] Flauzino, J. M. R., et al. Label-Free and Reagentless Electrochemical Genosensor Based on Graphene Acid for Meat Adulteration Detection. Biosensors and Bioelectronic, 195, 113628, 2022.

signal-to-noise ratio, the selectivity and sensitivity of the devices, and ultimately the final biosensing reading.

As already introduced in the previous sub-sections, graphene derivatives are most often combined with either graphene-based electrochemical or FET biosensors for applications related to AD. However, there are also cases, where graphene derivatives can also be coupled with other materials for addressing the same challenge[113].

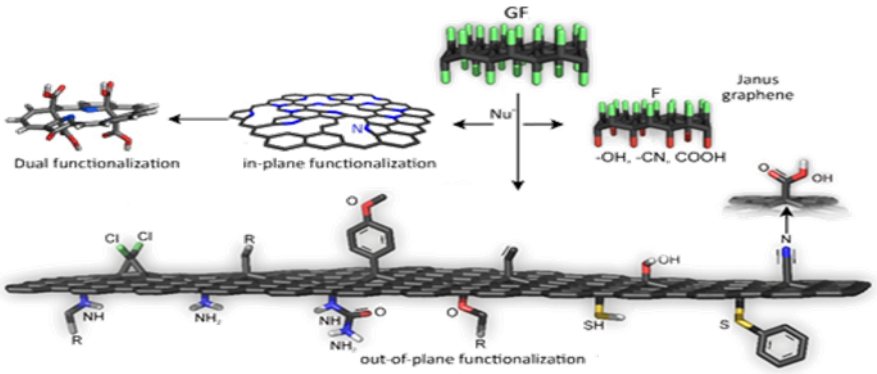

Figure 12. Fluorographene chemistry - pioneered and established by UP-CATRIN - leading to selectively and densely functionalized graphene derivatives, tailored for high electrochemical activity.

There are other categories of graphene (and non-graphene) PoC IVD technologies. However, we introduced the core technologies that will be developed during 2D-BioPAD. More information about the underlying technologies and their benefits compared to other state-of-the-art solutions will be covered under WP3.

5.3 Magnetic Nanoparticles for sample purification, flow control, and signal amplification

Materials in the nanoscale exhibit unique properties beyond conventional materials, that make them ideal for several biomedical applications. Magnetic Nanoparticles (MNPs) are one of the most widely researched and applied nanomaterials in life sciences. MNPs stand out as one of the most extensively studied and utilized nanomaterials in the field of life sciences. MNPs possess a multitude of distinctive characteristics including, but not limited to, their high surface-to-volume ratio, superparamagnetic nature, excellent biocompatibility, low toxicity, and ability for site-specific targeting. Moreover, their cost-effectiveness and sustainable manufacturing processes further enhance their suitability for a wide range of biomedical applications[114] .

When integrated with biosensors or aptasensors, they can significantly enhance sensitivity and reliability[115]. MNPs play a crucial role in sample purification, minimizing non-specific signals, and

[113] Palley, B. F., et al., (2023). Electrochemical Biosensors Composed of Polyethylenimine (PEI) and Graphene Derivatives for Rapid Detection of Alzheimer's Disease. In Electrochemical Society Meeting Abstracts 244 (No. 63, pp. 3006-3006).
[114] Chavan, N., Dharmaraj, D., Sarap, S., & Surve, C. (2022). Magnetic nanoparticles–A new era in nanotechnology. Journal of Drug Delivery Science and Technology, 77, 103899.
[115] Le, T. D., Suttikhana, I., & Ashaolu, T. J. (2023). State of the art on the separation and purification of proteins by magnetic nanoparticles. Journal of Nanobiotechnology, 21(1), 363.

regulating flow at various stages of the bioassay process, such as bioreceptor incubation, purification, recognition, and signal acquisition[116,117].

Magnetically responsive nanoparticles capable of targeting and interacting either directly (via appropriate surface functionalisation) or indirectly (via conjugation with probes such as antibodies or aptamers) with specific proteins can be easily controlled with high precision by an external magnetic field. This magnetic interaction can support the quantification of the bound target/analyte (e.g., a protein) either through supporting fine-tuned separation (leaving only the target/analyte in a known buffer) or by reading out magnetic signals (i.e., magnetic resistance, magnetic induction, nonlinear magnetization, etc.), as there is little magnetic background signal from biological samples, minimizing noise and interference[118]. The combination of these attributes can be valuable tools for bioassays, especially for PoC IVDs[119,120].

Up to date a variety of nanoparticles (NPs) have been employed in biosensing techniques for AD, including gold and silver NPs, quantum dots, graphene oxide NPs, Prussian Blue NPs, carbon nanostructures, and various forms of MNPs[121]. Their unique properties have been used to identify blood biomarkers, such as Aβ40, Aβ42 and p-Tau[122,123], with good selectivity and specificity, fast response and low LOD, with several applications across the AD continuum.

A more detailed exploration of the literature as well as benchmarking related to the 2D-BioPAD MNPs will be presented under WP2 activities. AI for PoC IVD design and operation.

5.4 AI for PoC IVDs

The application of Artificial Intelligence (AI) in material sciences has gained significant momentum in recent years, with traditional manual and human-intensive processes being augmented by Artificial Intelligence (AI)-driven simulation and experimental automation[124]. Focusing on the applications within 2D-BioPAD some preliminary findings are showcased below.

5.4.1 Aptamer modelling and aptamer-target interaction prediction

In silico/computational methods to select aptamer candidates for specific targets have been employed widely for over two decades[125], using structural information to run various simulations to identify the structure with the highest affinity[126]. Such solutions include but are not limited to RNAfold[127],

[116] Esmaeili, E., Ghiass, M. A., Vossoughi, M., & Soleimani, M. (2017). Hybrid Magnetic-DNA Directed Immobilisation Approach for Efficient Protein Capture and Detection on Microfluidic Platforms. Scientific reports, 7(1), 194.
[117] Zhu, N., et al., (2004). DNA Hybridization at Magnetic Nanoparticles with Electrochemical Stripping Detection. Electroanalysis, 16(23), 1925-1930
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[119] Hou, F., et al., (2023). The application of nanoparticles in point-of-care testing (POCT) immunoassays. Analytical Methods.
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[121] Abdullah, S. A., et al., (2023). Functional Nanomaterials for the Diagnosis of Alzheimer's Disease: Recent Progress and Future Perspectives. Advanced Functional Materials, 33(37), 2302673.
[122] Devi, R., et al., (2020). Au/NiFe 2 O 4 nanoparticle-decorated graphene oxide nanosheets for electrochemical immunosensing of amyloid beta peptide. Nanoscale Advances, 2(1), 239-248.
[123] Chiu, M. J., et al., (2020). Nanoparticle-based immunomagnetic assay of plasma biomarkers for differentiating dementia and prodromal states of Alzheimer's disease–A cross-validation study. Nanomedicine: Nanotechnology, Biology and Medicine, 28, 102182.
[124] Pyzer-Knapp, E. O., et al., (2022). Accelerating materials discovery using artificial intelligence, high performance computing and robotics. npj Computational Materials, 8(1), 84.
[125] Rost, B., & Sander, C. (1993). Prediction of protein secondary structure at better than 70% accuracy. Journal of molecular biology, 232(2), 584-599.
[126] Chushak, Y., & Stone, M. O. (2009). In silico selection of RNA aptamers. *Nucleic acids research*, *37*(12), e87-e87.
[127] http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi

CONTRAfold[128], PSIRRED[129] prediction models/engines, from the University of Vienna and Standford, respectively. These models have been employing AI-based approaches to increase performance, reduce computational resources required, and expand the range of applications (e.g., Neural networks to predict protein secondary structure based on the position-specific scoring matrices generated by PSI-BLAST, applied in PSIRRED[130]).

However, with the exponential growth of AI technology and even wider access to AI services, research (and commercial applications) has significantly shifted to the use of more advanced AI-driven approaches for predicting protein secondary structure, docking and selecting aptamers[131]. Some recent examples on aptamer modelling and predicting aptamer-target interaction using either machine or deep learning models are AptaNet[132], DAPTEV[133], APIPred[134], AptaTrans[135], and AptaBERT[136]. Their results, showcase that the combination of a large, curated dataset and modern deep learning models yields accurate aptamer-target interaction predictions, with performance that exceeds ROC-AUC of 95%.

Although no direct findings on AI-assisted aptamer selection for AD have emerged, additional research will be incorporated under WP2 activities along with most appropriate AI models.

5.4.2 2D material design

According to Huan, Li, and Zhu in their recent work about AI and graphene[137], remarkable progress has been made in properties prediction (electrical, mechanical, thermal, cytotoxicity), structure recognition (atomic structure, microscopic dimensions/shapes), inverse design (composition, microstructure), and task recognition (chemical recognition, motion recognition, 3D imaging) of graphene and its composites. The other way, i.e., the use of graphene for AI progress, has also been exponentially growing, with graphene being the main material of the novel neuromorphic computing hardware/chips, which is believed to be a promising way around the limits of Moore's Law.

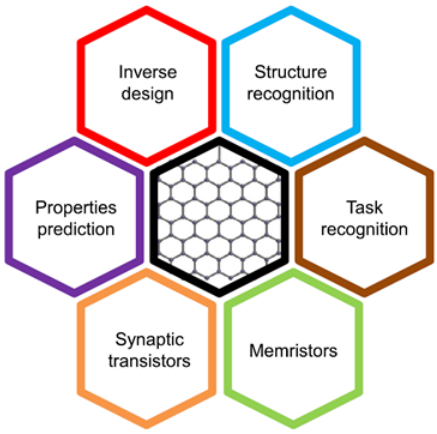

Figure 13. Graphene-incorporated artificial intelligence[137]

[128] http://contra.stanford.edu/contrafold/
[129] http://bioinf.cs.ucl.ac.uk/psipred/
[130] Jones, D. T. (1999). Protein secondary structure prediction based on position-specific scoring matrices. J. Molecular Biology, 292(2), 195-202.
[131] C Lee, S. J., et al., (2023). Design and prediction of aptamers assisted by in silico methods. Biomedicines, 11(2), 356.
[132] Neda Emami and Reza Ferdousi. AptaNet as a deep learning approach for aptamer–protein interaction prediction. Scientific Reports, 11(1), 2021.
[133] Andress, C., et al., (2023). DAPTEV: Deep aptamer evolutionary modelling for COVID-19 drug design. PLOS Computational Biology, 19(7), e1010774.
[134] Fang, Z., et al., (2023). APIPred: An XGBoost-Based Method for Predicting Aptamer–Protein Interactions. J. Chemical Information and Modeling.
[135] Shin, I., et al., (2023). AptaTrans: a DNN for predicting aptamer-protein interaction using pretrained encoders. BMC Bioinf., 24(1), 447.
[136] Morsch, F., et al., (2023). AptaBERT: Predicting aptamer binding interactions. bioRxiv, 2023-11.
[137] Huang, M., Li, Z., & Zhu, H. (2022). Recent advances of graphene and related materials in artificial intelligence. Advanced Intel. Sys., 4(10), 2200077.

AI-based approaches are already employed in graphene research to accelerate the computational processes involved, extending to various different applications as mentioned above (Figure **13**). Indicative examples include the work of Singh and Li[138,139], who employed AI models for molecular dynamics simulation to evaluate thermal-mechanical properties of graphene, showcasing comparable results to density-functional theory (DFT) simulations. This was also supported by Manti et al.[140], who explored ML models for structural instabilities of 2D materials, using data from the Computational 2D Materials Database[141], showcasing that the classification model developed can drastically reduce computational efforts in high-throughput studies (such as the ones required in material sciences).

On the other hand, Patel et al.[142] used a linear regression model to estimate the absorption values of graphene-based biosensors, delivering evidence of the model's high performance. Extending on the interaction with other materials, Zhang et al.[143] showed how an active ML model effectively reveals the microscopic processes involved in substrate-catalysed growth, and in particular graphene growth on Cu(111).

There are already several literature findings of the significant benefits AI can have in material science, and specifically for 2D materials, such as graphene. Under the work of WP3, the functionalization of the graphene design and fabrication process will be optimized via multimodal neural networks that will combine features such as sensitivity, number of defects, conductivity as well as structural data from the actual real-world fabrication and functionalization experiments.

[138] Singh, A., & Li, Y. (2022). ML potentials for Graphene. In ASME Int. Mechanical Eng. Congress & Exposition (Vol. 86656, p. V003T03A036).
[139] Singh, A., & Li, Y. (2023). Reliable ML potentials based on artificial neural network for graphene. Computational Materials Science, 227, 112272.
[140] Manti, S., et al. (2023). Exploring and machine learning structural instabilities in 2D materials. npj Computational Materials, 9(1), 33.
[141] Gjerding, M. N., et al., (2021). Recent progress of the computational 2D materials database (C2DB). 2D Materials, 8(4), 044002.
[142] Patel, S. K., et al. (2023). Graphene-based H-shaped biosensor with high sensitivity and optimization using ML-based algorithm. Alexandria Engineering Journal, 68, 15-28.
[143] Zhang, D., Yi, P., Lai, X., Peng, L., & Li, H. (2024). Active machine learning model for the dynamic simulation and growth mechanisms of carbon on metal surface. Nature Communications, 15(1), 344.

6. Ethical Consideration Roadmap

Important ethical points of attention are the novelty of the 2D-BioPAD system in combination with project activities involving human participants, human tissue, and processing of data to validate and develop the 2D-BioPAD system as well as understanding the societal perspectives, clinical needs, and challenges to implement the 2D-BioPAD system.

Hence, 2D-BioPAD (led by EVNIA) drafted an Ethical Consideration Roadmap (ECR) that has the purpose to detail the ethics management principles, forthcoming actions, and responsibilities to ensure that the ethics requirements are met within the 2D-BioPAD project. The ECR is a strategic document describing the fundamental ethical perspectives relevant to the 2D-BioPAD project and defining the procedures to be followed by the 2D-BioPAD consortium.

The ECR has been prepared using appropriate sections from the European Commission's guide "*EU Grants: How to complete your ethics Self-Assessment Version 2.0 13 July 2021*"[144] and adopting the ethical principles described by the UK Statistics Authority to guide the scope and methodology of ethics application in the 2D-BioPAD project. The ECR may be updated during the lifecycle of the project if needed, introducing further information as the project progresses and/or unexpected ethic issues arise.

The full (standalone) version of the ECR has been upload as a public resource on the 2D-BioPAD website (here).

6.1 European Code of Conduct for Research Integrity

All the 2D-BioPAD's consortium activities should be carried out in compliance with fundamental principles of research integrity described in "The European Code of Conduct for Research Integrity"[145] as follows:

- Reliability in ensuring the quality of research, reflected in the design, methodology, analysis, and use of resources.
- Honesty in developing, undertaking, reviewing, reporting, and communicating research in a transparent, fair, full, and unbiased way.
- Respect for colleagues, research participants, research subjects, society, ecosystems, cultural heritage, and the environment.
- Accountability for the research from idea to publication, for its management and organization, for training, supervision, and mentoring, and for its wider societal impacts.

6.2 Applied Ethical Principles in the 2D-BioPAD Project

In addition to the above-mentioned Research Integrity principles, 2D-BioPAD's consortium activities shall be carried out in compliance with ethical principles developed by the UK Statistics Authority[146] that are the following (Figure 14):

[144] Grants E. How to complete your ethics self-assessment Version 2.0, 13 July 2021.
[145] The European Code of Conduct for Research Integrity REVISED EDITION 2023.
[146] Authority US. Ethical considerations associated with Qualitative Research methods.

- Legal/regulatory compliance[146]: adhere to laws and regulations during product design, development, validation, and use minimize the risks of ethical issues.
- Public Good[146]: the use of data has clear benefits for users and serves the public good.
- Data Security and Confidentiality: data processing methods transparent and according to recognized standards. Quality Assurance (QA) and Quality Control (QC) activities must be an integral part of data management methodology and are implemented prior to the publication of any data, safeguarding the transparency, consistency, comparability, completeness, and accuracy of the data.
- Methodological Quality[147]: clinical activities shall be conducted in compliance with ISO 14155:2020 and ISO 20916:2019 that address good clinical practices for the design, conduct, recording, and reporting of clinical investigations carried out in human subjects to assess the safety and performance of medical devices and *in vitro* medical devices.
- Public Views and Engagement[148]: the views of the public are considered in light of the data used and the perceived benefits of the research.
- Transparency[146]: the access, use and sharing of data is transparent, and is communicated clearly and accessibly to the public.

Ethical perspectives and points of attention related to 2D-BioPAD project activities will be outlined in Section 6.5 according to each of the above-mentioned ethical principles.

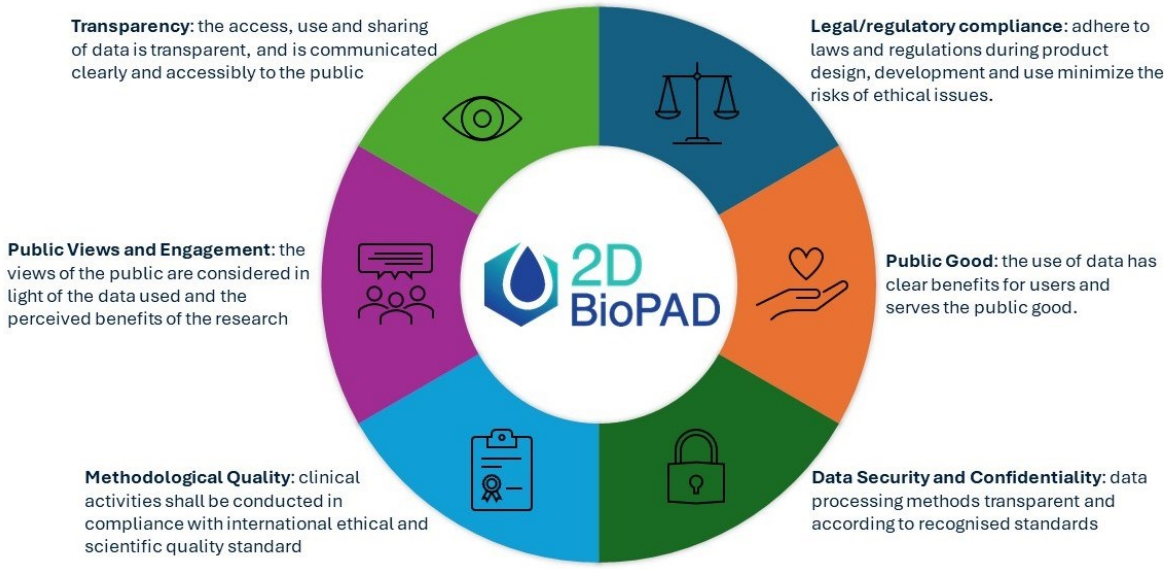

Figure 14: 2D-BioPAD's ethical principles.

[147] Vijayananthan A, Nawawi O. The importance of Good Clinical Practice guidelines and its role in clinical trials. Biomed Imaging Interv J. 2008;4(1):e5.
[148] Authority US. Considering public views and engagement regarding the use of data for research and statistics.

6.3 Ethical Requirements – Regulations and Guidelines

6.3.1 Global Requirements

All the 2D-BioPAD's consortium activities shall comply with:

- WMA Declaration Of Helsinki – Ethical Principles For Medical Research Involving Human Subjects[149].
- Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine[150].

6.3.2 European Requirements

European Regulations

All the 2D-BioPAD's consortium activities shall comply with the following European Regulations:

- Regulation (EU) 2016/679 of the European Parliament and of The Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation)[151].
- Regulation (EU) 2017/745 of the European Parliament and of the Council of 5 April 2017 on medical devices, amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/2009 and repealing Council Directives 90/385/EEC and 93/42/EEC[152].
- Regulation (EU) 2017/746 of the European Parliament and of the Council of 5 April 2017 on *in vitro* diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU[153].

European directives, standards, guidelines, conventions, and codes

In addition to the above-mentioned regulations, all the 2D-BioPAD's consortium activities will comply with the following European directive, standards, guidelines, conventions, and codes:

- The European Code of Conduct for Research Integrity[145].
- ICH E6 (R3) Guideline on good clinical practice (GCP)[154].
- Charter of Fundamental Rights of the European Union 2012/C 326/02[155].
- European Convention on Human Right as amended by Protocols Nos. 11, 14 and 15; supplemented by Protocols Nos. 1,4, 6,7, 12,13 and 16[156].

[149] WMA Declaration Of Helsinki – Ethical Principles For Medical Research Involving Human Subjects.
[150] Convention for the protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine (ETS No. 164).
[151] Regulation (EU) 2016/679 of the European Parliament and of The Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (GDPR).
[152] Regulation (EU) 2017/745 of the European Parliament and of the Council of 5 April 2017 on medical devices, amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/2009 and repealing Council Directives 90/385/EEC and 93/42/EEC.
[153] Regulation (EU) 2017/746 of the European Parliament and of the Council of 5 April 2017 on in vitro diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU.
[154] ICH E6 (R3) Guideline on good clinical practice (GCP).
[155] Charter of Fundamental Rights of the European Union 2012/C 326/02.
[156] European Convention on Human Rights as amended by Protocols Nos. 11, 14 and 15; supplemented by Protocols Nos. 1,4, 6,7, 12,13 and 16.

- Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells[157].
- Directive (EU) 2022/2555 of the European Parliament and of the Council of 14 December 2022 on measures for a high common level of cybersecurity across the Union, amending Regulation (EU) No 910/2014 and Directive (EU) 2018/1972, and repealing Directive (EU) 2016/1148 (NIS 2 Directive)[158].
- Ethics By Design and Ethics of Use Approaches for Artificial Intelligence Version 1.0, 25 November 2021[159].
- ISO 14155:2020: Clinical investigation of medical devices for human subjects — Good Clinical Practice
- ISO 20916:2019: In vitro diagnostic medical devices — Clinical performance studies using specimens from human subjects — Good study practice.

6.3.3 National Requirements

Because the 2D-BioPAD research activities will be conducted in 3 clinical centres in Finland, Greece, and Germany, the corresponding activities will comply also with the following national requirements:

- Finland:
  - The ethical principles of research with human participants and ethical review in the human sciences in Finland, Finnish National Board on Research Integrity TENK guidelines 2019[160].
  - The Medical Research Act and Decree (488/1999)[161].
- Germany:
  - The Act on Medical Devices (Medical Devices Act) (Medizinproduktegesetz – MPG) (especially §§19-24)[162].
  - (Model) Professional Code for Physicians in Germany - MBO-Ä 1997 -The Resolutions of the 121st German Medical Assembly 2018 in Erfurt as amended by a Resolution of the Executive Board of the German Medical Association on 14/12/2018[163].
- Greece:
  - Law 3418/2005 Code of Medical Ethics, GG A. 287/28.11.2005 (Κώδικας Ιατρικής Δεοντολογίας)[164].

[157] Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells.
[158] Directive (EU) 2022/2555 of the European Parliament and of the Council of 14 December 2022 on measures for a high common level of cybersecurity across the Union, amending Regulation (EU) No 910/2014 and Directive (EU) 2018/1972, and repealing Directive (EU) 2016/1148.
[159] Ethics By Design and Ethics of Use Approaches for Artificial Intelligence Version 1.0, 25 November 2021
[160] The ethical principles of research with human participants and ethical review in the human sciences in Finland, Finnish National Board on Research Integrity TENK guidelines 2019.
[161] The Medical Research Act and Decree (488/1999).
[162] The Act on Medical Devices (Medical Devices Act) (Medizinproduktegesetz – MPG) (especially §§19-24).
[163] (Model) Professional Code for Physicians in Germany- MBO-Ä 1997 -The Resolutions of the 121st German Medical Assembly 2018 in Erfurt as amended by a Resolution of the Executive Board of the German Medical Association on 14/12/2018.
[164] Law 3418/2005 Code of Medical Ethics, GG A. 287/28.11.2005 (Κώδικας Ιατρικής Δεοντολογίας).

6.4 2D-BioPAD Ethical Procedures and Responsibilities

6.4.1 General Assessment and Oversight – EVNIA

EVNIA (with Efstathios Vassiliadis as Task Leader of T1.2) has the overall responsibility of keeping oversight of ethical considerations made and applied throughout the entire life cycle of the project in accordance with the ECR. Taking on this role EVNIA strives to provide leadership by promoting and supporting a culture that builds ethics and integrity consciousness into the project activities.

EVNIA' specific responsibilities in carrying out oversight are as follows:

- Request and remind all partners of 2D-BioPAD's consortium to read and review the ECR;
- Ensure relevant partners of 2D-BioPAD's consortium complete the Self-Assessment and continuously update status of Self-Assessments in the 2D-BioPAD Consortium SharePoint Site;
- Monitor and review the status of the ECR and its activities i.e., Self-Assessments provided by partners of the 2D-BioPAD's consortium;
- Evaluate possible emerged ethical issues during the project activities and advice on the necessary corrective actions;
- Provide status and general assessment of ethics in the project in Steering Committee meetings to ensure open discussion, priority of and handling of ethical related questions and issues raised during the project.

6.4.2 Self-Assessment – Consortium Members

The Self-Assessment (see Annex III) process offers a framework for consortium partners to review and document the ethics of the project activities throughout the research cycle. The Self-Assessment must cover all ethical considerations made and applied in the 2D-BioPAD project's design, development/experimentation, and deployment phases. The Self-Assessment provides a timely means to identify ethical issues for the research planned and conducted.

The Self-Assessment is not intended to be performed by consortium members alone, but to be performed as a group, discussed, and documented by each Task leader representing different partners in the Consortium. The Self-Assessment method does not resolve the ethical issues, however, strives to identify ethical risks and shape future discussions that enable the prevention of ethical harms and the improvement of ethics in project activities.

The 2D-BioPAD consortium members' specific responsibilities in carrying out Self-Assessment are as follows:

- Complete the Self-Assessment per Task and archive in the 2D-BioPAD shared repository for EVNIA to review;
- React to emerged ethical issues during the project activities and communicate to the relevant responsible partner and EVNIA if needed.

In the stage of preparing D1.1 the 2D-BioPAD consortium members' were engaged to read and comment on the ECR and to understand their responsibility in reporting ethical incidental findings that might compromise the security or integrity of the research and involved participants. The Self-Assessment should be performed in two timepoints per task by the relevant consortium partner that is the main responsible for that task.

In respect to timepoints,

- The first Self-Assessment per task should be made as an initial assessment in month 1-4 after kick-off of the task focusing on the planned activities.
- The second Self-Assessment is a final assessment to be made when the task is finalized i.e., in the end of each task that will depict what was done in the conduct of the task e.g., provide the evidence/documentation for the activities.

For tasks that comprise more than one delivery (or versions) per task, only two Self-Assessments are required i.e., an initial assessment when initiating activities for the first delivery (or 1st version) and an end assessment when finalizing the last task/delivery (or 2nd or 3rd version).

Annex II depicts the planned timing of the Self-Assessment related activities along relevant milestones.

6.5 2D-BioPAD Main Ethical Principles

Ethical perspectives and guidance on points of attention will be outlined in the following sections and should be incorporated in 2D-BioPAD project activities that are depicted in the figure below and that should be followed by Consortium partners and members.

Figure 15: 2D-BioPAD project activities

6.5.1 Public Good

Main ethical scope

This principle focuses on ensuring the project will strive to serve the public good, is relevant to the communities involved, the project benefits outweigh the risks of the project and has objectives that

are not harmful or prejudicial to participants. Thus, the project activities should ensure human rights, health, and safety and introduce minimal harmful environmental impact throughout the project.

Application of the principle in the 2D-BioPAD project

2D-BioPAD project activities aim to introduce a fast and cost-effective IVD system that strives to support the early diagnosis and progression monitoring of AD directly at primary healthcare settings reducing costs of screening processes and increasing the public accessibility. The 2D-BioPAD project activities plan to: (1) Provide and improve evidence bases that will support the development of healthcare service delivery, and (2) Guide critical decision-making with anticipated benefits for economy, society, and quality of people life.

In this context, the 2D-BioPAD project must strive to:

- Not use data or research outcomes to directly identify data subjects or specific populations.
- Provide a significant public good in line with best practice guidance.
- Apply public goods to the entire population.
- Present negligible potential harm to anyone involved, including the public.
- Identify in the planned methods any possible outcomes bias and mitigate them as far as possible.

2D-BioPAD project deliverables that incorporate the ethical principle:

- Exploitation and Sustainability Plan (WP6 D6.4/D6.5/D6.6) provides the outline of the use that the 2D-BioPAD consortium intends to make of its Key Exploitable Results (KERs) along with the respective action plans and time frame for exploitation. This includes any further activities aimed at the dissemination, use, and sustainability of 2D-BioPAD's KERs, along with any findings concerning IP issues. The plan envisages 2D-BioPAD final strategy for exploitation, management of Intellectual Property Rights (IPR) and sustainability, including also any selected commercialization path if applicable.
- **Clinical Study Protocol** (WP5 D5.1/D.5.2/D5.3/5.4) describes design, deployment, evaluation, and validation of clinical studies.

Ethics Self-Assessment in the 2D-BioPAD project

In order to ensure that 2D-BioPAD project comply with the principle of Public Good it is advised to evaluate the impact of the activities on people, communities, organisations, and companies.

To do that, the balance between the potential positive impact of projects (i.e., the public good benefit) and any potential risks (direct, or indirect) to groups or individuals that may arise from, or is related to, the project, will be evaluated throughout a Self-Assessment.

The self-assessment will evaluate:

- Voluntary participation, Informed consent and privacy of individual's information;
- Security in regard to identification of participants;
- Methodological quality in data collection, analysis, and outputs;
- Potential harm (including stigmatization) or distress related to a project and its outcomes for those who have participated in the research;
- Potential benefits of the project.

6.5.2 Data Security and Confidentiality

Main ethical scope

This principle focuses on the protection of data subject's identity (whether person or organisation), and on maintaining confidentiality and security of the data collected during the research project. Researchers should be transparent in their approach to data collection, validation of data collection methods, methods to secure data and ensure participants' confidentiality in data management and anonymity in reporting of results.

Application of the principle in the 2D-BioPAD project

All 2D-BioPAD project data collection and data management activities shall strive to ensure that personal data is:

- Processed lawfully, fairly and in a transparent manner in relation to the data subject;
- Collected for specified, explicit and legitimate purposes relative to project's objectives and not further processed in a manner that is incompatible with those purposes;
- Adequate, relevant and limited to what is necessary in relation to the purposes for which they are processed;
- Accurate and, where necessary, kept up to date;
- Kept in a form which permits identification of data subjects for no longer than is necessary for the purposes for which the personal data are processed;
- Processed in a manner that ensures appropriate security of the personal data in compliance with GDPR requirements.

Moreover, the Consortium members must pay attention to the principle of proportionality, the right to privacy, the right to the protection of personal data, the right to the physical and mental integrity of persons, the right to non-discrimination, the need to ensure protection of the environment and high levels of human health protection.

2D-BioPAD project deliverables that incorporate the ethical principle:

In order to ensure that 2D-BioPAD project comply with the principle of Data Security and Confidentiality, a Data Management Plan (DMP) is available for all the consortium's partners. The DMP sets out the overall methodological principles pertaining to the management of the data that will be collected, generated and/or re-used in the framework of the project, safeguarding sound and ethical data management along the entire duration of the project. The DMP will be updated three times during the 2D-BioPAD project period to ensure that that all relevant data collection and data management aspects are planned and documented (i.e., D7.2 in M3, D7.3 in M24, and D7.4 in M48).

- Data Management Plan (WP7 D7.2/D7.3/D7.4) describes:
    - The data management lifecycle for the data to be collected, generated and/or re-used in the framework of 2D-BioPAD, serving as the key element of good data management.
    - The methodology employed is to safeguard the sound management of the data collected, and/or generated as well as to make them Findable, Accessible, Interoperable and Re-usable (FAIR).
    - Information on the data that will be collected, generated and/or re-used and the way in which it will be handled during and after the end of the project along with the standards applied to this end.
    - Details on how the data will be made openly accessible and searchable to interested stakeholders as well as its curation and preservation.

- The management of any research outputs other than data in line with FAIR principles.
- Information on the resources to be allocated so as to make data FAIR clearly identifying responsibilities pertaining to data management, while addressing data security and ethical aspects.

Ethics Self-Assessment in the 2D-BioPAD project

In addition, any potential risk related to data security and confidentiality that may arise from, or is related to, the project, will be evaluated throughout a Self-Assessment. Self-Assessment will evaluate potential risks related to:

- Processing of personal data;
- Informed Consent and privacy of individual's information;
- Bias, fairness and transparency in data collection, data management, data analysis, and outputs;
- Measures taken to avoid bias in input data and algorithm design where Artificial Intelligence will be used;
- Ethical standards in Cyber Security, put in place to ensure the trustworthiness, accuracy, and reliability of the systems and operations and to maintain data privacy and reduce the chances of a security breach.

6.5.3 Methodological Quality

Main ethical scope

This principle emphasizes the importance of ensuring suitable methodologies are applied in all research activities throughout the entire project phases while following applicable standards, and clinical guidelines, ensuring precision, reproducibility, and quality of research outputs, while also safeguarding the rights, integrity, and confidentiality of involved participants. From an organizational standpoint, this practice enhances resilience to public scrutiny and plays a crucial role in building public trust and confidence.

Application of the principle in the 2D-BioPAD project

2D-BioPAD project's activities shall strive to be:

- Conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with the applicable regulatory requirement(s) and the standards ISO 14155:2020, ISO 20916:2019 that address good clinical practices for the design, conduct, recording and reporting of clinical investigations carried out in human subjects to assess the safety and performance of medical devices and *in vitro* medical devices; and
- Initiated and continued only if the anticipated benefits justify the risks and if the rights, safety, and well-being of the involved subjects are the most important considerations and prevail over interest of science and society;
- Conducted by researchers skilled in the chosen methodology;
- Undergo a careful assessment of the chosen methods and subsequent analyses to ensure they effectively address the research questions and that methods are appropriately described in the study protocols;
- Supported by the available non-clinical- and clinical information available as state of the art;
- Conducted with products manufactured, handled, and stored in accordance with applicable Good Manufacturing Practice (GMP) and used in accordance with the approved protocol;

- Conducted in compliance with recognized standards of methodological integrity and quality and ensuring that research results are transparent by providing access to data, algorithms, or other results needed for replicating and validating findings.

2D-BioPAD project deliverables that incorporate the ethical principle:

- **Management and Quality Plan** (WP7 D7.1) defines the overall project management principles and procedures applied to 2D-BioPAD and the quality assurance (QA) provisions for safeguarding high-quality project outcomes. It describes the roles and responsibilities of each project participant, with emphasis on work breakdown and management, progress reporting, financial monitoring, payment processes, risk identification and change management.
- **Data Management Plan** (WP7 D7.2/D7.3/D7.4) see Section 6.5.2.
- **Clinical Study Protocol** (WP5 D5.1/D.5.2/D5.3/5.4) describes design, deployment, evaluation, and validation of clinical studies.

Ethics Self-Assessment in the 2D-BioPAD project

Any quality risk that may arise from, or is related to, the project, will be evaluated throughout a Self-Assessment.

The self-assessment will evaluate:

- The application of ethical principles originating from regulations and standards;
- The application of clinical guidelines or other state of the art information;
- The quality of data;
- Methods used to collect, process and visualize the data, and any assumptions made during those processes;
- Validity of the conclusions;
- Potential bias in data collection, analysis, and outputs;
- Measures taken to avoid bias in input data and algorithm design where Artificial Intelligence will be used;
- Ethical standards in Cyber Security, put in place to ensure the trustworthiness, accuracy, and reliability of the systems and operations and to maintain data privacy and reduce the chances of a security breach;
- Research activities involving human cells or tissues;
- Staff required expertise to undertake the research specified;
- Quality of methods used to safeguard of research governance process and human oversight;
- The potential of the quality methods in realising research benefits or mitigate risks.

6.5.4 Legal/regulatory Compliance

Main ethical scope

Prior to commencing their research, researchers need to carefully consider any legal obligations pertinent to their work. These requirements may vary based on the nature of the research and the context in which it is conducted. For instance, different countries may impose distinct legal obligations that researchers must take these into account. All research activities should adhere to laws and regulations during product design, development, and use, minimize the risks of ethical issues.

Application of the principle in the 2D-BioPAD project

2D-BioPAD project activities shall strive to:

- Be conducted in compliance with Global requirements listed in Section 6.3.1;

- Be conducted in compliance with European requirements listed in Section 6.3.2;
- Be conducted in compliance with National requirements listed in Section 6.3.3;
- Follow harmonized protocols;
- Obtain the required approvals (i.e., ethics, legal, etc.).

2D-BioPAD project deliverables that incorporate the ethical principle:

- Regulatory Affairs Plan (WP6 D6.4) outlines the European requirements for the registration and approval of the 2D-BioPAD system;
- Data Management Plan (WP7 D7.2/D7.3/D7.4) see Section 6.5.2

Ethics Self-Assessment in the 2D-BioPAD project

Any legal/regulatory risk that may arise from, or is related to, the project, will be evaluated throughout a Self-Assessment.

The Self-Assessment will evaluate if:

- Project activities and methods applied have been cleared against all relevant legislation and requirements;
- Copies of ethics approvals (if required by law or practice) are available and properly recorded;
- Informed consent forms and information sheets are available and properly recorded.

6.5.5 Public Views and Engagement

Main ethical scope

This principle emphasizes the importance of considering the views of the public in light of the data used and the perceived benefits of the research. Taking into account public opinions on the utilization of their data for research and statistics is crucial for upholding public trust and acceptance in the research work and the data gathered and utilized. Efficiently understanding and anticipating public attitude can also aid in designing more efficient and inclusive methodologies for data collection.

Application of the principle in the 2D-BioPAD project

2D-BioPAD project activities shall strive to:

- Ensure project findings reflect the experiences and opinions of the participant group;
- Identify project's contribution to the already existing information.

2D-BioPAD project deliverables that incorporate the ethical principle:

- Dissemination and Communication Plan (WP6 D6.1/D6.2/D6.3) outlines the overall communication activities and awareness-raising, dissemination of project results, management of all relevant activities, and partners' responsibilities in this respect. It includes specific actions and activities that will be carried out by the 2D-BioPAD consortium members in order to ensure success and maximum publicity for the project and its results. The Dissemination and Communication Plan will be updated three times during the 2D-BioPAD project period to ensure that that all dissemination and communication activities are planned and documented (i.e., D6.1 in M3, D6.2 in M24, and D6.3 in M48).

Ethics Self-Assessment in the 2D-BioPAD project

Any public view and public engagement risk that may arise from 2D-BioPAD project activities will be evaluated throughout a Self-Assessment evaluating if:

- The research involves engagement with the public stakeholders;
- The public is supportive of the project.

6.5.6 Transparency

Main ethical scope

This principle focuses on the crucial importance for a researcher of upholding ethical standards by transparently communicating about the methodology of data collection, data management, data analysis, results, and decision-making processes employed in research projects. This transparency supports to assess the research and its procedures effectively.

Application of the principle in the 2D-BioPAD project

2D-BioPAD project's activities shall strive to:

- Enable participants to be able to ask questions throughout the research process to ensure that they are accurately informed, and researchers should seek to answer these questions quickly and clearly;
- Provide participants information that should be accessible and tailored appropriately to the individual;
- Give access soon after the work is complete to an explanation of the outputs and recommendations arising from the project and the researchers should consider how this can be effectively delivered to different audiences, for maximum impact;
- Provide access to data, algorithms, or other results needed for replicating and validating our findings;
- Consider the ethical implications of gaining consent from adults who have impaired decision making and consult with the appropriate individuals, such as caregivers or support workers if patients involved lack capacity to consent to their participation.

2D-BioPAD project deliverables that incorporate the ethical principle:

- Dissemination and Communication Plan (WP6 D6.1) see Section 6.5.5.
- Data Management Plan (WP7 D7.2/D7.3/D7.4) see Section 6.5.2.

Ethics Self-Assessment in the 2D-BioPAD project

Any transparency risk that may arise from 2D-BioPAD project activities will be evaluated throughout a Self-Assessment if:

- The research outcomes are openly available to the public;
- Both methods and tools are widely available to the public;
- Informed consent form and information sheets are written in a language and in terms participants can understand;
- Informed consent form and information sheets describe the aims, methods and implications of the project activity, the nature of the participation and any benefits, risks or discomfort that might ensue;

- Informed consent form and information sheets explicitly state that participation is voluntary and that anyone has the right to refuse to participate and to withdraw their participation, samples or data at any time and without any consequences;
- Informed consent form and information sheets state how biological samples and data will be collected, protected during the project and whether they will be destroyed or reused afterwards;
- Informed consent form and information sheets state what procedures will be implemented in the event of unexpected or incidental findings.

7. Fine-tuning with Expert Stakeholders

7.1 Overview

To validate previous findings and further develop a comprehensive understanding of the requirements, challenges, barriers, drivers and enablers of PoC IVDs for AD, we carefully selected a diverse group of 26 interviewees from the SIAB and beyond (with nominations from the consortium). This sample included five technology providers, nine HCPs, seven patients/caregivers and five decision makers related to healthcare systems. Examining all stakeholder groups was particularly necessary for eliciting ethical aspects.

