finally lead to a loss of neurons as observed in the hippocampal sector CA1 in APP23 and APP48 mice [16,17]. Although these mechanisms for Aβ-related neurodegeneration have been found under artificial conditions in Aβ producing mouse models it is tempting to speculate that similar mechanisms occur in AD. APP-related production of extra- and/or intracellular Aβ, thereby, appears to be critical for neuritic and synaptic degeneration. As such, for the development of therapeutic strategies aimed at protecting neurons from AD-related degeneration it appears important to consider both types of Aβ-related neurodegeneration.

Additional files

Additional file 1: Types of commissural neurons in the frontocentral cortex as previously described [18].

Additional file 2: Statistical analysis.

Additional file 3: Western blot analysis of soluble, dispersible, membrane-associated, and insoluble (plaque-associated) Aβ in wildtype, APP48 and APP23 mice. Original blots related to Figure 3b.

Additional file 4: Mitochondrial alterations in peripheral neurites. **a-c**: Percentage of altered mitochondria (**a**), volume densities of altered mitochondria (**b**), and volume densities of all mitochondria (**c**) in peripheral neurites did not show significant changes among frontocentral neurons in wild type (WT), APP23 and APP48 mice. **d-f**: No significant differences in the percentage of altered mitochondria (**d**), volume densities of altered mitochondria (**e**), and volume densities of all mitochondria (**f**) in peripheral neurites of the CA1 sector of the Ammon's horn were found between APP48 mice and wild type controls. 3-month-old APP23 mice had less morphologically altered mitochondria (**d, e**) than wild type controls. In 15-month-old animals the trend was still visible but did not reach significance (**d, e**). *$p < 0.05$ (Further statistical analysis: Additional file 2).

Competing interests

D. R. Thal received consultant honorary from Simon-Kucher and Partners (Germany), and GE-Healthcare (UK).
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Authors' contributions

Neuropathology and immunohistochemistry: ARU, IK, JG, SK, HY, JW, DRT; animal breeding, genotyping and brain removal: DA, MS, DRT; Tracing and Laser Scan Microscopy: ARU, SL, DRT; Electron microscopy, immunoelectron microscopy and quantitative analyses: FS, IK, DRT; manuscript preparation: ARU, FS, MS, DRT; critical review of the manuscript: IK, DA, JG, SK, SL, HY, JW; study design, coordination and fund raising: MS, DRT. All authors read and approved the final manuscript.

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Author details

[1]Institute of Pathology - Laboratory of Neuropathology, University of Ulm, Ulm, Germany. [2]Novartis Institutes for Biomedical Research, Basel, Switzerland. [3]Department of Neurology, University of Bonn, Bonn, Germany. [4]Institute of Anatomy and Cell Biology, University of Ulm, Ulm, Germany. [5]Gunma University School of Health Sciences, Gunma, Japan.

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ORIGINAL PAPER

Pathology of clinical and preclinical Alzheimer's disease

Dietmar Rudolf Thal · Christine von Arnim · W. Sue T. Griffin · Haruyasu Yamaguchi · Robert E. Mrak · Johannes Attems · Ajeet Rijal Upadhaya

