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New Frontiers in Alzheimer's Disease Diagnosis

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1. Introduction

Alzheimer's disease (AD) is the most prevalent form of dementia accounting for 60-70% of all cases worldwide. As the world's population ages the incidence of AD is expected to increase rapidly turning into a global epidemic disease with incalculable sociological and economic consequences. In 2006, the prevalence of AD worldwide was calculated in 26.6 million and it is estimated that by 2050 current prevalence will be triplicated or quadruplicated, affecting 1 out of 85 persons worldwide [1, 2]. An accurate diagnosis and a timely detection are critical for improving the physical, clinical, emotional and financial impacts of the disease. However, this aim is far to be achieved and several studies indicate that less than 35 percentage of people living with AD or related dementias are correctly diagnosed [3, 4]. As a consequence, between 18% and 67% of the dementia patients are treated with a potentially inappropriate medication [5].

In this dramatic scenario, new technical, methodological and notional approaches are explored in order to overcome the inherent limitations in AD clinical diagnosis. Indeed, the identification of reliable diagnostic tools in AD remains impeded by the clinical, neuropathological and molecular overlap existing between AD and other types of dementia such as Mild Cognitive Impairment (MCI), or mixed forms of dementia, such as Vascular Dementia (VaD), Frontotemporal Lobar Degeneration (FTLD) or Lewy Body Dementia (LBD), and by the high AD heterogeneity according to disease onset, progression and duration [6-8].

Since the complexity of this scenario impairs the use of current diagnostic tools for a correct data interpretation, in the recent years, new strategies such as the integrated and combined use of neuropsychological profiles, imaging and biological fluids biomarkers have been developed, improving current diagnosis classification [9-11] and predicting the conversion from MCI to AD [12, 13].

Despite recent solid advances in the topic, up to date, no single diagnostic tool or combination of diagnostic tools can unequivocally confirm AD diagnosis. Indeed absolute confirmation and definite AD diagnostic still requires histopathologic analysis on the post-mortem brain certifying the presence of the pathologic disease hallmarks such as β -amyloid plaques and neurofibrillary tangles.

Since AD is a progressive disease and no treatment is available to recover neuronal integrity, the inaccuracy of AD early diagnosis and prognosis makes early therapeutic intervention difficult and impedes the prevention of neurodegeneration and cognitive dysfunction.

Identification of disease specific clinical, imaging and biochemical-based tools at early stages will help to greater extent to an early treatment which may restrain the disease progression. Additionally, a thorough understanding of the role of biomarkers in AD disease and their modulated levels in AD patients will facilitate the comprehension of their role in AD etiopathology and would help to establish a link between diagnostic and therapeutic fields. Therefore, the ultimate goal is to develop early and reliable diagnosis methods to establish an appropriate and prompt treatment. Indeed this aspect is imperative to maximize the efficiency of potential therapies and decrease symptomatology before pathological changes spread throughout the brain and massive death of neurons has already occurred. Finally, it should also be taken into consideration that the development of successful epidemiological risk assessment and diagnosis programs, including a routinely monitoring of disease progression, will need to be established through the development of new methodologies and protocols at low cost and with non-invasive approaches.

The present chapter summarizes the most recent findings in the field of AD, including neuropsychological profiles and brain and biological fluids biomarkers, which are currently paving the way for new focused approaches in AD diagnosis and prognosis.

2. Diagnostic criteria/Clinical and research criteria

According to the International Classification of Diseases (ICD-10) and the Diagnostic and Statistical Manual of Mental Disorders of the American Psychiatric Association (DSM IV) dementia is defined as a a worsening of cognitive function from a preexisting individual level. The major symptom is decline in memory and should be followed by at least one dysfunction in another major cognitive core skill, severe enough to impair a person's ability to perform everyday activities. The cognitive impairments should be irreversible and not be attributable to e.g. a delirium or another psychiatric disorder and must be present for at least 6 month.

Moreover, the German Society of Psychiatry, Psychotherapy and Neurology (DGPPN) as well as the German Neurological Society (DGN) refer to a subtle change in personality and behavior in the process of dementia [14]. The criteria of the American National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's disease and Related Disorders Association (NINCDS/ADRDA) are more often referred to in the literature, which differentiate the degree of diagnostic accuracy in "possible" or "probable dementia" [15, 16].

Based on the latter, commonly accepted dementia criteria, a “probable Alzheimer’s dementia” (AD dementia) is diagnosed by signs of dementia on clinical examination and neuropsychological tests whereby the memory impairment should be followed by another deficit in an additional cognitive skill. In alternative there is impairment in two cognitive skills with a recognized progression and without evidence of a reduced consciousness.

The age at onset should range between 40 and 90 years and other reasons for the cognitive decline, e.g. treatable causes, should have been carefully ruled out in the diagnostic work up.

The clinical criteria of a “possible Alzheimer’s dementia” comprise a dementia syndrome of untypical clinical presentation or duration in absence of other recognizable factors causative of dementia, or if there is a progressive cognitive deficit without a recognized underlying cause.

Exclusion criteria are referred to as sudden onset, focal neurological signs (hemiparesis, hemianopsia) at onset as well as early appearance of gait disorders or epileptic fits.

This categorization is kept according to different revisions of the NINCDS/ADRDA-criteria [16, 17]. Additionally, next to deficits in episodic memory, detection of specific biomarkers in the cerebrospinal fluid (CSF) and imaging (Magnetic Resonance Imaging (MRI) and/or Emission Computed Tomography (PET) is suggested which can increase sensitivity of AD diagnosis.

Further supporting results are, e.g., a progressive worsening of specific cognitive function, disabling in all-day activities, and occurrence of behavioral changes, a positive family history of AD (especially if neuropathologically confirmed), a normal CSF result (basic analysis) and unspecific electroencephalogram (EEG) changes.

A diagnosis of AD is compatible with plateaus during disease course, side symptoms as depression, aggressive behavior, paranoia etc., neurological symptoms in progressed disease state (myoclonus, gait problems, epileptic fits) and a normal Computerized Tomography (CT)-scan [14].

While both terminologies: “probable AD”, “possible AD” are proposed for the clinical setting, a third category of “probable and possible AD” was suggested for research purposes. Recent research criteria for clinical AD diagnosis include next to mnemonic deficits an occurrence of deficits in non-mnemonic function, e.g., language, visual-spatial orientation, executive function. Furthermore, an early diagnosis of AD is proposed already during prodromal stages of dementia, which refer to the clinical picture of a mild cognitive impairment (MCI) [18].

A MCI is a recognized risk factor for AD. Yet, there are presently no commonly agreed criteria [14]. According to international consensus criteria, MCI is considered a condition between normal and demented, a worsening of cognitive function (on self-observation or observation by others) that can be demonstrated on neuropsychological tests, a worsening of cognitive function during an observational time period during disease as well as conserved or only minimally impaired dysfunction in complex all-day activities [19]. The difference between MCI and dementia is based mostly on well-functioning in all-day activities. Standard meas-

measurements for cognitive function comprise 1-1.5 standard deviation below the age- and education-matched age group and a mini-mental status test of 24 or above points [18, 20].