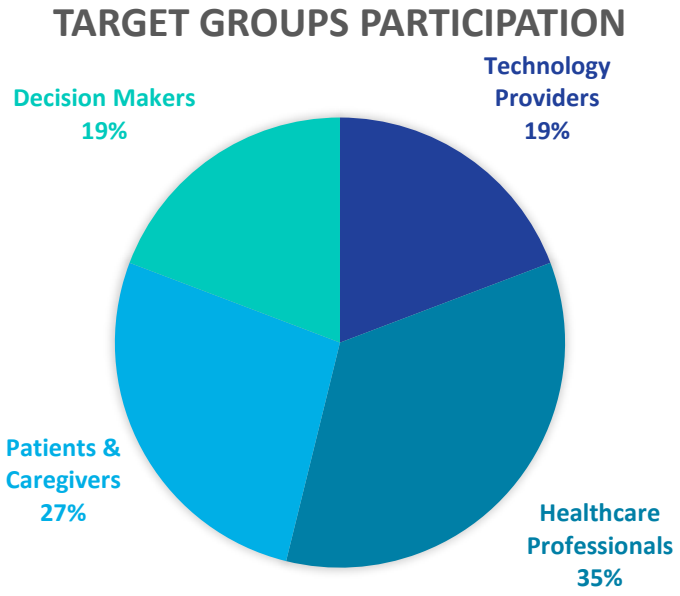

Figure 16. Stakeholder groups' participation in the Semi-structured Interviews.

The interviews were performed by Consortium members as follows:

Table 2. Semi-structured interviews performed by consortium partner

	Tech Providers	Patients & Caregivers	HCPs	Decision Makers	Total
UP-CATRIN	1	-	-	-	1
Q-PLAN	2	-	-	1	3
ICN2	1	-	-	-	1
AUTH	-	-	-	2	2
UEF	1	2	2	-	5
GAADRD	-	3	2	-	5
EVNIA	-	-	2	-	2
ZI	-	2	3	1	6
NUID UCD	-	-	-	1	1
Total	5	7	9	5	26

The geographic coverage of the stakeholders' engaged was also extended, covering 9 countries in Europe, i.e., Czech Republic, Germany, Greece, Finland, Ireland, Italy, Poland, Spain, and the UK.

Due to the limited number of participants per stakeholder groups, all the answers have been aggregated per group to outline the main findings of the discussions that took place.

7.2 Technology Providers

7.2.1 Level of experience & demographics

Five experts from five different countries (i.e., Spain Czech Republic, Finland, Italy, and the UK) mainly from academia (4 out of 5) were interviewed. Only one of them had extensive experience in AD biomarkers, whereas 3 out of 5 had a good experience in PoC IVDs.

7.2.2 Identification of AD blood-derived (plasma) biomarkers

Technology Providers that had experience with AD biomarkers discussed in more detail their importance and Intended Use in clinical practice. Based on the average ratings, certain indications can be extracted:

- Aβ40, Aβ42, Aβ42/Aβ40 are more fit for prognosis.
- p-Tau181 and p-Tau217 are considered more valuable for early diagnosis.
- p-Tau231, GFAP and TDP-43 are not suggested for any intended use.
- NfL, AOβ42, t-Tau are suggested equally for all intended uses.

Table 3. Rating of AD Biomarkers per Intended Use by HCPs during the Semi-structured Interviews.

Biomarker	Prognosis	Early Diagnosis	Progression Monitoring
Aβ40	6.0	5.5	5.5
Aβ42	7.0	6.7	6.7
Aβ42/Aβ40 ratio	6.0	5.5	5.5
p-Tau181	6.5	7.5	6.5
p-Tau217	6.5	8.5	6.5
p-Tau231	3.0	3.0	3.0
NfL	7.0	7.0	7.0
GFAP	3.0	3.0	3.0
TDP-43	2.0	2.0	2.0
AOβ42	8.0	8.0	8.0
t-Tau	8.0	8.0	8.0

Additional comments suggested:

- AOβ42 (soluble oligomer), recognized as a molecular biomarker and therapeutic target of AD due to its high brain toxicity, and it is correlated much more strongly with AD than the insoluble Aβ monomers.
- Aβ molecules, particularly important in CSF, plasma.
- TDP-43 is not an AD-Biomarker.

Current practices for biomarker selection for new biosensing technologies:

1. Literature Review: Review current research to identify gaps and emerging needs.
2. Clinical Assessment: Gather insights from healthcare professionals to understand real-world needs.

3. Stakeholder Collaboration: Partner with academia, industry, and healthcare organizations for additional insights.

7.2.3 Clinical Testing Procedure and Intended Use of AD Biomarkers

The core needs that technology providers identified are aligned with the desk research findings, with the main items being as follows:

- Early, accurate, and cost-effective diagnostics at scale, particularly in primary care (e.g., GPs, family doctors, etc.).
- Non-invasive testing through easily accessible biological fluids, offering a patient-friendly alternative to current diagnostic procedures.
- Facilitating rapid on-site testing without the need for specialized lab equipment and personnel.
- Enabling timely intervention and personalized treatment strategies.
- Better management and reduction of the progression rate of the disease.

However, technology providers went a bit further highlighting:

- the value of taking advantage of graphene's exceptional sensitivity and specificity to be able to detect AD biomarkers at low concentrations, as well as
- the necessity for further development of diagnostics.

7.2.4 Biosensing Performance

Biorecognition units/ probes / binding agents

1. Antibodies:
   - Advantages compared to aptamers:
     - High recognition performance.
   - Disadvantages/Limitations:
     - More expensive to manufacture.
     - Variability between batches.
     - Less controlled post-production modification.
     - Less stable and robust to ambient conditions.
     - Larger in size compared to aptamers.
2. Aptamers:
   - Advantages:
     - Less expensive to manufacture compared to antibodies.
     - Batch-to-batch consistency compared to antibodies.
     - More controlled post-production modification compared to antibodies.
     - More stable and robust to ambient conditions compared to antibodies.
     - Smaller in size compared to antibodies.
     - Synthetic nature avoids the use of animals in production.
     - Can be chemically modified to enhance stability and functionality.
     - Can be denatured and renatured multiple times without losing binding properties.
     - Tailorable for high affinity to various targets (suitable for detecting AD biomarkers like amyloid-beta peptides, tau proteins, and neurofilament light chains).

Finally, Technology Providers stated that both antibodies and aptamers can be designed to bind to a wide variety of targets with high specificity and affinity. On that regard, when asked to elaborate a bit more on the binding challenges, technology providers outlined:

- Surface Functionalization: Graphene's hydrophobic and chemically inert nature requires prior functionalization to facilitate stable probe binding while maintaining material conductivity.
- Orientation and Activity of Probes: Ensuring probes retain their three-dimensional structure and activity upon binding to graphene to maintain binding affinity and specificity.
- Non-specific Binding: Mitigating nonspecific adsorption of molecules from biological samples on the graphene surface to prevent false signals.
- Reproducibility and Consistency: Achieving consistent probe attachment across multiple graphene substrates to ensure biosensor reliability.
- Stability and Durability: Ensuring long-term stability and durability of probe-graphene binding under various environmental conditions to maintain biosensor sensitivity and specificity.

Use of Magnetic Nanoparticles

- **Increased Surface Area**: Nanoparticles on graphene create more binding sites, boosting sensitivity.
- **Signal Amplification**: Gold, silver, or magnetic nanoparticles enhance signal strength.
- Improved Conductivity: Metal nanoparticles enhance electron transfer, improving sensor performance.
- **Enhanced Specificity**: Functionalized nanoparticles increase biosensor specificity and accuracy.
- **Dual-Functional Platforms**: Nanoparticles aid in target capture, while graphene facilitates detection.
- **Multiplexing Capability**: Different nanoparticles enable simultaneous detection of multiple targets.
- **Stability and Durability**: Nanoparticle coating protects graphene, ensuring long-term stability.

Performance metrics

Main performance metrics for a graphene-based PoC IVD system:

1. Sensitivity (True Positive Rate): Ability to correctly identify individuals with the disease or target analyte.
2. Specificity (True Negative Rate): Ability to correctly identify individuals without the disease or target analyte.
3. Accuracy: Overall reliability of the system in making correct predictions.
4. Limit of Detection (LOD): Smallest concentration of the target analyte reliably detectable.
5. Precision/Reproducibility: Consistency of test results under the same conditions.
6. Robustness: Ability to maintain performance across varied environmental conditions.
7. Usability: Ease of use and portability of the device for PoC contexts.

Potential cut-offs for risk assessment:

- Low, intermediate, and high-risk categories based on clinical studies correlating biomarker levels with disease stages or outcomes.

Variability among bodily fluids and Intended Uses:

- **Choice of bodily fluid impacts**: biomarker concentration, presence of interferents, and ease and invasiveness of sample collection.
- **Intended Use** influences biosensor performance requirements, with sensitivity, specificity, or overall accuracy prioritized based on clinical needs.
- **User Feedback**: Feedback from clinicians, GPs, and family doctors is crucial for determining performance metrics and emphasizing key aspects in biosensor design and evaluation. Focus should be on metrics that are easy to understand and explain for clinical use.

Artificial Intelligence

As 2D-bioPAD employs AI for the design of the biosensing system, Technology Providers were asked to express their opinion on how AI technologies could support a PoC IVD systems. Their responses showcase interesting directions for the use of AI:

- Analyse diagnostic data in real-time to support treatment decisions, risk assessment, and disease progression monitoring, particularly useful for chronic conditions like AD.
- Identify patterns and correlations in complex datasets, aiding in the identification of new biomarkers or combinations of biomarkers for AD.
- Estimate the risk of developing AD based on various factors, improving early diagnosis and personalized treatment plans.
- Simulate and predict the performance of biosensor configurations, optimizing design for sensitivity, specificity, and stability.
- AI-powered interfaces can simplify results and guide users through testing, interpret results, and provide recommendations, making PoC IVD devices more accessible.
- AI should be employed with caution and taking into account ethical aspects.

7.2.5 Challenges

Development

Development of a new PoC IVD comes with several challenges which are also applicable in the case of AD:

- **Technical Complexity**: Finding the appropriate biomarker combination and designing sensitive and specific devices for low-abundance biomarkers.
- **Manufacturing and Scalability**: Ensuring cost-effective production without compromising quality.
- **User-Friendliness**: Creating devices easy to use for various users.
- **Cost Constraints**: Developing affordable systems for diverse healthcare settings.
- **Stability and Shelf Life**: Ensuring functionality over time and varied storage conditions.
- **Regulatory Challenges:** complex and costly process requiring extensive validation studies that might be different among different countries.
- **Variation of Results**: Minimizing false positives and negatives.
- **Potential Bias and Discrimination**: Addressing gender-specific (e.g. changes due to menstrual cycle and menopause) or genetic factors.
- **Interpretation of Results**: Ensuring appropriate understanding across healthcare providers.

Commercialisation

When it comes to commercialising a PoC IVD, the challenges appear to slightly differ. The following factors have been outlined by the technology providers:

- **Human factor**: familiarization of medical personnel with the new methodologies
- **Regulatory Approval**: Complex and costly process requiring extensive validation studies that might be different among different countries.

- **Financial Investment**: Securing funding for development, approval, and market launch. Reimbursement challenges, varying by healthcare system and country.
- **Manufacturing and Scale-up**: Ensuring consistency, competitive cost and quality while scaling production.
- **Market Adoption**: Convincing healthcare providers of advantages over existing solutions.
- **Competition**: If the hospital laboratories can have results within hours, what is the value proposition of such a device?
- **User Training and Support**: Providing comprehensive training for effective device use.

Ethics

When asked about ethical challenges and considerations for using an PoC IVD for AD, technology providers listed the following aspects:

- **Informed Consent**: Patients must be fully informed about the test, its purpose, implications, and associated risks.
- **Privacy and Confidentiality**: Strict protocols must protect patient data and ensure confidentiality.
- **Accuracy and Reliability**: Rigorous validation and quality control measures are needed to prevent false results.
- **Access and Equity**: The device should be accessible to all, addressing disparities in healthcare access. Gender bias and discrimination in data handling should be avoided.
- **Human factor**: Responsibility of doctors to interpret and communicate results. Consideration of extra workload for primary HCPs handling the device and communicating the results to patients.

Especially when it comes to safety, technology providers explained:

- **Sample Collection**: Ensure safe and sterile techniques.
- **Analytical Accuracy**: Rigorous validation for correct results (avoid false results).
- **Cross-Contamination**: Prevent between-sample contamination.
- **Chemical and Biological Safety**: Handle hazardous materials safely.
- **User Safety**: Design for safe use, comply with safety standards.

7.2.6 Other projects, solutions, data or information

Competition

All of the Technology Providers interviewed stated that they are not aware of any PoC IVD solution for AD.

Datasets / Databases

Technology Providers provided a range of databases

AD Related Databases

- Alzheimer's Disease Neuroimaging Initiative (ADNI): Provides a comprehensive dataset that includes MRI and PET images, biomarker data (CSF and blood), genetics, and clinical assessments.
- National Alzheimer's Coordinating Center (NACC): Purpose: Offers a database of clinical and neuropathological data collected from participants across the US, supporting research in AD and related disorders.

- Global Alzheimer's Association Interactive Network (GAAIN): Acts as a global data-sharing platform providing access to a vast array of AD patient data from contributing partners worldwide.

Material Sciences and Biosensing Design Resources

- Materials Project: Provides open data on material properties, including crystal structures, electronic structures, and thermodynamic properties, useful for designing novel materials for biosensing applications.
- Protein Data Bank (PDB): Serves as a repository for the 3D structural data of large biological molecules, such as proteins and nucleic acids, crucial for designing aptamers or other molecular recognition elements in biosensors.

Other general remarks

Technology providers highlighted the following aspects when asked to complement their viewpoint regarding topics that were not covered by the interview:

- Implementation of good manufacturing practices and quality systems to ensure reliability and consistency of the PoC IVD device.
- Utilizing the device for patient stratification to identify specific patient groups.
- Exploring market analogies with established tests like glucose or diabetes testing for developing a robust business model.
- Target mainly laboratory staff and not clinical staff as they already have a foul amount in their hands.
- If possible, use a very small amount of capillary blood to be less invasive.

7.3 Patients & Caregivers

7.3.1 Level of experience & demographics

Seven individuals representing patients and caregivers from the three countries of the 2D-BioPAD clinical centres (i.e., Finland, Germany, and Greece) were interviewed. Although none of the interviewees had a good experience in AD, 2 of them had extended experience with PoC IVDs.

7.3.2 Acceptance and trust

After elaborating more about the project and its envisioned solution, Patients and Caregivers were asked what would make such a solution easier to trust.

Out of the responses gathered the majority of the interviewees stated that their trust is related to their doctor's trust in the technology. Hence, the perception of their doctor about a technology is enough for trusting the technology from their side as well, highlighting the importance of the HCP in their care journey early on.

In addition, some of the interviews stated that it would help if they had additional information on:

- The reliability of the device (compared to other standards)
- The functionality of the device ("how it works").

Finally, one interesting remark introduced the perspective that such a device should be used only as a screening method for additional examinations. Meaning that they would trust the device, only if it was a gateway to additional tests.

7.3.3 Willingness to use a PoC IVD

Patients and Caregivers were asked to provide their opinion on whether they would be willing to perform a blood test with their doctor, instead of going to an external lab.

All Interviewees replied positively, with a misalignment on the appropriate timing and conditions for performing the test. Some of them being willing to run such a test after being symptomatic and some of them willing to take it even when asymptomatic. However, most of them were affirmative in taking the test regularly, as often as their doctor advised, extending to annual or bi-annual tests.

Important additional notes extracted from the discussion:

- Patients and Caregivers would like the test to be able to indicate whether the disease has progressed or stayed the same as before, each time they get examined.
- There should be mandatory screenings for certain age groups, to contribute to early identification.

Continuing the discussion, and seeing their positive attitude to use a PoC IVD, Patients and Caregivers were asked whether they would be willing to cover the cost of such examination. All of them replied positively, however their replies on the amount varying:

- Max of 50€
- 10€ (for a blood test)
- 50-150 €
- Up to 200€ (they would also pay 1000€ if the test guaranteed a certain diagnosis)
- Up to 100€, but would prefer a lower cost (they would also pay 500€ if the test guaranteed a certain diagnosis, but they would have to rely on financial support from their family)

Additional notes extracted from the discussion:

- It is important to consider that most people with dementia are retired with small pensions, so covering the cost by themselves would be a burden.
- A cost-free test would be preferable.

7.3.4 Current (perceived) healthcare burden for Alzheimer's Disease

Patients and Caregivers were asked their perceived healthcare burden of AD in terms of cost and time. Their responses varied, showcasing a significant disparity in terms of both time and costs.

- Time: 2 hours to a week (which could extent 2 more weeks for result collection)
- Cost: 100€ to 1.000€ (depending on the detail and amount of tests)

Additional notes extracted from the discussion:

- It is important to also consider travel costs. Especially for Patients and Caregivers who do not live in an urban environment with no direct access to labs, travel costs would be substantial in the overall process.
- It is quite often that the costs vary among HPCs and labs (even for the same test), which makes it even more challenging for Patients and Caregivers.

In respect to what kind of information and support they would need assuming they would have a real-time blood test that could aid a doctor in their decision-making about AD, Patients and Caregivers responded:

- Direct and understandable results with simple language from the diagnostic test, focusing on prognostic value and disease severity assessment.
- Access to clear and simple conclusions instead of scientific data. Some even requested a binary (yes/no) result with a colour gradient for the stage in the AD continuum.
- Include results on the rate of progression, again without necessary numerical values, but with coloured binary indications (green = healthy, red = pathology).
- In case numerical values are presented, clear thresholds to evaluate normal vs pathological condition.
- Clear explanation/interpretation of results and reassurance for the next steps (if any).

Furthermore, when asked about the appropriate timing for receiving this information, their responses covered all possible options:

- Before the test:

Patients and Caregivers seek understanding of what the test examines and its significance, in simple language, emphasizing the importance of early diagnosis. They also want to know about the potential positive impact the test may have on their lives.

One Patient expressed a preference for receiving information about possible therapy and further care before undergoing the test.

- After receiving test results:

Patients and Caregivers desire guidance on whether other types of dementia are excluded and what steps to take to address disease progression or further investigation needs. They also want to seek advice on lifestyle modifications to prevent or delay disease progression, such as reading, interventions, diet changes, and lifestyle habits like smoking and alcohol consumption.

- Post-diagnosis:

Patients and Caregivers require information on necessary steps, treatment options, including medication and social work advice, as well as whether they can still live independently and what specialists to consult. They also need reassurance and encouragement through information as well as psychological support to cope with the diagnosis.

7.3.5 Challenges

Patients and Caregivers were asked to provide their perception of challenging aspects for seeking and getting healthcare services. Through the discussion, several dimensions emerged:

- Financial challenges/barriers
  - Financial burden associated with seeking and receiving healthcare services and treatments for AD.

- Geographical challenges/barriers:
  - Long distances between diagnostic centers pose logistical challenges for patients and caregivers.
  - Limited availability of specialized healthcare facilities outside of major city centers.

- Complex planning and diagnosis:
  - Diagnostic planning for AD is multifaceted and requires coordination among various healthcare professionals.
  - Elderly individuals with cognitive impairment may struggle to navigate the complexities of the diagnostic process.
  - Identifying the appropriate healthcare provider who specializes in Alzheimer's diagnosis can be challenging.

- Monitoring of disease:
  - Annual monitoring might be insufficient and could lead to the patient thinking everything is well when it might not be.

- Stigma and delayed diagnosis:
  - Social stigma surrounding Alzheimer's Disease can lead to delays in seeking diagnosis and treatment. Stigma also causes isolation to patients, even the ones that are still in working-age.
  - Patients may not be taken seriously by healthcare providers, leading to trivialization of symptoms and delayed diagnosis.

- Mental stress:
  - Patients and Caregivers experience significant mental stress due to the challenges associated with seeking and obtaining a diagnosis. Uncertainty about the future, financial concerns, and the emotional toll of caregiving contribute to mental health burdens.

While covering these challenges, Patients and Caregivers also provided input on additional needs that require attention:

- Diagnostics should be faster, simpler, with shorter distances to doctors and faster appointments.
- Memory Clinics/Associations have important role (information, peer support).
- Occupational health covering the costs is an enabler in early diagnosis, as it shifts the barrier of cost.
- Along with the annual monitoring it is important to also have meetings with a memory instructor and/or a therapist to help you cope with the mental stress and suggest helpful activities.
- Having an assigned support person for the patients could help with isolation of patients.

Ethics

When asked about ethical dimensions and concerns for AD healthcare services Patients and Caregivers provided quite diverse answers, with almost all of them providing a different perspective on the topic. Their opinions are summarised below:

- Trust in HCP: Patients and caregivers are highly dependent on their HCP. Thus, the perceived impact is closely related to the trust in their HCP. As stated, if there is trust then there is less likely (to unlikely) to have adverse ethical issues;
- Data protection and confidentiality is considered quite important. Patient and Caregivers expressed their preference that information should store in their medical files but shielded

from public view, with limited access granted to their offsprings only for health-related purposes. This is also the case for the workplace, with interviewees stating that employers should not have access to their medical information without consent.

- AD Stigma: As there is no effective treatment yet, individuals may be reluctant to share information, communicate symptoms, or even visit an HCP.
- Need to address discrimination and ensure fair use of data, especially in case of scientific work, HCPs should be trained accordingly.
- Fear of misdiagnosis (false positive or false negative results), with Patients and Caregivers expressing their hesitation about undergoing testing without proven reliability and credibility (or text quality and accuracy).

7.3.6 Other projects, solutions, data or information

Complementing their answers to the questions, some additional remarks regarding accessibility of the test are documented:

- Level of accessibility: There is a debate on whether such a device should be available directly to patients or not. Some of them stated that AD Diagnosis should be exclusively performed by HCPs, not in pharmacies or by patients or family members at home, whereas others expressed the opinion that such a development would allow for wider outreach (more people would be able to perform the test at their discretion).
- Government-led funding: There should be state mobilization to promote early diagnosis, covered by public funds, and ensure widespread acceptance of testing.
- Access at Primary Care: Patients need early diagnosis, continuity of treatment, referral to specialists, and monitoring through their family doctor after a positive test result.

7.4 Healthcare Professionals/Practitioners

7.4.1 Level of experience and demographics

Nine HCPs from five different countries (i.e., Finland, Germany, Greece, Poland, and Scotland) with 4 from primary care, 2 in hospitals, 2 from academia and 1 from governmental position. The majority of the interviewees had a very high experience in AD Biomarkers and PoC IVDs, with only three stating limited experience in AD biomarkers and similarly two for PoC IVDs.

7.4.2 Clinical Testing procedure and Intended Use of AD biomarkers

Clinical or research protocols

In an effort to capture the current clinical practice and expectations in terms of AD biomarkers, HCPs were asked how they currently use fluid-derived AD biomarker results. Their answers, which varied, are outlined below:

- Clinical practice currently does not routinely use biomarkers, relying on clinical criteria for provisional diagnostics. There are certain exceptions, which are limited, and others that rely

on biomarkers mainly for research purposes and not for their day-to-day clinical practice. Usually, clinical criteria are employed for provisional diagnostics.

- Insufficient test quality (of CSF biomarkers) in the past has led some HCPs to discontinue their use.

On the other hand, their expectations were more aligned to the fact that Primary Care HCPs need "tools" that will allow them to deliver precise diagnosis, in the shortest possible time, following a simple and reliable process that will also support their interpretation and practical use.

Focusing more on the protocol, HCPs provided additional information regarding the procedures followed.

In particular HCPs mentioned that current evaluation of biomarkers is based mainly on standardized procedures, standard materials, rapid processing after blood sampling, standard operating procedure (SOP) for centrifugation and freezing. However, depending on the equipment and the re-agents employed results may vary.

On the other hand, it was stressed that any kind of fluid-based analysis (either CSF or blood) needs to be carefully re-evaluated regarding the way that blood is handled and stored, as not much attention is given to protein and RNA degradation.

The above protocols are mainly employed in specialised care, with CSF sampling being labour intensive, by highly trained personnel (as lumbar puncture is demanding).

Intended Use

Following, HCPs were asked to envision the role of blood (plasma) AD biomarkers, as most of them are not using their clinical practice. All of them agreed on a beneficial role highlighting the following key points:

- Simplification and acceleration of diagnostics
- Easier sample collection
- Broader application by less qualified healthcare providers
- Beneficial for all Intended Uses discussed, i.e., early diagnosis, differential diagnosis, and possibly progression monitoring , although currently limited in this regard (monitoring).

Extending the discussion to the added value such biomarkers would have in Primary healthcare all HCPs agreed that the main intended use that holds the most value is the early diagnosis. On top of that additional feedback included:

- Significant value by facilitating earlier diagnosis, particularly in primary care settings where screening many individuals with memory symptoms is challenging.
- They may aid in screening individuals with mild symptoms and referring them to specialist care, helping to target services and treatments effectively.
- Important to support general practitioners to have knowledge on the disease progression to better help patients navigate their next steps.
- Addressing the need for predicting disease development and monitoring progression, especially in anticipation of new drug treatments, is highlighted as valuable for improving patient care and resource allocation within healthcare systems.

7.4.3 Identification of AD blood-derived (plasma) biomarkers

Some of the biomarkers that were mentioned during the interviews included p-Tau 181, tau 217, NFL, and GFAP, Amyloid and tau. Following, as HCPs were asked to rate biomarkers per intended use, HCPs provided more elaborate discussion, as depicted below.

Based on the average rating values, certain indications can be extracted:

- Aβ42, Aβ42/Aβ40 ratio, p-Tau181, p-Tau217, p-Tau231, TDP-43, rt-quic-based analyses, Progranulin, and sTREM2 seem to have more value for early diagnosis. Out of these rt-quic-based analyses and p-Tau217 seem to have the highest rating.
- Aβ40, NfL, GFAP, and Progranulin are reported to have more value for Prognosis, with Aβ40 having a significantly lower rating.
- Beta synuclein, Progranulin, and sTREM2 are reported to have equally rated value for Progression monitoring.

Table 4. Rating of AD Biomarkers per Intended Use by HCPs during the Semi-structured Interviews

Biomarker	Prognosis	Early Diagnosis	Progression Monitoring
Aβ40	4.3	4.0	1.5
Aβ42	6.3	6.7	2.7
Aβ42/Aβ40 ratio	7.0	7.8	2.5
p-Tau181	7.0	7.3	3.8
p-Tau217	7.5	8.0	4.5
p-Tau231	7.0	7.5	7.0
NfL	6.8	6.0	5.0
GFAP	6.8	5.8	4.8
TDP-43	5.8	6.3	5.0
rt-quic-based analyses*	6.0	10.0	7.0
Beta synuclein	6.0	0.0	7.0
Progranulin	7.0	7.0	7.0
sTREM2	6.0	7.0	7.0

* From the additional biomarkers suggested, there is interest in the "rt-quic-based analyses" proposed due to the high ranking received. However, *rt-quick is an analysis method for biomarkers and not a biomarker itself*. Hence, it cannot be considered for further evaluation.

In addition to the above, one of the HCPs, a Neuroscience Professor/ Medical Doctor with high experience in biomarkers provided the following info:

- microRNA biomarkers at the CSF level of MCI to AD and cytokines (TNF alpha for example).

Comments on the biomarkers of this list:

- "Tau Protein 181, Tau Protein 217, Tau Protein 231, Neurofilament Light chain, Glial Fibrillary Acidic Protein, TDP-43" are only used in clinical studies and have not made it to the clinic.
- All TAU proteins are not specific to AD.
- There is no biomarker that gives info of the actual condition or progression of the patient's AD in terms of cognitive profile.

Specific comments on each biomarker:

- Aβ40: strong evidence only for screening and not for progression
- Aβ42: strong evidence only for screening and not for progression
- Aβ42/Aβ40 ratio: superior in value compared to individual markers
- p-Tau181: not specific for AD can also be found for other dementia, its specificity is an issue, for screening it may be ok but not for a proper diagnosis
- p-Tau217: not specific for AD can also be found for other dementia, its specificity is an issue, for screening it may be ok but not for a proper diagnosis
- p-Tau231: screening in elderly also in earlier stages- if someone is having cognitive problems it can be an aid in the diagnosis - based on its utility - promising but it also has specificity issues
- NfL: it has value in early diagnosis; however, you can also see it in other neurodegenerative pathologies
- GFAP: as above
- TDP-43: you can see it in other conditions (e.g., ALS and in TDP proteinopathies), what is unique about it is that it shows the advanced stage of the disease. A study found that 8 patients who had >tdp than the rest of patients experienced a different progression of AD.

7.4.4 Clinical Testing Performance

Following the discussion on the identification and rating of biomarkers, HCPs were requested to elaborate on specific cut-offs and other performance criteria for the assessment of the biomarkers.

Although they acknowledged the importance of clear cut-off values, specific values were not elaborated as specific cut-offs are not yet established in the field, they are highly dependent on the equipment and re-agents used, calling for modelling and big data studies.

On the other hand, performance criteria are clear, with sensitivity, specificity, and reliability (robustness) recognised as crucial factors for diagnostic certainty in clinical practice. One of the HCPs also introduced AUC potentially the most important metric for clinical practice.

Beyond technical performance, HCPs mentioned the need to simplify the sample/fluid collection process.

7.4.5 Clinical Assessment

HCPs were asked to consider receiving AD blood-derived (plasma) biomarker results directly on a mobile phone or tablet in real-time, and to specify what kind of information would assist them in their decision-making process based on that. Their responses included:

- Biomarkers such as APOE status, Tau, and APOE alleles for prognostic use.
- Clear visualization of results with relevant cut-off values.
- Diagnostic value and credibility of results for accurate diagnosis.
- Tutorial for the physician on how to use the device.
- Statistical prognosis (disease probability) based on biomarkers with high sensitivity and specificity.
- Exact values, stage, and severity of the disease.
- Simplified and easy-to-understand interpretations, possibly in color-coded format.
- CIS Integration of results into patient medical record systems in real-time.

Furthermore, when asked about the appropriate timing for receiving this information, their responses covered:

- All stages:
  - guidelines on what particular biomarkers mean and what is the next step.
  - comprehensive treatment plan
- Before Test:
  - Information about the test itself: what it examines and potential outcomes.
  - Guidance on implications of diagnosis and potential scenarios.
  - Reassurance and education on healthy lifestyle choices.
  - Knowledge of potential causes of symptoms even if no biomarkers are found.

- After Test (if MCI/AD):
  - Detailed explanation of results and their implications.
  - Support with psychological stress, including risk of depression or suicidal tendencies.
  - Discussion of treatment options and further care, including medication and rehabilitation.
  - Guidance on prevention and health-promoting behaviours.

- After Test (if no biomarkers are found):
  - Further clarification of symptoms and potential causes.
  - Explanation of increased risk of dementia and need for further screening.
  - Reassurance and support for maintaining a regular and meaningful life.
  - Education on healthy lifestyles and preventive measures.

Along the way, during the discussion on the information sharing, several core benefits have been outlined:

- Quick diagnosis confirmation.
- Simplified, non-invasive diagnostics with broader coverage.
- Potential for GP screening, faster patient appointments.
- Enhanced screening during patient visits, reducing costs and referrals.
- Facilitated treatment initiation, awareness of limitations, and specialized referrals.
- Improved care: cost-effectiveness, speed, safety, patient acceptance, and increased usage

7.4.6 Challenges

Cost and Time

HCPs were asked about the average cost and time needed for getting fluid-derived biomarker results for assisting diagnosis. Their responses varied:

- Cost ranges from 100 to 700 €
- Time ranges from 1 to 2 weeks

Time for diagnosis might depend on the doctor's experience and access to equipment. With that in mind some HCPs mentioned a range from 0,5 h to - 2 h.

Provision of healthcare services

When asked to consider the most challenging aspect in terms of healthcare services' provision for patients and their caregivers, HCPs provided an extensive list of challenges starting with the fact, that there is no treatment yet for AD. Beyond that their feedback included the following:

- Lack of specialized HCPs available through public means. Specialised care is mainly focused on challenging cases.
- High cost and accessibility issues of advanced imaging and biochemical tests, which are often not covered by insurance policies.
- Invasiveness of current diagnostic procedures, such as cerebrospinal fluid (CSF) collection, prompting the need for non-invasive alternatives like saliva-based tests.
- Lengthy, costly, and often discouraging diagnostic journey for patients and caregivers.
- Difficulty in identifying the accuracy/specificity/sensitivity of biomarkers for AD.
- Lack of approved biomarkers with clear clinical relevance to AD.
- Psychological burden and ethical considerations arise regarding diagnosis without symptoms, especially when no treatment options are available.
- Varying practices among doctors.
- Working-age individuals potentially underserved and requiring better screening and referral processes in primary care settings.

PoC IVD deployment

Moreover, HCPs were asked to describe main challenges and/or barriers to deploying a PoC IVD for detecting the biomarkers in primary healthcare settings. Several different aspects have been presented as outlined below:

- Cost and Clinical Impact:
  - Concerns about cost-effectiveness and clinical impact (especially considering absence of treatment for specific diseases).
  - Need for biomarkers to predict treatment response.

- Patient Acceptance and Education:
  - Patient trust and education on test accuracy.
  - Preference for seeking second opinions.

- Validation and Accuracy:
  - Importance of accurate diagnosis and validation.
  - Technical challenges regarding the quality of diagnosis so that there are no false-positive results.

- Ethical and Psychological Concerns:
  - Ethical considerations that the patient stress will increase for people that are positive before experiencing symptoms.
  - Need for comprehensive treatment concepts.

- Training and Interpretation:
  - Doctor training in result interpretation.
  - Patient could benefit from quick answers and differential diagnosis support.

Ethics

HCPs were requested to delve a bit more on the ethical considerations related to AD diagnosis. A number of ethical concerns were introduced:

- Stigma: Address societal attitudes towards AD to prevent discrimination.
- Privacy: Ensure confidentiality of test results, protecting against discrimination by insurers.
- Clinical Indication: Perform tests only when clinically necessary to avoid harm.
- Comprehensive Treatment: Use tests when a comprehensive treatment plan exists.
- Right to Know: Respect patients' right not to know diagnosis or prognosis.
- Safety: Ensure test accuracy and safety to prevent misdiagnosis.
- Informed Consent: Educate patients and obtain consent before testing.
- Avoid Over/Misdiagnosis: Balance benefits and risks of early detection.
- Doctor-Patient Relationship: Conduct tests in patients' best interests, fostering trust.

To build and maintain trust with HCPs:

- Diagnostic benefits
- Economically worthwhile for doctors
- Certainty of diagnosis/prognosis, test reliability

And with patients:

- Competence of the physicians
- Good doctor-patient relationship

Age specific, or other issues affecting trust and acceptance:

- intellectuals are more likely to fear loss of their autonomy
- people of low social status may fear needing financial help and becoming a burden on their family etc.

Specifically for safety

- Potential transmission of amyloid, requiring caution in storage and analysis of samples.
- Ensuring safety protocols similar to those for blood tests to prevent false results and ensure accuracy.
- Quality of diagnostics and the associated risks with blood tests, although few, ensuring that test results do not mislead.
- Ensuring ease of application, minimally invasive procedures, and non-infectiousness.

Specifically for trust/acceptance

- Comparison and cross-checking with the Gold standard method of biomarker identification.
- Endorsement and communication of usefulness by opinion leaders in healthcare.
- Ensuring data monitoring and confidentiality to address cybersecurity challenges.
- Demonstrated sensitivity and specificity accuracy.
- Backing by science and credibility.
- Diagnostic benefits and economic efficiency.
- Safety and accuracy.
- Strong scientific evidence supporting approval.

Additional needs

Finally, HCPs introduced additional aspects related with the challenged encounter by patients and caregivers, as well HCPs and the rest of the clinical and scientific communities.

- Better public awareness and access to healthcare structures, particularly for later stages of the disease requiring home management.
- Preliminary examinations and availability/accessibility to testing and healthcare (especially in rural areas).
- Better planning of diagnostic steps, prognostic implications, with empathy and understanding.
- Patients with a hereditary predisposition to find out whether they are also affected.
- Information, education and comprehensibility.

7.4.7 Other projects, solutions, data or information

Competition

- Research by the University Medical Center Göttingen in the field of cerebrospinal fluid diagnostics.

Other aspects

- Clinical correlation: biomarkers could reflect on the level of the disease helping treatment decision.
- What about patients that are biomarker positive but never develop AD?

7.5 Decision Makers

7.5.1 Level of experience and demographics

Five healthcare decision makers from three countries (i.e., Germany, Ireland, and Spain) were interviewed, covering academia, government, public and private clinics. The experience of the interviewees was almost evenly distributed both for AD and PoC IVD experience, presenting a diversity of answers.

7.5.2 Clinical Testing Procedure and Intended Use of AD biomarkers

Clinical or research protocols

In an effort to capture the current clinical practice and expectations in terms of AD biomarkers, Decision Makers were asked how they currently use fluid-derived AD biomarker results in their clinical settings. Decision Makers provided very distinct answers:

1. PET tracers can detect the load of Aβ fibrils in the brain and Aβ and tau levels can be measured in the CSF.
2. In research: Standardized procedures for collection and preparation (e.g. centrifugation, rapid freezing)
3. For ensuring integrity and reliability of data in sample collection, analysis of biomarkers, transfer, and storage:
   - Sample Collection: Standardized protocols, Proper labelling, Adequate volume, Timeliness
   - Analysis of Biomarkers: Validated assays, Quality control, Calibration, Standardization Procedure
   - Transfer: Proper handling, Temperature control, Secure packaging

- Storage: Temperature control, Stability testing, Proper labelling, Inventory management
- Ethical considerations: Informed consent, Privacy protection, Compliance
- Data management: Record-keeping, Data security, Data sharing

4. The protocol starts in primary healthcare, where cognitive or memory problems are detected and then, the patient is sent to the neurology specialist for a better diagnosis. After that:
   - Further cognitive or behavioural tests,
   - blood analysis that discards a deficit of B12 vitamin or hypothyroidism as a cause for dementia,
   - patients go through neuroimaging, MRI, etc.,
   - neuropsychological study,
   - biomarker analysis, if dementia is in an early stage, where usual treatments are forbidden (the new trends in monoclonal antibodies can be very helpful).

7.5.3 Clinical Testing Performance

When asked which performance metrics, for AD biomarkers in plasma, are the most important to support the discussed intended uses, Decision Makers responded:

- Sensitivity and specificity: Flagged as the most important metrics.
- Overall accuracy: to avoid false positives or negatives.
- Disease specific benchmarking: Finding specific biomarkers for this illness and validate the results of the PoC device with other tests.
- Universally accepted cut-offs: Currently cut-offs exist but are not actively used in clinical practice, due to different laboratories having disparities in how they treat the samples. Thus, results come back with an associated interpretation.

Focusing more on factors that are most important for their decision making, Decision Makers were given the opportunity to elaborate further:

- Pricing: Affordability and accessibility of diagnostic tests.
- Precision of the diagnosis: Accuracy and reliability of the test results.
- Practicability, User-friendliness, ease of access and non- invasive techniques.
- Complementarity with other tests.
- Robustness and re-use.

7.5.4 Clinical Assessment

Decision Makers were asked to consider receiving AD blood-derived (plasma) biomarker results directly on a mobile phone or tablet in real-time, and to specify what kind of information would assist them in their decision-making process based on that. Their responses included:

- Comparative results with the same cohorts in terms of age, sex, ethnicity, etc.
- Absolute values, ranges and a measure of certainty in the diagnosis.
- Training HCPs on the use of the device and its specifications, technical details, operation, troubleshooting, support and safety.
- CIS Integration: The results should be able to integrate with the interfaces of the hospitals' IT frameworks. Ideally directly linked to the patient's record.
- Would be useful to be able to use anonymized results for research.

Furthermore, when asked about the appropriate timing for receiving this information, their responses covered:

- Before Diagnosis:
  - General information about how the tests work.
  - Information about the disease.
  - Consequences of a diagnosis.
- After Diagnosis:
  - Exact diagnosis.
  - Information about progress.
  - Prognosis.
  - Treatment options.
- If AD/No Biomarkers are Found:
  - Guidance on when to repeat the test.

During the discussion with the Decision Makers, it was argued that only doctors/nurses should have access to the results and be able to share the information with patients and families.

Additionally, it's essential to provide protocols to discriminate between AD patients and normal subjects with high sensitivity and specificity, as well as contextual information to General Practitioners to facilitate their understanding and decision-making process.

7.5.5 Need for a PoC IVD for AD – Intended Use

To assess the need for a PoC IVD for AD, Decision Makers were asked about the core benefits they perceive such a device could offer in a primary healthcare setting for accurately detecting AD blood-derived (plasma) biomarkers. Decision Makers provided a range of responses that extended across all intended uses, however with different rationale.