The prevalence and conversion rates are variable according to the distinct examination setting. In the clinical setting the annual conversion rate from MCI to AD has been calculated at around 10 percent [14, 21].

At present 4 different MCI subtypes are characterized: amnesic single domain, amnesic multiple domains, non-amnesic single domain and non-amnesic multiple domains [20], whereas the probability to develop AD is highest in MCI with memory deficits [14, 21].

3. Neuropsychological profiles

3.1. The neuropsychological profile of AD

AD is generally characterized by a slowly progressive preclinical (pre-symptomatic) state over several years, an approximately 1-2 years lasting pre-dementia phase until development of dementia, which can be categorized into 3 states (mild, medium, severe) [22, 23].

The progressive cognitive deficits hereby parallel neuropathological changes in the brain, whereby cognitive deficits vary individually. The degree of disease severeness refers to cognition and life skills, whereby transition of states can merge. A mild dementia is considered when complex tasks cannot be performed anymore, but an independent life organisation is still possible. A medium-state dementia is referred to if an independent life organisation is impaired but possible with help and observation of family and care-givers. In severe dementia constant guidance and help is required, an independent life organisation is not possible anymore.

At early stages of AD deficits are predominantly characterized by impairment of declarative memory, visual-spatial orientation and lexical-semantic language. Emotionally, in social contacts as well as in personality, patients with AD appear normal for a long period of time ("facade"). They tend to trivialize their deficits. When they recognize cognitive dysfunction, AD patients often describe themselves to be more forgetful without further specification.

Memory impairment (representative brain areas: hippocampus, gyrus parahippocampalis and adjacent temporomedial areas) affects the ability to encode and recapitulate novel memory contents for a longer period of time, whereas the short time memory and the working memory are mostly unaffected in early stages. The procedural memory often keeps unaffected. In the clinical setting progressive memory deficits often appear in forgetfulness of novel information, in repetitive phrases, difficulties in maintaining complex tasks (strands), e.g. forgetting where the keys/money have been stored, which can lead to paranoid reactions. Neuropsychological characterisations are a slow learning curve, rapid forgetfulness, recency-effects due to deficits in encoding, impaired and prolonged memorising, intrusions and a reduced discrimination-ability, non-profit of context cues as well as deficits in orientation in time [22].

In further disease progression according to a time-associated gradient (first in- last out) also long term memories (semantic and biographical memory) are impaired, with affection of identity and personality in medium and severe AD stages [23].

Deficits of the *visual-spatial orientation* (representative brain areas: parietal lobes) are often associated with important all-day activities: writing, calculating, reading the clock, getting dressed or basic orientation in space. This can overlap with memory deficits and deficits in planning skills. Firstly affected are untrained complex skills, e.g., drawing, clock drawing (mispositioning of the minute hand, confusion of hour/minute hand), reading street maps, orientation in unknown buildings, filling in documents. Drawings can show simplifications, repetitions, altered angles, "closing-in" and loss of perspective. Well established and trained skills, e.g., reading, signing a paper, getting dressed, are mainly affected in medium disease stage. A sensitive parameter that can be valuable in early AD diagnosis but also as a parameter of disease progression is clock reading as a trained skill [24, 25].

Deficits in visual-spatial orientation with massive impairment in complex visual awareness are the main characteristic and leading symptom of the *posterior cortical atrophy* (affected brain region: atrophy of the parietal and occipital lobe). The posterior cortical atrophy is a recognised variant of AD with early onset, early visual agnosia and prosopagnosia, whereas memory is less impaired in the beginning [23]. Depending on the affected projection system (occipital-parietal or occipital-temporal) problems of analysing visual-spatial information: e.g., space, depth, movement, position and orientation (dorsal visual route/"where-system") or problems in analysing of shapes/structures, colours, objects, faces and complex space-topographical scenes (ventral visual route/"what system") can occur. Both systems are tightly connected [26, 27].

Affection of *language* (affected brain region: Wernicke area) is characterized by initial difficulties in finding the right words, which is compensated by strategies of avoidance and paraphrases as well as by difficulties in naming of less frequently used objects. The patients tend to make semantic-superior and semantic-associative mistakes (dog=animal, pyramid=Egypt or also volcano=vesuvius). Syntax, articulation and prosody are unaffected.

Material that they read is less often understood, the understanding of complex facts or contents in the figurative sense (collocations) is declining. Verbal fluency is reduced, whereas the semantic is more affected than the phonematic [28]. During disease, language becomes progressively poor of content, stays however relatively fluent with difficulties in word finding as well as with imprecise, diffuse and less informative comments, drifting from topic, talking cross purposes and setting phrases. This results in abrupt sentences, mistakes of syntax, phonematic paraphrases and in problems of speech comprehension for simple comments. In the final stages a total loss of speech occurs [23].

Next to the 3 main symptoms, disturbances in *executive function* (affected brain region: prefrontal cortex) appear. Executive functions comprise: problem solving thinking, monitoring, planning and conducting of complex tasks, working memory, cognitive fluidity and flexibility. Besides a reduced word fluidity and flexibility also abilities in planning can be

impaired early. Especially the so-called set-shifting abilities, that require a permanent shift in alertness, are affected at early stages [29].

Attention is tightly associated with executive function. This is especially required in complex tasks. Deficits in attention initially present very discretely, e.g. in dual task-questions (pre-frontal cortex, anterior cingulum).

During disease progression, also alertness is impaired which presence of a more rapid exhaustion.

During medium stages all components of attention are majorly affected [22]. Last but not least, apraxia (affected brain region: parietal lobe) and agnosia (affected brain region: occipital lobe and both basal temporal neocortex) can occur already during early and middle stages of dementia. Simple movements are not possible any longer, inaccurate moves cannot be corrected, this can present as, e.g., body-part-as object-mistakes (ideomotoric apraxia), impairment of planning and conducting of sequential tasks (ideatoric apraxia), recognition of line drawing is inhibited.

Cognitive-related *impairment of all-day activities* affects complex instrumental skills in early stages of dementia, e.g. using new instruments, filling in written documents, later on using familiar devices and basal all-day abilities deteriorate progressively.

Psychiatric side symptoms such as anxiety, agitation, excitability, aggressive behaviour or paranoia are not frequently present in early stages, but appear more often in middle and late stages of disease. There is a higher vulnerability for states of disorientation already in the preclinical stage, e.g. after hospital admissions, drug intolerance, malnutrition. Also depressive mood changes as well as reduction of daily activities are considered early signs [23].

Depression is the most frequent psychiatric side symptom and accounts for about 30% of the patients, especially during early disease stage and here from the degree of presentation rather mild. However, depression is considered a main psychiatric disease in the elderly. In general, depressed patients can articulate their symptoms more precisely; they can manage their all-day activities in a better way and demonstrate during neuropsychological testing self-doubts and complain about deficits in concentration. The mood is continuously suppressed and a lack of motivation is more exhibited.

The onset of cognitive deterioration is more distinct in patients with depression, whereas in patients with AD this occurs more gradually. The deficits can affect the whole spectrum of cognition, whereby executive dysfunction (predominantly flexibility) and problems with attention dominate. However, also memory deficits are described [30]. In detailed observation of single tasks aspects, e.g. in recalls of wordlists, primacy more than recency effects are shown, and recall is generally better.

While demented patients guess more often and describe things, depressed patients react with omissions and hesitant answers. Orientation is widely intact and confabulation, aphasic and apractic elements don't occur [22, 31].

3.2. The neuropsychological examination

Major tasks and aims of a neuropsychological examination comprise 1) determination and quantification of impaired cognitive function and resources as well as their consequences for maintaining all-day life, 2) assessment on changes of cognitive dysfunctions in progressive or reversible disease conditions, 3) differential diagnosis and securing of diagnosis as well as 4) evaluation of therapeutic benefits.

An important detail of the examination is a thorough interview with exploration of the clinical history, self-observation and observation of others, orientation, current mood situation (psychiatric side symptoms), as well as observation of behaviour during both interview and test situation. A final judgement is built from the test results with reference to emotional and motivational processes, a qualitative mistake analysis, and observation of behaviour during tests and interview, the resulting information derived from the interview and an evaluation of all-day competences during course of disease.

Neuropsychological testing represents an essential diagnostic tool in dementia diagnosis. It should be thoroughly performed and comprise the essential key competences. An "overtesting" should be avoided. In general, the choice of tests should orientate according to the individual differential diagnosis that is being questioned, the capacity of the individual patient and the time that is available.

Consecutively, a choice of test procedures is presented, that have been established in dementia diagnosis. As some of them cannot be administered solely for securing the diagnosis, a combination of several test procedures should be used.

For assessment of cognitive deficits in AD both screening methods as well as standardized psychometric tests are applied. Presumably, the most practical screening test in the clinical setting is the MMSE (Mini-Mental-State-Examination) according to Folstein et al. [32]. It comprises the examination of orientation in time and space, retentiveness and memory, attention and working memory, language (reading, writing, naming, speech comprehension, reading and meaning comprehension) as well as visual-spatial competences. The test takes usually approximately 10-12 minutes, the analysis results from a simple summation of points. At maximum 30 points can be achieved. The specificity ranges at 87 percent and the sensitivity at 82 percent [33]. However screening tests- as the mentioned MMSE- are only suitable, using cut off levels, for overviewing and determining severity of the dementia and for follow-up during disease course.

The MMSE is not acceptably sensitive in early onset dementia and does not allow, amongst other due to missing age and education correction, a satisfactory differentiation between "healthy" and "ill". For quantification of disease severity standard values of interpretation are provided, that can vary easily. Alternatively also CDR (Clinical Dementia Rating) or GDS (Global Deterioration Scale) can be applied. A general drop in points of around 3 MMSE points per year substantiates the suspected diagnosis of AD [34].

The *DemTect* (*Dementia Detection Test*), likewise a screening test, focuses more precisely on Alzheimer-specific impairments with its task of word-list learning and delayed recall.

Furthermore it comprises more tasks on executive functions (working memory, word fluidity and cognitive flexibility). At maximum 18 points can be obtained. The DemTect is economic in time (8-12 minutes), it encloses a rough age correction ($< 60 / \geq 60$ years) and presents with a high sensitivity for early stage AD and MCI [35].

After introduction in 1986 in the USA from the *Consortium to Establish a Registry for AD*, the newly established *CERAD test battery* has received great acceptance also in German-speaking countries. This novel neuropsychiatric testing tool has developed into a standardized dementia test procedure which aims to decipher cognitive dysfunction typical of AD [34, 36]. Analysed skills are: semantic fluidity (naming animals), naming of black and white drawings, verbal compliance and retentiveness (word list), delayed recall and recognition as well as constructive praxis (to copy something) and figural memory. The test battery also includes the MMSE. The results of a huge multicentre-validation study performed in German-speaking countries ($n=1100$), show that the variables: verbal fluidity, word list, memory, recall of wordlist, discrimination ability and recall of constructive praxis majorly contributed to the discriminability from healthy elderly persons to AD patients with a sensitivity of 87 percent and a specificity of 98 percent. Severe differences in profiles of AD patients, patients with vascular dementia and mixed dementia could not be obtained. A better discrimination was attained between AD patients, patients with depression and mild impairments. Both patients with depression and MCI ranged between Healthy and AD [36, 37].

To trace better on subcortical dysfunction, since 2005 additional tasks were included that aim to quantify on cognitive processing speed and flexibility (Trail making test A and B) as well as phonematic word fluidity tasks (words with initial letter "s") (CERAD-Plus). The whole test duration ranges between 30-45 minutes. The raw score are age- and education-matched (school and professional education) and also gender-matched. They are designated as z-levels as a measure of deviation to normal. The CERAD-Plus test battery allows a qualitative assessment on cognitive ability, on evaluation of disease severity and a follow up on repeated testing.

However, at present a parallel test version is not available, thus it is recommended to use an alternative test for memorising word lists when test intervals are on short-term. In suspicion of other underlying dementia causes further psychometric tests can be applied.

As an additional screening instrument for calculation of disease severity and for follow-up, the *clock-drawing test* is often recommended [38]. Next to visual-spatial abilities the test requires abilities in planning and semantic memory. The assessment includes, e.g. the integrity of the clock face, the presence of the clock hands, problems of drawing and conceptual difficulties. The sensitivity accounts for 90 percent, the specificity ranges at 56 percent. A qualitative evaluation is reasonable as well as the observation while drawing the clock face. In a qualitative feature analysis for securing the AD diagnosis in differentiation to patients with depression and healthy subjects ($n=205$, patients of a memory clinic) only errors occurred solely in patients with AD (with exception of one): in disorganised stereograms, only one clock-hand, mixing of numbers (1-12 with 12-24), mixing of minutes- and clock-hands, false or altered order of numbers and inability to write numbers [39].

In mind of the low specificity of the clock drawing test, Schmidtke et al. suggest an additional *clock reading test* with respect that it doesn't require higher executive function. The clock reading test is culture-, language-, education- and gender-independent, however shows a slight age-effect. It is easy to use and quickly analysable by a simple point system. Both in AD and LBD abnormalities are detected early and in comparison to healthy persons the sensitivity ranges at 82 percent, the specificity at 70 percent [24, 25].

In suspicion of an *apractic dysfunction*, a corresponding examination is informally possible, while allowing the patient to mimic easy gestures or mimic using distinct utensils (e.g. hammer, saw and scissors). As long as the patient is unable to perform the movements according to verbal request, one should allow him (to exclude problems with language comprehension) to imitate the demanded movements. For assessment of an ideatoric apraxia the patient is asked, e.g. to prepare a letter for shipment.

In order to examine *all-day competences* there are different tests available, e.g. the ADL-/ IADL-scale (Activities of Daily Living /Instrumental Activities of Daily Living), the Bayer-ADL-scale (Bayer Activities of Daily Living) or the FAQ (Functional Activities Questionnaire) which evaluate distinct functions partially very detailed. These tests are completed in general by relatives or by the interviewer [40-42]. Hereby, the FAQ has proven more sensitive compared to the IADL (85% to 57%) in the differentiation of "demented" and "normal". The specificity ranged at 81 percent [42].