- Enable early detection of AD, allowing treatment to start before irreversible brain damage occurs, helping individuals become aware of potential cognitive decline and access treatments as they become available.
- Simplify and speed up the diagnostic process, making it less invasive and requiring fewer preliminary examinations.
- Enable the facilitation of screenings for a wider cohort compared to analysing CSF.
- Provide a means for monitoring disease progression, allowing for timely interventions and adjustments in treatment plans.

7.5.6 Challenges

Considering the different hierarchy, and thus priorities, Decision Makers were asked to consider the most challenging aspect in terms of healthcare services' provision by HCPs under their department and/or division. Their responses included:

- Accurate diagnosis: especially before treatment initiation.
- Interpretation of biomarker results: The need for specialized expertise to correctly interpret rapid test results.
- Expensive, time consuming, invasive and complex processes: If the device is easy to operate and has easily accessible results it would be easily accepted by the GPs.
- Reliability with standard of care: Providing the same level of evidence, specificity, and accuracy as higher technologies at a lower cost.

Accordingly, Decision Makers were asked to consider the most challenging aspect in terms of healthcare services provision for patients and their caregivers? Their responses slightly differed:

- Monitoring treatment effect: Ensuring rapid evidence of treatment effectiveness post-diagnosis.
- Easy to understand interpretation of results: Understanding the diagnosis and coping with psychological stress for patients and caregivers.
- Accurate and reliable diagnosis: via appropriate IVDs.
- Stabilizing protocols that ensure quality of results in blood-based tests.
- Expensive, time consuming, invasive and complex processes: Addressing long waiting times, travel requirements, and invasive procedures for patients during diagnosis.
- Implementing effective management and utilization of diagnostic devices within healthcare systems.
- Proper counselling and referral processes by GP teams.

Cost and Time

Decision Makers were asked about the average cost and time needed for getting fluid-derived biomarker results for assisting diagnosis. The responses converged to the fact that the process is expensive. More expensive and invasive than a blood-based test for AD.

The following are indicative in Spain (only the Spanish interviewee provided this information):

- Cost:
  - For an amyloid PET scan, the cost is 1400€.
  - For a more general PET scan, the cost is 700€.
- Time:
  - CSF (the less costly method), the average time is three weeks.
  - For neuroimaging, the average result time is 10 days.

Echoing the above once more, when asked about the core benefits of a PoC IVD in a primary healthcare setting that could accurately detect AD biomarkers in plasma, Decision Makers stated:

- ease of use
- less invasive
- faster, so the process towards treatment would be sped up
- cheaper

PoC IVD deployment

Furthermore, Decision Makers were asked to describe main challenges and/or barriers to deploying a PoC IVD for detecting the biomarkers in primary healthcare settings. Responses presented:

- PoC IVD must be affordable, easy to use, require a small amount of blood, have high sensitivity and specificity.
- Incorrect interpretation, if the results are not embedded in overall clinical context.
- Difficulties from the different ways of measuring and the lack of standard protocols and thresholds.

Ethics

Extending on ethical consideration, Decision Makers provided their opinion regarding the use of a PoC IVD device for detecting AD biomarkers in plasma.

- Confidentiality: Medical results must be kept confidential, with access limited to the medical team caring for the patient.
- Communicating Diagnosis: Communicating the diagnosis of AD carries significant responsibility, considering its impact on various aspects of the patient's life, such as professional and insurance matters.
- Informed Consent: Patients should be provided with information about the use of their data and given the opportunity to provide informed consent.

According to Decision Makers, HCPs will trust the device if the Test Quality is high and if they have know-how on interpretation. They will also appreciate accuracy, cost savings, practicability.

On the other hand, Patients will trust the procedure if there is a good Doctor-Patient Relationship.

Trust and Acceptance

- Scientific proof of the test's effectiveness and reliability. The results should also be validated with other data (e.g. proteomic data).
- Cost savings
- Practicability and ease of use
- Proper documentation for HPCs with informed consent for patients.

Safety

- The device needs to be simple and sharp parts must not be easily accessible.
- Safety concerns related to blood samples (e.g. stability of the reagents, contamination in the blood extraction, degradation of blood or the components of the PoC with time or temperature).

Two of the Decision Makers expressed the opinion that since it is not an invasive method, there are no safety concerns.

7.5.7 *Other projects, solutions, data or information*

Many are currently investigating blood biomarkers (e.g. in Spain, Alberto Lleó, Institut de la Recerca Sant Pau and Gemma Salvadó from Barcelona Beta Brain Research center).

Competition

- Research in this area by:
  - companies like Fujirebio, Roche Diagnostics
  - Research teams like the work of Dr. Kaj Blennow in the University of Gothenburg.
- UK: Researchers from Alzheimer Research UK and Alzheimer Society, directed by Fiona Carragher are developing a blood test for dementia detection (Nov 2023).
- Newcastle: work on biomarkers but not close enough to a mature solution or something close to be commercialized.

Other aspects

- It would be important to have as many biomarkers used for diagnosis as possible, even if it is from different tissues.

Interesting remark: It would be important to start considering early on "Manufacturing at scale" (manufacturing for the market), so that we have a tool that is readily scalable and not just a prototype.

7.6 Main findings/results

The engagement of the four stakeholder categories (i.e., Technology Providers, Patients and Caregivers, HCPs, and Decision Makers) during the Semi-structured Interviews spurred interesting discussions that led to the extraction of several insights and recommendations. Involved stakeholders had the opportunity to actively engage and provide feedback to the project, while also learning more about its activities and in overall raising awareness on AD, biomarker research and PoC IVD technologies.

The sample of interviewees was quite balanced across all four categories, with a slightly higher participation of HCPs. The interviews also had a good EU coverage in terms of participating countries, with 9 being represented in the sample of interviewees. Emphasis was given to the 3 countries of the clinical centers (i.e., Germany, Greece, and Finland), from where 3 our 4 categories were interviewed. Following, even though the experts were "handpicked" by the consortium partners, based on their experience and expertise, half of them (n=13/26) had limited knowledge on AD, with a smaller number having a higher knowledge base (n=6/26). On the other hand, their experience on PoC IVD systems was quite balanced with 12 reporting a limited experience level, 12 an extended one, and 2 with an intermediate level.

7.6.1 End-user Needs and Challenges

All the stakeholders that were interviewed reflected, to some degree, upon the needs and challenges of end-users related to AD. The needs and challenges identified are listed below:

- **Early, Accurate, Cost-Effective Diagnostics**: Ensuring timely and affordable testing, especially in primary care.
- **Streamlined and Affordable Diagnosis**: Simplifying the diagnostic procedure (especially for elderly people) by making it faster and by reducing the cost of diagnosis. Working-age individuals are often underserved and require better screening, counselling and referral in primary care settings.
- **Non-Invasive Testing**: Utilizing easily accessible biological fluids for diagnostics.
- **Rapid On-Site Testing**: Enabling quick testing without specialized lab equipment and personnel.
- **Accessible Diagnostics**: Making diagnostics faster and geographically and financially accessible for patients.
- **Public healthcare**: Specialized HCPs available through public means.
- **Timely Intervention and Personalized Treatment**: Facilitating prompt and tailored treatment strategies, based on the needs of each patient.

- **Financial Support for Diagnostics**: Insurances should cover the costs of advanced imaging, biochemical tests etc., so that the cost barrier is shifted from the patients and their families when seeking and receiving treatment for AD.
- **Mental Stress Management**: Psychological Support for Patients and Caregivers (with memory instructor or therapist), addressing stigma around AD, uncertainty about the future, financial concerns, and the emotional toll of caregiving. Assigned Support Persons could also provide companionship to combat patient isolation. Finally, it is important to address the psychological burden arising in cases of diagnosis without symptoms, especially when no treatment options are available.
- **Enhanced Public Awareness around AD**: Memory Clinics/Associations spreading information, offering peer support and helping eliminate the stigma around AD (especially in rural areas).
- **Access to Specialized Healthcare Providers**: Ensuring availability of diagnostic centers, facilities and specialized professionals (outside big cities as well).
- **Reliable Biomarkers for Diagnosis**: Identifying accurate and approved biomarkers with clear clinical relevance to AD.
- **Improved Diagnostic Planning**: Streamlining diagnostic processes with empathy and understanding.
- **Monitoring Treatment Efficacy**: Ensuring effectiveness of post-diagnosis treatment as well as mental health support of patient and caregiver.
- **Quality Assurance in Diagnostics**: Ensuring reliability in blood-based tests.
- **Effective Counselling and Referral**: Establishing robust counselling and referral systems.

7.6.2 Clinical Practice

Protocols

Although HCPs and Decision Makers recognise that **there are standardised approaches to biomarker assessment**, most of them were **not aware of employing fluid-derived biomarkers in clinical practice**. In fact, it was discussed that they rely on clinical criteria for provisional diagnostics, which is especially the case for primary healthcare settings, which has been enhanced by insufficient testing quality in the past. Those that were aware, stated that such protocols are employed mainly in **specialised care for research purposes**, by dedicated trained personnel. On top of that, a number of mentioned protocols employ additional diagnostic methods (i.e., neuroimaging) to extract a more reliable outcome, which increases significantly the cost of the diagnosis.

On the other hand, even in research, results are not harmonised as they are highly dependent on the equipment and re-agents used, limiting replicability and cross-studies comparison and assessment.

However, the interviewees recognise the need in primary care for “tools” that will allow them to deliver **precise diagnosis, in the shortest possible time, following a simple and reliable process that will also support their interpretation and practical use**.

Further discussing on who should have access to this protocol, most stakeholders agreed that **HCPs should be the ones performing the tests**, and not patients or caregivers themselves.

Benefits of Blood AD Biomarkers in Primary Care

All stakeholder groups agree that blood-based AD biomarkers would benefit clinical practice and the provision of healthcare services by HCPs in primary healthcare setting. Some core benefits include:

i. **simplification and acceleration of diagnostics**,
ii. **less-intrusive sample collection**,
iii. **cost-effective**,
iv. broader application by **less qualified HCPs**,
v. **added value for prognosis**, **diagnosis**, **progression monitoring** and future **treatment effect** assessment,
vi. improved **health management** and **follow up to specialised care**,
vii. **better resource allocation** within healthcare systems.

Performance Criteria

Perhaps one of the very few aspects that all stakeholders agree on. **Sensitivity**, **specificity**, and **reliability** (**robustness**) are recognised as crucial factors for diagnostic certainty in clinical practice. In addition, two additional elements were discussed: (i) the need for disease specific benchmarking of the diagnostic test, and (ii) the standardization of cut-off values, both perceived as important aspects for the reliability / credibility of the examination.

Testing Process

Different access to information needs have been flagged by the stakeholders regarding the testing process. Decision Makers flagged that at any given point, **guidelines and information about the biomarkers examined should be available**, along with **potential implications and treatment plans**. This was also echoed by Patients and Caregivers, who emphasised on clarity and simplicity of the information provided, covering the diagnosis, the prognosis, and the rate of progression.

More detailed opinions on the information shared from HCPs to Patients and Caregivers before and after the test are as follows:

- **Before Test:**

Patients and caregivers should be provided with **clear and easy-to-understand information on the test itself**: how it works, what it examines, and how the process will unfold. Following the information shared should focus on the **results of test**. What are the implications of a positive or a negative test; what is the potential impact in their lives, etc. In some cases, the need to delve deeper into the disease, causes of symptoms without or without “positive” testing, potential therapies, treatment options or interventions were suggested.

- **After Test (if MCI/AD):**

Again, there is a consensus among responses. Primary concern across all responders Is the **explanation of the results and their implications** for the **patient and caregiver**. **Detailed guidance** was also mentioned, both for supporting any **adverse mental effect**, but also for discussing treatment options and further health and social care steps (e.g., medication, rehabilitation, lifestyle modifications, health-promoting behaviour, etc.). In order to be able to better handle the information provided, the need to disclose additional information about the disease progression mechanism, rate of deterioration, etc. are deemed necessary. Finally, there is also a need for psychological support to cope with the diagnosis and to address other aspects as potential issues such independent living.

- **After Test (if no "positive" biomarkers are found):**

Decision Makers and HCPs provided additional information for the case that the test results are "negative". In such a case, additional clarification on existing symptoms (if any) should be discussed along with potential causes, along with guidance for future examinations based on sound reasoning and risk assessment facts. And as always, the advice for health living and everyday activities that can act as preventive measures.

Cost & Time

Highly diverse results were documented in terms of costs and time for running a fluid-derived biomarker test.

HCPs, Patients and Caregivers roughly agreed on the range of cost (i.e., **100 – 1000€**), which varies significantly for such tests. In terms of time, again the two groups agreed that depending access to the equipment, the time required to get results on a biomarker test is currently in the range of **1-2 hours** (if the HCP has direct access to the equipment) or **1-3 weeks** (if they collaborate with an external lab).

Patients and Caregivers also raised the issue of **travel costs**, which is directly linked with wider challenge of accessibility to healthcare.

7.6.3 AD Biomarkers and Intended Use

From all the discussions that took place, it is not possible to draw clear conclusions but rather some indications on the AD biomarkers and their value per Intended Use. The two stakeholder groups that provided feedback on the biomarkers are Technology Providers and HCPs, with some of the biomarkers (blue) having only one answer. The table below shows the average values extracted:

Table 5. Aggregated average rating of AD biomarkers per Intended Use across all stakeholder groups.

Biomarker	Prognosis	Early Diagnosis	Progression Monitoring
Aβ40	6.0	5.5	5.5
Aβ42	**7.0**	6.7	6.7
Aβ42/Aβ40 ratio	6.0	5.5	5.5
p-Tau181	6.5	**7.5**	6.5
p-Tau217	6.5	**8.5**	6.5
p-Tau231	3.0	3.0	3.0
NfL	**7.0**	**7.0**	**7.0**
GFAP	3.0	3.0	3.0
TDP-43	2.0	2.0	2.0
AOβ42	**8.0**	**8.0**	**8.0**
t-Tau	**8.0**	**8.0**	**8.0**
Beta synuclein	6.0	0.0	**7.0**
Progranulin	**7.0**	**7.0**	**7.0**
sTREM2	6.0	**7.0**	**7.0**

if we were to analyse results considering a Net Promoter Score, then we would only consider values above 8 as "promoting" values, which would be indeed the case in terms of perceived value from the stakeholders interviewed. For the biomarkers that received a lot of answers we can observe that only

p-Tau217 is more widely accepted as a "good" biomarker, and that's only in the case of **early diagnosis**.

AOβ42 and t-Tau have also received high numbers, however having only one response the results cannot be generalized. However, t-tau has been analysed thoroughly in the desk research and further assumptions could be drawn.

Considering a lower threshold (i.e., 7.0), the following mapping of biomarkers per intended use can be derived:

Prognosis: Aβ42 and NfL (potentially also AOβ42, t-Tau and Progranulin)

Early Diagnosis: p-Tau181, p-Tau217, and NfL (potentially also AOβ42, t-Tau, Progranulin and sTREM2).

Progression Monitoring: NfL (potentially also AOβ42, t-Tau, Beta Synuclein, Progranulin and sTREM2).

7.6.4 The role of PoC IVDs

Technology Providers predominantly addressed the role of a PoC IVD system for AD, complemented by insights from other stakeholder groups.

Starting with the technological perspective, emphasis was given on the value of taking advantage of graphene's exceptional **sensitivity and specificity** to address the challenges of current lab tests. Coupled with (i) **Aptamers**, which are found to have a long of benefits, especially compared to antibodies (smaller size, less expensive, more controlled chemical production, higher replicability and affinity to target analytes, etc.), and (ii) **Magnetic Nanoparticles**. which further improve the overall biosensing performance (amplified signal, improved conductivity and specificity, etc.), PoC IVDs are considered promising alternatives to a range of applications, including that of identifying AD biomarkers in bodily fluids, i.e., CSF and Blood. This feedback is quite important as it validates the original assumptions of 2D-BioPAD and brings forth the technological advancements that such an approach can induce to AD research. On the other hand, the use of **Artificial Intelligence (AI)** was focused more on the analysis of generated medical data, and not so much on the biosensor design. Considering the latter, two scenarios were introduced, the use of AI for (i) simulating the performance of the biosensor and (ii) for identifying new biomarkers.

With that in mind, the **accuracy** and **reliability** of such a device is considered crucial for its real-life application. Ensuring accurate and consistent results, with standardised and benchmarked cut-off values, is essential and should extend to not only **minimize false positives and false negatives** (high sensitivity and specificity) but also handle **sex, gender and cultural bias** (e.g., gender bias due to changes in hormones or genetic factors). And of course, the results should be presented to the end-user in a manner that they are easy to understand and interpret for clinical use, and of course needing the minimum amount of sample (i.e., blood) possible. Hence the PoC IVD should be **user-friendly**, both *easy to use* and *easy to understand*, accompanied with the appropriate training material and guidance.

Of course, such a technology also comes with limitations and challenges, from surface functionalization, orientation activity of the aptamers, to **reproducibility** and **consistency**, especially in terms of **stability** and **durability.** After production, the device should be able to operate as intended for a longer period of time and under various storage conditions, without degradation of the device and of course its results.

When developing such a device, several other factors should be taken into consideration. As a medical device, **regulatory compliance** is one of the most demanding, as it might be quite a challenge to receive approval and get to the market. But even then, clinical and market adoption are not ensured, hence considering **reimbursement** scenarios should be also considered before reaching the clinic.

Several stakeholders, beyond Technology Providers, highlighted that such a technology should be designed from the beginning, bearing in mind its **scalability** and **large-scale manufacturing**. Good manufacturing practices and quality systems should be considered to ensure the needed reliability and consistency of the PoC IVD device.

Large-scale manufacturing is also closely related to the market cost of the PoC IVD. All stakeholder mentioned that the deployed solution should be **affordable**, and ideally covered by government funding. However, Decision Makers raised the issue of **cost-effectiveness and clinical impact for health systems**, especially absent of a cure. When asked, Patients and Caregivers provided quite diverse answers in terms of willingness to cover the expenses of such a device. With the majority staying within the range of 50-200€, it is quite interesting that they would be willing to pay much more (500-1.000€) if the test could guarantee a certain diagnosis.

Finally, as any new technology, the deployment of an PoC IVD in (primary) healthcare settings should be accompanied by **education and training** of the stakeholders involved. HCPs should be trained on (i) how the system works (general principles), (ii) how to use it effectively, and (ii) how to interpret its results, whereas Patients and Caregivers should be educated on (i) the main principles of how the device works and (ii) on the test's accuracy and benchmarks.

Even though graphene-based biosensors have been in the biomedical domain for quite some time, there is a need for further development of IVD approaches, especially if we consider the biomarker challenges for early detection and progression monitoring of AD. Nevertheless, exploring market analogies with established tests like glucose or diabetes testing, would allow developing a robust business model, which will be analysed later in the project under T6.3.

7.6.5 The ethics dimension

All interviewed stakeholders engaged in ethical aspects related to AD, the existing clinical approaches and the potential implications of a PoC IVD towards improving the care journey.

Trust and Acceptance

A strong message across all stakeholders had to do with the issue of **trust**. Patients and Caregivers are highly dependent on their HCP, and this is also reflected in their trust to the process of the examination; if the HCP trusts it, then the Patients and Caregivers will also trust it. However, this entails that there is already established trust between the HCP and the Patient/Caregiver. Hence, the role of the HCP is crucial for establishing and maintaining trust to a new technology and the provision of a healthcare service. In addition, as mentioned by Decision Makers and HCPs, the reliability and credibility of the technology can also be a key factor in building and maintaining trust with HCPs (first) and Patient/Caregivers (secondly). In the case of HCPs, if there is additional information related to how to interpret the results (diagnostic benefits), scientific benchmarks in accuracy (including also comparison with the current "gold standards"), cost savings, and finally the practicability in its application.

In that regard, another important ethical concern often raised has to do with **data protection and confidentiality**. Patient and Caregivers expressed their preference that information should be stored

in their medical files but shielded from public view, with limited access granted to their offsprings only for health-related purposes, this is also the case for the workplace and healthcare, with interviewees stating that employers and insurers should not have access to their medical information without consent, as it may lead to discrimination. This could be easily resolved by a carefully drafted transparent informed consent. Patients and Caregivers should be well-informed about all related positive and negative aspects before testing, including their rights for the use of their data. This also extends to research, as the Patients and Caregivers expect that their HCPs will use their data appropriately and fairly, hence this should be covered in the informed consent form provided before any examination takes place.

AD Stigma & Misdiagnosis

One of the main challenges to maintaining trust is how the HCPs communicate their interpretation of the results, e.g., the diagnosis. **Communicating the diagnosis** of AD carries significant responsibility, considering its impact on various aspects of the patient's life, such as professional and insurance matters. Patients and Caregivers also expressed their concerns about a potential **misdiagnosis**, which is strongly linked with both the lack of awareness, the lack of standardised and universally accepted testing procedures, and of course, the lack of treatment. On the other hand, even if the diagnosis is accurate, all stakeholders highlighted the effect of the **AD stigma**, which is linked with social and professional isolation, but also healthcare risks, as insurers (as well as other groups) may discriminate in the presence of a positive diagnosis. Other perspectives raised related to the concept of "stigma", had to do with the **fear of loss of autonomy**, especially strong in "intellectual" individuals, and the **fear of economic burden**, especially in the case of individuals of "low social" status.

As a result of these concerns, some of the guidelines provided by the interviewees focused on (i) performing tests only when clinically necessary to avoid harm; (ii) respect the "right to not know" of Patients and Caregivers, but always having in mind the health risks and benefits of such a decision; (iii) balance benefits and risks of early detection to avoid over/misdiagnosis.

Safety

In terms of safety, responses collected were more straightforward and practical. The **biological sample collection**, **storage** and **handling** should carefully follow existing clinical guidelines and protocols to ensure that the use of the device is safe, minimally invasive and non-infectious. An interesting remark that requires further attention and potentially research has to do with the timing of the test, to ensure stability of the reagents, avoid contamination in the blood extraction and degradation of blood or the components of the PoC with time or temperature.

A summary of all the main findings extracted from the interviews is presented in the below figure.

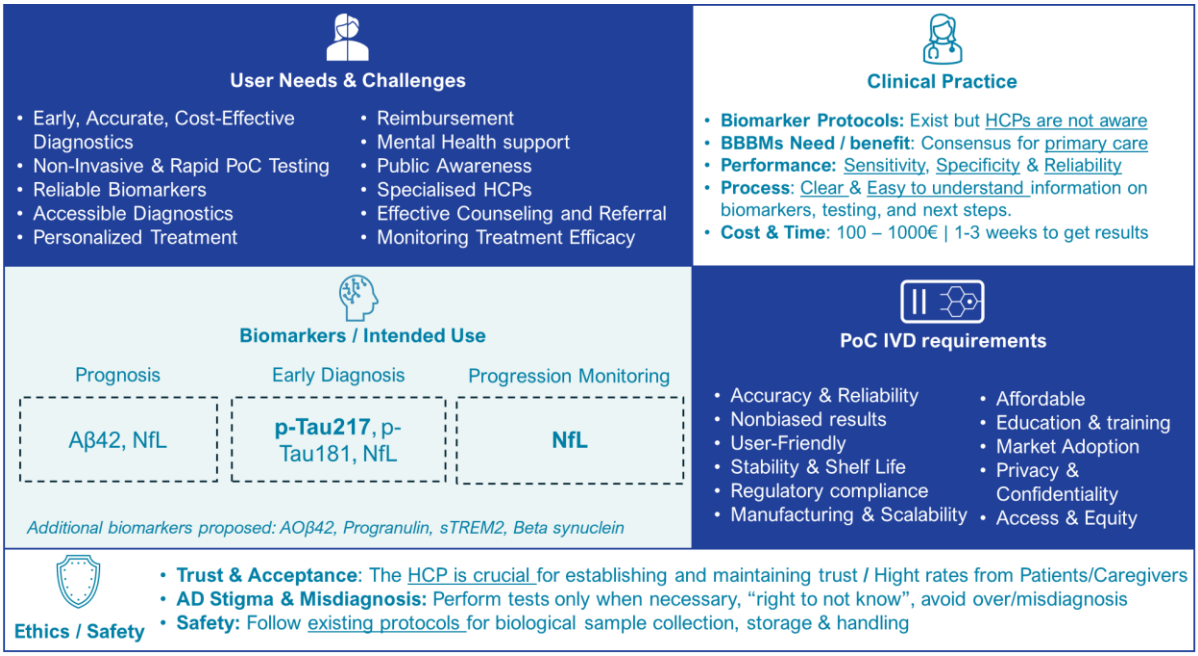

Figure 17. Main findings from the Semi-Structured Interviews

8. Wider Feedback via an Online Survey

8.1 Sample size

A total of 197 participants were enrolled in the Online Survey as of 06/03/2024, leading to a response rate of 45.68%. Out of the total 197 participants who enrolled, 107 did not complete the online survey, while 90 successfully completed it. The collected sample size turned out to be smaller than initially planned, however, no specific trends or patterns were identified for this outcome.

The respondents encompassed a diverse array of the initially targeted population groups, including Biomarker Experts (n=12), Decision/policy makers (n=13), Primary (n=8) and Specialized (n=18) HCPs, as well as AD patients (n=10) and Caregivers (n=29). To mitigate potential readability concerns, an additional question assessing English comprehension was incorporated into the Online Survey. No readability concerns were raised by the respondents and/or the non-respondents.

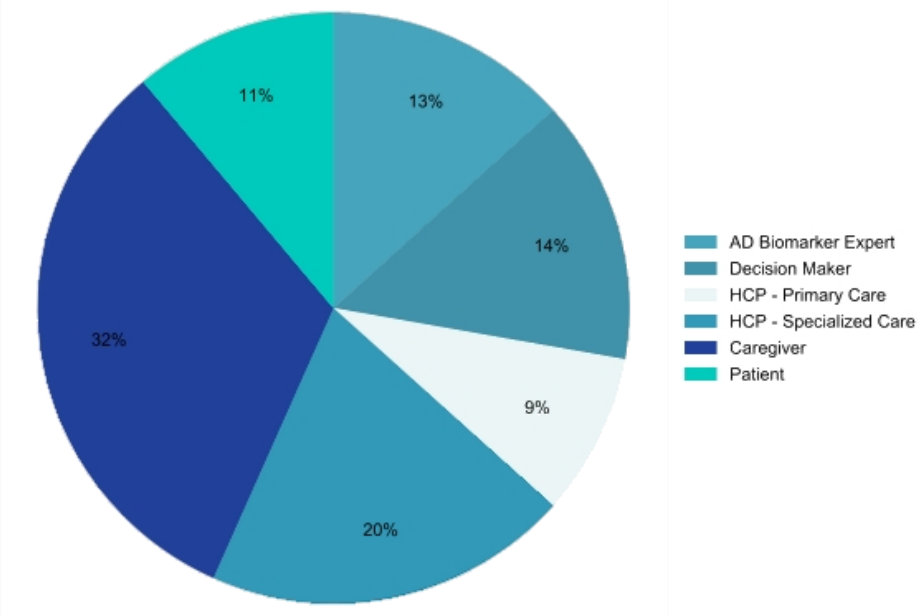

Figure 18: Profile of the 90 Respondents

As previously mentioned, no formal calculation was conducted to determine the sample size, which might have contributed to the lower-than-expected number of completed respondents. Due to the sample size gathered, there was not a comprehensive representation of all target population groups. Specifically, among the 90 respondents, a considerable majority of 68 (76%) were from Greece. This skewed distribution posed a challenge in interpreting the analysed results due to the disproportionate representation of Greek respondents within the total sample.

Table 6: Main country of residence of the 90 Respondents

Country	Frequency	Percentage
Denmark	1	1%
France	2	2%

Country	Frequency	Percentage
Germany	8	9%
Greece	68	76%
Ireland	2	2%
Other	5	6%
Spain	4	4%
Total	90	100%

8.2 Demographics

8.2.1 Level of English of the 90 respondents

An English proficiency question was included in the Online Survey to evaluate respondents' comprehension and understanding of English. The responses provided by the participants were subsequently interpreted to ascertain whether their answers aligned with the level of proficiency they had indicated. This evaluation helped ensure the accuracy and reliability of the data collected.

Among the 90 respondents, eight chose "native or near-native," 53 opted for "fluent" in English, 26 selected "basic" knowledge, and 3 indicated "very limited" proficiency.

8.2.2 Demographic Characteristics per Participant Profile

To obtain insights into the fundamental demographic characteristics requested for the current Online Survey, encompassing English comprehension level, age, primary country of residence, and education level across all 90 respondents from all participating target groups, a table was generated to offer a succinct overview of all pertinent result data (Annex VI).

The following sections will discuss the relevant result information per target participant group to gain a better understanding of the demographics within each group.

Patients

The overall number of enrolled Patients in the Online Survey was 10. Among these 10 AD Patients, no one was a native English speaker, leaving the English level divided among “Fluent” (n=3/10; 30%), “Basic” (n=5/10; 50%), and “Very limited” (n=2/10; 20%).

In addition, the Patients’ ages varied mostly between 65-74 (n=4/10; 40%) and 75 and over (n=4/10; 40%), with only two Patients identified in the ages between 45-64. Following their main residency, it was revealed that all Patients were residing in Greece.

The majority of Patients reported that they hold a “Bachelor’s degree” (n=6/10; 60%), while the remaining four reported either a “High-school degree” (n=1/10; 10%), an “Occupationally-specific program” (n=1/10; 10%), a “Master’s degree” (n=1/10; 10%), and a “PhD” (n=1/10; 10%.

Last but not least, Patients were also asked about their occupation, to evaluate any professional relevance to AD. It is revealed that occupation is being divided into different types of sectors including “Public sector or Education” (n=2/10; 20%), “Business and other services, Finance or Insurance” (n=2/10; 20%), and additional types (n=5/10; 50%) of occupation (i.e., “French teacher”, “Pensioner”, “Telecommunication Industry secretary”, “Retired”, “Household”).

Caregivers

The enrolled Caregivers in the Online Survey were 29. Among these Caregivers, 58.62% (n=17/29) reported being “Fluent” in English, while 31.03% (n=9/10) of them reported having basic English comprehension, and only 10.34% (n=3/29) were reported as native English speakers.

The Caregivers’ ages mostly varied among 45-54 (n=10/29; 34.48%) and 55-64 (n=11/29; 37.93%). However, Caregivers were also divided between other age groups such as 25-34 (n=3/29; 10.34%), 35-44 (n=3/29; 10.34%), and only one Caregiver selected being in the age group of 65-74 (n=1/29; 3.45%).

The vast majority of Caregivers reported residing in Greece (n=26/29; 89.66%), with only 3.45% of Caregivers residing in Germany (n=1/29), Ireland (n=1/29), and the UK (n=1/29) accordingly.

When asked about their education level, 41.38% (n=12/29) of Caregivers owned a bachelor's degree, followed by 24.14% (n=7/29) selecting a master's degree, 13.79% choosing a PhD (n=4/29), and 6.90% (n=2/29) holding a Post Doc. Additionally, 3.45% of Caregivers selected education levels that varied from high school diploma to medical degree and internship.

Finally, similarly with Patients, Caregivers were asked about their occupation. A similar pattern with Patients has been observed, with the addition of having Caregivers being part of the "Health or social care" sector as well (n=6/29; 20.69%). Furthermore, enrolled Caregivers seem also to be divided in additional types of occupations such as “Business and other services Finance or Insurance” (n=5/29; 17.24%), “Public sector or Education” (n=7/29; 24.14%), “Manufacturing, Construction or Agriculture” (n=3/29; 10.34%), and supplementary occupation types (“Other”; n=5/29; 17.24%) including “Information technology”, “Military”, “Journalist-Health editor”, and “Private sector”.

Decision Makers

Thirteen (n=13) Decision Makers were enrolled in the Online Survey. Among these 13 Decision Makers, 76.92% (n=10/13) were fluent in English, compared to 15.38% (n=2/13) and 7.69% (n=1/13) reporting having basic English comprehension and being a native speaker accordingly.

In addition, 38.46% (n=5/13) of the Decision Makers were aged from 25-34 years, followed equally by 23.08% (n=3/13) of them belonging to either the 35-44 or 45-54 age groups, with only 15.38% (n=2/13) of Decision Makers being aged among 55-64 years.

The vast majority (n=8/13; 61.54%) of Decision Makers were residing in Greece, with all remaining Decision Makers (n=5/13) residing in multiple countries (i.e., Denmark (n=1/13; 7.69%), Germany (n=2/13; 15.38%), Ireland (n=1/13; 7.69%), Austria (n=1/13; 7.69%)).

Last but not least, when asked for their education level, 23.08% (n=3/13) of Decision Makers reported having a PhD, compared to 15.38% (n=2/13) selecting having either a bachelor's degree, a master's

degree, a medical degree, or doing their residency. Only 7.69% (n=1/13) of Decision Makers stated having a Post Doc or completing an occupationally-specific program.

Primary HCPs

Eight (n=8) Primary HCPs participated in the Online Survey. Among them, 50% (n-4/8) reported to be fluent in English, while 37.5% (n=3/8) indicated having basic English comprehension. Only one (12.5%) Primary HCP reported "Very limited" English capacity.

The age distribution among Primary HCPs showed minimal variance, with 75% (6 out of 13) falling within the 45-54 age bracket, while only 12.50% (1 out of 13) fell into either the 25-34 or 35-44 age groups. Additionally, it was found that all Primary HCPs (n=8/8; 100%) were residing in Greece.

Among Primary HCPs, 37.50% (n=3/13) disclosed possessing either a medical or master's degree, while 12.50% (n=1/13) indicated having a PhD or being in residency.

Specialized HCPs

Of the 18 Specialized HCPs who participated in the online survey, 61.11% (n=11/18) stated that they were fluent in English, while 27.78% (n=5/18) claimed to have basic English proficiency. Merely 11.11% (n=2/18) of the Specialized HCPs identified themselves as native English speakers.

Age descriptions among Specialized HCPs varied, with the largest proportion (n=6/18; 33.33%) falling within the 45-54 years age range. Following this, 27.78% (n=5/18) were aged 35-44 years, 22.22% (n=4/18) were aged 25-34 years, 11.11% (n=2/18) were aged 18-24 years, and finally, 5.56% (n=1/18) were aged 65-74 years.

Specialized HCPs reported only two countries of residence, with Greece being the most commonly selected (n=14/18; 77.78%) followed by Germany (n=4/18; 22.22%).

In conclusion, seven Specialized HCPs (38.89%) indicated possessing a master's degree as their highest level of education, with six (33.33%) selecting a medical degree as their highest attainment. Additionally, 16.67% (n=3/18) mentioned they were currently undertaking their internship, while 5.56% (n=1/18) reported owning a Post Doc degree or being in residency.

Biomarker Experts

Twelve (n=12) Biomarker Experts participated in the Online Survey. Among them, 66.67% (n=8/12) revealed fluency in English, while 16.67% (n=2/12) stated being either native English speakers or having basic English proficiency.

Ages among Biomarker Experts also exhibited variability, with 33.33% (n=4/12) falling within either the 25-34 or 35-44 age brackets. Furthermore, 25% (n=3/12) belonged to the 45-54 age group, and only 8.33% (n=1/12) selected being in the 55-64 age range.

A few (n=4/12; 33.33%) of Biomarker Experts indicated they were from Spain, while 16.67% (n=2/12) reported being from both Greece and France. Additionally, 8.33% of Biomarker Experts stated they resided in either Germany, Malta, Poland, or the USA.

Lastly, 58.33% (n=7/12) of the Biomarker Experts either held or were pursuing a Postdoctoral degree, while 16.67% (n=2/12) possessed a master's or PhD degree. Only one (8.33%) Biomarker Expert held a bachelor's degree.

8.3 Patients and Caregivers

8.3.1 Level of Experience

Out of the 29 Caregivers[165] enrolled in the Online Survey, 22 indicated that they had "None" experience in AD biomarkers, while only one (3.45%) stated that they were "Competent," and another one (3.45%) "Advanced beginner" in this area. Additionally, 79.31% reported having no experience in PoC IVD, compared to five (17.24%) stating being "Novice," and only one (3.45%) Caregiver stated being "Competent" in the area.

Now, when it comes to the 10 Patients enrolled in the Online Survey, the vast majority of them (n=8/10; 80%) stated that they had no experience in AD biomarkers, similar to 90% of Patients (n=9/10) who stated the same regarding PoC IVD experience. Moreover, only one (10%) of the enrolled Patients was an "Advanced beginner" in AD biomarkers, followed by the same Patient stating being an "Advanced beginner" in PoC IVDs as well.

8.3.2 Acceptance and trust

To examine acceptance and trust, Patients and Caregivers were asked about their willingness to use a PoC IVD. The vast majority of both Caregivers (n=28; 96.55%) and Patients (n=9; 90%) (Figure 19) responded that they would be willing to have a digital blood test that could provide immediate quantitative results (e.g., a finger test for blood sugar) with their primary healthcare doctor instead of going to a lab.

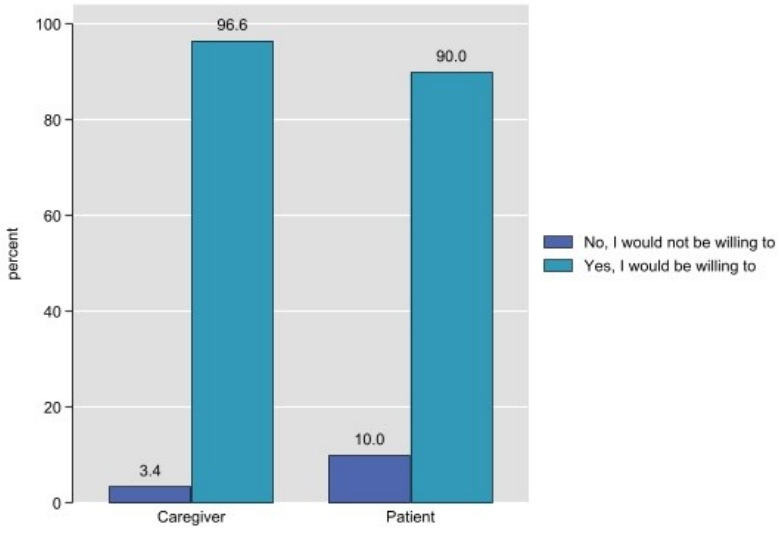

Figure 19: Caregivers' and Patients' Willingness to use PoC IVD

[165] The Caregiver who indicated advanced experience in AD biomarkers was employed in the public sector or education and held a PhD, whereas the one who indicated being "Competent" was engaged in "Hospitality, Catering, or Leisure Services" and held a bachelor's degree. Additionally, the Caregiver who reported being "Competent" in PoC IVD held a master's degree and was occupied in the "Transport, Retail, or Wholesale" sector.

Furthermore, Patients and Caregivers were asked to specify when during the disease process they would be comfortable to let their doctor use a blood test as a part of their clinical assessment. The results (Figure 20) revealed that the largest proportion of 19 Caregivers (67.86%) and six Patients (66.67%) answered 'Before any symptoms', and 28.57% of Caregivers and 33.33% of Patients answered 'After first cognitive complaints/symptoms appear (primary care)', and one Caregiver answered, 'After getting diagnosed with cognitive impairment (specialized care)'.

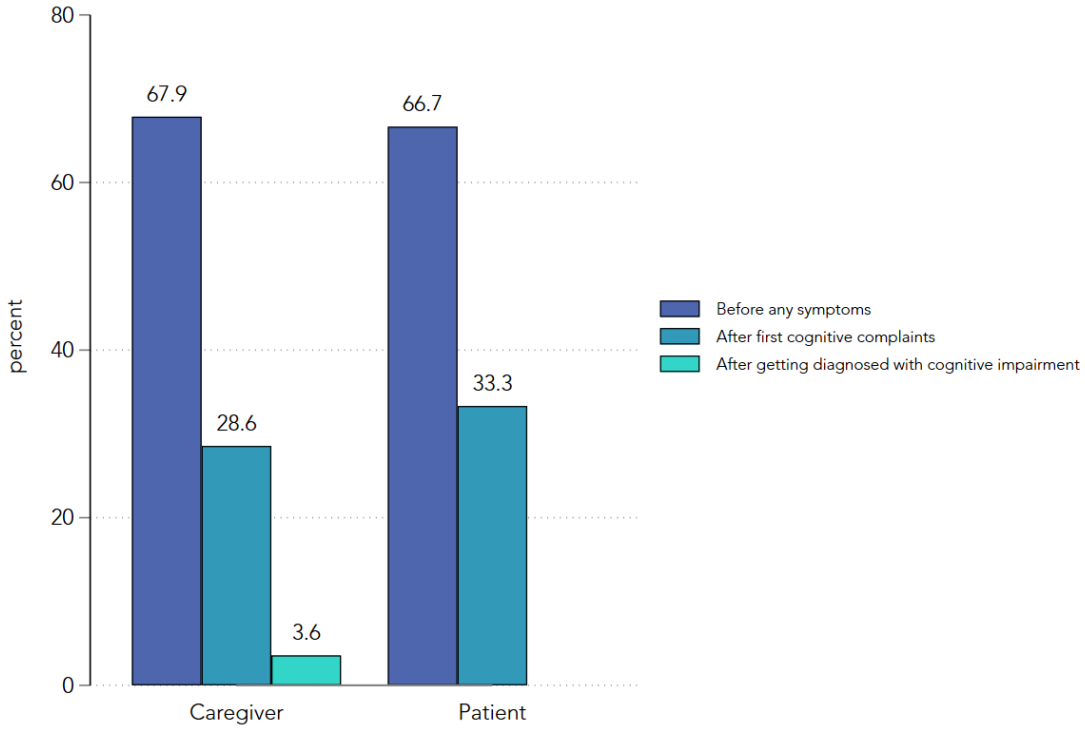

Figure 20. Patients' and Caregivers' Specification on when during the Disease Process they would be comfortable to let a Doctor use such a Blood Test

In terms of frequency of willingness to take a blood test, most Caregivers (n=13; 46.43%) and Patients (n=4; 44.44%) reported 'every year' followed by every 6 months for Caregivers (n=10; 35.71%) and 'every 2 years' by Patients (n=3; 33.33%). Fewest of Caregivers answered less often than every 6 months (n=5; 17.86%), whereas 33.33% among Patients were willing to take the blood test only every 2 years (n=3) (Figure 21).