Psychiatric side symptoms, e.g. *depression*, are assessed early during the neuropsychiatric interview. As needed additional depression scales can be used, e.g. the Geriatric Depression scale (GDS) or the Beck Depression Inventar (BDI), that are available also in short profile [43]. The input of depression scales depends on each situation and on the cognitive capacity of the patient. It should not lead to extend the usual time of the whole neuropsychiatric test situation.

4. Diagnostic imaging methods in AD

4.1. Computerized Tomography (CT)

Computerized Tomography (CT) is helpful in the detection of atrophy as well as other focal processes in brain and spinal cord, however it is not sufficient to substantiate AD diagnosis. Based on the low tissue contrast in comparison to magnetic resonance imaging (MRI), CT serves well in the diagnostic classification of dementia syndromes. Advantages compared to MRI include a shorter time of investigation, low costs and a broad distribution [44]. In addition, CT allows an uncomplicated monitoring of critically ill patients.

With progressing age, brain volume decreases due to dying neurons and decline in water content. The annual atrophy rate ranges at around 0.24 % of total brain volume and is visible by the expansion of the ventricular system [45].

In AD, patients show a progressive brain atrophy in advancing disease which lies above the age matched population. This is demonstrated by enlargement of sulci and a dilatation of the

ventricular system. Hereby, the dilatation of the ventricular system points to a subcortical tissue loss whereas the enlargement of the outer CSF interspaces points to cortical tissue loss [46]. The senso-motoric and the primary-visual cortex stay unaffected.

4.2. Magnetic Resonance Imaging (MRI)

MRI allows a high-contrast presentation of neuro-anatomical structures, pathological processes as well as of functional changes in brain activity. With progressive age a higher exchange rate of fluids exists between the ventricular system and the brain parenchyma. This is visible in T2- and Fluid-attenuated inversion recovery (FLAIR)-sequences by signal alterations in the ependyma of the anterior horns [47]. Intermittent, subcortical and central signal increases in the white matter (white-matter-lesions) increase with progressive age. Additionally brain iron accumulation can be detected in basal ganglia by increasing signal changes in T2-sequences.

Already in early AD stages MRI can display brain atrophy patterns. These can predominantly be located in the medial temporal lobe, in the hippocampus and the gyrus parahippocampalis. Also, the entorhinal cortex, the amygdala, basal ganglia as well as thalamus and the parietal cortex can be involved [44]. An important role in the early detection of AD plays the Nucleus basalis Meynert. The voxel-based morphometry (VBM) reduces the weaknesses of predominantly investigator-dependent manual volumetry [48]. Modern computer techniques allow the spatial recognition of specific brain regions or the whole brain [49]. Hereby the volume of the typically affected brain region is exactly displayed and is comparable to that of other control groups. The majority of published studies show that patients with a MCI present with a smaller hippocampal volume than healthy controls and patients with AD have a smaller hippocampal volume in comparison to patients with MCI [50]. Patients with MCI hold an elevated risk for the development of AD [51]. Typical AD changes can also occur after brain trauma and long-lasting epilepsy.

Functional MRI (fMRI) has the potential to demonstrate cerebral blood flow as well as oxygen use of certain brain areas in response to specific stimuli or while processing certain cognitive tasks.

Due to the inherent magnetic properties of blood, represented by hemoglobin and deoxyhemoglobin, different patterns of activation are visible [44].

Despite of the high spatial resolution, this method presents with a high sensitivity for minor head movements. Studies of AD patients show a decrease of activity in the hippocampus, the parahippocampal areas as well as in the parietal and pre-frontal cortex in comparison to healthy control groups. Furthermore, fMRI is useful in monitoring of medical treatment in AD patients.

4.3. Emission computed tomography (SPECT and PET)

Imaging via single photon emission computed tomography (SPECT) and positron emission computed tomography (PET) allows the detection of local hemodynamic and metabolic dysfunction. After intravenous injection of a radioactive tracer and uptake in brain, the tracer

localizes at the region of regional activity and images are taken. As the tracers often have short radioactive half life, the radioactive decay (emission of positrons) can be measured.

SPECT imaging shows the regional cerebral blood flow (rCBF) at rest by the regional uptake of glucose as an expression of neuronal activity. Hereby functional abnormalities can already be detected before symptom onset. The tracers ^{99m}Tc -HMPAO and ^{99m}Tc -ECD are mostly used in clinical practise. Due to their lipophilic character the tracers reach the cells in the first minutes after injection proportionately to rCBF [52]. The typical SPECT image in AD is characterized by a reduced rCBF in the medial and superior temporal lobes as well as in the posterior cingulum and precuneus without a reduced striatal DAT-binding [53]. Due to a very low spatial resolution of SPECT the diagnostic accuracy is lower than PET [54]. However application can be meaningful in clinical practise in order to differentiate other dementia causes.

PET imaging illustrates a regional dysfunction of glucose metabolism via application of ^{18}F -FDG. Patients with AD demonstrate here, according to SPECT, a typical nuclide-distribution pattern of neuronal loss. Over 85 % of PET diagnosed AD patients could be neuropathologically verified [53]. At early AD disease stage and before symptom onset, a temporoparietal metabolic dysfunction is visible by voxel-based (volumetric pixel) analysis. Also patients with a genetic risk for development of AD show early decreases in signal activity [55]. As PET is the most efficient method for diagnostic verification of an AD, it has meanwhile established to a standard tool in dementia research [56].

For further diagnostic approaches the tracer ^{11}C -PIB was developed, which allows detection and distribution of A β -plaques *in vivo* [57]. Next to an efficient diagnostic procedure and early disease recognition the dimension of AD dementia can be illustrated.

5. Biomarkers in peripheral tissues

Biomarkers are used as indicators of normal and pathogenic processes in a broad range of tissues, especially in peripheral tissues, which facilitates the accessibility of testing samples with minimal invasive methods. Despite substantial progress has been made in the area of biomarker development to confirm the diagnosis at early-clinical AD stages, less is known about the potential role of biomarkers in peripheral tissues in the prediction of AD [58]. Since it has been demonstrated for decades the existence of biochemical changes in the brain preceding the clinical AD onset (up to 20 years in advance) [59, 60] it is suggested that these changes may be also indirectly reflected in biological fluids. However, no tests are currently available to confirm an early AD diagnosis prior to clinical or symptomatic manifestations. The ongoing standardization efforts and quality control programs in biomarkers analysis, the development of tests in fully automated instruments, the combined detection of the well-established core biomarkers, the discovery of new regulated molecules improving current sensibility and sensitivity and the analysis of novel promising biomarkers in large independent cohorts will boost biomarker's performance and facilitate the introduction of new AD diagnosis and prognosis tests in biological fluids in clinical routine.

5.1. CSF

CSF is the prime target among biological fluids in the search of specific biomarkers related to neurological disorders. The easy accessibility to this biofluid and its singular biophysicochemical characteristics make CSF ideal for biomarkers investigation. On one hand, CSF is not a very complex fluid, being composed of a restricted amount of metabolites [61], which facilitates technical screening for regulated molecules. On the other hand, the direct contact between CSF and the extracellular space of the brain puts CSF in a valuable position to be considered as a potential indicator of the pathological processes occurring in the brain during different disease stages. This last aspect has not been analysed in depth since real comparisons and correlations are cumbersome and can only be formally made when using CSF and brain tissues from the same patients and the same disease stages.