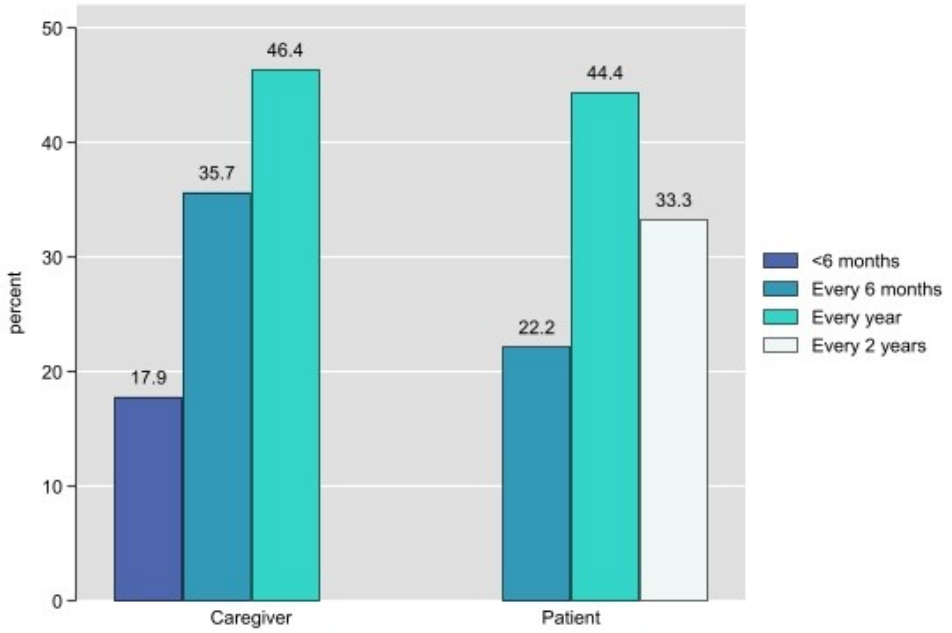

Figure 21: Caregivers' and Patients' Specification on how often they would be willing to take a Blood Test

Results on Caregivers' willingness to cover the costs of having a blood test taken showed that around half of Caregivers were willing to pay (n=13; 46.43%) and the other half were not willing to pay (n=15; 53.57%). A clearer contrast was found in Patients responses showing that most Patients were not willing to pay for having a blood test taken (n=8; 88.89%) (Figure 22).

In specification for reasons for not being willing to cover the costs, nine Caregivers referred to potential "high costs" of the blood test, four Caregivers answered that costs "should be covered by the public healthcare system" and two Caregivers used other arguments. Among patients, four Patients answered that they would not be willing to or able to cover the costs and two Patients responded lack of willingness due to the uncertainty regarding the blood test's reliability.

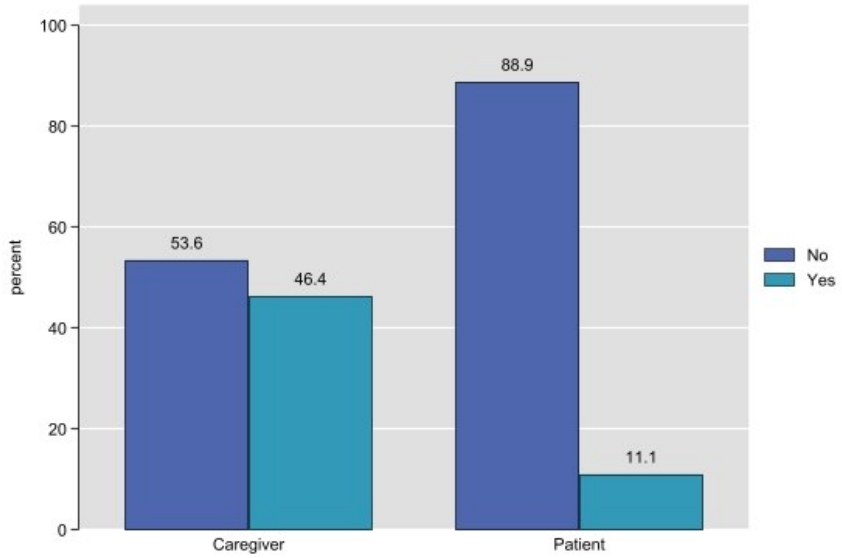

Figure 22: Caregivers' and Patients' Willingness to cover the Costs of a Blood Test

In terms of willingness in what to pay for having a blood test taken, Caregivers and Patients were asked to specify this in categories ranging from <20€ to >200€ (Table 7). Most Caregivers (53.85%) responded that they would pay 20–50€ and three Caregivers (23.08%) would pay less <20€ whereas one Caregiver would be willing to pay 50–100€ and two Caregivers >200€. Only one Patient was willing to cover the cost of the test and was willing to pay 50-100€.

Table 7: Costs Caregivers are willing to pay for a Blood Test

Q: Please specify how much you would be willing to pay for such a test:		
	Frequency	Percentage
<20€	3	23.085
20–50€	7	53.85%
50–100c	1	7.69%
>200€	2	15.38%
Total	13	100%

8.3.3 Willingness to use a PoC IVD for AD

Caregivers and patients were asked a set of questions in relation to their willingness to use a PoC IVD for AD. In respect to what kind of information and support they would need assuming they would have a real-time blood test that could aid a doctor in their decision-making about AD, Caregivers most frequently responded *'guidance on available treatment options'*, followed by *'Guidance for intervention and management of symptoms'*, *'Communication with your treating physician and confirmation of results' credibility'*, *'Support Groups/Mental Health Support'*, *'Caregiver Training'*, and *'Engagement in meaningful activities that stimulate cognitive, social, and physical functions'*.

For the same question, most Patients responded, *'Communication with your treating physician and confirmation of results' credibility'*, followed by *'Guidance for intervention and management of symptoms', 'Guidance on available treatment options', 'Meaningful activities that stimulate cognitive, social, and physical functions'* (Table 8).

Table 8: Information and support Caregivers and Patients would need for AD decision-making

Q: Assuming you would do a real-time blood test that could aid your doctor in their decision-making about Alzheimer's Disease, what kind of information and support would you need?[166]						
	Frequency		% of responses		% of cases	
	Caregivers	Patients	Caregivers	Patients	Caregivers	Patients
Communication with your treating physician and confirmation of results' credibility	17	7	15.18%	21.88%	58.62%	70%
Guidance for intervention and management of symptoms	19	6	16.96%	18.75%	65.52%	60%
Guidance on available treatment options	20	5	17.86%	15.63%	68.97%	50%
Educational Resources	8	1	7.14%	3.13%	27.59%	10%
Support Groups/Mental Health Support	15	2	13.39%	6.25%	51.72%	20%
Caregiver Training	12	4	10.71%	12.5%	41.38%	40%
Medication Management	9	2	8.04%	6.25%	31.03%	20%
Engagement in meaningful activities that stimulate cognitive, social, and physical functions	12	5	10.71%	15.63%	41.38%	50%
Other	0	0	0%	0%	0%	0%
Total	112	32	100%	100%	386.21%	320%

Furthermore, Patients and Caregivers were asked to specify when they would like to get information about blood test to aid doctors in decision-making about AD (Figure 23). The results revealed that both Caregivers and Patients prefer having communicated the test result in a doctor visit compared to having access to the result online. This was shown by most Caregivers (n=15; 51.72%) and Patients (n=4; 40%) answered 'After the test during the visit' and 'Before the test during the visit' (Caregivers 31.03%; Patients 30%), and fewest Caregivers and Patients answered, 'Somewhere online that I could find relevant information at any time' (Caregivers 17.24%; Patients 20%). Only one Patient answered 'other' (n=1; 1%).

[166] Note: This is a multiple response question. Percent of response is the percentage of each response out of total responses from the given question. Percentage of cases in the percentage of participants out of the total participant number that chose that option.

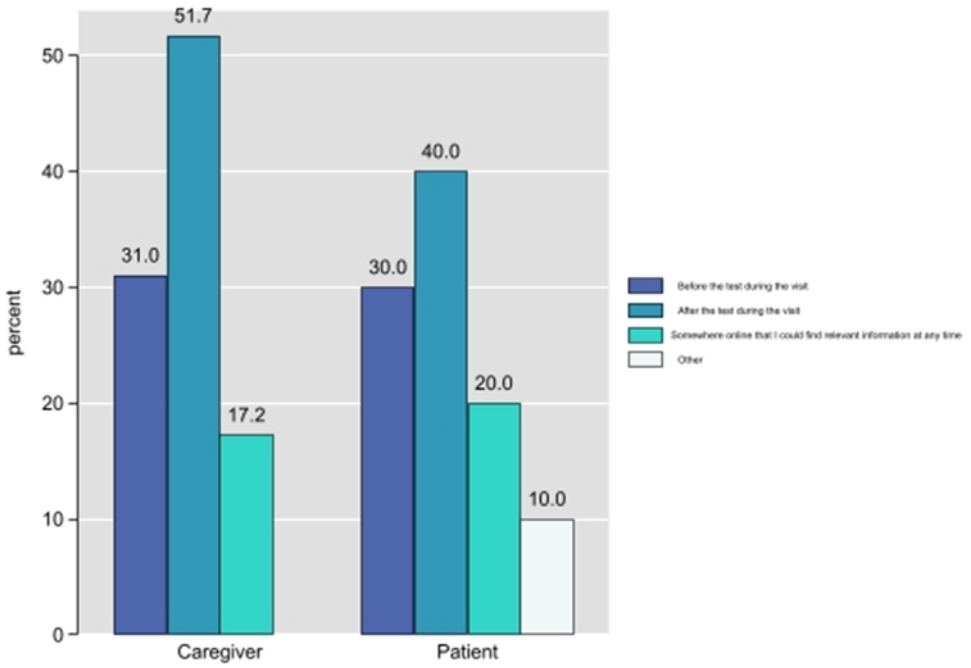

Figure 23. Caregivers' and Patients' Specification on when they would like to get Information about the Test

In respect to assuming having a real-time blood test, Caregivers and Patients were asked what kind of information they would be interested in having from the test if they received the results on a mobile phone or tablet. For this question, most Caregivers responded, 'Alzheimer's Disease stage based on biomarker metrics' (n=24; 82.7%) (Table 9), whereas most Patients responded 'Prognosis/Risk assessment for progression' (n=8; 80%). Both Caregivers and Patients answered least frequently getting information on 'Biomarker Metrics (including e.g. biomarker range values, overall accuracy of biomarker results)' (Caregivers: n=17; 58.62%; Patients: n=2; 20%).

Table 9: Caregivers' preference for test information received on a mobile phone or tablet.

Q: Assuming you would do a real-time blood test that could aid your doctor in their decision-making about Alzheimer's Disease, what kind of information would you be interested in having from the test if you were receiving the results on a mobile phone or tablet?						
	Frequency		% of responses		% of cases	
	Caregivers	Patients	Caregivers	Patients	Caregivers	Patients
Biomarker Metrics (including e.g. biomarker range values, overall accuracy of biomarker results)	2	17	12.5%	27.42%	20%	58.62%
Alzheimer's Disease stage based on biomarker metrics (e.g., early stage, middle stage, late stage)	6	24	37.5%	38.71%	60%	82.76%
Prognosis/Risk assessment for progression	8	21	50%	33.87%	80%	72.41%
Other	0	0	0%	0	0%	0
Total	16	62	100%	100%	160%	213.79%

Out of 29 Caregivers, 7 Caregivers (24.14%) replied that they had concerns about doctors and the healthcare system using a blood test for measuring AD related biomarkers and using the results to make recommendations and decisions about your healthcare. Among these respondents, three Caregivers raised concern regarding the reliability of the test and how the data would be used, one emphasized lack of trust to the healthcare system, another lack of experience, and in addition that test results should be used for decision making.

Among the 10 patients, 2 patients raised concerns (20%) about the overall credibility of test and treatment following the test result whereas the other patient questioned the HCP's knowledge and competence in respect to the test (Figure 24).

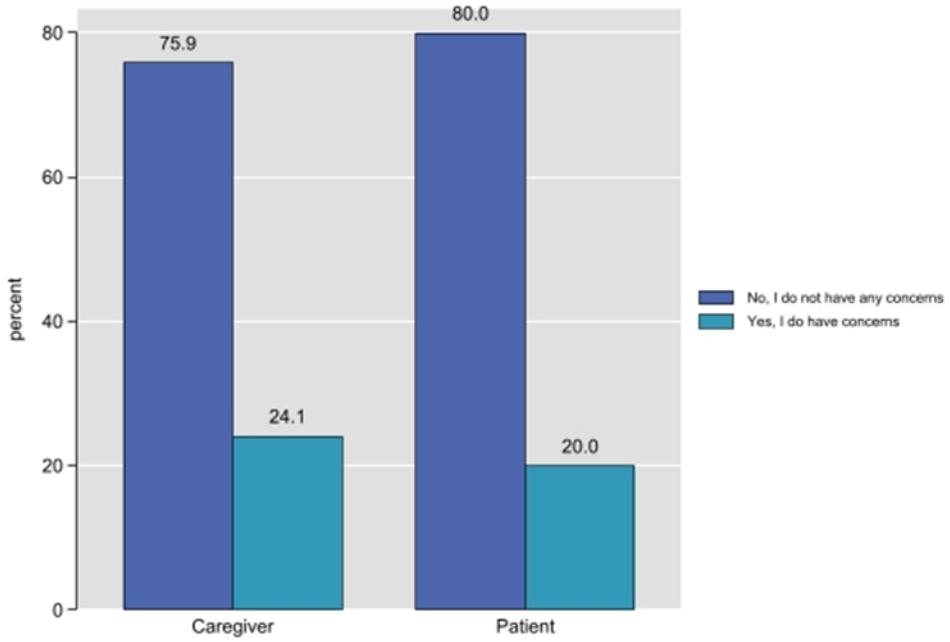

Figure 24: Caregivers' and patients' concern regarding HCPs using the blood test results to make recommendations and decisions about your healthcare

8.3.4 Current (perceived) healthcare burden for Alzheimer's Disease

Caregivers and patients were then asked a set of questions in relation to their perceived healthcare burden of AD in terms of cost and time, but also challenging aspects for seeking and getting healthcare services.

The majority of Caregivers 21/29 (72.41%) responded that they were not aware of the needed costs. Among the remaining eight Caregivers, two answered ≤500 € (6.9%), four answered >500-1000 € (13.79%), one answered >1000-2000 € (3.45%), and one answered >2000 € (3.45%).

On the same topic, the majority of Patients 8/10 (80%) responded that they were not aware of the needed costs whereas the two remaining Patients answered ≤500 € (20%).

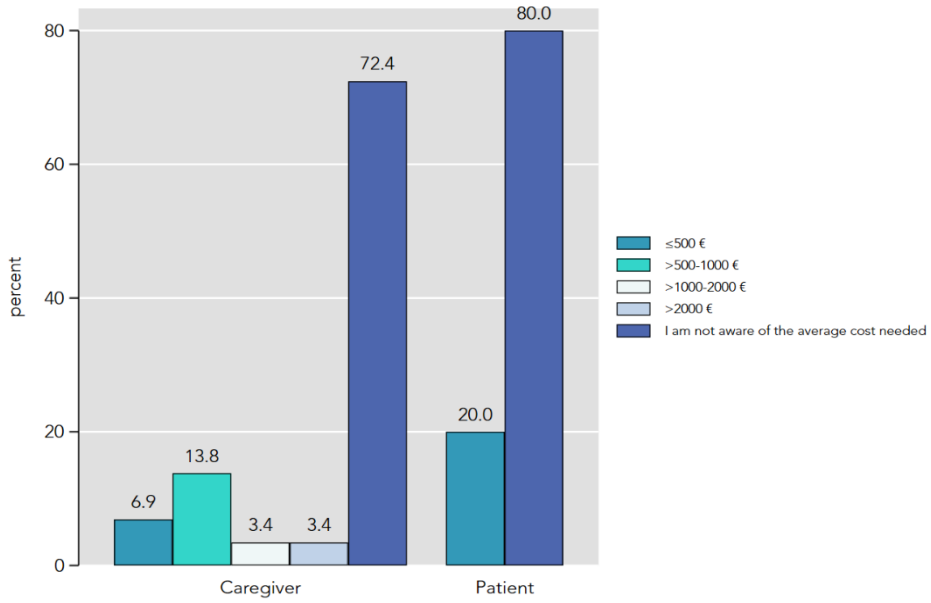

Figure 25: Caregivers' and Patients' perception of average costs for an accurate AD diagnosis

In terms of needed time for an accurate AD diagnosis, out of the 29 Caregivers, 12/29 (41.38%) responded that they were not aware of the average time needed, whereas five Caregivers replied ≤3 months (17.24%), seven replied >3-6 months (24.14%), four replied >6 months-1 year (13.79%), and one replied >1 year (3.45%).

Out of the 10 Patients, 6/10 (60%) responded that they were not aware of the average time needed, whereas one Patient replied ≤3 months (10%), another replied >6 months-1 year (10%), and two replied >1 year (20%).

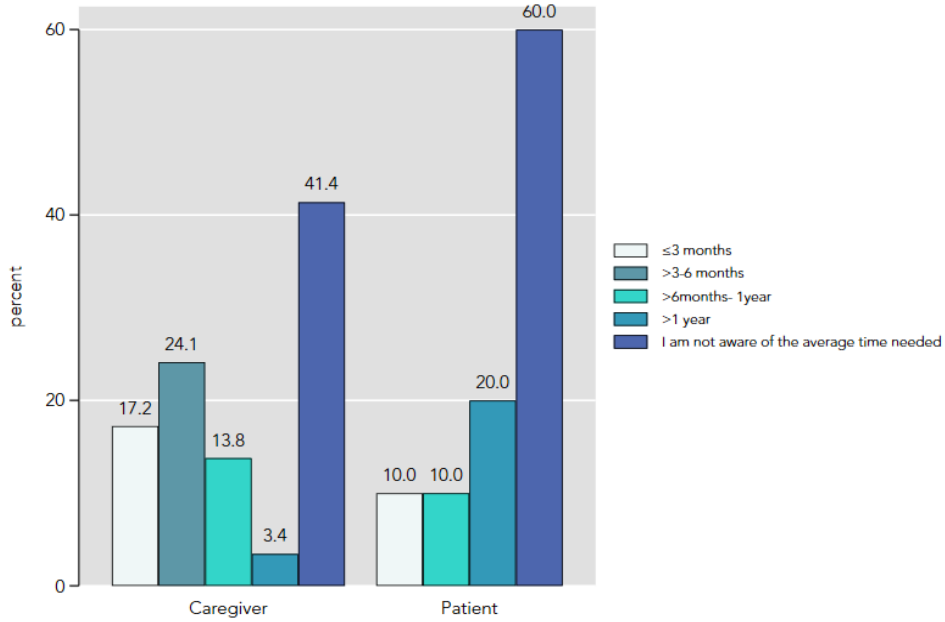

Figure 26: Caregivers' and Patients' perception of average time required for making an accurate AD diagnosis

The Caregivers were asked to provide their perception of challenging aspects for seeking and getting healthcare services for AD (Table 10). The majority of Caregivers (n=21; 72.41%) responded

'Healthcare system prioritization of treatable versus non-treatable diseases', followed by 'Lack of symptom education/Alzheimer's Disease education at primary healthcare settings' (n=18; 62.07%) and 'Cost' (n=18; 62.07%), 'Alzheimer's Disease Stigma' (n=16; 55.17%), 'Lack of awareness/knowledge for the public' (n=18; 44.83%), 'Extended wait times' (n=12; 41.38%) and 'Lack of balance between primary and specialized healthcare services for Alzheimer's Disease' (n=11; 37.93%), 'Over-specialization of professionals in specialized centers for Alzheimer's Disease' (n=6; 20.69%), 'Sex, gender and cultural bias in provision of healthcare services' (n=2; 6.9%), and one (3.45%) answered "Not enough specialized centers for AD".

Accordingly, the majority of Patients (n=7; 70%) responded 'Lack of awareness/knowledge for the public', followed by 'Alzheimer's Disease Stigma' (n=4; 40%) and 'Cost' (n=4; 40%), 'Lack of symptom education/Alzheimer's Disease education at primary healthcare settings' (n=3; 30%), 'Sex, gender and cultural bias in provision of healthcare services' (n=3; 30%), 'Healthcare system prioritization of treatable versus non-treatable diseases' (n=2; 20%), 'Extended wait times' (n=1; 10%), 'Over-specialization of professionals in specialized centers for Alzheimer's Disease' (n=1; 10%), and 'Lack of balance between primary and specialized healthcare services for Alzheimer's Disease' (n=1; 10%).

Table 10: Caregivers' perception challenges for seeking and getting AD healthcare services

Q: Which aspects are challenging for seeking and getting healthcare services for Alzheimer's Disease?						
	Frequency		% of responses		% of cases	
	Caregivers	Patients	Caregivers	Patients	Caregivers	Patients
Alzheimer's Disease Stigma	16	4	13.56%	15.38%	55.17%	40%
Cost	18	4	15.25%	15.38%	62.07%	40%
Extended wait times	12	1	10.17%	3.85%	41.38%	10%
Healthcare system prioritization of treatable versus non-treatable diseases	21	2	17.8%	7.69%	72.41%	20%
Over-specialization of professionals in specialized centers for Alzheimer's Disease	6	1	5.08%	3.85%	20.69%	10%
Lack of balance between primary and specialized healthcare services for Alzheimer's Disease	11	1	9.32%	3.85%	37.93%	10%
Lack of awareness/knowledge for the public	13	7	11.02%	26.92%	44.83%	70%
Lack of symptom education/AD education at primary healthcare settings	18	3	15.25%	11.54%	62.07%	30%
Sex, gender and cultural bias in provision of healthcare services	2	3	1.69%	11.54%	6.9%	30%
Other	1	0	0.85%	0%	3.45%	0%
Total	118	26	100%	100%	406.9%	260%

8.4 Decision Makers

8.4.1 Level of Experience

Out of the 13 Decision Makers enrolled in the Online Survey, four indicated that they were “Proficient” in AD biomarkers, while three stated that they were “Competent” in this area. Additionally, 38.46% reported having no experience in PoC IVD, whereas four Decision Makers (30.77%) reported that they do not have any experience in PoC IVDs.

8.4.2 Clinical Testing Procedure and Intended Use of AD Biomarkers

Clinical or research protocols

The majority of Decision Makers (i.e., 92.31%), indicated that they were not aware of any clinical or research protocols currently utilized for collecting and analysing AD fluid-derived biomarkers.

Intended Use

In addition, Decision Makers were asked about the intended use they envisioned for AD blood-derived (plasma) biomarker results, with all of them (n=13) indicating that early diagnosis would be of utmost importance. Furthermore, five (38.46%) Decision Makers also selected prognosis and progression monitoring as additional pertinent uses for these biomarker results (Figure 27)

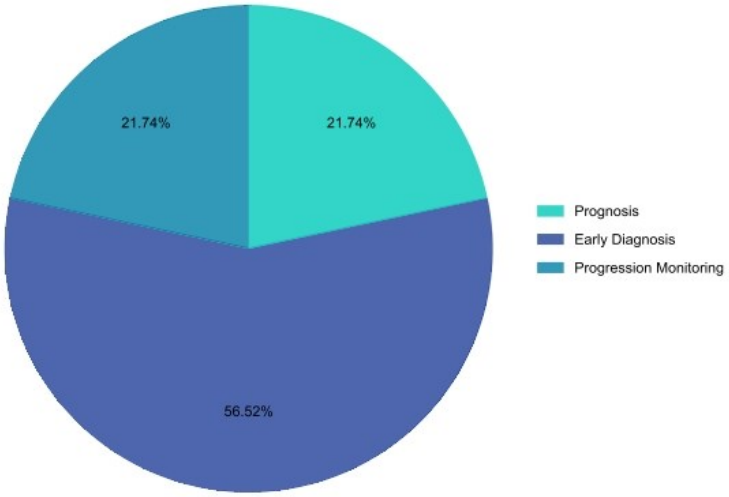

Figure 27: Intended use envisioned for AD blood-derived biomarkers among Decision Makers

Patient’s Care Journey Stage

As per the responses from Decision Makers, the stage in the patient’s care journey deemed most appropriate to assess AD blood-derived biomarkers is as follows (Figure 28):

- Early detection of "at-risk" healthy individuals at primary healthcare: 46.15% (n=6);
- Early detection of AD onset (i.e., Subject Cognitive Impairment (SCI) or Mild Cognitive Impairment (MCI)) at primary healthcare: 30.77% (n=4);
- Differential diagnosis and treatment selection at specialized care: 15.36% (n=2);
- Treatment response at specialized care: 7.69% (n=1).

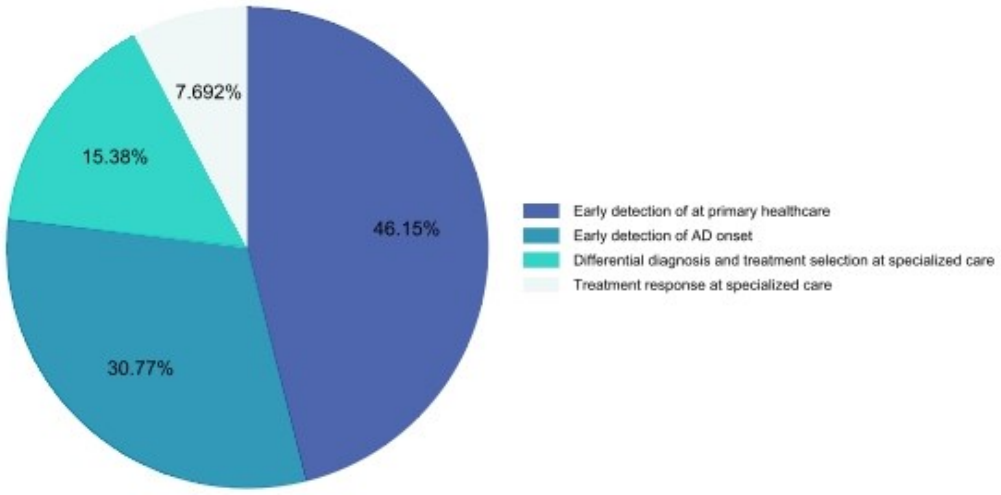

Figure 28: Decision Makers' envision of patient's care journey stage for assessing AD blood-derived biomarkers

8.4.3 Clinical Testing Performance

Decision Makers were asked which factors are important for their decision-making process regarding AD. The results indicate that “Pricing/cost” 53.85% (n=7/13), “Availability” 53.85% (n=7/13), “Minimal or Non-Invasiveness” 53.85% (n=7/13), and “Complexity/User-friendliness” 38.46% (n=5/13) were among the most commonly chosen options. "Robustness/Reliability" and "Credibility" were also among the most chosen options, to a slightly lower extent, with each selected by 30.77% (n=4/13) of Decision Makers (Figure 29).

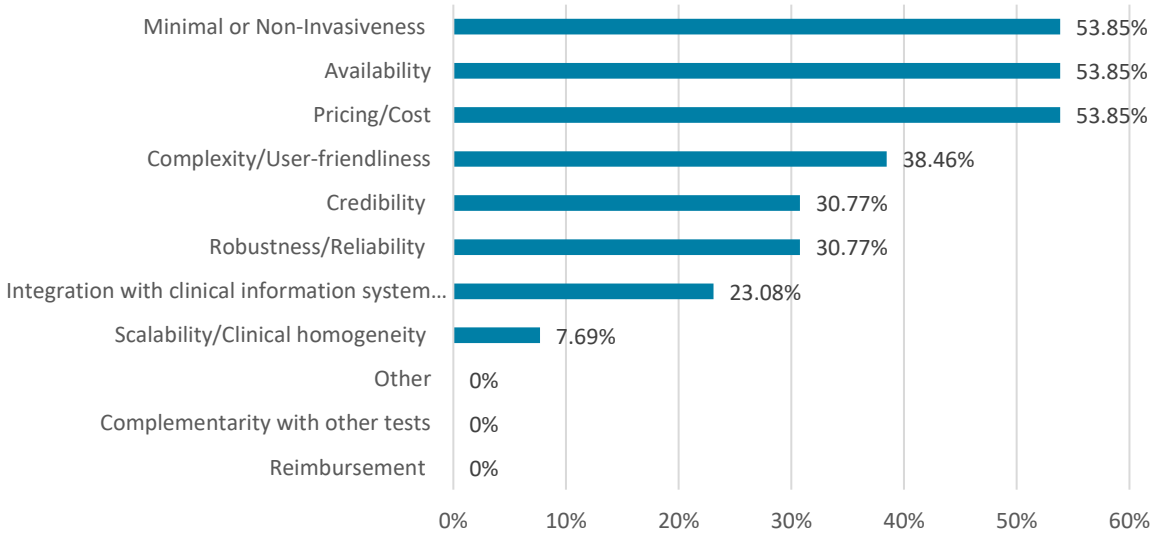

Figure 29: Factors considered important for AD Decision Making among Decision Makers

8.4.4 Clinical Assessment

In terms of clinical assessment, Decision Makers were asked how they would envision receiving AD blood-derived (plasma) biomarker results directly on a mobile phone or tablet in real-time, as well as how they would envision integrating or transferring these results to the Clinical Information System (CIS). In particular, among the Decision Makers surveyed, 53.85% (n=7/13) selected "Direct and secure access from the mobile app to the CIS" as their envisioned method for receiving AD blood-derived biomarker results, 23.08% (n=3/13) selected "File storage (e.g., extract files and store them in the CIS) Error Handling", while 23.08% (n=3/13) selected "Dedicated app (extension of the CIS) to interact directly with the device" (Figure 30).

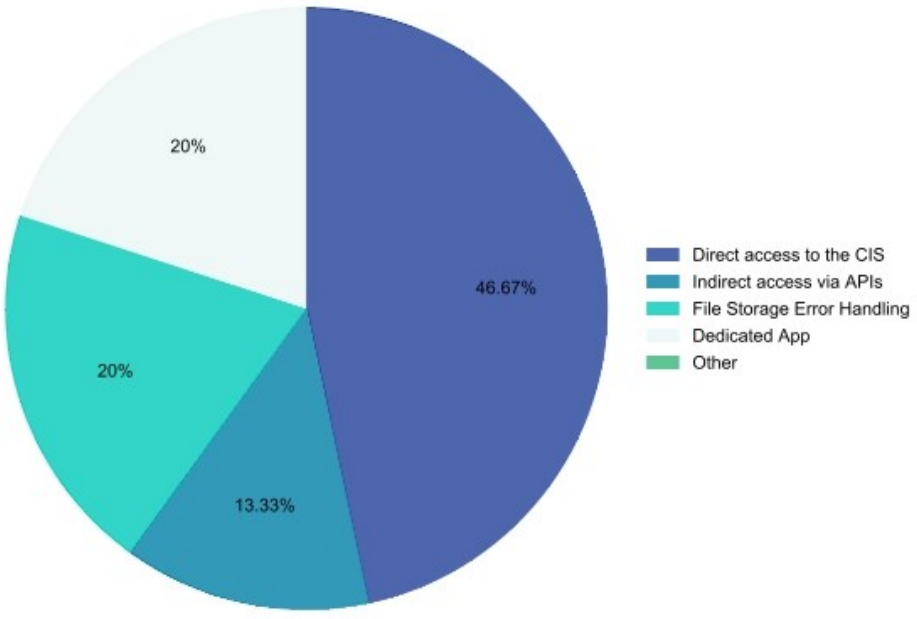

Figure 30: Envision of receiving in a phone/tablet AD blood-derived biomarkers among Decision Makers

8.4.5 Need of a PoC IVD for AD

To assess the need for a PoC IVD for AD, Decision Makers were asked about the core benefits they perceive such a device could offer in a primary healthcare setting for accurately detecting AD blood-derived (plasma) biomarkers. Among the Decision Makers surveyed 100% (n=13/13) indicated "Early diagnosis and intervention, potentially slowing down disease progression" as a core benefit of a PoC IVD for AD biomarkers in a primary healthcare setting.

Additionally, 46.15% (n=6/13) selected both "Reduced healthcare costs, resulting from early diagnosis and early preventative measures that could lessen the need for expensive long-term care" and "Quality of Life Improvement as a result of early intervention and appropriate management based on biomarker results" as potential benefits. Lastly, "Patient convenience and accessibility (e.g., avoid appointment downtime with secondary/specialized care, use of already existing blood test protocols, etc.)" and "Preventive health measures such as lifestyle modifications, cognitive exercises, or targeted medications" were each selected by 38.46% of cases (n=5/13) (Figure 31).

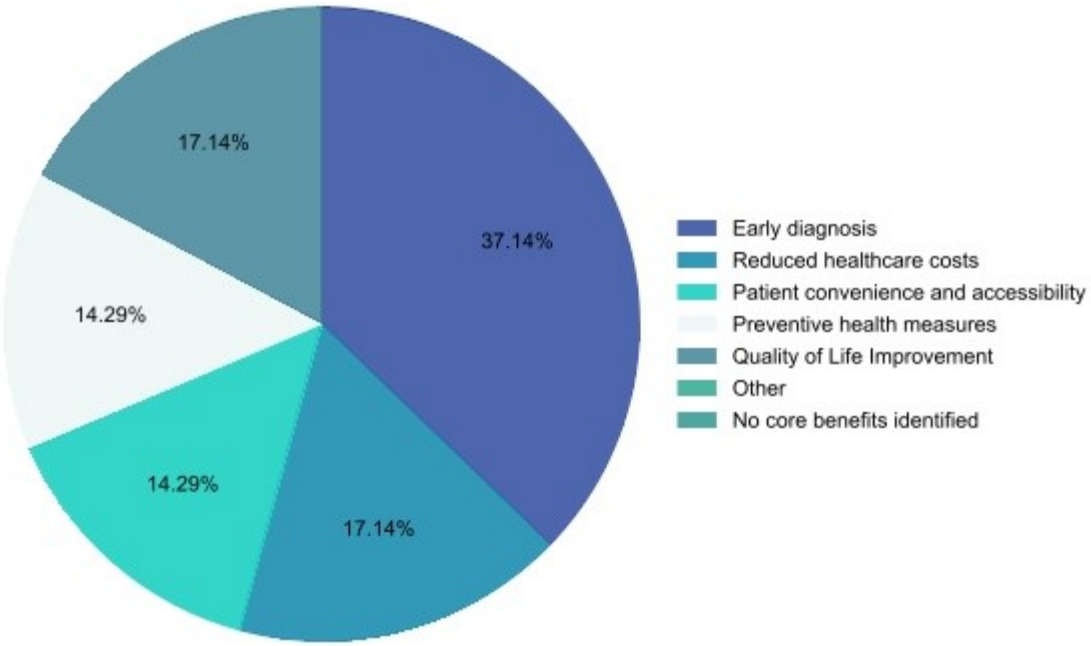

Figure 31: Core benefits of a PoC IVD in a Primary healthcare setting

8.4.6 Challenges

Cost and Time

To evaluate the challenges around AD, Decision Makers were asked about the average cost needed for obtaining AD fluid-derived results to assist in diagnosis. From the 13 Decision Makers surveyed, two (15.38%) of them chose "None" when asked about their experience in AD biomarkers.

Therefore, the relevant cost and time questions regarding biomarkers were directed to the remaining 11 Decision Makers. Among the respondents, 63.64% (n=7/11) did not provide information regarding the average cost needed for obtaining AD fluid-derived results, whereas 27.27% (n=3/11) did not provide information when asked about the time required for obtaining these results. In addition, among the respondents, 54.55% (6/11) stated that the average time needed for such a test is two weeks or longer. Additionally, six Decision Makers shared the same perspective regarding the average time needed for such testing, indicating a consensus on the duration of the process.

To gain an understanding of the overall cost challenge for AD diagnosis, Decision Makers were also asked about the average cost needed to accurately diagnose the patient's health status in terms of AD. This inquiry aimed to gather insights into the financial implications associated with AD diagnosis from the perspective of Decision Makers.

Among the respondents, 46.15% (n=6/13) replied that they were not aware of the average cost needed for accurately diagnosing the patient's health status in terms of AD, indicating a lack of information or estimates on this aspect. Additionally, 23.08% (n=3/13) replied that the average cost is around ">500-1000 €", suggesting some awareness of the potential costs associated with AD diagnosis among a portion of the respondents.

PoC IVD Deployment

Furthermore, Decision Makers were asked to provide their familiarity with the main challenges and/or barriers to deploying a PoC IVD for detecting AD blood-derived (plasma) biomarkers in primary healthcare settings. This inquiry aimed to gather insights into the perceived obstacles or difficulties that Decision Makers anticipate in implementing such technology in primary healthcare settings.

Among the respondents, 76.92% (n=10/13) identified "Lack of knowledge/expertise from Healthcare Providers (HCPs)" as the most common challenge to deploying a PoC IVD for detecting AD blood-derived (plasma) biomarkers in primary healthcare settings. Following this, "High Testing Cost (Pricing/Cost)" was identified as the most common challenge by 46.15% (n=6/13) of the respondents. Likewise, "Lack of awareness from patients" was also chosen by 38.46% (n=5/13) of the respondents as a significant challenge (Figure 32).

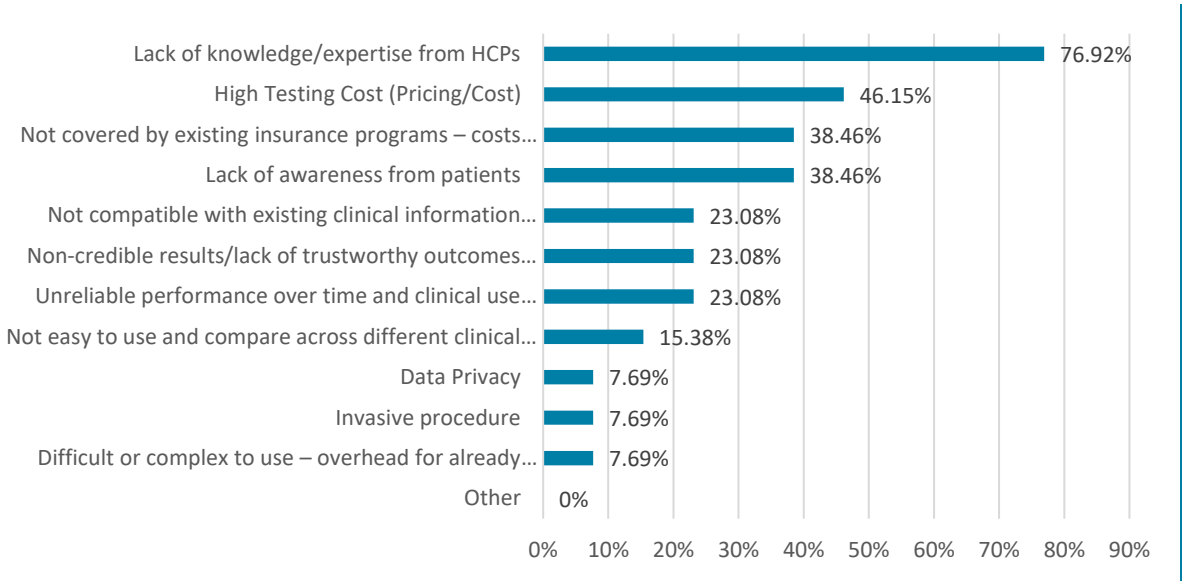

Figure 32: Main challenges and/or barriers to deploying a PoC IVD for detecting AD blood-derived (plasma) biomarkers in primary healthcare settings among Decision Makers

8.4.7 Other projects, solutions, data or information

To identify any other relevant projects, Decision Makers were asked if they were aware of any PoC IVD solutions for AD. All of them (n=13, 100%) replied that they were not aware of any such solutions. Exactly, the lack of awareness among Decision Makers regarding existing PoC IVD solutions for AD when blood-derived biomarkers are involved potentially indicates a gap in the availability of such solutions.