The performance of CSF biomarkers as a diagnostic tool has greatly improved in parallel with the improvement of detection methodologies such as new generation proteomic technologies and high-throughput transcriptomic methodologies (deep-sequencing, microarrays and quantitative PCR panels), which eased and expanded the possibilities to measure full expression signature in a single assay enabling the inference of networks and biological functions associated to deregulated datasets. Indeed, current data indicate the existence of deregulated levels of proteins, peptides, small RNAs, mitochondrial DNA and a broad range of metabolites in the CSF of AD samples. In addition new outcomes are expected from worldwide undergoing large longitudinal studies in very-well defined cohorts [62].

5.1.1. Protein biomarkers

In recent years, a number of reports have exploited proteomic techniques to study the levels of selected proteins and peptides in the CSF of healthy and diseased individuals. Current data indicate that proteins and peptides such as β -amyloid ($A\beta_{1-42}/A\beta_{42}$ and $A\beta_{1-40}/A\beta_{40}$), total tau and phosphorylated tau (p-tau) meet the criteria to discriminate AD from individuals suffering from other types of dementias, as well as from healthy individuals and are considered as the core AD biomarkers [63]. According to different studies these biomarkers meet the consensus recommendations on AD biomarkers that should have >80% sensitivity and >80% specificity [64]. Importantly, core AD biomarkers molecules correlate with neuropathological hallmarks of AD, such as the presence of extracellular amyloid plaques ($A\beta$ peptides), axonal degeneration (tau protein) and neuronal tangles (p-tau).

Three main observations unveil the clinical relevance of these molecules. Firstly, their appropriate sensibility and sensitivity have been successfully validated by independent large-scale multicentre studies [65-69], although these studies also point out that biomarkers measurements present significant inter-laboratory variations [70]. Secondly, $A\beta_{42}$, tau and p-tau have been validated as predictors of AD in patients with MCI [71-74]. Lastly, longitudinal studies indicate that, at least, Tau and $A\beta_{42}$ in CSF reflect the underlying disease state in early clinical and late stages of AD.

5.1.1.1. A β peptides

A β 42 along with A β 40 is secreted into the extracellular space and biological fluids, including CSF, as consequence of the proteolytic activity of proteinases on the Amyloid precursor protein (APP). Both peptides are found in senile plaques but their intracellular production, aggregation rates and proposed pathogenic functions are significantly distinct [75-77].

A consistent decrease in A β 42 levels has been observed in the CSF of patients suffering from AD in several studies [78-80] but also in Subcortical White-matter Dementia (SWD) [81] and in Down Syndrome (DS) [82]. Reduced A β 42 levels in AD are suggested to reflect either sequestration of A β 42 in senile plaques, since A β 42 CSF levels inversely correlate with the presence of senile plaques [83], or due to non-detectable A β 42 oligomers in the assay, although alternative explanations may be plausible. In FTD, A β 42 levels are significantly lower than in control samples, but higher than in AD cases [81, 84]. A β 42 sensitivity and specificity in AD samples ranges from 78 to 100% (mean 85,6%) and from 67 to 100% (mean 88,5%), respectively [78]. A recent meta-analysis of 50 analytical studies indicates that CSF A β 42 concentrations are significantly lower in AD when compared to MCI, FTD, PD and VaD but only moderately lower when compared to LBD [85].

Contrary to what is observed with A β 42, A β 40 and A β 38 levels are not altered in the CSF of AD patients [79, 86, 87], but a significant decrease in A β 40 levels is observed in FTLD when compared to AD and control cases [88]. In addition, A β 40 levels, and more markedly A β 38 levels, are decreased in FTD when compared to control samples [89].

A growing body of evidence suggests the superior performance of A β 42/A β 40 ratio when compared to A β 42 alone using different analytic assays [79, 90, 91]. Importantly A β 42/A β 40 ratio is able to predict the conversion from MCI patients to AD when compared to cognitively stable MCI patients and MCI patients who developed other forms of dementia [79]. A β 42/A β 40 ratio is also able to discriminate better AD from VaD, LBD and non-AD dementia than A β 42 alone and equally AD from FTD and non-AD dementia than the combination of A β 42, p-tau and total tau [92]. Multiple studies also show an increased sensitivity and specificity in the use of A β ratio when compared to A β 42/tau ratio, although the performance of combined biomarker analysis in AD diagnosis and prognosis is still a matter under discussion [93-96].

In addition to the regulated levels of monomeric A β species in the CSF of AD patients, encouraging observations have been reported in the potential diagnostic and prognostic role of BACE1, one of the main enzymes involved in the pathological cleave of the APP. Several independent observations indicate the presence of higher BACE1 levels and activity in the CSF of MCI and AD samples when compared to controls [97-100]. BACE1 activity is also increased in CJD samples [101] suggesting common pathological mechanisms among both diseases. Importantly, BACE1 correlate with classical AD biomarker's profile, brain atrophy in AD cases [102] and ApoE4 genotype [99], the latter being associated with an increased A β peptide *ex vivo* production [103]. In addition, specific BACE1 inhibitors dramatically reduce the presence of A β peptides in the CSF of AD patients [104] pointing out for a direct correlation between brain A β peptide processing and A β CSF levels.

5.1.1.2. *A β oligomers*

Recent studies demonstrated the presence of increased levels of A β oligomeric species in the CSF of AD patients when compared to controls using a broad range of methodological approaches [105-110]. Indeed, the analysis of individual A β oligomeric species is gaining experimental momentum due to their potential specific role in AD pathogenesis. A β 40 oligomers levels are found to be increased in the CSF of AD patients at different disease severity stages, and a combined analysis of A β 40 oligomers and monomeric A β 42 greatly improved sensitivity and specificity to 95% and 90%, respectively [108]. Although the pathogenic role of A β 40 in AD is still under discussion A β 40 deposits have been reported both in control and AD brains [111, 112]. A β 40-positive senile plaques with amyloid core are frequently associated with microglia in contrast to A β 42-positive plaques [111], suggesting a role of microglia in the generation and aggregation of A β 40 species in diseased brain. However, the different ability of A β fibrils and oligomers to react with microglia suggests a more complex scenario [113].

A β 42 oligomers are increased in the CSF of AD patients [114] and the ratio of A β oligomers to A β 42 is significantly elevated in AD patients [115]. Interestingly, the increased levels of A β 42 oligomers in the CSF of MCI and AD samples may explain decreased levels of monomeric A β 42. The recent development of the protein misfolding cyclic amplification assay (PMCA), based on the seeding activity of A β oligomers catalysing the polymerization of the monomeric A β , permits the discrimination of AD samples from other neurodegenerative non-degenerative neurological diseases with a sensitivity of 90% and specificity of 92% [109]. The use of A β -PMCA as a prognostic tool for detection of MCI still needs to be established. Importantly, detection of A β oligomers in the CSF is highly dependent on the native or disaggregated state of these oligomers [114, 116].