Lastly, Decision Makers were also asked whether they were aware of any public datasets or databases that are relevant to AD fluid-derived (plasma) biomarker research. 92.31% (n=12/13) stated that they were not aware of any such datasets or databases. The one Decision Maker respondent (7.69%) that was aware, specified EDIAN as a relevant database/dataset.

8.5 Healthcare Professionals – Primary & Specialised Care

8.5.1 Level of Experience

Out of the 8 Primary HCPs enrolled in the Online Survey, two indicated that they were “Proficient” in AD biomarkers, while three stated that they were “Novice” or did not have any experience in this area. In addition, three (37.5%) Primary HCPs indicated that have “None” or “Novice” experience in PoC IVDs accordingly, while only two (25%) stated that they are “Proficient” in that area.

On the other hand, out of the 18 Specialized HCPs enrolled in the Online Survey, seven indicated that they have “None” reflecting no experience in AD Biomarkers or were “Novice” in AD biomarkers, while two stated that they were “Advanced beginner” in this area. In addition, 16 Specialized HCPs indicated that they have “None” (66.67%) or “Novice”(22.22%) experience in PoC IVDs accordingly, while only one (5.56%) stated “Competent” and another (5.56%) “Proficient” in that area.

8.5.2 Clinical Testing procedure and Intended Use of AD biomarkers

Clinical or research protocols

The majority of Primary and Specialised HCPs, comprising 87.5% and 83.3% respectively, indicated that they were not aware of any clinical or research protocols currently utilized for collecting and analysing AD fluid-derived biomarkers. The Primary HCP did not provide adequate information about their protocol, whereas the three Specialised HCPs presented the following protocols:

Table 11: AD fluid-derived biomarker Protocols by Specialised HCPs

AD fluid-derived biomarker Protocols
Clinical trials for Biomarker development.
Protocol for study "Noselab": Sample collection, transfer and storage for nasal secrete, Plasma and cerebrospinal fluid.
Lumbar puncture and CSF analysis. Additionally, an experimental research protocol for collection of nasal samples via "nosecollect".

For these protocols, HCPs were requested to also identify relevant limitations, introducing challenges related to (i) invasive or highly demanding sample collection; (ii) logistics in biomarker analysis, as in many cases the samples are send to external labs for analysis; and (iii) sample storage, as the sample “has to be frozen in max. 10 minutes and shipped in 4 to 7 days this may be problematic for clinical diagnostic procedure”.

Intended Use

Furthermore, Primary and Specialised HCPs were asked about the intended use they envisioned for AD blood-derived (plasma) biomarker results. Five Primary HCPs (62.5%) and eleven Specialised HCPs (61.1%) indicated that early diagnosis would be of utmost importance. Furthermore, four Primary HCPs (50%) and five Specialized HCPs (27.78%) selected prognosis as additional pertinent use for these biomarker results. In addition, five Specialized HCPs (27.78%) also selected progression monitoring as additional pertinent use for these biomarker results (Figure 33).

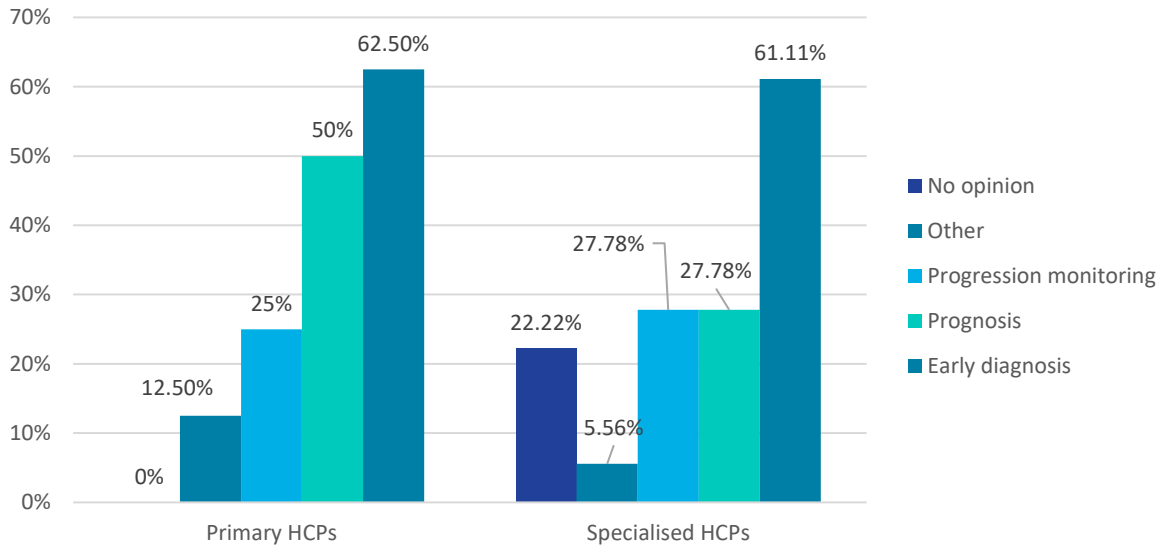

Figure 33. Intended use envisioned for AD blood-derived biomarkers among Primary and Specialised HCPs

From the HCPs that responded Other, only one Specialised HCP elaborated more, stating that “recruitment for clinical trials” could be an additional /complimentary intended use.

Following, to gain insights into how HCPs envision the support of AD blood-derived (plasma) biomarkers in prognosis, early diagnosis, and progression monitoring, they were asked separate questions for each of these aspects based on what they chose to envision as important.

In terms of prognosis, Primary HCPs who chose it, divided their answers among the options of "Support diagnosis", "Predict and guide clinical decisions", and "Guide treatment options", with all of these options being selected 3 times each (75%). On the other hands, Specialized HCPs who chose it, divided their answers among the options of "Predict and guide clinical decisions" (n=5, 35.7%), "Support diagnosis" (n=3, 21.4%), and "Guide treatment options” (n=3, 21.4%).

When asked for early diagnosis, Primary HCPs showed a preference for "Support clinical decision making" with four of them (80%) choosing this, with "Improve reliability for detecting preclinical AD" and "Reduce costs" following, both being selected equally by two different Primary HCPs (40%). Specialized HCPs showed a preference for "Support clinical decision making" with eight of them (72.7%) choosing this, with "Improve reliability for detecting preclinical AD" and seven with "Support clinical decision making".

Lastly, both Primary and Specialised HCPs, who selected progression monitoring showed an equal preference for all provided options (i.e., "Diagnostic confirmation", "Assessing the effectiveness of treatment", "Personalized Medicine").

Patient's Care Journey Stage

Having a better view on the intended use, Primary and Specialised HCPs were asked to assess the stage in the patient's care journey that would be most appropriate to assess AD blood-derived biomarkers. Most of the answers of Primary HCPs were divided among "Early detection of 'at-risk' healthy individuals at primary healthcare", with 50% (n=4) choosing it over "Differential diagnosis and treatment selection at specialized care", with 25% (n=2) (Figure 34).

Accordingly, Most of the answers of Specialised HCPs were divided among "Early detection of 'at-risk' healthy individuals at primary healthcare", with 55.5% (n=10) choosing it over "Early detection of AD onset (i.e., Subject Cognitive Impairment (SCI) or Mild Cognitive Impairment (MCI)) at primary healthcare", with 27.78% (n=5) (Figure 35).

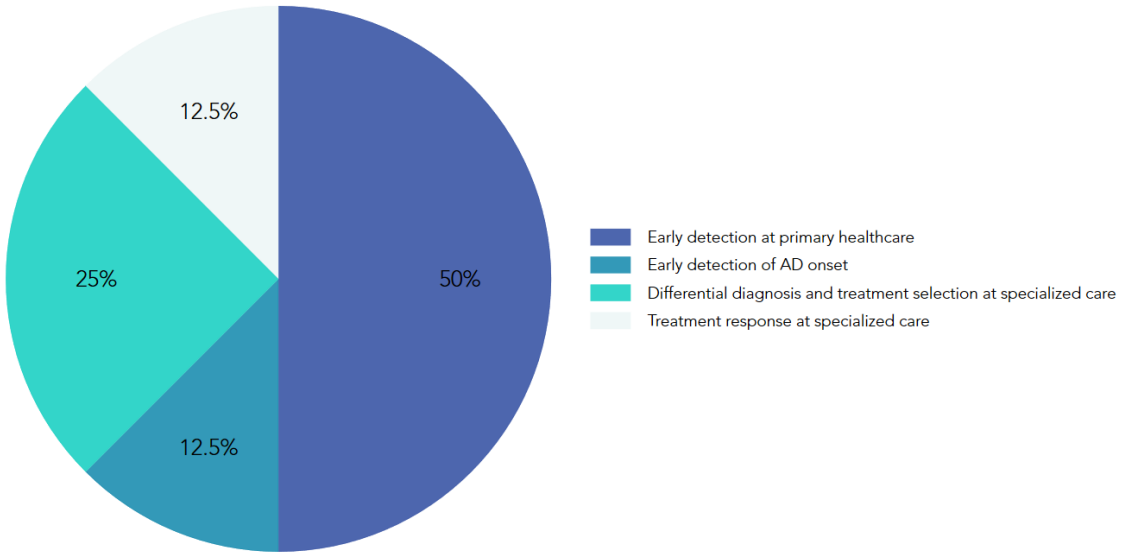

Figure 34: Envision of stage in the patient's care journey that would be most appropriate to assess AD blood-derived biomarkers among Primary HCPs

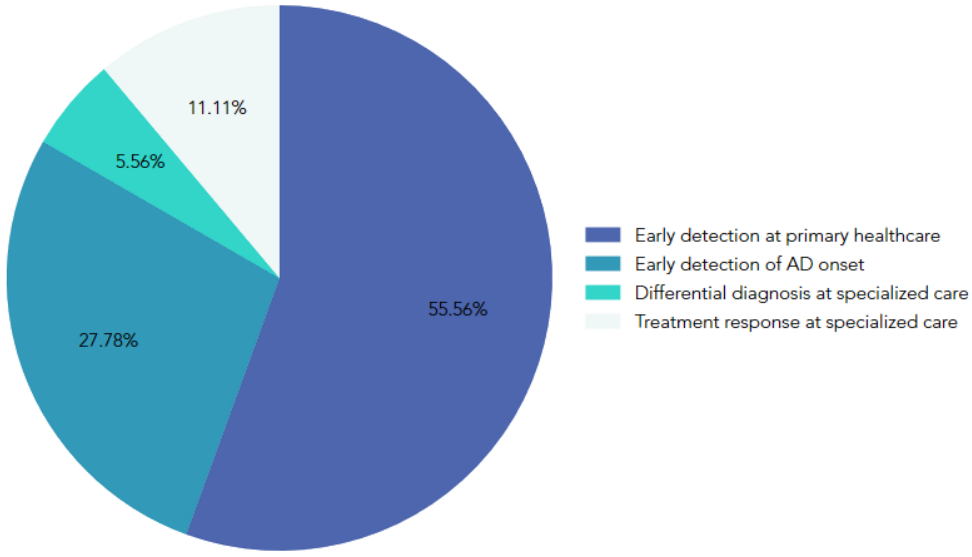

Figure 35: Envision of stage in the patient's care journey that would be most appropriate to assess AD blood-derived biomarkers among Specialized HCPs

8.5.3 Identification of AD blood-derived (plasma) biomarkers

The Online Survey asked whether Primary and Specialised HCPs were aware of any AD blood-derived (plasma) biomarkers in an attempt to confirm and/or evaluate their experience or involvement with this topic. Almost all of the Primary HCPs, 87.5% (n=7), stated that they were not aware of any AD blood-derived (plasma) biomarkers. The remainder Primary HCP rated both Beta amyloid and Tau Protein as "Very Important" for prognosis and "Important" for early diagnosis, however their profile does not warrant credibility to this response.

The case with Specialised HCPs, as anticipated, was different, with 11 Specialised HCPs (61%) (previously replied that they had some experience in AD biomarkers) asked to rate nine different AD blood-derived (plasma) biomarkers as per intended use in providing a prognosis, early diagnosis, and perform progression monitoring.

In terms of Amyloid Beta (Aβ) 1-40, out of the 11 HCPs, six HCPs answered that Amyloid Beta (Aβ) 1-40 would be "important" for providing an early diagnosis (54.5%) and five for providing a prognosis (45.4%), whereas only three HCPs (27.2%) rated Amyloid Beta (Aβ) 1-40 to be important for progression monitoring (Figure 36).

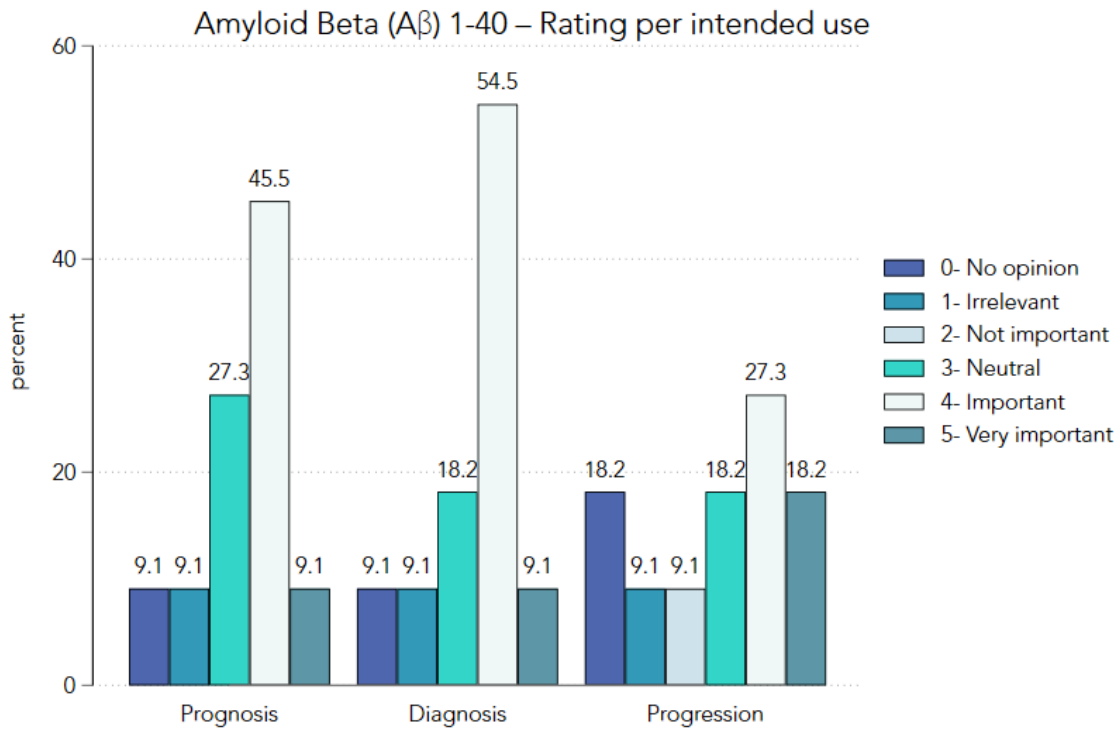

Figure 36: Rating per intended use of Amyloid Beta (Aβ) 1-40 among Specialized HCPs

Moreover, when asked regarding Amyloid Beta (Aβ) 1-42, similarly five HCPs rated this biomarker to be "important" for prognosis (45.4%), and six HCPs rated "important" in providing early diagnosis and for progression monitoring (54.5%) (Figure 37).

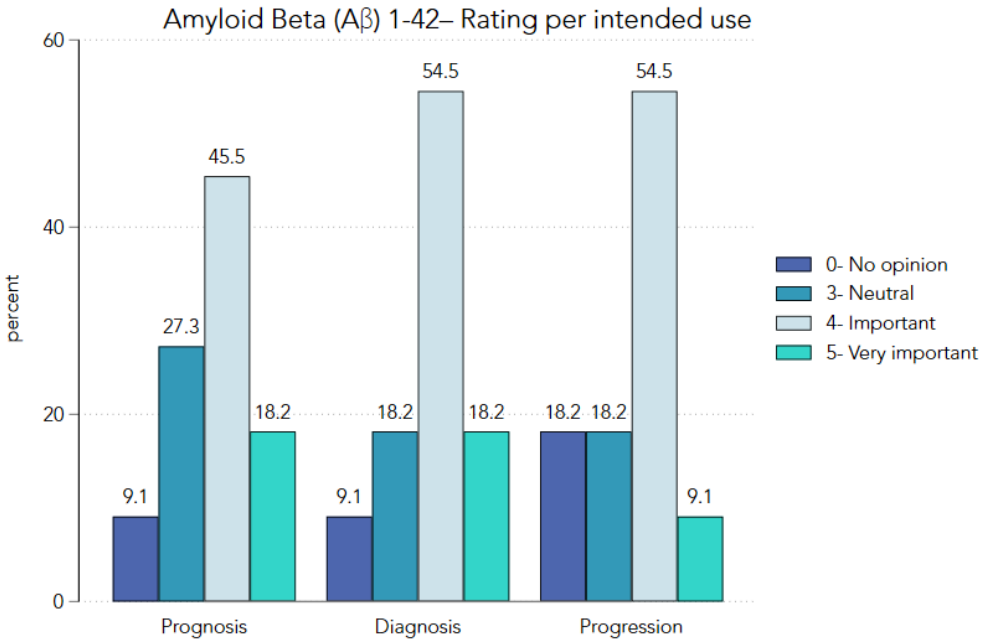

Figure 37: Rating per intended use of Amyloid Beta (Aβ) 1-42 among Specialized HCPs

In respect to Aβ42/Aβ40 ratio, the 11 HCP's answers were divided their answers among the options of "important"(n=4, 36.3%) and "very important" (n=4, 36.3%) for providing a prognosis, and similarly for five out of the 11 HCPs answered "important" (n=5, 45.4%) and "very important" (n=4, 36.3%) for providing an early diagnosis whereas seven HCPs noted "important" (63.6%) in progression monitoring (Figure 38).

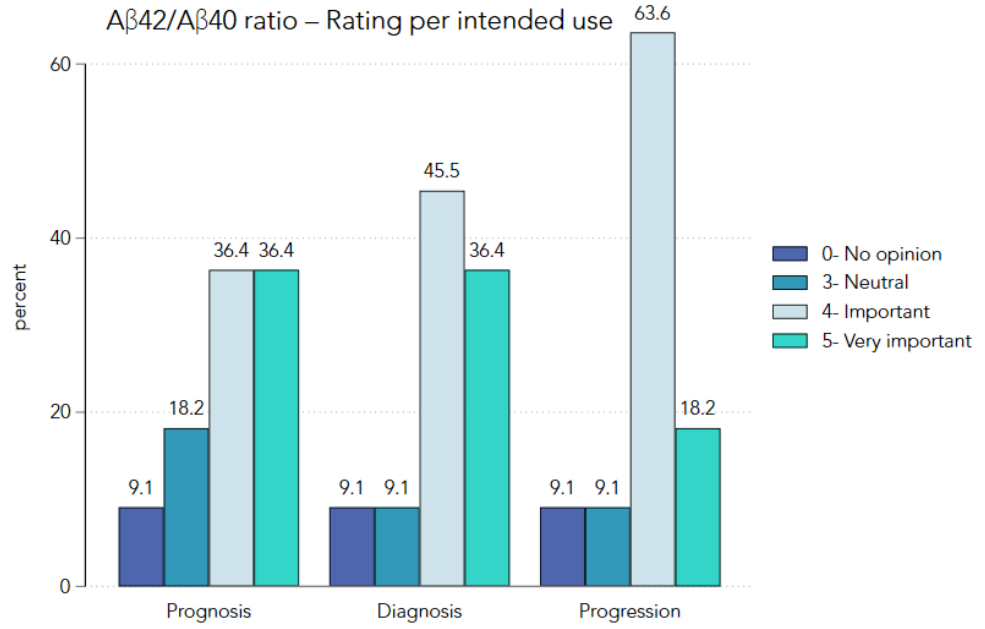

Figure 38: Rating per intended use of Aβ42/Aβ40 ratio among Specialized HCPs

Regarding Tau Protein 181, out of the 11 HCPs, six HCPs answered that Tau Protein 181 would be "important" for providing both a prognosis and an early diagnosis (54.5%), whereas seven of the HCPs (63.6%) noted the perspective in using Tau Protein 181 for assessing progression monitoring (Figure 39).

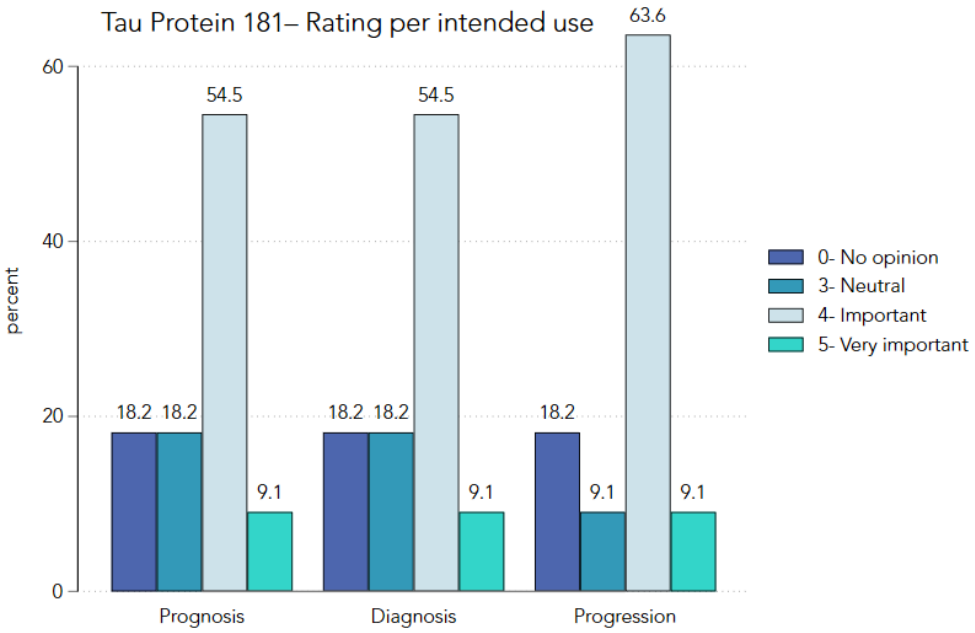

Figure 39: Rating per intended use of Tau Protein 181 among Specialized HCPs

Moreover, when asked regarding Tau Protein 217, seven Specialized HCPs rated this biomarker to be "important" for prognosis and early diagnosis (63.6%), and six HCPs rated "important" for progression monitoring (54.5%) (Figure 40).

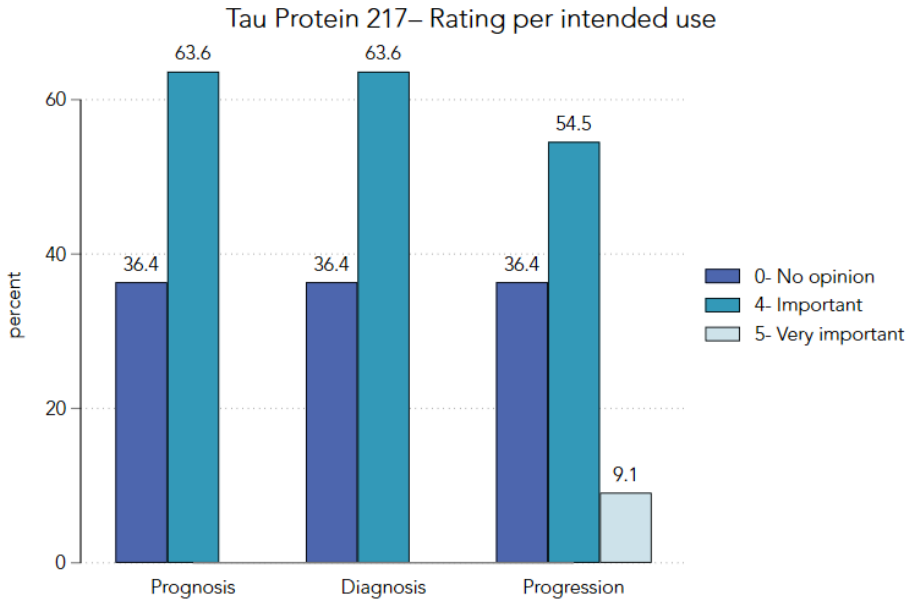

Figure 40: Rating per intended use of Tau Protein 217 among Specialized HCPs

Lastly, Specialized HCPs were asked to rate Tau Protein 231. For rate Tau Protein 231, most HCPs divided their answers among the options of "no opinion"(n=5, 45.45%) and "important"(n=4, 36.3%) for providing a prognosis and an early diagnosis. Similarly, five HCPs out of the 11 HCPs answered, "no opinion" (n=5, 45.4%) in terms of use of Tau Protein 231 in progression monitoring (Figure 41).

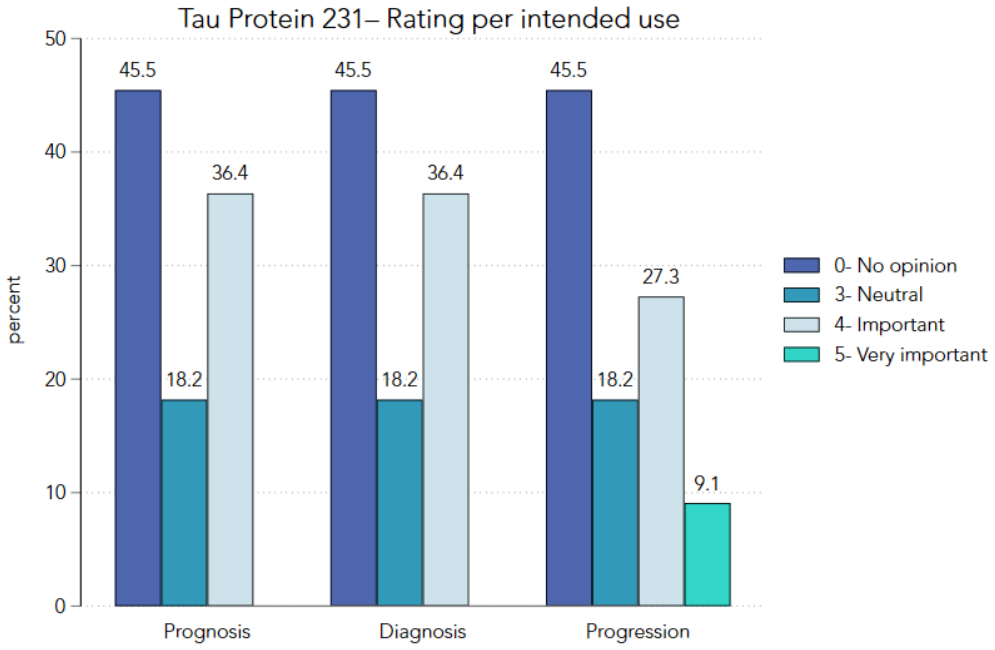

Figure 41: Rating per intended use of Tau Protein 231 among Specialized HCPs

Finally, Specialized HCPs were asked to rate Neurofilament Light chain, Glial Fibrillary Acidic Protein, and TDP-43. In respect to these three biomarkers, the majority of the 11 Specialized HCPs answered "no opinion"(n=5-7; 45.45%-63.64%) for using the biomarkers for providing a prognosis, an early diagnosis and assessing progression monitoring. However, when asked about the intended use of Neurofilament Light chain, five HCPs (45.45%) indicated use for progression monitoring.

The 11 Specialised HCP (61%) who replied that they had some experience in AD biomarkers and who rated the intended use of nine different AD blood-derived (plasma) biomarkers were additionally asked if they were aware of cut-offs and/or concentration of the nine AD blood-derived (plasma) biomarkers. Ten out of the 11 HCPs (90.91%) answered that they were not aware of cut-offs and/or concentration of the nine AD blood-derived (plasma) biomarkers and the only HCP who answered 'yes' did not provide any cut-off values when asked to specify this.

In addition, the 11 Specialized HCP with some experience in AD biomarkers answered that they were not aware of other AD blood-derived biomarkers than the biomarkers presented in the Online Survey. Similarly, the seven Specialized HCP who reported having no experience in AD biomarkers responded that they were not aware about any other AD blood-derived biomarkers.

8.5.4 Clinical Testing Performance

In an attempt to evaluate which factors are important for the decision-making process regarding AD, Primary HCPs selected: "Robustness/Reliability" (n=5/8; 62.5%), "Availability" (n=4/8; 50%), "Minimal or Non-Invasiveness" (n=4/8; 50%), "Pricing/Cost" (n=3/8; 37.5%), "Credibility" (n=3/8; 37.5%), and "Complexity/User-friendliness" (n=3/8; 37.5%) as the most chosen options. With a slightly different focus, Specialised HCPs selected: "Pricing/Cost" (n=11/18; 61.11%), "Availability" (n=11/18; 61.11%), “Credibility” (n=9/18; 50%), "Complementarity with other tests" (n=9/18; 50%), and “Minimal or Non-Invasiveness” (n=8/18; 44.44%) as the most chosen options (Figure 42).

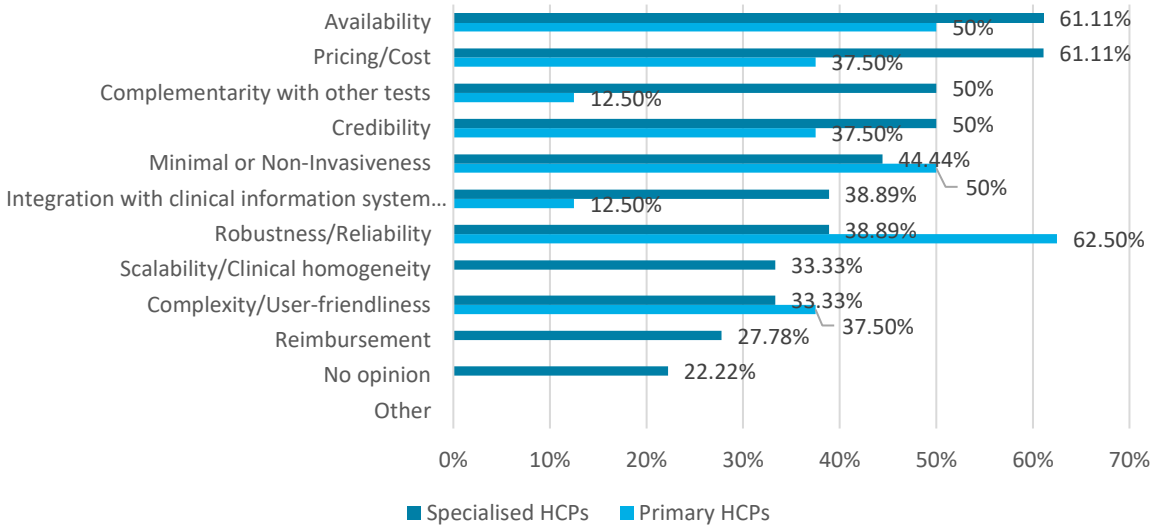

Figure 42: Factors considered important for AD decision making among Primary and Specialised HCPs

8.5.5 Clinical Assessment

In terms of clinical assessment, Primary and Specialised HCPs were asked to consider receiving AD blood-derived (plasma) biomarker results directly on a mobile phone or tablet in real-time, and to specify what kind of information would assist them in their decision-making process based on that.

Among the HCPs surveyed, 75% of Primary HCPs and 65.22% of Specialised HCPs stated "Diagnostic value" and another 50% of Primary HCPs and 34.78% of Specialised HCPs indicated "Biomarker Metrics" as the most desired information that would assist them in this hypothetical scenario for their decision-making process.

8.5.6 Challenges

Cost and Time

To gain an understanding of the overall cost challenge for AD diagnosis, Primary and Specialised HCPs were also asked about the average cost needed to accurately diagnose the patient’s health status in terms of AD. This inquiry aimed to gather insights into the financial implications associated with AD diagnosis from the perspective of Primary and Specialised HCPs.

Among the Primary HCP respondents, 75% (n=6/8) replied that they were not aware of the average cost needed for accurately diagnosing the patient's health status in terms of AD, indicating a lack of information or estimates on this aspect. Additionally, 25% (n=2/8) of Primary HCPs replied that the average cost is around ≤500€, suggesting some awareness of the potential costs associated with AD diagnosis among a portion of the respondents.

Among the Specialised HCP respondents, 72.22% (n=13/18) replied that they were not aware of the average cost needed for accurately diagnosing the patient's health status in terms of AD, indicating a lack of information or estimates on this aspect. On the other hand, two Specialised HCPs (11.11%) replied that the average cost is ≤500€, another two (11.11%) around >500-1000€, whereas one Specialised HCP (5.56%) responded that the average cost exceeds >2000€.

As Specialised HCPs are more aware of AD biomarkers, they were also asked about the average cost and time needed for getting AD fluid-derived results for assisting accurately diagnosis in terms of AD. This inquiry aimed to gather insights into the additional resources' implications associated with AD diagnosis from the perspective of Specialized HCPs.

Among the Specialized HCPs respondents, who had experience in AD Biomarkers, 81.82% (n=9/11) replied that they were not aware of the average cost needed for getting AD fluid-derived results to accurately diagnose the patient in terms of AD, indicating a lack of information or estimates on this aspect. Out of the two Specialised HCPs that were aware of the costs, one (9.09%) replied that the average cost is ≤300€, whereas another one (9.09%) stated that the average cost is >300-500 €.

In terms of time, the majority of Specialized HCPs (n=7/11; 63.64%) answered that they were not aware of the time needed getting AD fluid-derived biomarker results. The remaining four answers stated ≤ 1 week (n=1, 9.09%) and ≥2 weeks (n=3, 27.27%).

PoC IVD Deployment

Furthermore, Primary HCPs were asked to provide their familiarity with the main challenges and/or barriers to deploying a PoC IVD for detecting AD blood-derived (plasma) biomarkers in primary healthcare settings. This inquiry aimed to gather insights into the perceived obstacles or difficulties that Primary HCPs anticipate in implementing such technology in primary healthcare settings.

Among the Primary HCP respondents, 62.5% (n=5/8) identified "Not covered by existing insurance programs – costs will be covered by the patient (Reimbursement/Health Insurance coverage)" as the most common challenge to deploying a PoC IVD for detecting AD blood-derived (plasma) biomarkers in primary healthcare settings. Following this, "High Testing Cost (Pricing/Cost)" was identified as the most common challenge by 50% (n=4/8) of the respondents. Likewise, " Lack of knowledge/expertise from HCPs" was also chosen by 50% (n=4/8) of the Primary HCP respondents as a significant challenge.

Among the Specialised HCP respondents, 55.56% (n=10/18) identified "High Testing Cost" as the most common challenge to deploying a PoC IVD for detecting AD blood-derived (plasma) biomarkers in primary healthcare settings. Following this, "Lack of knowledge/expertise from HCPs" was identified as the most common challenge by 44.44% (n=8/18) of the Specialised HCP respondents. Likewise, "Lack of awareness from patients" was also chosen by 44.44% (n=8/18) of the Specialised HCP respondents as a significant challenge.

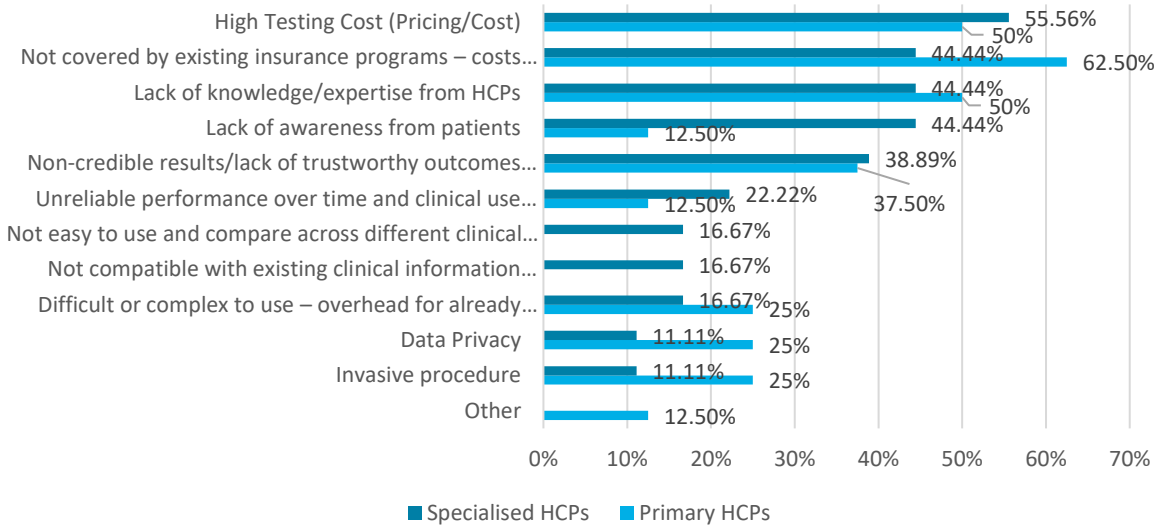

Figure 43: Main challenges and/or barriers to deploying a PoC IVD for detecting AD blood-derived (plasma) biomarkers in primary healthcare settings among Primary and Specialised HCPs

8.5.7 Other projects, solutions, data or information

To identify any other relevant projects, HCPs were asked if they were aware of any PoC IVD solutions for AD. All Primary (n=8, 100%) and Specialised HCPs (n=18, 100%) replied that they were not aware of any such solutions.

Lastly, HCPs were also asked whether they were aware of any public datasets or databases that are relevant to AD fluid-derived (plasma) biomarker research. All Primary (n=8, 100%) and most Specialised HCPs (n=17, 94.4%) stated that they were not aware of any such datasets or databases. The one Specialised HCP respondent specified “ADNI, Biofinder I + II, DCN biobank, and individual databases of memory clinics across Europe”.

8.6 Biomarker Experts

8.6.1 Level of Experience

Out of the 12 Biomarker Experts enrolled in the Online Survey, six indicated that they are “Competent”, while two stated that they were “Proficient” and “Expert” in this area reflecting high experience in AD Biomarkers in this group of survey participants. In addition, Biomarker Experts indicated somewhat different experiences in PoC IVDs varying from being a “Novice” (n=4/12; 33.33%) and having “None” (n=1/12; 8.33%) to highest level of experience being an “Expert” (n=3/12; 25%).

8.6.2 Clinical Testing Procedure and Intended Use of AD Biomarkers

Clinical or research protocols

The majority of Biomarker Experts, comprising 75%, indicated that they were not aware of any clinical or research protocols currently utilized for collecting and analysing AD fluid-derived biomarkers,

whereas three Biomarker Expert responded that they were aware of protocols, which are presented below:

Table 12: AD fluid-derived biomarker Protocols mentioned by Biomarker Experts

AD fluid-derived biomarker Protocols
Clinical trials with beta amyloid and PET
Beta Amyloid protein quantification in CSF
ELISA

For these protocols, Biomarker Experts were requested to also identify relevant limitations, introducing challenges related to (i) invasive sample collection; (ii) complexity in biomarker analysis, as specificity, selectivity, reproducibility (variation between patients) are quite challenging due to low concentrations of biomarkers; (iii) sample transfer, due to the time and cost associated to it; and (iv) sample storage, because of challenges with proper storage, contaminations, and time.

Intended Use

In addition, Biomarker Experts were asked about the intended use they envisioned for AD blood-derived (plasma) biomarker results, with eight of them (34.78%) indicating that both prognosis and early diagnosis would be of utmost importance. Furthermore, five (21.74%) Biomarker Experts highlighted progression monitoring as additional pertinent use for these biomarker results (Figure 44).