The finding that regulated levels of A β oligomer species are present in the CSF of AD patients' biofluids has a tremendous translational interest, since growing evidences indicate that soluble A β oligomers rather than aggregated A β plaques are more likely to be the main pathogenic agents of disease [117-119]. Consequently, preliminary data indicate that the analysis of A β oligomers, combined with levels of soluble A β peptides, may be relevant disease predictors and valuable tools for the analysis of AD progression.

5.1.1.3. *Tau*

The levels of total tau in the CSF, contrary to A β 42 levels, increase with age [120]. Increments in tau levels have also been described in the CSF of AD and MCI patients in a broad range of several studies [121, 122] ranging from moderate to severe depending on the methodology and cohort used [78]. It is believed that deregulated tau may be reflecting the neuronal and axonal damage present in brain tissue and, as a consequence, the presence of increased tau levels is not a specific event for AD. Accordingly, transient tau increments have been also reported in acute stroke [123], and the most increased tau levels are observed in prion diseases such as in CJD, where massive neuronal cell death is present [124, 125]. Higher CSF tau is also associated with smaller brain volume in individuals with AD [126]. On the other hand, neurological diseases with minor neuronal loss and other dementias such as VaD, LBD and alcoholic

dementia reflect minor or no significant changes in the levels of tau protein in the CSF, and tauopathies such as FTD also present inconsistent data [121, 127, 128].

A meta-analysis from different studies comparing tau levels in different dementia samples found that, although tau levels in AD are significantly increased when compared to controls, tau concentrations are moderately elevated in LBD, FTLD and VaD impeding a clear stratification between disease groups. Nevertheless, tau levels are useful to differentiate VaD from stroke [129] and, as expected, only CJD is characterized by extremely increased tau values, resulting in a sensitivity and specificity over 90% [130].

The improved performance of tau when analysed together with other AD biomarkers has been widely demonstrated [131, 132]. The combined use of A β 42 and tau discriminates better between controls and AD and is very useful to predict MCI progression [69, 133]. A recent study also showed that decreased A β 42 and increased tau levels are able to discriminate LBD from PD patients in spite of both being synucleopathies [134]. In the same line of evidences, combination of α -synuclein levels and A β 42/tau ratios improves the diagnostic accuracy of PD [135].

A broad range of studies also demonstrated the helpfulness of the combined analysis of tau with non-AD core biomarkers. Assessment of tau and neuronal thread protein raises specificity and sensitivity for AD when compared to the individual analysis of both proteins [136]. Similarly, integrated analysis of tau and the regional cerebral blood flow in the posterior cingulate cortex discriminates MCI progressing to AD from non-progressive MCI [137]. The combined analysis of tau is also valuable for discriminating other diseases besides AD. As an example, the merged analysis of tau and midbrain-to-pons atrophy is reported to be useful for early identification of progressive supranuclear palsy (PSP), discriminating PSP cases from controls and patients suffering from corticobasal syndrome (CBS) and FTD [138].

5.1.1.4. *Phospho-tau*

Similarly to total tau, p-tau levels are increased in AD samples, although higher variability on its specificity and sensibility is reported when compared to the non-phosphorylated tau form [78, 127]. Several considerations should be done in this regard.

On one hand, the number of studies analysing p-tau levels is not as large as those performed for its non-phosphorylated form. In addition, sensitivity and sensibility may depend on the analysed phosphorylation site, although sensitivity for AD seems equal for at least the three main epitopes used in clinical diagnosis [139]. Importantly, results from a meta-analysis study indicate that tau phosphorylated at the Threonine 181 levels are able to discriminate AD from other dementias and MCI [140].

On the other hand, the utility of p-tau in the differential AD diagnosis against other neurodegenerative diseases is advantageous over total tau since p-tau levels reflect AD pathogenesis [141]. Indeed, p-tau levels in the CSF may reflect the levels of tau phosphorylation in AD brains. Tau is more increased in the CSF of sCJD patients than in AD, while p-tau is only modestly increased in sCJD [142]. In addition, tau levels are increased in neurological diseases such as in acute ischemic stroke, while p-tau levels remains unaltered [123]. Indeed, tau phosphory-

lation is physiologically regulated during several biological processes such as neuronal development, while tau levels usually remain more stable. Therefore, a direct correlation between total tau and p-tau levels cannot be established, and several lines of evidence indicate that p-tau levels are differently regulated, not only in AD, but also in other neurodegenerative diseases. In this regard, the main tau kinase, Glycogen synthase kinase 3 (GSK3) is assumed to be hyperactivated in AD brain, inducing pathogenic tau hyperphosphorylation, aggregation and formation of the intracellular NFTs. Although a direct correlation between GSK3 activity and tangle formation in AD is still under discussion [143], GSK3 levels and activity are markedly reduced in sCJD brain [144]. Thus, the distinct regulation of tau phosphorylation in the brain of AD and CJD, may explain the different p-tau/tau ratios observed in both diseases, which permits a differential diagnosis [145].

Recently it has been observed that patients suffering from rpAD present highly increased p-tau levels in the CSF [146] when compared to controls and classical AD patients. Since it is estimated that rpAD may be accounting for 10-30% of all AD cases, it is urgently needed to establish if lack of disease stratification may lead to misinterpretation of p-tau analysis between rapidly progressive and classical AD forms. In this regard, a combination of high CSF tau without proportionally elevated p-tau-181 is associated with a faster rate of cognitive decline [147]. In this regard, longitudinal studies indicates that a combination of low A β 42 and high tau and p-tau levels is highly predictive of MCI progression and cognitive decline rate [74, 148].

5.1.1.5. *Inflammatory cytokines*

A common feature in the Central Nervous System of neurodegenerative diseases is the presence of chronic neuroinflammation associated with an exacerbated gliosis [149]. The role of a chronic and sustained inflammation in neurodegeneration is still a matter of debate as neuroinflammation has been suggested to play both detrimental and protective functions depending on disease stage, brain region, activation of anti-inflammatory mechanisms and cellular milieu among others [150]. Besides these considerations point out a critical role of neuroinflammation in the molecular mechanisms linked to AD pathology [151] and a broad range of inflammatory cytokines and immune response mediators are increased in the CSF of AD patients. A correlation between inflammatory markers and biomarkers of neurodegeneration has been described [152], and consequently, neurodegenerative disorders with high inflammatory chronic profiles such as prion diseases [153] present higher inflammatory-related deregulations in the CSF [154, 155]. However, the specific inflammatory profile observed in different types of dementia and at different disease stages indicates that inflammatory biomarkers could be used as surrogate markers for AD diagnosis and prognosis.

The anti-inflammatory cytokine TGF β -1 is consistently upregulated in AD cases [156, 157]. Interestingly, during the progression from MCI to AD, a pro-inflammatory state is proposed since MCI patients who progressed to AD showed higher TNF α and lower TGF β -1 and A β 42 levels than control individuals or those non progressing to AD [158]. These data are in agreement with increased levels of the acute-phase C-reactive protein (CRP) and IL-6 in the CSF of MCI patients when compared to AD patients, indicating that inflammatory mechanisms

are already progressing even before changes in core AD biomarkers such as A β 42 and tau can be detected in the CSF [159].