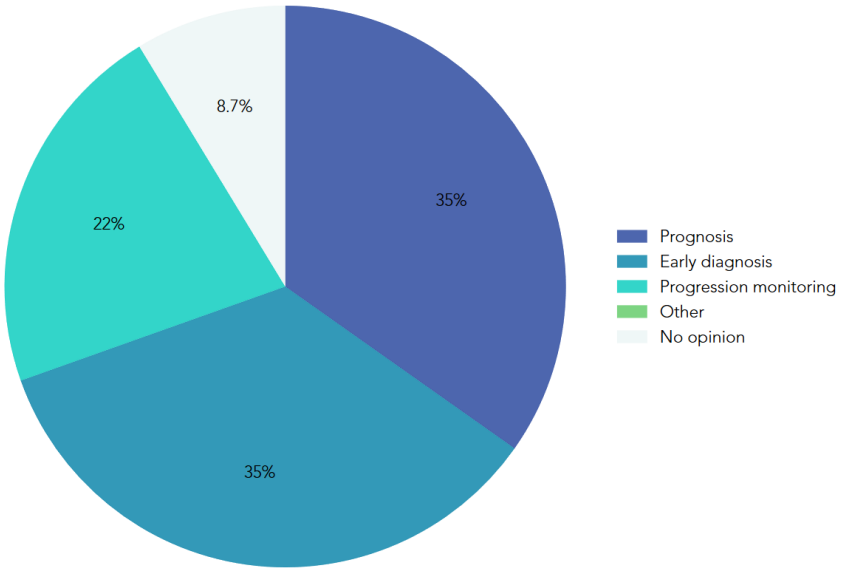

Figure 44: Intended use envisioned for AD blood-derived biomarkers among Specialized HCPs

To gain insights into how Biomarker Experts' envision the support of AD blood-derived (plasma) biomarkers in prognosis, early diagnosis, and progression monitoring, they were asked separate questions for each of these aspects based on what they chose to envision as important. In terms of prognosis, six Biomarker Experts who chose it, divided their answers among the options of "Support diagnosis", "Predict and guide clinical decisions", "Guide treatment options", and "Guide policy

making for health programs". When asked for early diagnosis, Biomarker Experts showed a preference for "Improve reliability for detecting preclinical AD" and "Support clinical decision making" with six of them (62.5%) choosing this, and five with "Help with risk assessment", "Optimize the benefits of available and emerging treatments", and "Reduce patient discomfort ". Lastly, Biomarker Experts who selected progression monitoring showed an almost equal preference for all provided options (i.e., "Diagnostic confirmation", "Assessing the effectiveness of treatment", "Personalized Medicine").

Patient's Care Journey Stage

Last but not least, to assess the envision of Biomarker Experts in terms of the stage in the patient's care journey that would be most appropriate to assess AD blood-derived biomarkers, they were asked this question accordingly. The majority of Biomarker Experts (75%, n=9/12) opted for "Early detection of AD onset," while only 16.67% (n=2/12) selected "Differential diagnosis and treatment selection at specialized care," and 8.33% (n=1/12) chose "Early detection of "at-risk" healthy individuals at primary healthcare" (Figure 45).

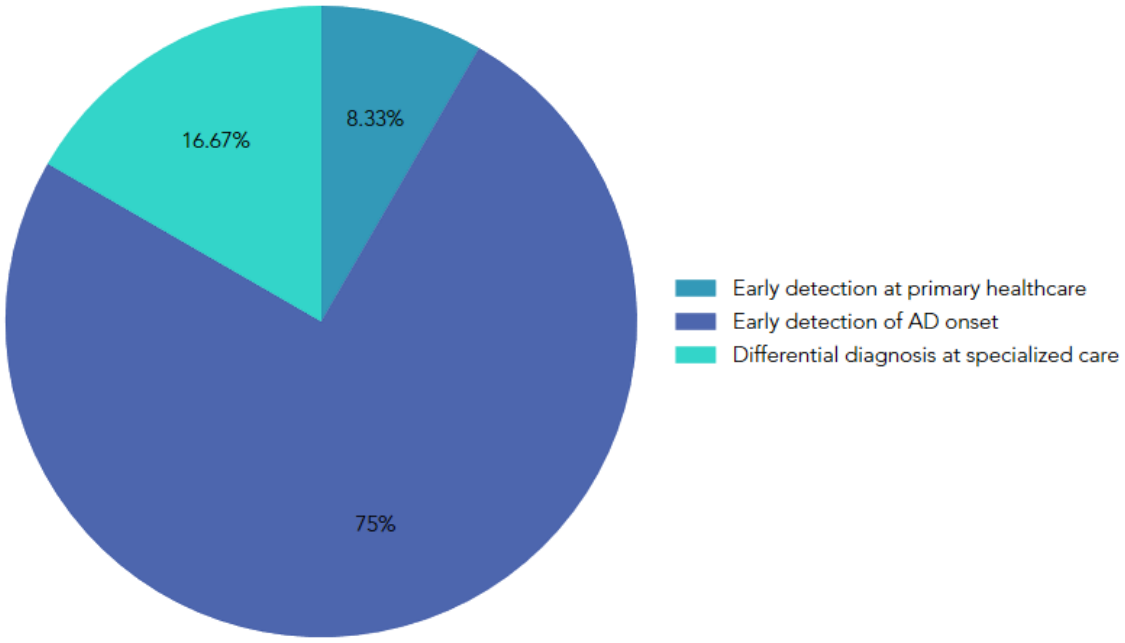

Figure 45: Envision of stage in the patient's care journey that would be most appropriate to assess AD blood-derived biomarkers among Biomarker Experts

8.6.3 Identification of AD blood-derived (plasma) biomarkers

Among the 12 Biomarker Experts surveyed, 91.67% (n=11/12) reported having some experience in AD biomarkers. These 11 experts were then asked to rate nine different AD blood-derived (plasma) biomarkers based on their intended use in providing prognosis, early diagnosis, and facilitating progression monitoring.

Regarding Amyloid Beta (Aβ) 1-40, out of the 11 Biomarker Experts surveyed: 45.45% (n=5/11) rated it as "Important" for providing a prognosis, 27.27% (n=3/111) rated it as "Very important" for early diagnosis, and 45.45% (n=5/11) rated it as "Important" for progression monitoring (Figure 46).

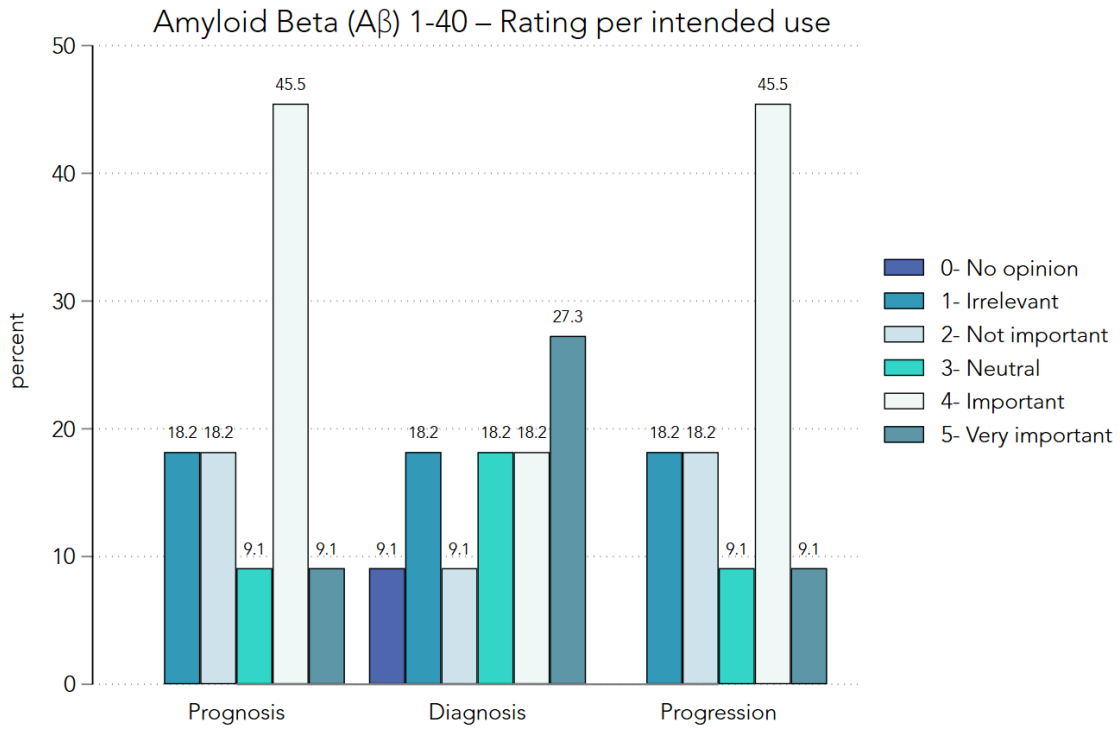

Figure 46: Rating per intended use of Amyloid Beta (Aβ) 1-40 among Biomarker Experts

Furthermore, when queried about Amyloid Beta (Aβ) 1-42: 81.82% (n=9/11) of Biomarker Experts deemed it "important" for prognosis, 63.64% (n=7/11) considered it "important" for early diagnosis, 54.55% (n=6/11) rated it as "important" for progression monitoring (Figure 47).

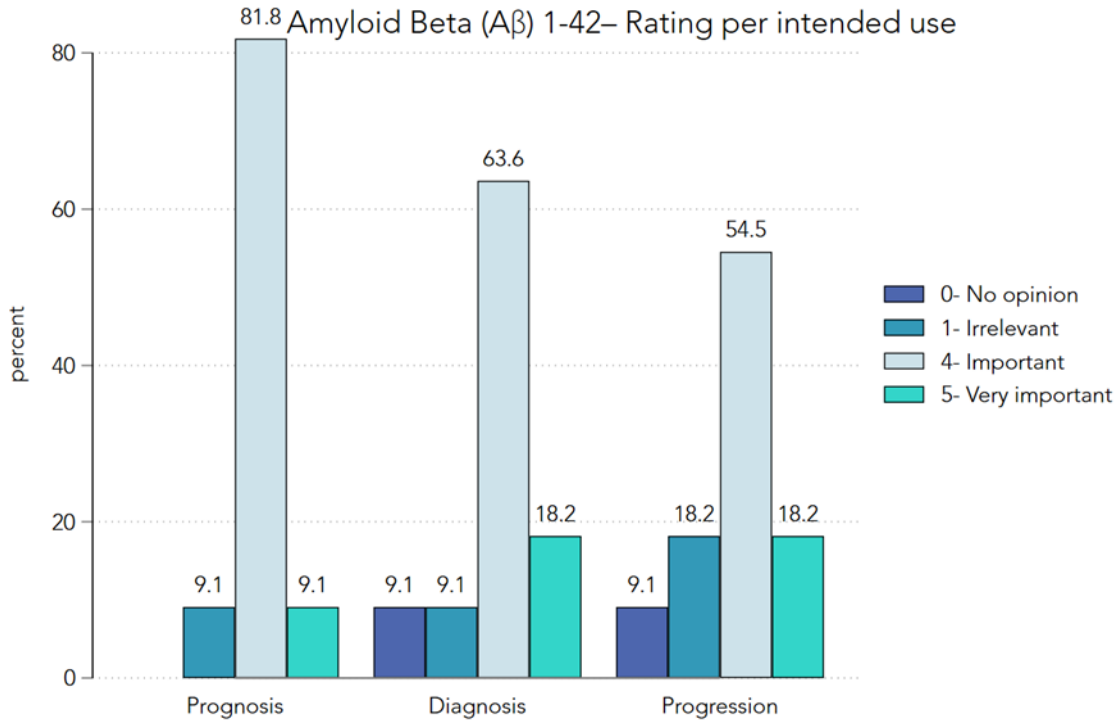

Figure 47: Rating per intended use of Amyloid Beta (Aβ) 1-42 among Biomarker Experts

Regarding the Aβ42/Aβ40 ratio: 72.73% (n=8/11) of Biomarker Experts rated it as "Important" for prognosis, 63.64% (n=7/11) rated it as "Important" for early diagnosis. Similarly, 54.55% (n=6/11) of Biomarker Experts rated it as "Important" for progression monitoring (Figure 48).

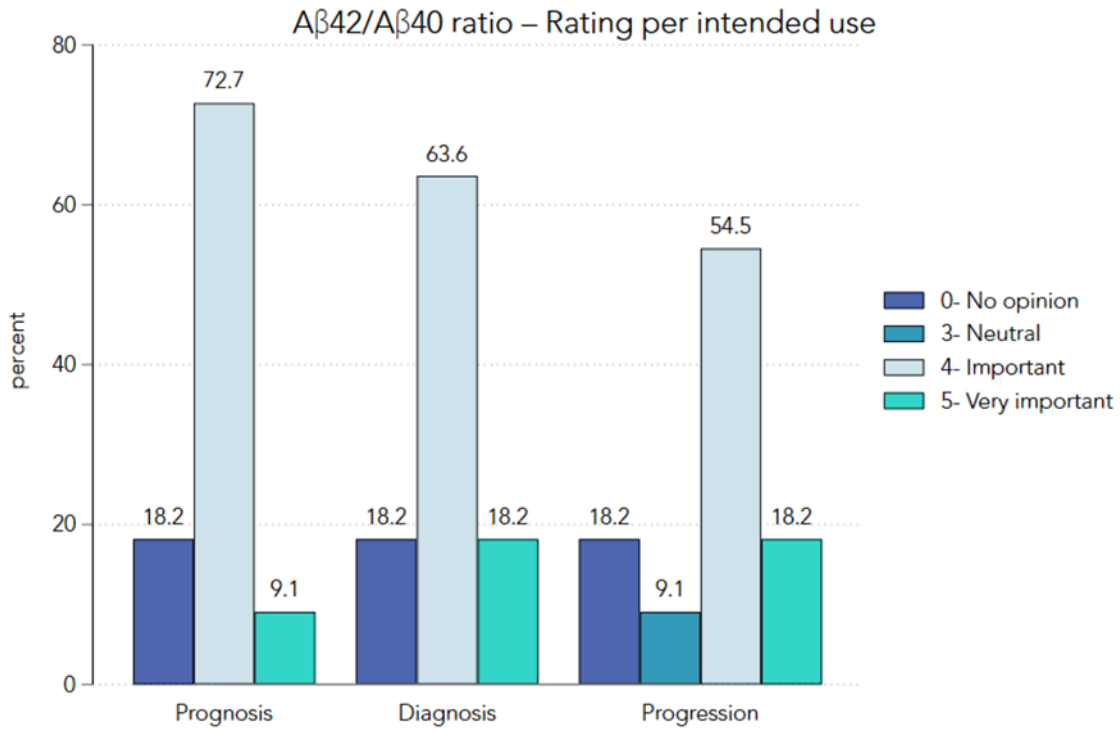

Figure 48: Rating per intended use of Aβ42/Aβ40 ratio among Biomarker Experts

Regarding Tau Protein 181, out of the 11 Biomarker Experts surveyed 54.55% (n=6/11) considered it "Important" for providing a prognosis, 72.73% (n=8/11) noted its importance for early diagnosis, 63.64% (n=7/11) of Biomarker Experts opted for its relevance in progression monitoring (Figure 49).

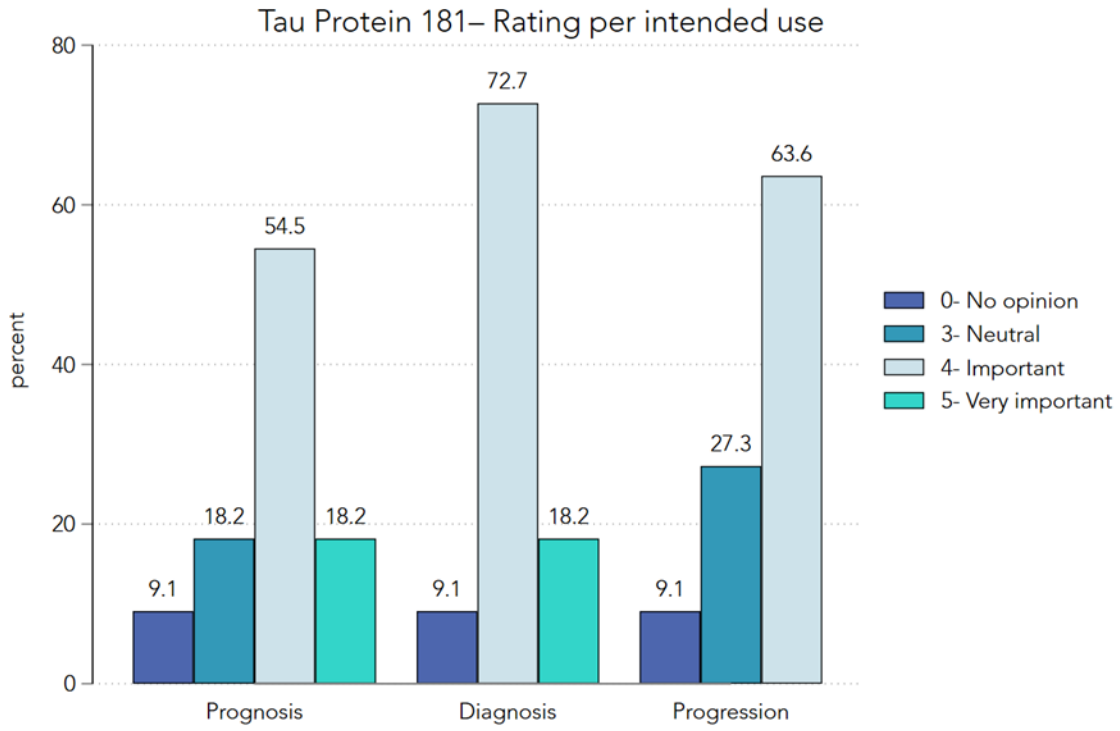

Figure 49: Rating per intended use of Tau Protein 181 among Biomarker Experts

Moreover, when queried about Tau Protein 217: 54.55% (n=6/11) of Biomarker Experts rated it as "Important" for prognosis, 63.64% (n=7/11) rated it as "Important" for early diagnosis, 81.82% (n=9/11) of Biomarker Experts considered it "Important" for progression monitoring (Figure 50).

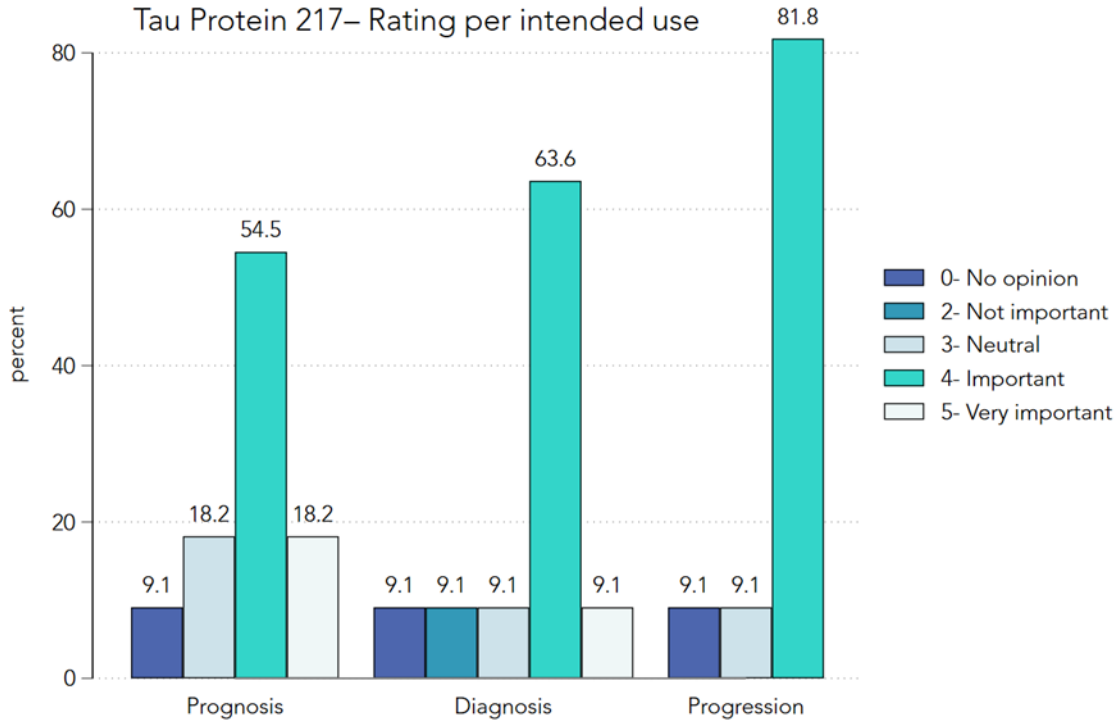

Figure 50: Rating per intended use of Tau Protein 217 among Biomarker Experts

Lastly, Biomarker Experts were asked to rate Tau Protein 231. Out of the 11 Biomarker Experts surveyed 54.55% (n=6/11) rated it as "Important" for prognosis, 63.64% (n=7/11) rated it as "Important" for providing early diagnosis, 72.73% (n=8/11) considered it "Important" for progression monitoring accordingly (Figure 51).

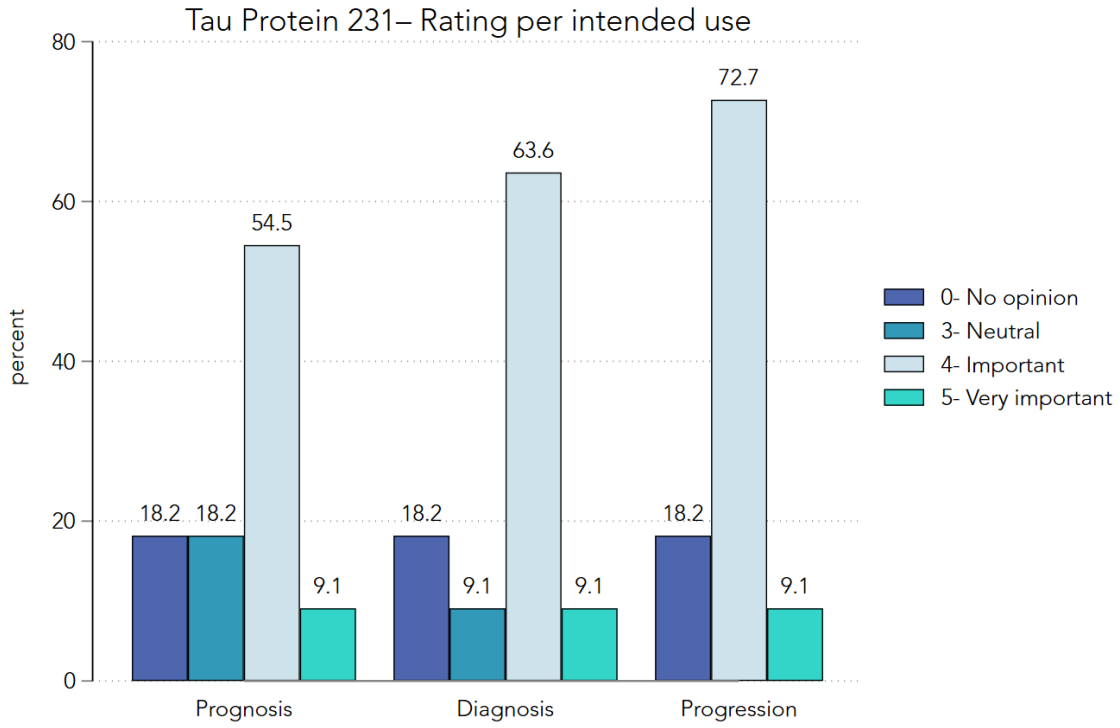

Figure 51: Rating per intended use of Tau Protein 231 among Biomarker Experts

In respect to Neurofilament Light chain, 45.45% (n=5/11) of Biomarker Experts rated it as “Important” for prognosis, while 36.36% (n=4/11) rated it as “Important” for both early diagnosis and progression monitoring. Additionally, 27.27% (n=3/11) of Biomarker Experts expressed “No opinion” for all three

different AD clinical stages for this biomarker.

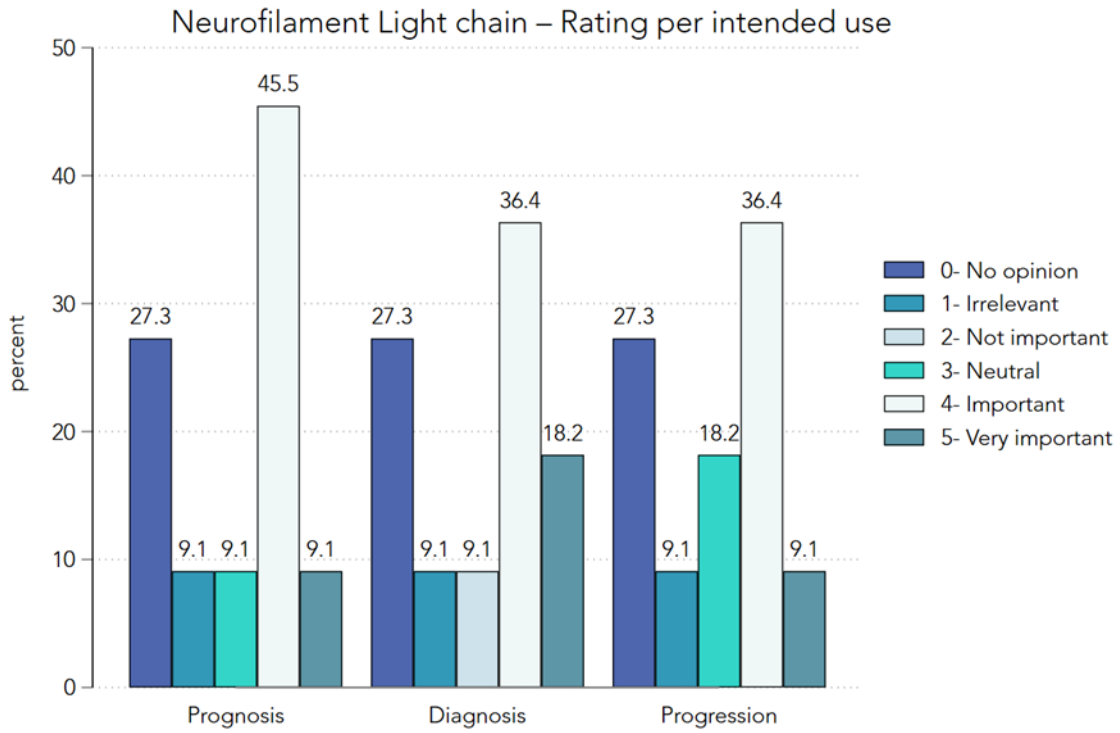

Figure 52: Rating per intended use of Neurofilament Light chain among Biomarker Experts

Moreover, 36.36% (n=4/11) of Biomarker Experts rated Glial Fibrillary Acidic Protein as “Important” for prognosis, while 45.45% (n=5/11) and 27.27% (n=3/11) considered it “Important” for early diagnosis and progression monitoring, respectively. Additionally, 36.36% (n=4/11) of Biomarker Experts expressed “No opinion” when asked to rate Glial Fibrillary Acidic Protein.

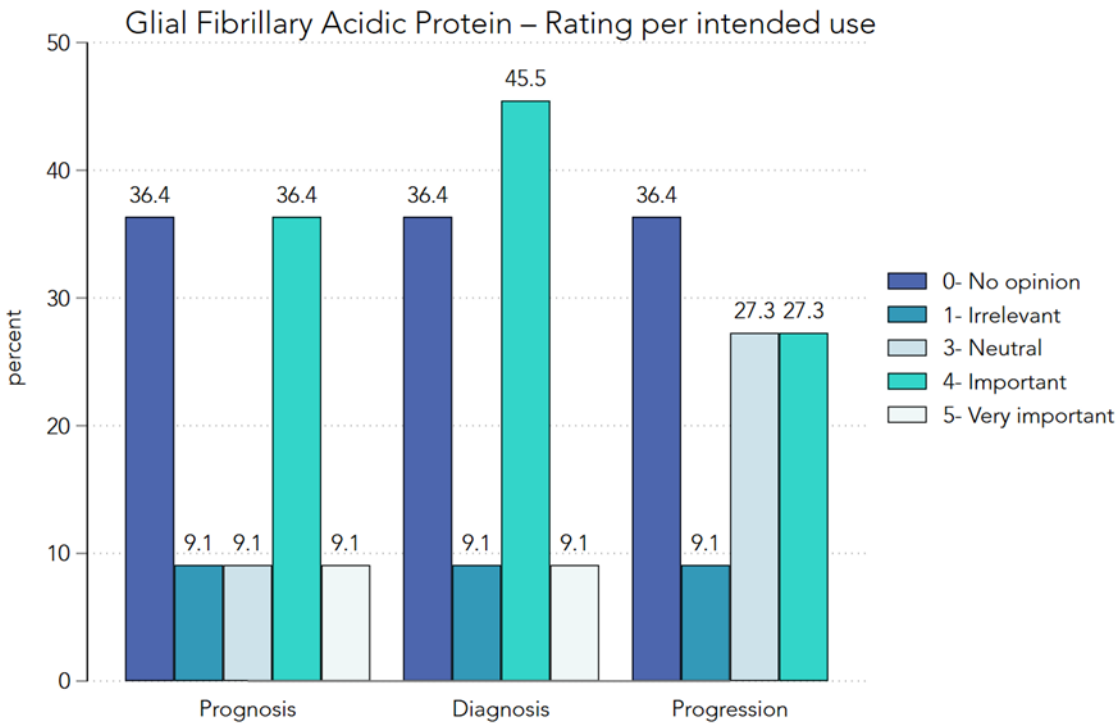

Figure 53: Rating per intended use of Glial Fibrillary Acidic Protein among Biomarker Experts

When it comes to the rating of the TDP-43 biomarker 27.27% (n=3/11) of Biomarker Experts were equally divided between "Neutral" and "Important" for prognosis, 54.55% (n=6/11) rated it as "Important" for early diagnosis, 27.27% (n=3/11) rated it as "Important" for progression monitoring. Additionally, 36.36% (n=4/11) of Biomarker Experts did not express an opinion regarding rating this biomarker for all related stages.

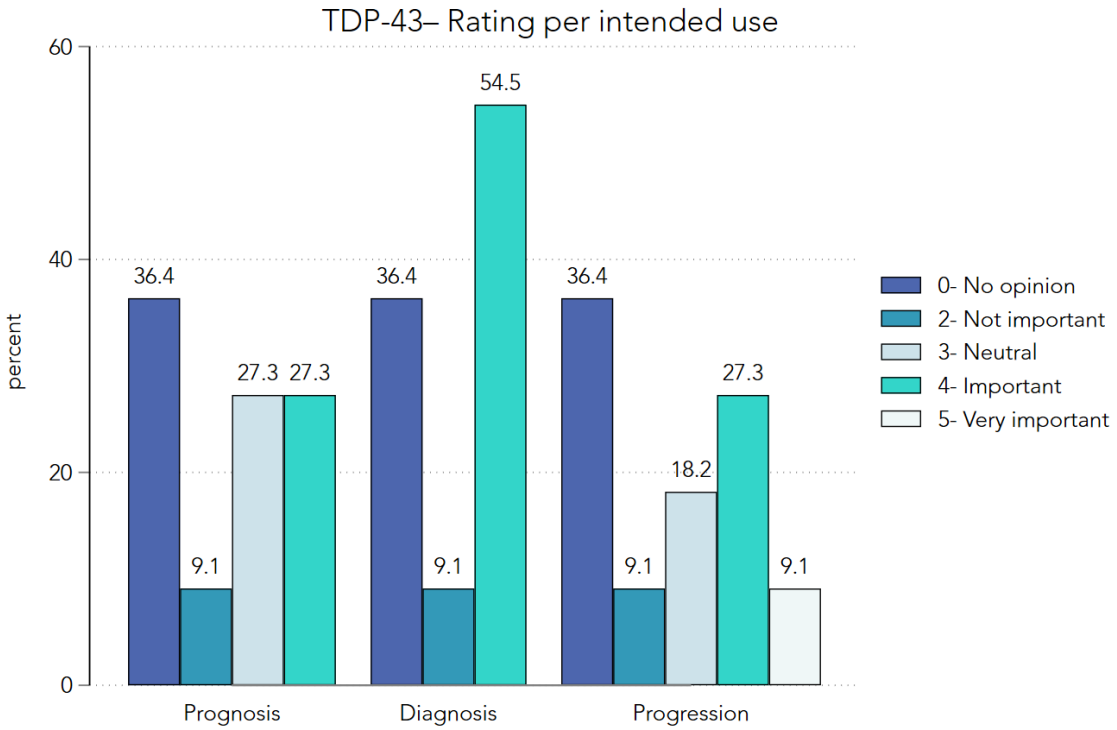

Figure 54: Rating per intended use of TDP-43 among Biomarker Experts

When it comes to awareness of cutoffs, none of the Biomarker experts were knowledgeable about any cutoffs for the aforementioned biomarkers.

In addition, 10 out of 11 Biomarker Experts with some experience in AD biomarkers answered that they were not aware of other AD blood-derived biomarkers beyond the ones presented in the Online Survey. The one Biomarker Expert who was aware of additional AD blood-derived biomarkers shared that "exosome size and count," "alpha synuclein," "syntenin-1," "Alix," and "TSG101" were either "Important" or "Very important" for prognosis, early diagnosis, and/or progression monitoring.

8.6.4 Challenges

Cost and Time

To gain an understanding of the overall cost challenge for AD diagnosis, Biomarker Experts were also asked about the average cost required to obtain AD fluid-derived results for assisting accurate diagnosis in terms of AD. This inquiry aimed to gather insights into the financial implications associated with AD diagnosis from the perspective of Biomarker Experts.

Among the respondents, i.e., Biomarker Experts with experience in AD Biomarkers, 91.67% (n=11/12) of them mostly replied with either ≤300€ (n=4/11; 36.36%), or >300-500 € (n=3/11; 27.27%). Similarly, 27.27% (n=3/11) of respondents replied that they were not aware of the average cost needed for obtaining AD fluid-derived results to accurately diagnose the patient in terms of AD, indicating a lack of information or estimates on this aspect.

Furthermore, Biomarker Experts were asked to provide the average time needed for obtaining AD fluid-derived biomarker results to assist in diagnosis. In this regard, the replies of Biomarker Experts

were divided among "No opinion" (n=4/11; 36.36%), "≥2 weeks" (n=4/11; 36.36%), and "≤1 week" (n=3/11; 27.27%) in terms of the time needed to obtain AD fluid-derived biomarker results.

To gain an understanding of the overall cost challenge for AD diagnosis, all Biomarker Experts were also asked about the average cost needed to accurately diagnose the patient's health status in terms of AD. Among the respondents, 33.33% (n=4/12) replied that they were not aware of the average cost needed for accurately diagnosing the patient's health status in terms of AD, indicating a lack of information or estimates on this aspect for some of them. Meanwhile, "≤500 €" and ">500-1000 €" were equally chosen by Biomarker Experts as the average cost (n=3/11; 25%). Lastly, 16.67% (n=2/12) of Biomarker Experts indicated ">1000-2000 €" as the average cost.

PoC IVD Deployment

Furthermore, Biomarker Experts were asked to provide their familiarity with the main challenges and/or barriers to deploying a PoC IVD for detecting AD blood-derived (plasma) biomarkers in primary healthcare settings. This inquiry aimed to gather insights into the perceived obstacles or difficulties that Biomarker Experts anticipate in implementing such technology in primary healthcare settings.

Among the respondents, 50% (n=6/12) identified "Lack of knowledge/expertise from HCPs", "High Testing Cost (Pricing/Cost)", and "Unreliable performance over time and clinical use (Robustness/Reliability)" as the most common challenges to deploying a PoC IVD for detecting AD blood-derived (plasma) biomarkers in primary healthcare settings. Following this, "Lack of awareness from patients", "Not covered by existing insurance programs – costs will be covered by the patient (Reimbursement/Health Insurance coverage)", and "Not easy to use and compare across different clinical settings (Scalability/ Clinical homogeneity)" were identified as the most common challenges by 41.67% (n=5/12) of the respondents. Likewise, "Non-credible results/lack of trustworthy outcomes (Credibility/ Trustworthiness)", "Difficult or complex to use – overhead for already overburdened personnel (Complexity/User-friendliness)", and "Not compatible with existing clinical information systems (interoperability)" were also chosen by 33.33% (n=4/12) of the respondents as significant challenges (Figure 55).

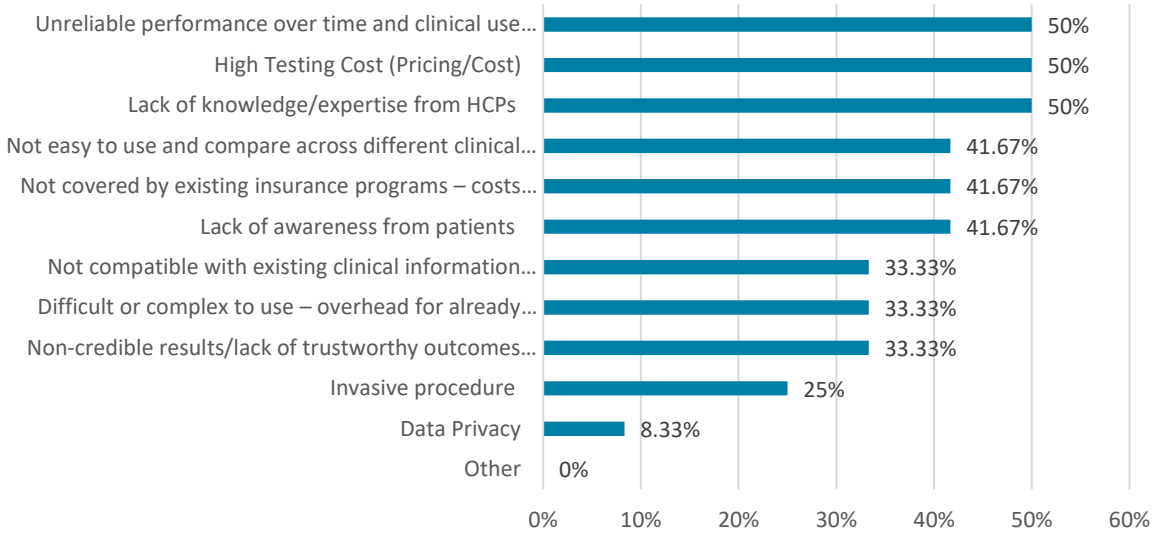

Figure 55: Main challenges and/or barriers to deploying a PoC IVD for detecting AD blood-derived (plasma) biomarkers in primary healthcare settings among Biomarker Experts

8.6.5 Other projects, solutions, data or information

To identify any other relevant projects, Biomarker Experts were asked if they were aware of any PoC IVD solutions for AD. All of them (n=12; 100%) replied that they were not aware of any such solutions.

Lastly, Biomarker Experts were also asked whether they were aware of any public datasets or databases that are relevant to AD fluid-derived (plasma) biomarker research. 100% (n=12/12) stated that they were not aware of any such datasets or databases.

8.7 Respondent Groups Comparative Analysis

To gain deeper insights and understanding of the responses provided for specific sets of questions related to research, clinical aspects of AD, PoC IVDs, as well as socioeconomic preferences and challenges, a direct result comparison was conducted among respondents who were presented with the same questions. This approach allowed for a direct assessment of any potential differences, preferences, or patterns across the responses, that will potentially enable 2D-BioPAD researchers to identify key trends, challenges, and areas of interest within the dataset.

By conducting this comparative analysis, 2D-BioPAD researchers can indeed pinpoint areas for further investigation and develop targeted interventions or strategies to address the identified needs and preferences more effectively. However, it's crucial to keep in mind the limitations of the Online Survey discussed in Section 8.1 and Section 8.2. These limitations include factors such as the small sample size and potential biases due to the sample composition. Therefore, while the comparative analysis can provide valuable insights, 2D-BioPAD researchers should interpret the findings with caution and consider these limitations when designing their future 2D-BioPAD studies or interventions.

8.7.1 Clinical Testing Procedure and Intended Use of AD Biomarkers

Depending on the context of specific questions tailored to the targeted participant groups, which included Primary and Specialized HCPs, Decision Makers, and Biomarker Experts, inquiries were made concerning the clinical testing procedures for AD and the intended use of AD biomarkers. The total number of enrolled respondents for the Online Survey questionnaire category concerning the clinical testing procedure was 51. Depending on the context, questions were asked or omitted accordingly for these participants.

The questions concerning clinical protocols, their constraints, and the anticipated intended use for AD blood-derived biomarkers were presented to all 51 respondents enlisted in this category, determined by their participant profiles. However, it's important to highlight that the questions addressing how AD blood-derived (plasma) biomarkers could aid HCPs in prognosis, early diagnosis, and progression monitoring of AD were not posed to Decision Makers within this category. Given the limited sample size and the exclusion of certain groups from these specific questions within this category, no comparative analysis was undertaken for this set of questions.

Out of the 51 respondents surveyed, 43 were not aware of any clinical protocols for AD fluid-derived biomarkers, leaving only 8 respondents who were aware. Among these 8 respondents, Biomarker Experts demonstrated the highest level of awareness compared to all participants, followed by Decision Makers. Additionally, Specialized HCPs exhibited greater awareness of such clinical protocols compared to Primary HCPs.

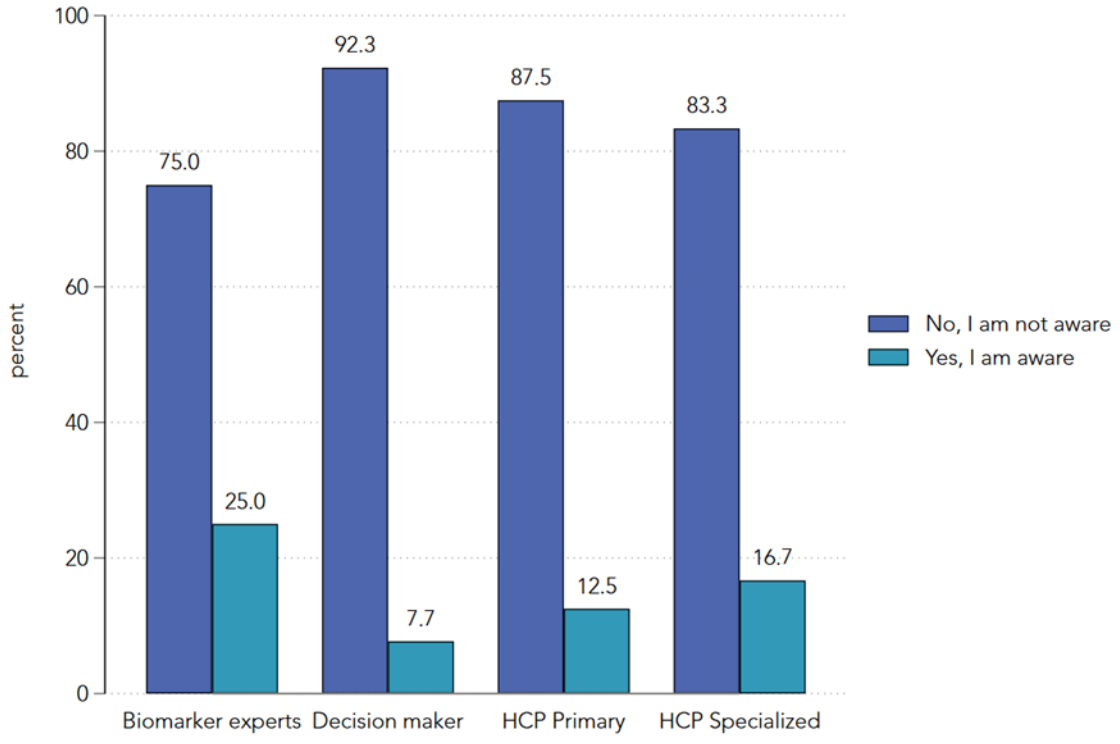

Figure 56. Awareness of Clinical Protocols for AD fluid-derived biomarkers among inquired respondents.

Among the 51 respondents, only 6 (11.76%) withheld their opinion regarding the anticipated application of AD blood-derived (plasma) biomarkers. The remaining 22 respondents highlighted early diagnosis (72.55%) as the primary envisioned use, with prognosis (43.14%) and progression monitoring (33.33%) trailing closely behind. Prognosis was predominantly favoured by Biomarker Experts (66.7%), followed by Primary HCPs (50%), Decision Makers (38.5%), and Specialized HCPs (27.8%), with the latter groups displaying comparatively less enthusiasm. Early diagnosis garnered unanimous support from all Decision Makers (100%), closely followed by Biomarker Experts (66.7%), Primary HCPs (62.5%), and Specialized HCPs (61.1%). In contrast, progression monitoring received minimal preference from all respondents, resulting in larger percentages indicating a lack of consideration for AD blood-derived biomarkers at this stage.

In conclusion, within this category, a comparative analysis was conducted for the question concerning the most appropriate stage in the patient's care journey to assess AD blood-derived biomarkers. The majority of respondents (21/51; 41.18%) indicated "Early detection of 'at-risk' healthy individuals at primary healthcare" as the preferred stage, followed closely by "Early detection of AD onset (i.e., Subject Cognitive Impairment (SCI) or Mild Cognitive Impairment (MCI)) at primary healthcare" (19/51; 37.25%). "Differential diagnosis and treatment selection at specialized care" garnered support from 7 out of 51 respondents (13.73%), while "Treatment response and/or disease progression monitoring at specialized care" was selected by 4 out of 51 respondents (7.84%).

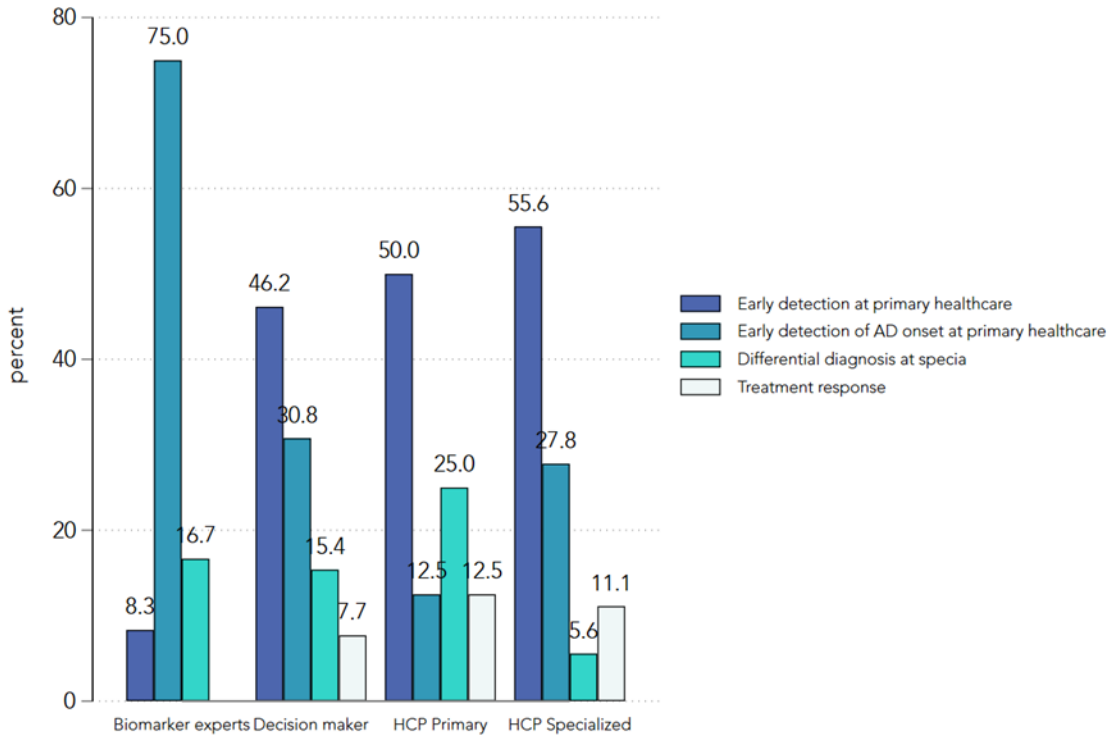

Figure 57. Envision of stage in the patient's care journey that would be most appropriate to assess AD blood-derived biomarkers among inquired respondents

8.7.2 Identification of AD blood-derived (plasma) biomarkers Comparative Analysis

Likewise, the Category of AD biomarkers identification was exclusively presented to Specialized HCPs and Biomarker Experts who indicated a level of experience other than "None" in the demographics section. The total count of experienced participants enrolled for this category/set of questions amounted to 22.

The AD biomarkers assessed for rating in the stages of prognosis, early diagnosis, and progression monitoring included Aβ40, Aβ42, Aβ42/Aβ40 ratio, p-Tau181, p-Tau217, p-Tau231, NfL, GFAP, and TDP-43. Interestingly, all AD biomarkers, with the exception of NfL, GFAP, and TDP-43, were consistently rated as "Important" by the majority of both Specialized HCPs and Biomarker Experts across all three stages of AD, i.e., prognosis (Figure 58), early diagnosis (Figure 59), and progression monitoring (Figure 60).

More specifically, when Specialized HCPs and Biomarker Experts were asked to rate the abovementioned biomarkers for the three AD stages, Aβ40, Aβ42, Aβ42/Aβ40 ratio, p-Tau181, p-Tau217, and p-Tau231 emerged as preferred choices with a tendency towards the "Important" option across all stages.

Aβ40 was predominantly rated as "Important" by 45.45% (n=10/22) for prognosis, 36.36% (n=8/22) for early diagnosis, and 36.36% (n=8/22) for progression monitoring, compared to only 13.64% (n=3/22) who deemed it irrelevant across all three stages. Similarly, Aβ42 received high ratings as "Important" by slightly more Specialized HCPs and Biomarker Experts, with 63.64% (n=14/22) for prognosis, 59.09% (n=13/22) for early diagnosis, and 54.55% for progression monitoring. Only 4.55% (n=1/22) identified it as "Irrelevant" for prognosis and early diagnosis, and 9.09% (n=2/22) for progression monitoring. The Aβ42/Aβ40 ratio was also predominantly rated as "Important" by respondents, with 54.55% (n=12/22) for prognosis and early diagnosis, and 59.09% (n=13/22) for progression monitoring, with no respondents selecting it as "Irrelevant" for all three stages.

p-Tau181 followed a similar pattern, with 54.55% (n=12/22) rating it as "Important" for prognosis and 63.64% (n=14/22) as "Important" for early diagnosis and progression monitoring, with no one considering it as "Irrelevant". Additionally, p-Tau217 was rated as "Important" for all stages, with 59.09% (n=13/22) for prognosis, 63.64% (n=14/22) for early diagnosis, and 68.18% (n=15/22) for progression monitoring, with no one rating it as "Irrelevant" for any stage. Finally, p-Tau231 received similar "Important" ratings from most respondents, with 45.45% (n=10/22) for prognosis, 50% (n=11/22) for early diagnosis, and progression monitoring, and no one rating it as "Irrelevant" for any stage.

Additionally, when Specialized HCPs and Biomarker Experts were asked to rate the NfL biomarker per AD stage, opinions were inconclusive. Approximately 36.36% of respondents presented no opinion for all stages, while an equal percentage (36.36%) considered it important for prognosis. For early diagnosis, 31.82% deemed it important, and for progression monitoring, 40.91% considered it important). Similarly, the same pattern emerged for the rating of GFAP across AD stages. Forty point ninety-one percent (40.91%) of respondents had no opinion, while 31.82% considered this biomarker important for both prognosis and early diagnosis. For progression monitoring, 27.27% selected it as important. Lastly, when asked to rate the TDP-43 biomarker, most respondents in this category expressed no opinion on the prognosis and progression monitoring stages (50% for each), while

45.45% equally distributed their answers between "No opinion" and "Important" for the early diagnosis stage.

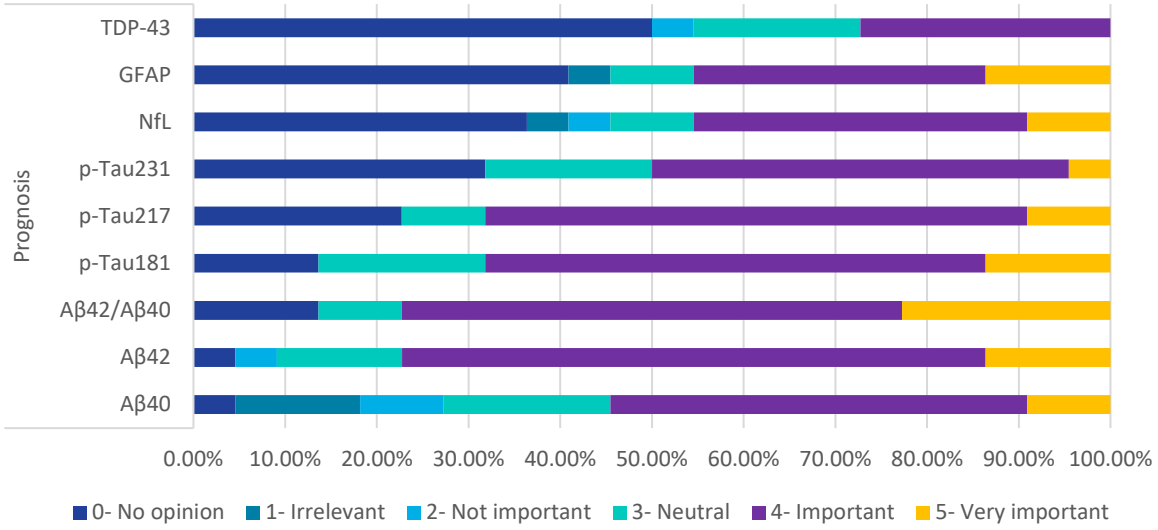

Figure 58. Importance rating of AD blood-derived (plasma) biomarkers for prognosis of AD.

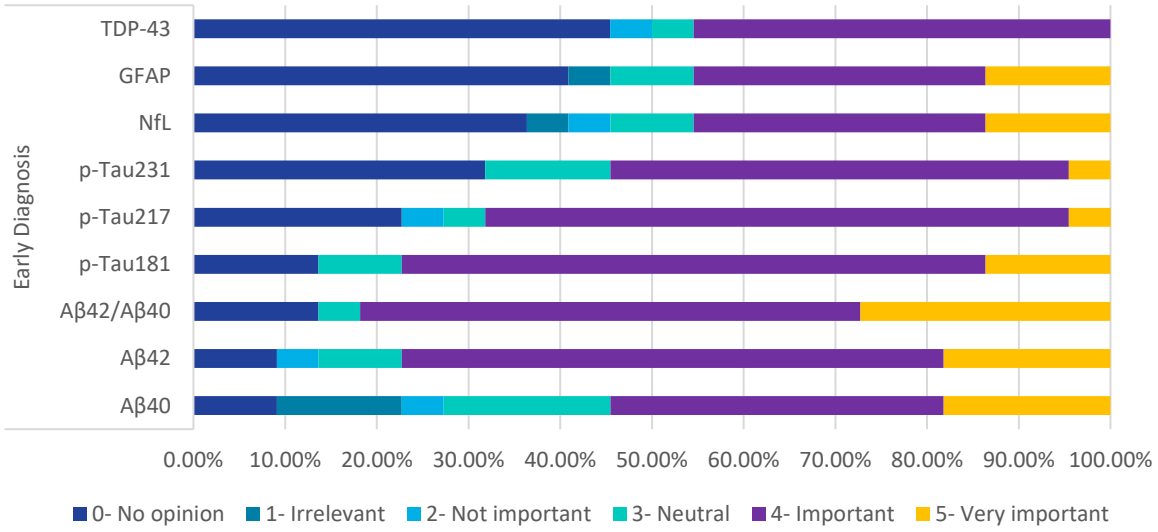

Figure 59. Importance rating of AD blood-derived (plasma) biomarkers for early diagnosis of AD.

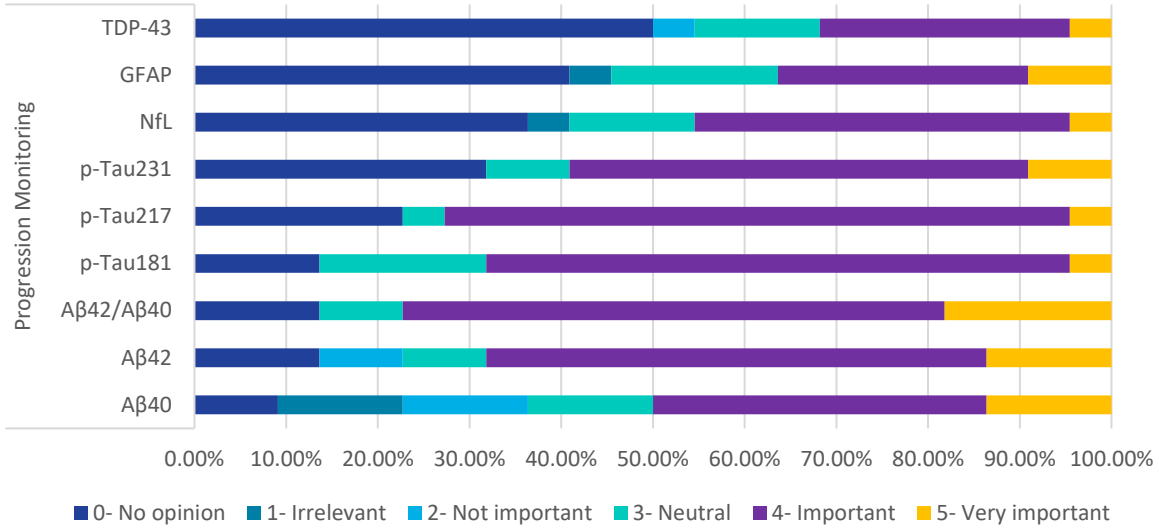

Figure 60. Importance rating of AD blood-derived (plasma) biomarkers for progression monitoring of AD.

8.7.3 Challenges

In an effort to obtain direct and clearer insights into various challenges and barriers experienced by patients, caregivers, specialists, and relevant stakeholders in their daily healthcare settings, a comparative analysis was conducted. These questions were presented to all targeted participants, although their specific content might vary based on individual profiles.

When asked about their familiarity with the average cost required for obtaining AD fluid-derived biomarker results to diagnose AD, the majority of respondents (n=19/33; 57.58%), including Decision Makers, Specialized HCPs, and Biomarker Experts, expressed no opinion. The remaining 14 respondents provided responses as follows: "≤300 €" (n=7/33; 21.21%), ">300-500 €" (n=6/33; 18.18%), and ">500-1000 €" (n=1/33; 3.03%). Notably, most of the respondents who were unaware of the average cost were either Specialized HCPs or Decision Makers. Similarly, when questioned about the average time needed, a similar proportion of respondents (42.42%, n=14/33) had no opinion, while 39.39% (n=13/33) selected "≥2 weeks" as the average time required. Among those who had no opinion, Specialized HCPs (63.6%) constituted the majority, with Biomarker Experts (36.4%) and Decision Makers (27.3%) following suit.

Similarly, when HCPs Specialized and Primary, Decision Makers, Biomarker Experts, and Caregivers[167] were asked about their familiarity with the average cost required to diagnose AD, the vast majority (n=50/80; 62.50%) indicated that they were not aware of the average cost needed. A few respondents (n=12/80; 15%) selected ">500-1000 €", while even fewer respondents chose "≤500 €" (n=10/80; 12.50%) or ">1000-2000 €" (n=5/80; 6.25%) or ">2000 €" (n=3/80; 3.75%). Once again, Primary HCPs (75%) and Specialized HCPs (72.2%), along with the addition of Caregivers (72.4%) were primarily those who were not aware of the average cost needed.

[167] For additional comparative analysis related to Patients and Caregivers (i.e., comparative analysis related to "Willingness to use a Point-of-Care In Vitro Diagnostic – Acceptance & Trust" and "Willingness to use a Point-of-Care In Vitro Diagnostic for Alzheimer's Disease – Acceptance & Trust"), please refer to **Section 8.3.2** and **Section 8.3.3.**

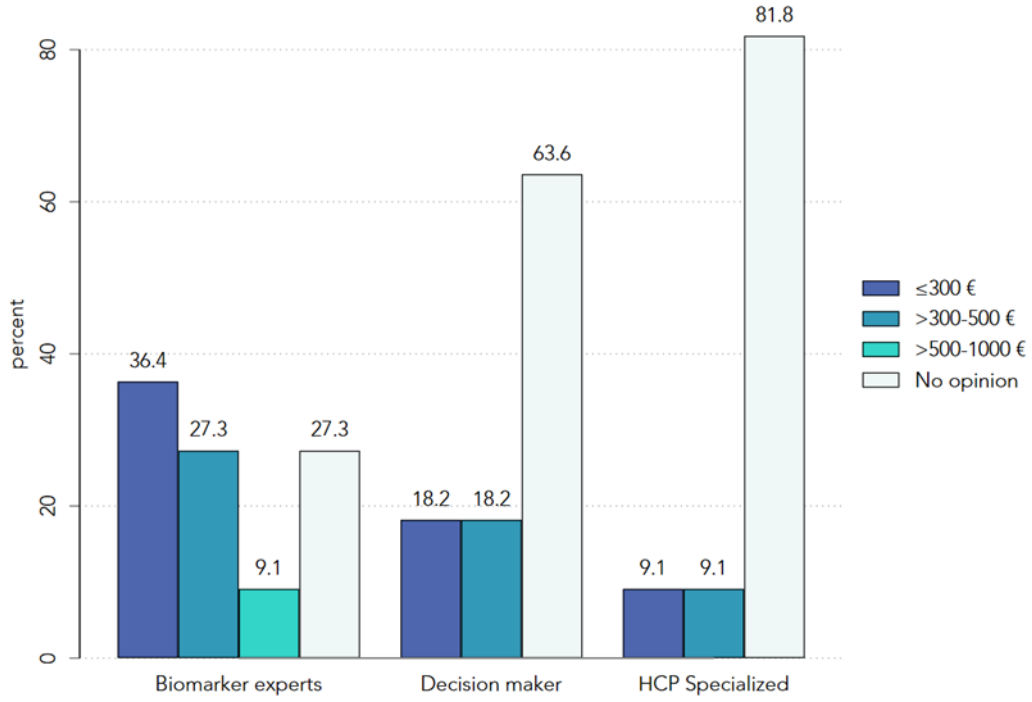

Figure 61. Familiarity with average cost needed for getting AD fluid-derived biomarker results to diagnose AD among inquired respondents.

In the comparative analysis aimed at elucidating the primary challenges and barriers to implementing a PoC IVD for detecting AD blood-derived (plasma) biomarkers in primary healthcare settings, several key findings emerged. The most commonly selected options among respondents were "Lack of knowledge/expertise from HCPs" (54.9%), "High Testing Cost (Pricing/Cost)" (50.98%), "Reimbursement/Health Insurance coverage" (45.1%), "Lack of awareness from patients" (37.25%), "Credibility/Trustworthiness" (33.33%), and "Robustness/Reliability" (27.45%). Moreover, "Complexity/User-friendliness", "Interoperability", and "Scalability/Clinical homogeneity" were equally chosen among respondents (19.61%), followed by "Invasive procedure" (15.69%) and "Data Privacy" (11.76%). Notably, "Pricing/Cost", "Reimbursement", "Credibility", and "Lack of knowledge/expertise from HCPs" were options that were almost equally selected by all respondent groups, compared to all other aforementioned options.

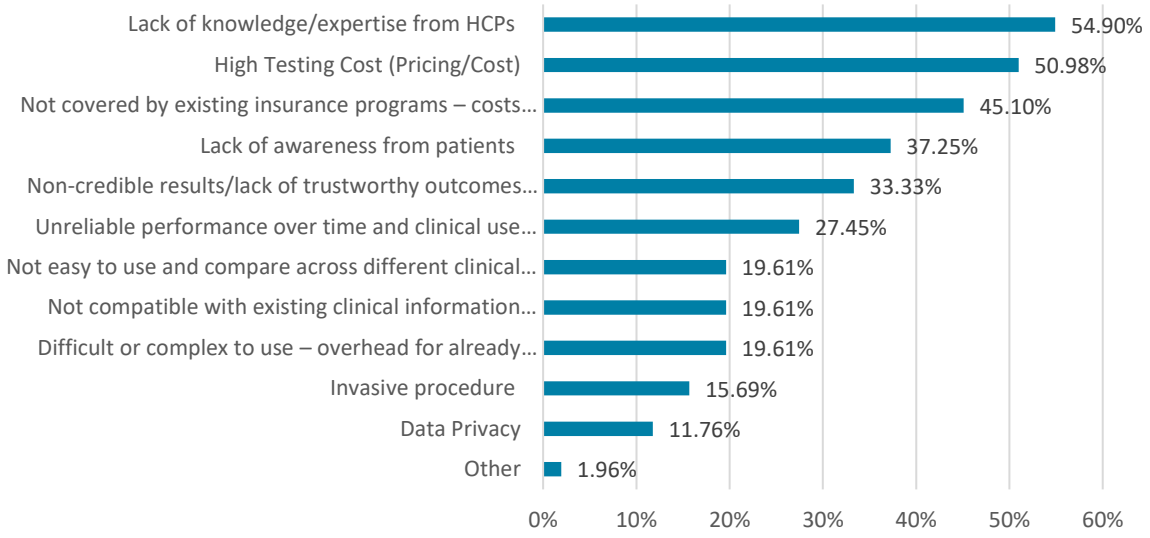

Figure 62. Main challenges and/or barriers to deploying a PoC IVD for detecting AD blood-derived (plasma) biomarkers in primary healthcare settings among inquired respondents

8.8 Main findings/results

The analysis of demographic data from all 90 respondents revealed a strong grasp of English among the majority and it reflected a diverse mix of targeted participant profiles. However, it's important to note that the distribution of groups within these respondents was not uniform, with Caregivers being the most represented group, and a significant number of respondents located in Greece (Table 6). Despite these variances, respondents, including Patients and Caregivers, exhibited generally high levels of education. Additionally, the occupational backgrounds of Patients and Caregivers revealed a mix, with some solely in the healthcare sector, ensuring a diverse representation of other occupations among the remaining respondents in these groups. Nonetheless, their responses were comprehensive and reflective, drawing from personal experiences within the context of AD. Moreover, due to the limited experience of most respondents in AD Biomarkers or PoC IVDs, opportunities for enhancing knowledge or validating existing concepts within the Online Survey, especially regarding AD biomarker ratings, cut-offs, and awareness of potentially overlooked biomarkers, were restricted. Nevertheless, respondents provided pertinent feedback, regardless of their familiarity with these topics (Section 8.3.1; Section 8.4.1; Section 8.5.1; Section 8.6.1). Age distribution varied across groups, with Patients typically representing the older demographic within the Online Survey sample.

8.8.1 End-user Needs and Challenges

Given the unique challenges posed by AD, respondents were asked to share their insights and opinions on various socioeconomic challenges associated with AD. These discussions primarily centered around costs, the time required for diagnosis, and the main obstacles for implementing a PoC IVD in healthcare settings, considering the current circumstances. It was uncovered that **costs associated with AD fluid-derived biomarkers were generally not within the awareness of most Decision Makers and Specialized HCPs**. Among Biomarker Experts, the predominant response indicated costs of

"≤300€". This lack of awareness extended to responses concerning the overall cost and average time required for diagnosing AD and receiving AD-related care, with the majority of participants, including Patients and Caregivers, **lacking knowledge on these matters**. Additionally, the main challenges identified for introducing a PoC IVD into healthcare settings include a **lack of knowledge** among HCPs and patients, **high costs**, and **lack of insurance coverage**.

These challenges underscore the urgent need for comprehensive action to address various aspects of AD diagnosis and care (extending across all elements explored in the Online Survey):

- **Improved Education, Cost-Effective Solutions, and Broader Insurance Coverage**: Efforts should be made to enhance education and awareness among HCPs and patients about AD diagnosis and care. Moreover, there is a need for the development of cost-effective solutions and broader insurance coverage to facilitate the adoption of PoC IVDs and improve AD diagnosis and management;
- **Complexity of Diagnosis Process**: The multifaceted nature of the AD diagnostic process, involving multiple examinations and varying across different healthcare systems, underscores the need for standardized protocols and guidelines to minimize inconsistencies and challenges in the diagnostic pathway;
- **Healthcare System Variations**: The differences in approaches to diagnosing AD across various healthcare systems globally highlight the importance of developing adaptable and universally applicable diagnostic strategies to ensure equitable access to care for all individuals affected by AD;
- **Limited Education and Training**: Addressing the lack of awareness and understanding among stakeholders, including Decision Makers and Specialized HCPs, regarding the costs associated with AD diagnosis and care requires targeted education and training initiatives to bridge knowledge gaps and promote informed decision-making;
- **Stigma and Misconceptions**: Combatting the significant stigma attached to AD and other forms of dementia is essential to encourage individuals to seek diagnostic testing and access available treatments without fear or misconceptions hindering their decision-making;
- **Limited Research Dissemination**: Enhancing the dissemination of research findings on AD diagnosis and biomarkers to non-specialist audiences, including decision makers and the general public, is vital for promoting awareness and understanding of the latest advancements in diagnostic techniques and associated costs.

Overall, addressing these multifaceted challenges is imperative for improving the diagnostic process for AD, ensuring individuals receive timely and accurate diagnoses, and ultimately leading to better outcomes for patients and caregivers. Collaboration among stakeholders, investment in research and education, and policy interventions are essential components of a comprehensive approach to tackling the complexities of AD diagnosis and care.

The needs and challenges identified from the Online Survey are significantly aligned with the results of the Semi-Structured Interviews. The few differences identified are focused on the need for a "rapid and less-invasive" solution (see also Section 8.8.4), as well as for "personalised treatment".

8.8.2 Clinical Practice

Protocols

During the analysis of the clinical testing procedure category within the Online Survey, which pertains predominantly to Primary and Specialized HCPs, Decision Makers, and Biomarker Experts, it became evident that a significant portion of respondents (n=41/53) lacked awareness of any clinical research protocols for AD biomarkers (which was also the case identified during the Semi-Structured Interviews). As a result, the majority of respondents did not offer substantial limitations concerning the protocols for AD fluid-derived biomarkers. This observation may indicate either a gap in the utilization of AD biomarkers/specific protocols for AD biomarkers in healthcare settings, highlighting areas for improvement in research- and decision-making processes, or a knowledge gap among the participants involved in the Online Survey.

In addition, the majority of respondents' perspective on utilizing biomarker results underscores the importance of **early diagnosis**, suggesting a potential need among stakeholders for biomarkers to offer **robust evidence supporting early diagnostic assessments** across various healthcare settings, including **primary** and **specialized** care. Additionally, this preference underscores the relevance of research experts to prioritize efforts in this direction. Decision makers could also leverage this insight to enhance approaches within healthcare settings more effectively.

Performance Criteria & Testing process

When asked to provide their opinion on factors important for decision-making regarding AD, there appeared to be better alignment between Specialized HCPs and Decision Makers compared to Primary HCPs. While Primary HCPs selected some similar options, they did not prioritize them to the same extent. Specifically, "**Pricing/Cost**" and "**Availability**" emerged as highly preferred factors for Specialized HCPs and Decision Makers, whereas Primary HCPs mostly prioritized "**Robustness/Reliability**". These preferences indicate a demand for solutions that are cost-effective, readily available for all patients, and deliver reliable results. Furthermore, there was a strong emphasis on the diagnostic value among HCPs for receiving AD blood-derived (plasma) biomarker results directly on a mobile phone or tablet in real-time. Similarly, Decision Makers supported the need for direct and secure access from the mobile app to the CIS. This alignment suggests a shared preference and recognition of the importance of having such a solution in healthcare settings to enhance the diagnostic process for patients. Indeed, it is noteworthy that Decision Makers have expressed support for the implementation of a PoC IVD for AD. They emphasized desired benefits such as early diagnosis and intervention, cost reduction, and improvement in patient quality of life (QoL). This underscores an emerging need to optimize costs in AD treatment while enhancing the efficiency and effectiveness of the diagnostic process, ultimately aiming to enhance the overall QoL for patients.

8.8.3 AD Biomarkers and Intended Use

In the Online Survey, respondents also provided insights on how they envision AD blood-derived (plasma) biomarkers could support HCPs in prognosis, early diagnosis, and progression monitoring of AD. For prognosis, the majority selected options related to diagnosis support, prediction and guidance of clinical decisions, and guidance of treatment options, indicating a consensus among respondents.

However, Primary HCPs showed a lesser inclination towards certain options compared to Specialized HCPs and Biomarker Experts, possibly reflecting a gap in their involvement in early AD diagnosis in primary care settings. In terms of early diagnosis, there was greater alignment between Specialized HCPs and Biomarker Experts, with Primary HCPs showing again less alignment, potentially highlighting differences in their roles and experiences. For progression monitoring, respondents generally agreed that AD blood-derived biomarkers could aid in diagnostic confirmation, assessing treatment effectiveness, and personalized medicine, underscoring the importance of this type of biomarkers in various stages of AD management.

Regarding the stage in the patient's care journey deemed most appropriate for assessing AD blood-derived biomarkers, opinions varied. Specialized HCPs leaned towards "Early detection of 'at-risk' healthy individuals at primary healthcare", followed by Primary HCPs and Decision Makers, while Biomarker Experts favoured "Early detection of AD onset". This diversity suggests that both stages are considered crucial across different stakeholders, however it doesn't necessary align with the level of care (primary, secondary or tertiary), but mainly the need for an accurate and reliable diagnosis.

Specialized HCPs and Biomarker Experts consistently rated all presented biomarkers in the Online Survey as "Important" across nearly all stages, with minimal variation in responses (which vastly differs from the results of the Semi-Structured Interviews). This uniformity in ratings may indicate a lack of distinction among biomarkers in terms of their importance for different stages of AD (Section 8.7.2). As previously mentioned, due to their limited experience with AD biomarkers, all Specialized HCPs and Biomarker Experts were unable to provide any cut-offs for the nine biomarkers they were asked to rate for prognosis, early diagnosis, and progression monitoring. Nevertheless, some slight preferences can be observed, which align with the results of the Semi-Structured Interviews.

In particular, for prognosis, Aβ42 and Aβ42/Aβ40 seem to be ranked the highest (77.28%) across all other biomarkers, with Aβ42/Aβ40 having a precedence (as it had a higher percentage under "Very Important" than AB42). p-Tau181 and p-Tau217 follow with 68.18% (with p-Tau181 being considered more important than p-Tau217 for Prognosis).

Similarly, for early detection, Aβ42/Aβ40 has been flagged as "Important" or "Very important" by 81.82% of the respondents, followed by Aβ42 and p-Tau181 with 77.28%. On the contrary, p-Tau217, which was identified as the most valuable for early diagnosis by both the Desk Research and the Semi-Structured Interviews, comes fourth with 68.19%.

Finally, for progression monitoring, once more, Aβ42/Aβ40 has been highlighted by 77.27% of the respondents, followed by p-Tau217 (72.73%), Aβ42 (68.18%) and p-Tau181 (68.18%).

It is remarkable that almost half of the respondents (~45%) had either "No opinion" or flagged as "Irrelevant" NfL, GFAP, and TDP-43, which of course contradict the findings from the Desk Research, and the discussion with the experts during the Semi-Structured Interviews. Furthermore, respondents lacked awareness of any new additional biomarkers for AD. This lack of awareness extended to similar projects or initiatives among all enrolled participants.

This indicates either a gap in the knowledge of the respondents enrolled in the Online Survey or a broader gap in the biomarkers commonly used in this field (or staying up to date with recent advances). Regardless, these questions did not yield new insights into cut-offs or new biomarkers through the Online Survey. Overall, these findings highlight the need for increased education and

training in the field of AD biomarkers to enhance knowledge and awareness among healthcare professionals and experts.

8.8.4 The role of PoC IVDs

Aggregating responses on challenges/barriers for deploying a PoC IVD in primary healthcare, we observe a clear alignment with the Semi-Structured Interviews' findings, however not with the same "priority". Almost half of the respondents (45%-55%) give emphasis on the **HCPs capacity to effectively use such technology** along with the accompanied financial concerns that have to do with the **cost of the solution** and its subsequent **reimbursement** from the government or an insurance company. Even though these elements were also identified during the interviews, their importance varied. This could be explained by the fact that during the Semi-Structured Interviews, there was room for providing additional information and answering questions regarding the technology and its envisioned use, which was not the case for the Online Survey.

In the same context, the **Accuracy and Reliability** of a PoC IVD is only covered by one third of the Online Survey respondents (33%) contrary to the Semi-Structured Interviews, during which it was mentioned as the most important requirement for such a device.

8.8.5 The ethics dimension

Patients and Caregivers expressed their **acceptance**, **trust**, and **willingness** to use a PoC IVD both overall and specifically for AD. The results indicated that both **Patients and Caregivers would be willing to use a PoC IVD overall, even before experiencing any symptoms**, and **would prefer to undergo such testing annually** (Section 8.3.2). However, the vast majority inclined to cover the cost of such a test on their own, with those willing to cover the cost mostly choosing amounts ranging from **20 to 50 €**. Regarding the willingness to use a PoC IVD specifically for AD, Patients and Caregivers expressed the need for **support in understanding available treatment options**, **interventions**, and **AD management**. They also highlighted the importance of **communication with treating physicians** and the **credibility of the test results**, as well as the need for cognitive stimulation.

Additionally, they emphasized the importance of receiving results related to prognosis, risk, and AD stage. Overall, the majority of Patients and Caregivers did not express any concerns about undergoing such testing, indicating an overall willingness and perceived need for PoC IVDs in the field of AD diagnosis and management (Section 8.3.3). These findings suggest that Patients and Caregivers are receptive to innovative diagnostic tools that can provide valuable insights into AD risk, prognosis, and management, provided that they are supported by HCPs and that the tool is accessible at an affordable cost.

In conclusion, the Online Survey successfully fulfilled its objective by garnering insights from a diverse range of participants. Furthermore, it successfully reached its target of 150 enrolled participants, comprising 90 respondents and 107 non-respondents, totalling 197 participants out of the intended 150. It revealed that various stakeholders involved in AD, including patients, caregivers, HCPs, decision-makers, etc., express a strong willingness to adopt a PoC IVD solution. This solution could potentially improve the diagnostic process, treatment methods, and decision-making related to AD. Moreover, the Online Survey highlighted common concerns shared across all groups, such as

cost/insurance, credibility, and knowledge, which serve as significant barriers to acceptance for AD diagnosis.

Focusing on the main differences compared to the Semi-Structured Interviews, the main findings of this overall exploration are updated as indicated in Figure **63**.

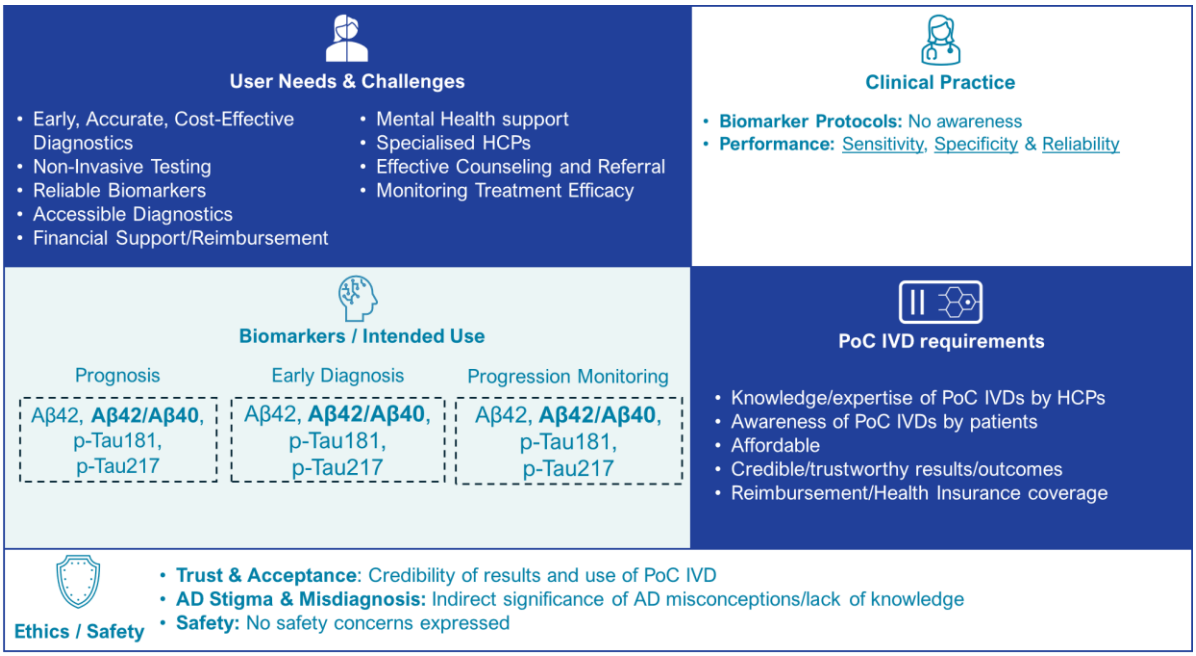

Figure 63. Main findings from the Online Survey.

9. Conclusions

This report provides a comprehensive overview of the Alzheimer's Disease landscape, covering the associated **needs and challenges** of several actors involved. Employing a user-centre methodology the analysis covered extensive desk research that was extended and fine-tuned by **26 semi-structured interviews** with key experts and citizens (i.e., Technology Providers, Decision Makers, HCPs, Patients and Caregivers), actively engaging with both the 2D-BioPAD SIAB. Following a wider **Online Survey** expanded the gathered knowledge collecting 197 participants, out of which **90 have fully completed** the Online Survey and their responses have been analysed in detail. Key insights for the aforementioned elements were included in *Section 7.6* and *Section 8.8* to provide context and enhance understanding.