In relation to this, a comparative analysis between Amnesic Mild Cognitive Impairment (aMCI) and MS patients indicated that pro-inflammatory cytokines and CD45+ lymphocytes are present in the same levels in both diseases. Taking into account that MS can be considered the most representative neuroinflammatory disease, these observations indicate that inflammatory mechanisms may be crucial for AD etiopathology.

In this regard, the pro-inflammatory cytokine osteopontin (OPN), also known as the secreted phosphoprotein 1 (SPP1) and involved in macrophage recruitment to inflammatory sites and cytokine production [160], is also elevated in the CSF of AD patients and in MCI patients developing AD. OPN levels correlate with cognitive decline and with increased levels in early disease phases [161, 162]. OPN has also been found elevated in the CSF during attacks of MS [163].

In addition, the major acute-phase protein SAP (Serum amyloid P component) has lower levels in MCI patients who progressed to AD than in those who did not progress to AD [164], suggesting that low SAP levels are linked to an increased risk of progression to AD.

Alternative promising inflammatory-biomarkers have been proposed. On one hand, lipocalin 2, whose levels are decreased in the CSF of MCI and AD patients and increased in brain regions with associated AD pathology [165]. On the other hand, the astrocytic marker YKL-40, has been reported to be increased in AD at early stages of the disease [166-169] and in FTD and aMCI patients [166]. In addition YKL-40 levels correlate positively with the classical core biomarkers tau and p-tau [166].

5.1.1.6. *MicroRNAs*

microRNAs (miRNA) are endogenous small non-coding RNAs (20-22 nucleotides) that are involved in post-transcriptional gene regulation by targeting mRNAs for cleavage or translational repression [170]. miRNAs have emerged as key regulators of various aspects of neuronal development and dysfunction. Deregulated small RNA signatures, especially miRNAs, have been observed in the brain of a broad range of neurodegenerative diseases such as AD, PD, HD or ALS [171, 172] and experimental evidences ascribe a functional role to miRNAs in the pathogenic molecular mechanisms leading to neurodegeneration [173-175]. With the advent of high-throughput technologies, full transcriptomic signatures can be provided not only from tissues, but also from samples with small amounts of starting material such as biological fluids and associated exosomes [176-178]. In this regard, more than 100 circulating miRNAs are deregulated in pathological conditions [179] and some of them have been proposed as potential biomarkers for disease diagnosis and prognosis, mainly in cancer and neurodegenerative diseases. Regarding the levels of circulating miRNAs in AD, several studies already reported changes when compared to control samples. A recent pilot study in two different cohorts showed that hsa-miR-27a-3p expression is reduced in the CSF of AD patients [180]. Decreased levels of this miRNA correlate with high tau and low A β amyloid levels. A second study analysed a selected group of miRNA candidates and observed that miRNAs 34a, 125b and

146a levels were significantly lower in the CSF of AD patients when compared to control cases, while the levels of the miRNAs 29a and 29b were significantly higher [181]. In an independent study low levels of miRNA-146a were also detected in the CSF of AD patients [182]. In this regard the expression of miRNA-146a is increased in AD [183] and CJD brains [184], in AD mice models [185] and in scrapie mice [184]. miRNA-146a expression in AD mice models also correlates with senile plaque density and synaptic pathology [185]. This miRNA is induced by the interleukin IL-1 β , modulating the expression of IL-6 and the cyclooxygenase COX-2 and acting as a negative regulator of the astrocyte-mediated inflammatory response [186, 187]. In addition miRNA-146a negatively regulates TLR signalling to prevent exacerbated inflammation, thus, it seems to play a key role in the modulation of the neuropathology associated to chronic inflammation in neurodegenerative diseases. Whether the regulation of miRNAs in CSF is a consequence of neuronal cell damage or a modulated pathogenic response is still a matter of discussion.

In summary, all preliminary studies argue for the presence of deregulated levels of miRNAs in the CSF of AD patients with potential translational value. Exclusion of blood contamination effects, standardization of the assays, together with cross-disease and technical validation in larger cohorts need to be carried out to assess the potential role of miRNAs signatures as specific diagnostic and prognosis biomarker tool in AD and to define new diagnostic therapeutic opportunities related to the miRNA field.

5.1.1.7. Mitochondrial DNA

A pioneering study demonstrated that asymptomatic patients at risk of AD and symptomatic AD patients exhibit a significant decrease in the levels of circulating cell-free mtDNA in the CSF [188]. Data were generated by qPCR and digital droplet PCR and validated in an independent cohort of patients. Interestingly, this decrease is disease-specific, as mtDNA levels in the CSF of FTLD patients remain unchanged. Since decreased levels of mtDNA precede the appearance of the classical AD biomarkers such as A β 42, mtDNA is an excellent potential pre-clinical AD biomarker. Further studies in larger cohorts including rpAD and CJD samples will determinate the clinical use of mtDNA analysis as a prognosis AD biomarker.

5.1.1.8. Metabolic profile

The use of analytic technologies such as Nuclear magnetic resonance and Liquid chromatography–mass spectrometry to analyse the metabolic signatures of biological fluids deserves special attention [189]. The metabolic profile in human CSF samples of AD patients and age-matched healthy controls unveils the presence of a significant presence of deregulated metabolites in AD cases [190]. Among them, higher corticols levels are found in AD cases, which correlate with AD progression and severity. In addition, the same study proved that combined analysis of different metabolites may increase sensitivity and specificity above 80%.

A second metabolic profile study identified the deregulated metabolic pathways in the CSF of MCI and AD patients [191]. The number of altered pathways increased with disease severity. Among them, Krebs cycle was significantly affected in MCI and cholesterol and sphingolipids

transport was altered in AD. A high percentage of altered pathways in the CSF were also deregulated in plasma from the same individuals (30% in MCI and 60% in AD, respectively). Deregulated pathways performing the best disease discrimination were biosynthesis and metabolism of cortisone and prostaglandin 2.

Finally, a third study using metabolomics in the CSF of MCI and AD patients demonstrated the presence of elevated methionine (MET), 5-hydroxyindoleacetic acid (5-HIAA), vanillyl-mandelic acid, xanthosine and glutathione levels in AD patients and elevated 5-HIAA, MET, hypoxanthine and other metabolites in MCI patients when compared to healthy controls. Metabolite ratios revealed changes within tryptophan, MET and purine pathways [192], showing a partial overlap between MCI and AD.

Metabolomics is a promising tool for AD diagnosis indicating a slightly lower or similar performance when compared to classical AD biomarkers such as tau and A β 42 depending on the study. Further analysis in large independent cohorts, technical updates as well as a combination of metabolic profiling with classical or alternative biomarkers will define the potential use of high throughput metabolic analysis in the AD diagnostic field. Besides, metabolite signatures may help to unveil the progression mechanisms and pathways leading to different dementia stages.