The summary of all the aforementioned aspects consists of user-driven recommendations for the design and development of the 2D-BioPAD system. Combined with the Ethical Consideration Roadmap (Section 6), user requirements that consider ethics, are briefly outlined below:

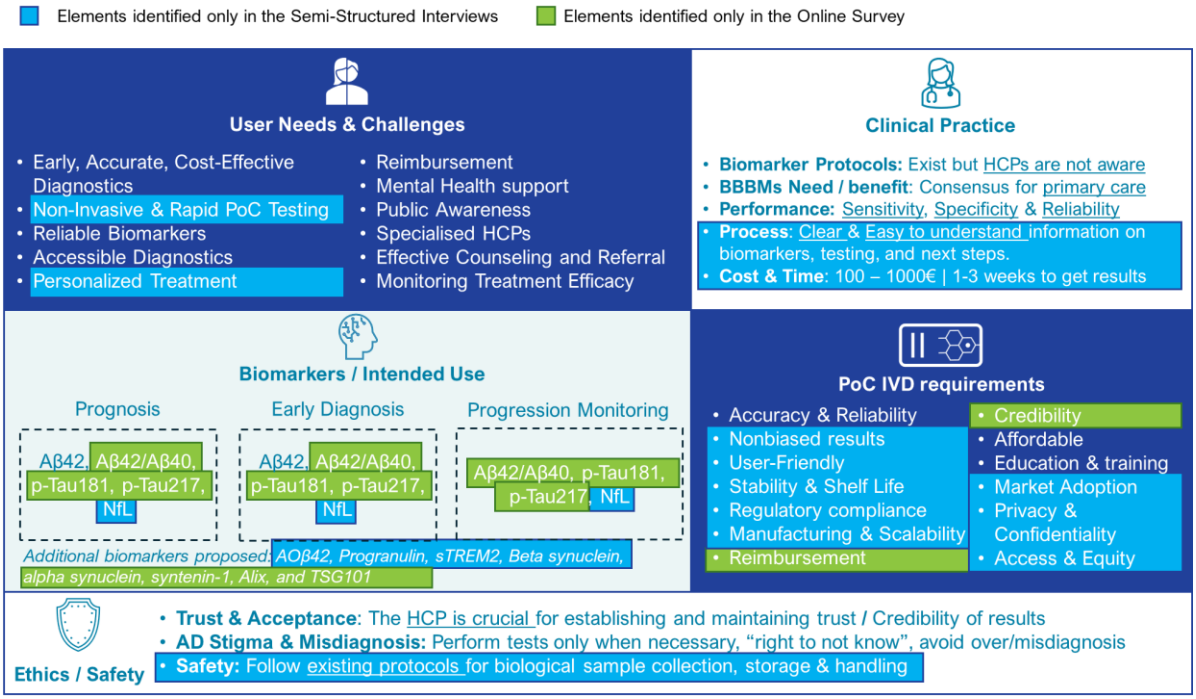

Figure 64. Main user needs, challenges and PoC IVDs requirements for AD.

The knowledge gained from this report will primarily fuel the activities of T1.3 and T5.1, which aims to identify 2D-BioPAD's technical and clinical requirements, respectively. The elements identified and validated with various external stakeholders will serve as the building blocks for the 2D-BiOPAD framework to develop a system that will address these topics, translating them into technical requirements, while designing the clinical pilot studies protocol.

Nevertheless, while these insights/recommendations offer guidance for the 2D-BioPAD solutions, it is important to note that they may evolve over time and require periodic updates to address emerging issues and changing circumstances.

In addition, the exploration of the core 2D-BioPAD enabling technologies that began in the context of T1.1 will continue through WP2, WP3, and WP4, where further emphasis will be placed on the development of the various components of the 2D-BioPAD PoC IVD.

Regarding T6.1, key insights of this report will feed the creation of dissemination and communication material, such as infographics, informative articles/news items and scientific publications. This material will help raise awareness of the topic and promote knowledge transfer, actively aiming to address one of the core challenges related to AD.

Under T6.5, discussions with projects and initiatives that were already identified mainly during the interview and online survey activities, will be followed-up, in order to establish fruitful synergies. Meanwhile, T6.3 will rely on market insights to inform business modelling and planning activities, whereas T6.4 will follow up with the self-assessments and the generation of clear evidence for addressing regulatory affairs and drafting the regulatory acceptability plan.

Finally, the Online Survey will continue collecting responses by the end of April 2024, thus the dataset hosted on the 2D-BioPAD Zenodo Community will be updated accordingly with all the new input after the survey is closed for responses.

Annex I – Biomarkers

The complete list of promising biomarkers that have been identified as candidates for the 2D-BioPAD system.

Amyloid Beta (Aβ) 1-40

Properties	
Concentration in Plasma	Scientific research: Health status unknown: 1-220 pg/ml Assay range: 0.38-280 pg/ml
Method	Simoa Neurology 4-Plex E Advantage Kit
Molecule Size	40 amino acids 4.33 kDa[168]
Requirements	
Transportation	Plasma samples: -
Storage	Plasma samples: -80°C
Sample Volume	100 µl (for current test – plasma)
Commercial Product	
Plasma (Simoa): https://www.quanterix.com/simoa-assay-kits/neurology-4-plex-e-ab40-ab42-gfap-nf-l-new/	

Amyloid Beta (Aβ) 1-42

Properties	
Concentration in Plasma	Scientific research: Health status unknown: 0.3-13 pg/ml Assay range: 0.14-100 pg/ml
Method	Simoa Neurology 4-Plex E Advantage Kit
Molecule Size	42 amino acids 4.51 kDa[169]
Requirements	
Transportation	Plasma samples: -
Storage	Plasma samples: -80°C
Sample Volume	100 µl (for current test – plasma)
Commercial Product	
Plasma (Simoa): https://www.quanterix.com/simoa-assay-kits/neurology-4-plex-e-ab40-ab42-gfap-nf-l-new/	

[168] https://pubchem.ncbi.nlm.nih.gov/compound/57339250
[169] https://pubchem.ncbi.nlm.nih.gov/compound/57339251

Tau Protein 181 – pTau

Properties	
Concentration in Plasma	Scientific research: Health status unknown: 1-100 pg/ml Assay range 0.62-1280 pg/ml
Method	Simoa pTau-181 Advantage V2.1 Kit
Molecule Size	48-67 kDa
Requirements	
Transportation	Plasma samples: -
Storage	Plasma samples: -80°C
Sample Volume	100 µl (for current test – plasma)
Commercial Product	
Plasma (Simoa) https://www.quanterix.com/simoa-assay-kits/p-Tau181-v2-new/	

Tau Protein 217

Properties	
Concentration in Plasma	Not available Expected range based on literature in the range of a few pg/ml
Method	Simoa® ALZpath p-Tau 217 Advantage PLUS
Molecule Size	48-67 kDa
Requirements	
Transportation	Plasma samples: -
Storage	Plasma samples: -80°C
Sample Volume	100 µl (for current test – plasma)
Commercial Product	
Plasma (Simoa) https://www.quanterix.com/news/diagnostic-accuracy-of-a-plasma-phosphorylated-tau-217-immunoassay-for-alzheimers-disease-pathology/	

Tau Protein 231

Properties	
Concentration in Plasma	Assay range 0.091-0.837 pg/ml
Method	Simoa® p-Tau 231 Advantage PLUS
Molecule Size	48-67 kDa
Requirements	
Transportation	Plasma samples: -
Storage	Plasma samples: -80°C
Sample Volume	100 µl (for current test – plasma)
Commercial Product	
Plasma (Simoa) https://www.quanterix.com/simoa-assay-kits/p-tau-231/	

Neurofilament Light (NFL) chain

Properties	
Concentration in Plasma	Diagnostic service and scientific research: Health status unknown: 3-250 pg/ml Assay range: 0.085-1440 pg/ml Healthy: BMI 25, age dependent[170] (https://doi.org/10.1016/S1474-4422(22)00009-6, serum values) 20-29 y, <7 pg/ml 30-39 y, <9 pg/ml 40-49 y, <11 pg/ml 50-59 y, <15 pg/ml 60-69 y, <19 pg/ml 70-79 y, <23 pg/ml
Method	Simoa NF-light ™ V2 Advantage Kit
Molecule Size	68 kDa
Requirements	
Transportation	Plasma samples: -
Storage	Plasma samples: -80°C
Sample Volume	100 µl (for current test – plasma)
Commercial Product	
Plasma, serum and CSF (Simoa) https://www.quanterix.com/simoa-assay-kits/nf-light/	

Glial Fibrillary Acidic Protein (GFAP)

Properties	
Concentration in Plasma	Scientific research: Health status unknown: 17-600 pg/ml Assay range: 0.44-20000 pg/ml
Method	Simoa Neurology 4-Plex E Advantage Kit
Molecule Size	432 amino acids 49.88 kDa[171]
Requirements	
Transportation	Plasma samples: -
Storage	Plasma samples: -80°C
Sample Volume	100 µl (for current test – plasma)
Commercial Product	
Plasma (Simoa): https://www.quanterix.com/simoa-assay-kits/neurology-4-plex-e-ab40-ab42-gfap-nf-l-new/	

[170] Benkert, P., et al. (2022). Serum neurofilament light chain for individual prognostication of disease activity in people with multiple sclerosis: a retrospective modelling and validation study. The Lancet Neurology, 21(3), 246-257.
[171] https://pubchem.ncbi.nlm.nih.gov/protein/P14136

TDP-43

Properties	
Concentration in Plasma	Scientific research: Health status unknown: 10-1300 pg/ml Assay range: 2.48-2000 pg/ml
Method	Simoa TDP-43 Advantage Kit
Molecule Size	43 kDa
Requirements	
Transportation	Plasma samples: -
Storage	Plasma samples: -80°C
Sample Volume	100 µl (for current test – plasma)
Commercial Product	
Plasma (Simoa): https://www.quanterix.com/simoa-assay-kits/tdp-43/	

Beta-synuclein

Properties	
Concentration in Plasma	Based on literature, expected ranges around a few pg/ml in Plasma
Method	-
Molecule Size	134 aminoacids, 19 kDa
Requirements	
Transportation	Plasma samples: -
Storage	Plasma samples: -80°C
Sample Volume	100 µl (for current test – plasma)
Commercial Product	
N/A	

Annex II – 2D-BioPAD Ethics Management Milestone Overview

Action Point	Description	Documentation	Timeline
1	Preparation of Ethics Plan (i.e., Ethical Consideration Roadmap) by Evnia.	Ethical Consideration Roadmap	Submission 19th of April 2024 (part of D1.1) project M7
2	Have workshop in the 2nd Semester Meeting to present and discuss the ECR	- 2nd Semester Meeting Agenda - PowerPoint ECR Presentation	17th and 18th of April 2024 project M7
3	Self-Assessment is be performed by each WP task leader representing different Consortium partners.	Self-Assessment Forms archived in the 2D-BioPAD SharePoint Site Ethical Self-Assessments - (See Annex III – **ECR - Self-Assessment**)	Two Self-Assessments should be performed i.e., 1) initial assessment in month 1-4 after kick-off of the task focusing on the planned activities, 2) final assessment when the task is finalized i.e., in the end of each task.
4	Evnia ensures monitoring and follow-up on Self-Assessments	- Status overview in the 2D-BioPAD SharePoint Site.	Throughout the project.
5	Preparation of Dissemination and Communication Plan	- Dissemination and Communication Plan (WP6 D6.1)	Submission project M3 v1, M24 v2, M 48 v3
6	Preparation of Regulatory Affairs Plan	- Regulatory Affairs Plan (WP6 D6.7)	Submission project M48
7	Preparation of Exploitation and Sustainability Plan	- Exploitation and Sustainability Plan (WP6 D6.4)	Submission project M48
8	Preparation of Management and Quality Plan	- Management and Quality Plan (WP7 D7.1)	Submission project M3
9	Preparation of Data Management Plan	- Data Management Plan (WP7 D7.2)	Submission project M3 v1, M24 v2, M 48 v3

Annex III – ECR - Self-Assessment

Purpose

The present Self-Assessment for the 2D-BioPAD offers a framework for Consortium partners to review the ethics of the project activities throughout the research cycle. The Self-Assessment must cover all identified possible ethics issues identified for the 2D-BioPAD project's design, development/experimentation, and deployment phases. The Self-Assessment provides a timely means to identify ethical issues for the research conducted. The method does not resolve the ethical issues, however, strives to identify ethical risks and shape future discussions that enable prevention of ethical harms and improvement of ethics in project activities.

Responsibility

- The Self-Assessment is not intended to be performed by consortium members alone, but be performed as a group, discussed, and documented by each WP task leader representing different partners in the Consortium.
- WP/Task leaders are responsible to complete, and archive completed Self-Assessment form in the project's online repository.

Procedure

The Self-Assessment shall be read through and then completed with information regarding the name of the Organization, Country, WP task leader name, Work Package and Task numbers.

- Notes for the WP task leader:
    - All passages/text in italics and highlighted in grey are intended to support the WP Task leader during Self-Assessment preparation. These passages shall be deleted prior to delivery of the document so the Self-Assessment only comprises results of the Self-Assessment.
    - Where the answer is YES or NO, please tick NO if NOT APPLICABLE.
    - Where a specific document is requested to be kept on file and provided on request, please tick "Document available" check box if available.

Respondent of Self-Assessment

Organization	Country	WP/Task Leader	WP/Task	Date	Signature
			WPX / TY.Z		

Public Good

Public Good: evaluation of potential Risks and Benefits of the project		YES	NO	Description
Is there potential for your work to be used to make decisions about individuals (e.g., as may be the case with predictive modelling projects) or to identify individuals?		☐	☐	*Please specify and, for each risk identified, please report any possible mitigations that could be applied to minimise it.*
If YES	What ramifications may this have for these individuals?	*Please specify.*		
Is there potential for your work to be used to make decisions about, or to identify, particular groups or communities within society?		☐	☐	*Please specify and, for each risk identified, please report any possible mitigations that could be applied to minimise it.*
If YES	What ramifications may this have for them?	*Please specify.*		
Are there any potential data gaps in your work that could lead to harm, stigmatisation or distress for individuals or groups who are under-represented in your analysis (i.e., those who may be missing from your data)?		☐	☐	*Please specify and, for each risk identified, please report any possible mitigations that could be applied to minimise it.*
If YES	How could this be mitigated?	*Please specify.*		

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Public Good: evaluation of potential Risks and Benefits of the project		YES	NO	Description
Is there potential for harm, stigmatisation or distress for individuals or groups who are (a) included as data subjects in your project or (b) may be impacted as a result of the findings of the research (including social, environmental, economic, physical or mental health impacts)?		☐	☐	*Please specify and, for each risk identified, please report any possible mitigations that could be applied to minimise it.*
If YES	How can these risks be minimised?	*Please specify.*		
Is there potential for negative impacts for organisations who are (a) included as data subjects in your project or (b) may be impacted as a result of the findings of the research (including reputational impacts)?		☐	☐	*Please specify and, for each risk identified, please report any possible mitigations that could be applied to minimise it.*
If YES	How can these risks be minimised?	*Please specify.*		
Is there potential for harm or distress to members of the research team, research facilitators, or other individuals involved in activities related to conducting the project?		☐	☐	*Please specify and, for each risk identified, please report any possible mitigations that could be applied to minimise it.*
If YES	How can these risks be minimised?	*Please specify.*		
Are there specific envisaged public benefits of your work?		☐	☐	*Please specify.*
If YES	How will you achieve these benefits?	*Please specify.*		
Is there any evidence-base behind your justification of potential benefits?		☐	☐	*Please specify.*
If YES	Is it peer-reviewed?	*Please specify.*		
	How confident are you that these benefits will be realised?	*Please specify.*		
Are there any limitations in your project approach that may limit the impact of potential benefits?		☐	☐	*Please specify.*

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Public Good: evaluation of potential Risks and Benefits of the project		YES	NO	Description
If YES	What are these and how have they been minimised?	*Please specify.*		
Is the work focused on enhancing trust in statistics or statistics producers (e.g., challenging or validating official statistics)?		☐	☐	*Please specify.*
If YES	By what means will it do this?	*Please specify.*		
Is the work addressing a topic that requires urgent or timely data to aid decision-making?		☐	☐	*Please specify.*
If YES	What is the rationale for this?	*Please specify.*		
Is the work addressing data gaps in statistics?		☐	☐	*Please specify.*
If YES	Which ones?	*Please specify.*		
Will your work effectively communicate findings so that public benefit can be maximised across different audiences who may engage with your project results?		☐	☐	*Please specify.*
If YES	What communication methods and channels will you use to ensure this?	*Please specify.*		
Does your project approach uphold the principles of trustworthiness, quality and value in statistics?		☐	☐	*Please specify.*
If YES	In what way?	*Please specify.*		

Data security and confidentiality

Data security and confidentiality	YES	NO	Description	Document available	Document available (tick if yes)
Does your activity involve processing of personal data?	☐	☐	*Please provide information as requested below.* *1) Details of the technical and organizational measures to safeguard the rights and freedoms of the participants/data subjects. These may include:* • *Project specific data protection policy and/or the contact details of the data protection officer (these must be provided to the participants)* • *The security measures to prevent unauthorised access to personal data* • *Anonymisation /pseudonymisation techniques.* *2) Provide details of the informed consent procedures with regard to the data processing (if relevant).*	1) Informed consent forms and information Sheets (if relevant).	☐
				2) Data management plan (if relevant).	☐

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Data security and confidentiality		YES	NO	Description	Document available	Document available (tick if yes)
				3) Provide explanation as to how all of the processed data is relevant and limited to the purposes of the project ('data minimisation' principle) *4) Provide justification of why personal data will not be anonymised/ pseudonymised (if relevant).* *5) Provide details of the data transfers (type of data transferred and country to which data are transferred).*	3) Data protection impact assessment (if relevant).	☐
If YES	Does it involve the processing of special categories of personal data (e.g. sexual lifestyle, ethnicity, genetic, biometric and health data, political opinion, religious or philosophical beliefs)?	☐	☐	*1)Provide justification for the processing of special categories of personal data (if relevant).* *2) Provide justification to why the project objectives cannot be reached by processing anonymised/ pseudonymised data (if applicable).*		

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Data security and confidentiality			YES	NO	Description	Document available	Document available (tick if yes)
	If YES	Does it involve processing of genetic, bio-metric or health data?	☐	☐		1) Declaration confirming compliance with the laws of the country where the data were collected.	☐
	Does it involve profiling, systematic monitoring of individuals, or processing of large scale of special categories of data or intrusive methods of data processing (such as, surveillance, geolocation tracking etc.)?		☐	☐	*1) Provide Details of the methods used for tracking, surveillance or observation of participants.* *2) Provide details of the methods used for profiling.* *1) Provide assessment of the ethics risks related to the data processing operations.* *2) Provide explanation as to how the rights and freedoms of the participants/data subjects will be safeguarded and harm will be prevented.* 3) *Provide explanation as to how the data subjects will be informed of the existence of the profiling, its possible consequences and how their*	1) Opinion of the data controller on the need for conducting data protection impact assessment under art 35 GDPR. (if relevant).	☐

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Data security and confidentiality	YES	NO	Description	Document available	Document available (tick if yes)
			fundamental rights will be safeguarded.		
Does your activity involve further processing of previously collected personal data (including use of pre-existing data sets or sources, merging existing data sets)?	☐	☐	*1) Provide details of the database used or of the source of the data.* *2) Provide details of the data processing operations.* *3) Provide explanation as to how the rights of the participants/data subjects will be safeguarded.* *4) Provide explanation as to how all of the processed data is relevant and limited to the purposes of the project ('data minimisation' principle)* *5) Provide justification of why the data will not be anonymised/ pseudonymised (if relevant).*	1) Confirmation that the data controller has a lawful basis for the data processing and that the appropriate technical and organisational measures are in place to safeguard the rights of the data subjects.	☐
				2) Permission by the owner/manager of the data sets (e.g. social media databases) (if applicable).	☐
				3) Informed Consent Forms + Information Sheets + other consent documents (if applicable).	☐

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Data security and confidentiality	YES	NO	Description	Document available	Document available (tick if yes)
Is it planned to export personal data (data transfer) from the EU to non-EU countries?	☐	☐	*1) Provide details of the types of personal data and countries involved.* *2) Provide explanation as to how the rights and freedoms of the participants/data subjects will be safeguarded*	1) Confirmation that data transfers will be made in accordance with Chapter V of the General Data Protection Regulation 2016/679.	☐
Is it planned to import personal data (data transfer) from non-EU countries into the EU or from a non-EU country to another non-EU country?	☐	☐	*1) Provide details of the types of personal data and countries involved. 1) Confirmation of compliance with the laws of the country in which the data was collected.*	1) Confirmation of compliance with the laws of the country in which the data was collected.	☐
Is it planned to use Artificial Intelligence in your project/activity?	☐	☐	*1) Provide details of type of data artificial intelligence will be processed by AI* *2) Provide mathematical, technical, and functional details of the AI models, software infrastructure. Different use cases, limitations of the AI models and how to avoid pitfalls.*	1) Study protocols or DMP (or both).	☐

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Data security and confidentiality		YES	NO	Description	Document available	Document available (tick if yes)
If YES	Are you going to inform participants about the use of AI?	☐	☐	*1) Provide explanation on how the participants and/or end-users will be informed.* *2) Provide details on the content of the documentation provided to end-users.*	1) Informed Consent Forms + Information Sheets + other consent documents (if applicable).	☐
	Is there any measure taken to avoid bias in input data and algorithm design?	☐	☐	*1) Provide details on how AI system will be developed and on the type of training data.* *2) Provide details on the analysis of measure distribution, noise, data generality of training data.*		☐
	Will the AI model contain data and parameters sensitive to people's personal and professional life?	☐	☐	*1) Provide details on type of data.*	1) Study protocol and DMP.	☐
	Have you assessed the main ethical risks for the use of AI technology?	☐	☐	*1) Provide details on the ethical risks foreseen for the use of AI technology.* *2) Provide details on how the risks are mitigated.*	1) Risk Management documents, Study protocol and DMP.	☐

Methodological Quality

Methodological Quality	YES	NO	Description	Documents to be kept on file and provided on request	Document available (tick if yes)
Is the activity conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with GCP and the applicable regulatory requirement(s).	☐	☐	*1) Details on applicable requirements.*		
Is the activity supported by non-clinical and clinical information available as state of the art and acquired during the first steps of the project?	☐	☐	*1) Provide a summary of available data*		
Is the activity conducted with products manufactured, handled and stored in accordance with applicable Good Manufacturing Practice (GMP) and used in accordance with the approved protocol.	☐	☐	*1) Provide a list of products used within the process*	1) A Material Safety Data Sheet (MSDS), Certificate of Analysis (COA), Study protocol or any other applicable.	☐
Is the activity conducted in compliance with recognised standards of data integrity and quality.	☐	☐	*1) Provide details on the applicable standards*		
Is the activity conducted by researchers skilled in the chosen methodology.	☐	☐	*1) Provide name and job title of the team members involved in this activity*	1) Team members Curricula Vitae.	☐
Does your activity involve interventions (physical also including imaging technology, behavioural treatments, tracking	☐	☐	*1) Specify which type of intervention*		

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Methodological Quality		YES	NO	Description	Documents to be kept on file and provided on request	Document available (tick if yes)
and tracing, etc.) on the study participants?						
Does your activity involve the use of human cells or tissues?		☐	☐	*Provide details on type of human cells or tissue and how they are going to be used*		
If YES	Are they available commercially?	☐	☐	*1) Details on cell types and provider (company or other).*	1) Copies of import licences (if relevant).	☐
	Are they obtained within this project?	☐	☐	*1) Details on cell types including the source of the material, the amount to be collected and the procedure for collection.* *2) Details on the duration of storage and what will be done with the material at the end of the activity.* *3) Confirmation that informed consent has been obtained.*	1) Copies of ethics approvals.	☐
					2) Informed consent forms and information sheets.	☐
	Are they obtained from another project, laboratory or institution?	☐	☐	*1) Details on cell types.* *2) Country where the material is stored.* *3) Details of the legislation under which material is stored.* *4) Details on the duration of storage and what will you do with it at the end of the project?* *5) Name of the laboratory/institution.* *6) Country where the laboratory/institution is located.* *7) Confirm that the material is fully anonymised or that consent for secondary use has been obtained.*	1) Authorisation by primary owner of cells/tissues (including references to ethics approvals).	☐
					2) Copies of import licences (if relevant).	☐
					3) Statement from the primary laboratory/institution that informed consent has been obtained.	☐

Legal/regulatory compliance

Legal/regulatory compliance	YES	NO	Description
Are the activity and methods employed consistent with Global legal requirements set up in ECR?	☐	☐	*1) Specify which are the Global requirements applicable to the activity.*
Are the activity and methods employed consistent with European legal requirements set up in ECR?	☐	☐	*1) Specify which are the European requirements applicable to the activity.*
Are the activity and methods employed consistent with National legal requirements set up in ECR?	☐	☐	*1) Specify which are the National requirements applicable to the activity. If not applicable put N/A*

Public Views and Engagement

Public Views and Engagement		YES	NO	Description
Is the public widely supportive of the project aim and method?		☐	☐	
If YES	Does the research involve regular engagement with the public and/or stakeholders?	☐	☐	*1) Specify how the research involve engagement with the public and/or stakeholders.*
	Do activities' findings reflect the experiences and opinions of the participant group?	☐	☐	*1) Specify how findings reflect the experiences and the opinions of the participant group.*

Transparency

Transparency		YES	NO	Description of the required characteristic	Documents to be kept on file and provided on request	Document available (tick if yes)
Does your activity involve human participants?		☐	☐			
If YES	Are they volunteers?	☐	☐	*1)Provide details on recruitment, inclusion and exclusion criteria and informed consent procedures.* *2)Provide details on unexpected findings policy.*	1) Copies of ethics approvals (if required by law or practice).	☐
					2) Informed consent forms and information sheets.	☐
	Are they healthy volunteers for medical studies?	☐	☐	*1) Details of the recruitment, inclusion and exclusion criteria and informed consent procedures.* *2) Details on incidental findings policy.*	1) Copies of ethics approvals (if required by law or practice).	☐
					2) Informed consent forms and information sheets.	☐
	Are they patients for medical study?	☐	☐	*1) Details on the disease/condition /disability*	1) Copies of ethics approvals (if required by law or practice).	☐

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Transparency		YES	NO	Description of the required characteristic	Documents to be kept on file and provided on request	Document available (tick if yes)
				2) Details on the recruitment, inclusion and exclusion criteria and informed consent procedures. *3) Details on incidental findings policy*	2) Informed consent forms and information sheets.	☐
	Are they potentially vulnerable individuals or groups?	☐	☐	*1) Details on the type of vulnerability.* *2) Details of the recruitment, inclusion and exclusion criteria and informed consent procedures.* *3) Procedures to ensure participants are not subject to any form of coercion and undue inducement.*	1) Copies of ethics approvals.	☐
					2) Informed consent forms and information sheets.	☐
Are informed consent form and information sheet required for your activity?		☐	☐			
If YES	Are they written in a language and in terms involved persons can fully understand?	☐	☐			
	Do they describe the aims, methods and implications of the project activity, the nature of the	☐	☐			

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Transparency		YES	NO	Description of the required characteristic	Documents to be kept on file and provided on request	Document available (tick if yes)
	participation and any benefits, risks or discomfort that might ensue?					
	Do they explicitly state that participation is voluntary and that anyone has the right to refuse to participate and to withdraw their participation, samples or data at any time — without any consequences?	☐	☐			
	Do they state how biological samples and data will be collected, protected during the project and whether they will be destroyed or reused afterwards?	☐	☐			
	Do they state what procedures will be implemented in the event of unexpected or incidental findings?	☐	☐			
	Are there other persons unable to give informed consent?	☐	☐	*1) Details on the procedures for obtaining consent from the guardian/legal representative.* *2) Procedures to ensure participants are not subject to any form of coercion and undue inducement.*		
Will research outcomes be openly available to the public?		☐	☐			
If YES	How will research outcomes be disseminated?			*1)Details on activity's dissemination plan*		

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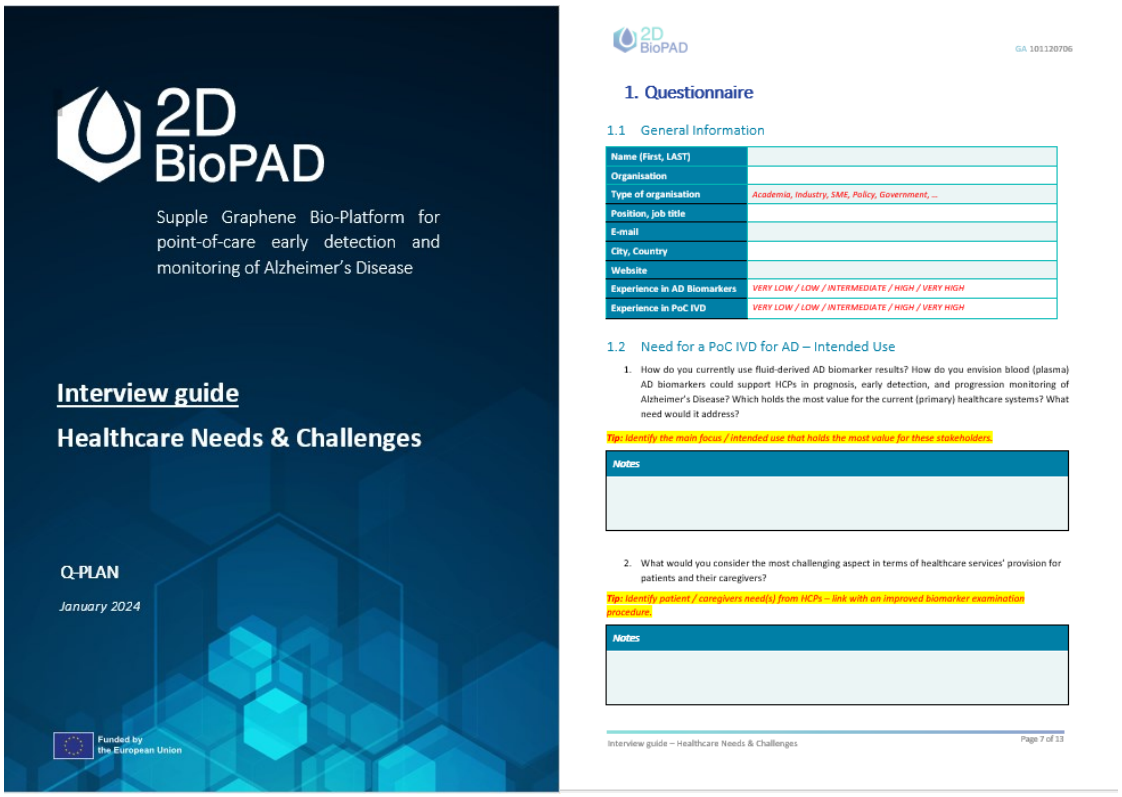
Need for self-assessment revision/addition

Need for self-assessment revision/addition	YES	NO	Reason for self-assessment revision/addition	Expected timepoint
Do you expect to make an ethics self-assessment again at a later stage in the project i.e., revision/addition to the ECR.?	☐	☐		

Annex IV – Interview Guide



2D BioPAD

Supple Graphene Bio-Platform for point-of-care early detection and monitoring of Alzheimer's Disease

Interview guide

Healthcare Needs & Challenges

Q-PLAN

January 2024

Funded by the European Union

2D BioPAD GA 101120706

1. Questionnaire

1.1 General Information

Name (First, LAST)	
Organisation	
Type of organisation	*Academia, Industry, SME, Policy, Government, …*
Position, job title	
E-mail	
City, Country	
Website	
Experience in AD Biomarkers	*VERY LOW / LOW / INTERMEDIATE / HIGH / VERY HIGH*
Experience in PoC IVD	*VERY LOW / LOW / INTERMEDIATE / HIGH / VERY HIGH*

1.2 Need for a PoC IVD for AD – Intended Use

1. How do you currently use fluid-derived AD biomarker results? How do you envision blood (plasma) AD biomarkers could support HCPs in prognosis, early detection, and progression monitoring of Alzheimer's Disease? Which holds the most value for the current (primary) healthcare systems? What need would it address?

Tip: Identify the main focus / intended use that holds the most value for these stakeholders.

Notes

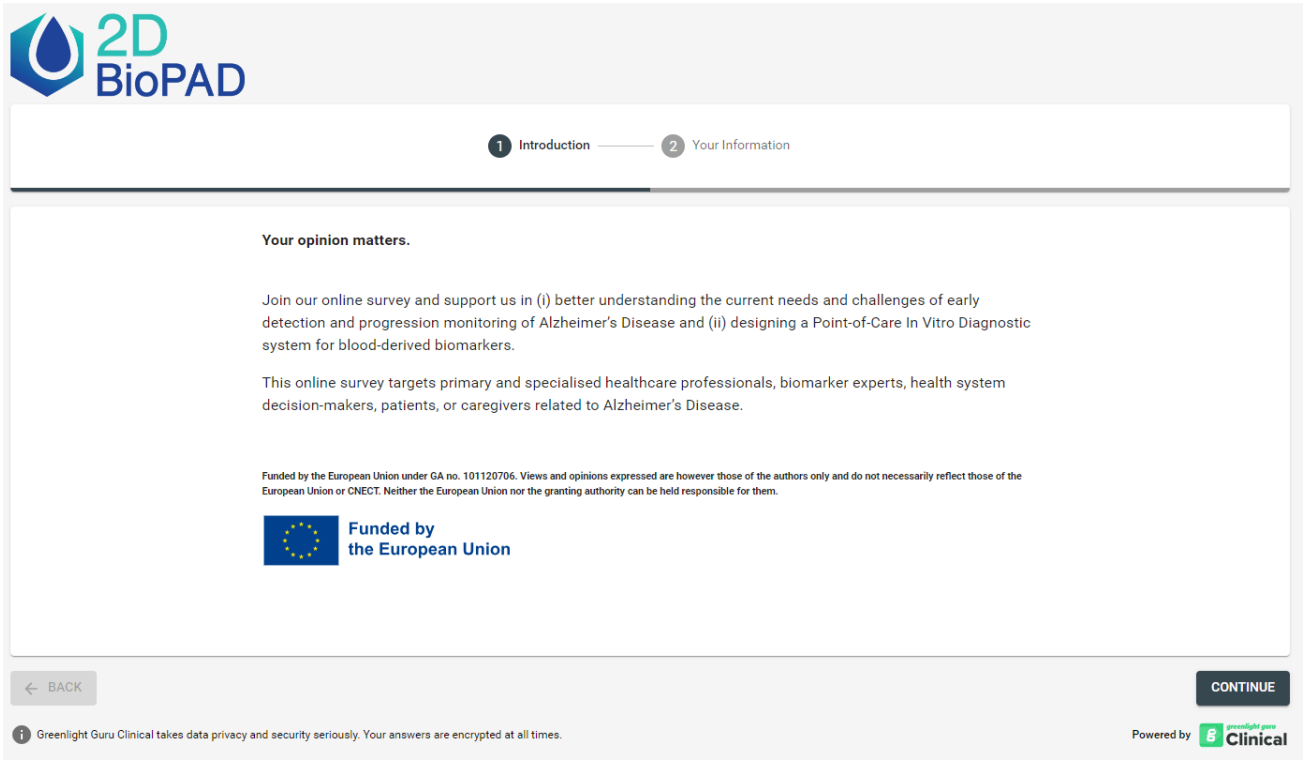
2. What would you consider the most challenging aspect in terms of healthcare services' provision for patients and their caregivers?

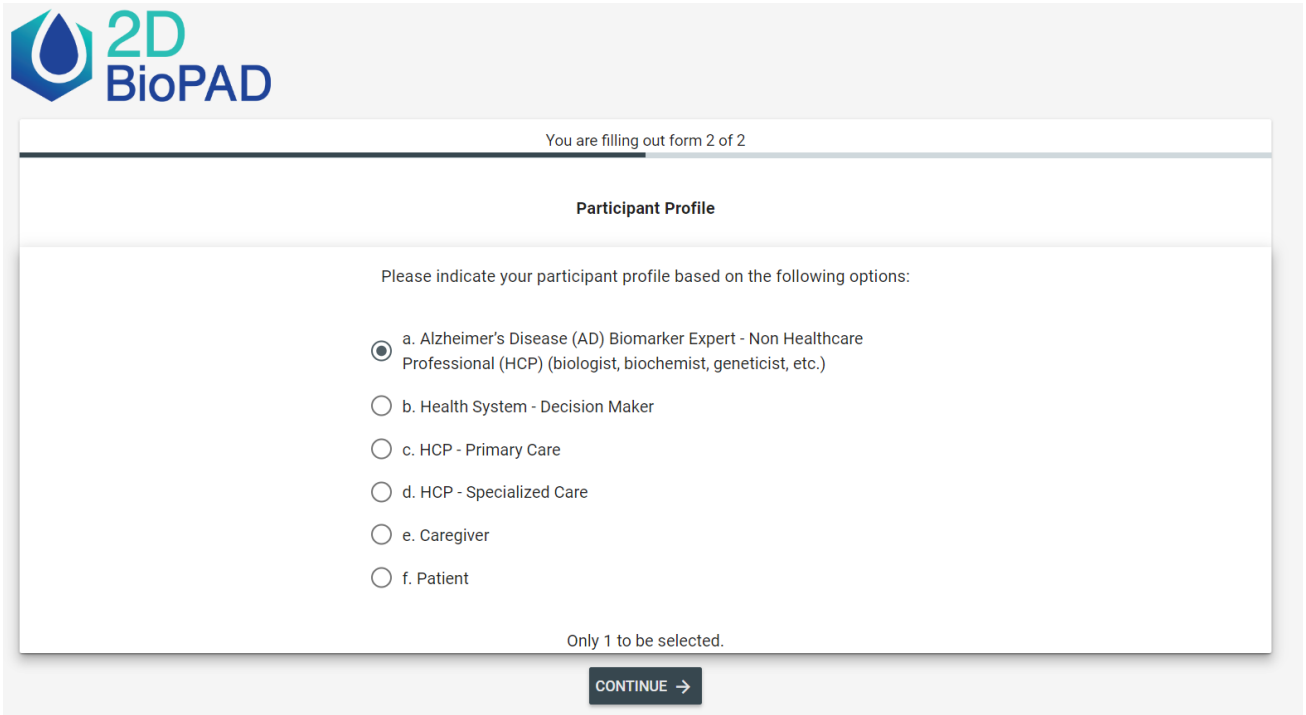
Tip: Identify patient / caregivers need(s) from HCPs – link with an improved biomarker examination procedure.

Notes

Interview guide – Healthcare Needs & Challenges Page 7 of 13

Annex V – Online Survey





Annex VI - Demographic Characteristics per participant profile

	Patients (N=10)		Caregivers (N=29)		Decision Makers (N=13)		HCPs Primary (N=8)		HCPs Specialized (N=18)		Biomarker Experts (N=12)	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
English Level												
Native or near-native	0	0%	3	10.34%	1	7.69%	0	0%	2	11.11%	2	16.67%
Fluent	3	30%	17	58.62%	10	76.92%	4	50%	11	61.11%	8	66.67%
Basic	5	50%	9	31.03%	2	15.38%	3	37.5%	5	27.78%	2	16.67%
Very limited	2	20%	0	0%	0	0%	1	12.5%	0	0%	0	0%
Age												
18-24	0	0	1	3.45%	0	0%	0	0%	2	11.11%	0	0%
25-34	0	0	3	10.34%	5	38.46%	1	12.50%	4	22.22%	4	33.33%
35-44	0	0	3	10.34%	3	23.08%	1	12.50%	5	27.78%	4	33.33%
45-54	1	10%	10	34.48%	3	23.08%	6	75%	6	33.33%	3	25%
55-64	1	10%	11	37.93%	2	15.38%	0	0%	0	0%	1	8.33%
65-74	4	40%	1	3.45%	0	0%	0	0%	1	5.56%	0	0%
75 and over	4	40%	0	0%	0	0%	0	0%	0	0%	0	0%
Main Country of Residence												
Czech Republic	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Denmark	0	0%	0	0%	1	7.69%	0	0%	0	0%	0	0%
Finland	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
France	0	0%	0	0%	0	0%	0	0%	0	0%	2	16.67%
Germany	0	0%	1	3.45%	2	15.38%	0	0%	4	22.22%	1	8.33%
Greece	10	100%	26	89.66%	8	61.54%	8	100%	14	77.78%	2	16.67%
Ireland	0	0%	1	3.45%	1	7.69%	0	0%	0	0%	0	0%

	Patients (N=10)		Caregivers (N=29)		Decision Makers (N=13)		HCPs Primary (N=8)		HCPs Specialized (N=18)		Biomarker Experts (N=12)	
Spain	0	0%	0	0%	0	0%	0	0%	0	0%	4	33.33%
Other*	0	0%	1	3.45%	1	7.69%	0	0%	0	0%	3	25%
Education Level												
Primary education	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
High-school degree	1	10%	1	3.45%	0	0%	0	0%	0	0%	0	0%
Occupationally-specific program	1	10%	1	3.45%	1	7.69%	0	0%	0	0%	0	0%
Bachelor's degree	6	60%	12	41.38%	2	15.38%	0	0%	0	0%	1	8.33%
Master's degree	1	10%	7	24.14%	2	15.38%	3	37.50%	7	38.89%	2	16.67%
Philosophy Doctorate (PhD)	1	10%	4	13.79%	3	23.08%	1	12.50%	0	0%	2	16.67%
Post Doctoral (Post-Doc)	0	0%	2	6.90%	1	7.69%	0	0%	1	5.56%	7	58.33%
Medical degree	0	0%	1	3.45%	2	15.38%	3	37.50%	6	33.33%	0	0%
Internship	0	0%	1	3.45%	0	0%	0	0%	3	16.67%	0	0%
Residency	0	0%	0	0%	2	15.38%	1	12.50%	1	5.56%	0	0%

*Other countries of residence include three Biomarker experts from Malta, Poland, and the USA. Additionally, one Decision maker was from Austria, and one Caregiver was from the UK.

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