5.2. Blood

Despite the description of altered levels of several molecules in the blood levels of several molecules in the blood of AD patients as AD clinical biomarkers. Direct analysis in blood or blood-derived serum or plasma samples presents a broad range of advantages over CSF analysis. Blood extraction is minimally invasive and sample is easily collected, processed and stored over time. However, variations in the levels of blood metabolites may be reflecting a broad spectrum of changes not directly related to the neurodegenerative process. In addition, the dynamic range of the changes are lower than in CSF obtaining, most of the times, inconsistent data. Additionally, contrary to CSF, blood is a very complex fluid composed of different types of metabolites and cell types that present significant oscillations in response to external factors not related to pathogenic events. The analysis of specific blood cells could be an alternative approach to link potential biomarkers levels with AD pathology, being a field under intense study.

5.2.1. Protein biomarkers

The core CSF AD biomarkers present minimal alterations in plasma. A β 40 levels are higher in AD than in controls, although a high overlap is observed between groups. No changes have been observed for A β 42, and A β 40 and both A β 40 and A β 42 levels showed no association with cognitive decline [86]. Albeit some partial overlap between groups, tau levels in plasma are increased in AD when compared to control and MCI patients. Interestingly, tau levels cannot differentiate non-progressive from AD progressive MCI patients and there is a lack of correlation between CSF and plasma tau levels [193].

High-throughput proteomic studies have tried to report the complex deregulated signatures between control and AD samples. A 2D-Mass spectrometry-based study detected a deregulated set of proteins in AD plasma complement factor H precursor and α -2-macroglobulin, which were validated and correlated with disease severity [194]. Independent multi-analyte profiling studies also demonstrated the presence of deregulated levels of proteins in MCI and AD samples when compared to controls both in serum and plasma. Among them some hits are related to AD pathogenesis such as the apoE [195, 196] as well as a broad range of inflammatory mediators [196, 197]. In an array-based ELISA study, 18 signalling proteins were able to distinguish AD from control samples with high accuracy (90%) and to predict MCI to AD progression [198], although the validation of this dataset has been ambiguous [199, 200]. The observation of a high variability between independent analyses indicates that further validations by independent methodologies in different cohorts need to be performed before resolving the clinical relevance of high-throughput blood-based analysis.

Alternative plasma biomarkers include the brain-reactive autoantibodies, present in sera irrespective of the presence of any pathology. This finding led to the analysis of the potential AD-specific autoantibody signature, which has been suggested to possess diagnostic value due to its ability to distinguish AD cases from controls, PD and breast cancer samples [201].

5.2.2. *microRNAs*

miRNA signature from CSF is only slightly more stable when compared to serum, suggesting that both biofluids are appropriate for the screening analysis of small RNAs [202]. Therefore, several studies addressed the potential deregulated miRNA signature in blood-derived AD samples. Using a microarray and qPCR validation approach the miR-125b, miR-23a and miR-26b were downregulated in the serum of AD cases when compared to non-inflammatory and inflammatory neurological controls and to FTD cases [203]. miR-125b presented the best accuracy discriminating AD from other groups. The same study observed that miR-125b and miR-26b levels were also diminished in the CSF of AD patients. An independent validation study was able to replicate downregulation of miR-125b in AD serum [204].

In a different approach, using RNA-sequencing and qPCR validation, downregulated levels of the miR-98-5p, miR-885-5p, miR-483-3p, miR-342-3p, miR-191-5p and let-7d-5p in the serum of AD cases were reported. The miR-342-3p showed the best sensitivity and specificity and correlated with cognitive decline [205]. However, downregulated levels of miR-342-3p in biological fluids are also a common hallmark in cancer [206]. Using a similar approach a 12 blood-based miRNA signatures was suggested to discriminate AD patients from controls and samples from patients suffering from different neurodegenerative diseases with high diagnostic accuracies [207]. Nonetheless, the different sample origin impedes a formal comparison between disease group's studies. The analysis of peripheral blood mononuclear cells identified upregulated levels of miR-34a, miR-181b in AD cells [208].

Despite the promising future of miRNA as biomarkers tools of clinical relevance, several considerations need to be done. Lack of validation among current available studies, even when using similar platform, indicates that sample collection and methodology needs further

standardization. In addition, high-throughput data need to be cross-validated in longitudinal studies using different cohorts and selected miRNAs validated in multicentre studies. Under these conditions miRNA in blood-related samples may serve as prognostic and diagnostic through the analysis of miRNA signatures alone or combined with the analysis of classical AD biomarkers.

6. Conclusion

The use of combined analysis of current AD diagnostic tools is gaining experimental momentum due to its demonstrated value as a better prognostic and diagnostic tool when compared to individual assessments. As most promising candidates, CSF markers as well as methods of in vivo neuroimaging have been identified. Among them, we can find structural MRI, ¹⁸F-FDG-PET and novel in vivo amyloid-PET imaging [209, 210]. In longitudinal studies it was shown that with the help of these biomarkers AD could be diagnosed already in mild symptomatic states with high accuracy allowing a predictability of its development [210]. Investigations of patients with genetic AD have demonstrated already 15 years prior to the onset of dementia significant pathological alterations in distinct biomarkers [211, 212].

Although these results are only assignable in a limited way to sporadic AD, the latter study provides impressive evidence on the long preclinical course of AD.

Current diagnostic concepts should therefore apply not at first when AD dementia has developed, but support explicitly the application of biomarkers at distinct stages of AD as it was shown that biomarkers become positive already at early and presymptomatic stages [213, 214].

In conclusion, differential diagnosis of a dementia syndrome requires besides clinical history and neuropsychological testing, analysis of metabolites in biological fluids as well as imaging methods. All these diagnostic approaches will not only allow an explanation towards the underlying cause of dementia but will also be useful in monitoring disease treatment and progression. The detection of AD at an early stage is hereby essential, as a further disease progression can be influenced positively by early initiation of treatment.

Integration of data generated during the last decades should be used to build up a worldwide rational algorithm based in the use of standardized, economically affordable methodologies and easily accessible samples.

Nomenclature

AD: Alzheimer's disease, rpAD: rapidly progressive Alzheimer's disease, CJD: Creutzfeldt-Jakob disease, aMCI: Amnesic Mild Cognitive Impairment, MCI: Mild cognitive impairment, FTLD: Frontotemporal Lobe Degeneration, FTD: Frontotemporal Dementia, CSF: Cerebrospinal Fluid, PD: Parkinson Disease, HD: Huntington Disease, ELISA: Enzyme-Linked Immunosorbent Assay, MS: Multiple Sclerosis, SWD: Subcortical White-matter Dementia, MMSE: Mini-mental state examination, APP: Amyloid precursor protein, DS: Down Syndrome, CRP:

C-reactive protein, PSP: progressive supranuclear palsy, CBS: corticobasal syndrome; NINCDS/ADRDA: American National Institute of Neurological and Communicative Disorders and Stroke /Alzheimer's disease and Related Disorders Association; DGPPN: German Society of Psychiatry, Psychotherapy and Neurology; DGN: German Neurological Society; MRI: Magnetic Resonance Imaging, PET: Emission Computed Tomography; SPECT: single photon emission computed tomography; EEG: Electroencephalogram; CT: Computerized Tomography; CDR: Clinical Dementia Rating; GDS: Global Deterioration Scale; ADL-/IADL: Activities of Daily Living /Instrumental Activities of Daily Living; VBM: voxel-based morphometry; FLAIR; Fluid-attenuated inversion recovery; rCBF: regional cerebral blood flow.

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