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Abstract Alzheimer's disease (AD) is characterized neuropathologically by the presence of amyloid plaques, neuritic plaques, and neurofibrillary tangles (NFTs). These lesions occur not only in demented individuals with AD but also in non-demented persons. In non-demented individuals, amyloid and neuritic plaques are usually accompanied with NFTs and are considered to represent asymptomatic or preclinical AD (pre-AD) pathology. Here, we defined and characterized neuropathological differences between clinical AD, non-demented pre-AD, and non-AD control cases. Our results show that clinical AD may be defined as cases exhibiting late stages of NFT, amyloid, and neuritic plaque pathology. This is in contrast to the neuropathological changes characteristic of pre-AD, which display early stages of these lesions. Both AD and pre-AD cases often exhibit cerebral amyloid angiopathy (CAA) and granulovacuolar degeneration (GVD), and when they do, these AD-related pathologies were at early stages in pre-AD cases and at late stages in symptomatic AD. Importantly, NFTs, GVD, and CAA were also observed in non-AD cases, i.e., in cases without amyloid plaque pathology. Moreover, soluble and dispersible, high-molecular-weight amyloid β-protein (Aβ) aggregates detected by blue-native polyacrylamide gel electrophoresis were elevated in clinical AD compared to that in pre-AD and non-AD cases. Detection of NFTs, GVD, and CAA in cases without amyloid plaques, presently classified as non-AD, is consistent with the idea that NFTs, GVD, and CAA may precede amyloid plaque pathology and may represent a pre-amyloid plaque stage of pre-AD not yet considered in the current recommendations for the neuropathological diagnosis of AD. Our finding of early stages of AD-related NFT, amyloid, and GVD pathology provides a more clear definition of pre-AD cases that is in contrast to the changes in clinical AD, which is characterized by late stages of these AD-related pathologies. The observed elevation of soluble/dispersible Aβ aggregates from pre-AD compared to that in AD cases suggests that, in addition to more widespread AD-related pathologies, soluble/dispersible Aβ aggregates in the neuropil play a role in the conversion of pre-AD to clinical AD.

D. R. Thal (✉) · A. Rijal Upadhaya
Institute of Pathology - Laboratory of Neuropathology, Center for Biomedical Research, University of Ulm, Helmholtzstrasse 8/1, Ulm, Germany
e-mail: Dietmar.Thal@uni-ulm.de

C. von Arnim
Department of Neurology, University of Ulm, Ulm, Germany

W. S. T. Griffin
Donald W. Reynolds Center on Aging, UAMS, Little Rock, AR, USA

W. S. T. Griffin
Geriatric Research Education and Clinical Center, Veteran's Affairs Medical Center, Little Rock, AR, USA

H. Yamaguchi
Gunma University School of Health Sciences, Maebashi, Gunma, Japan

R. E. Mrak
Department of Pathology, University of Toledo, Toledo, OH, USA

J. Attems
Institute for Ageing and Health, Newcastle University, Newcastle upon Tyne, UK

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Introduction

Alzheimer's disease (AD) is histopathologically characterized by amyloid plaques, neurofibrillary tangles (NFTs), neuropil threads, and neuritic plaques [20]. Cerebral amyloid angiopathy (CAA) and granulovacuolar degeneration (GVD) are further pathological changes associated with AD [22, 36, 40, 41, 47]. Amyloid plaques and CAA lesions consist of aggregates of the amyloid β-protein (Aβ) [16, 29]. In addition to amyloid plaques and CAA, soluble and dispersible Aβ can be found in brain homogenates [24, 26, 34, 35, 38]. NFTs and neuropil threads contain aggregated abnormal phosphorylated τ-protein [6, 18]. Neuritic plaques are amyloid plaques, which are associated with dystrophic neurites [13, 44]. GVD represents granules in the neuronal cytoplasm, which consist of abnormal phosphorylated τ-protein, phosphorylated transactive response DNA-binding protein TDP-43, casein kinases, and other proteins [14, 15, 19, 23, 37, 49]. All of these lesions are found not only in demented individuals with symptomatic AD but also in non-demented persons [3, 8, 33, 40, 41, 43].

The current National Institute of Aging–Alzheimer Association (NIA-AA) neuropathological consensus guidelines for the diagnosis of AD recommend reporting levels of AD pathology in the brain regardless of the clinical status of a given individual. AD pathology is considered whenever Aβ plaques are observed [20]. Although the clinical diagnosis of AD is restricted to demented individuals [30], it has been proposed to categorize non-demented patients who exhibit AD-related biomarker profiles as preclinical AD (pre-AD) [39]. Thus, the neuropathological identification of AD pathology in non-demented individuals serves as a biomarker for AD, and those non-demented individuals who have such AD pathology can be classified as pre-AD cases. The NIA-AA guidelines for the neuropathological diagnosis of AD define AD through the presence of Aβ plaques [20]. In an analogous fashion, pre-AD cases at the neuropathological level may be defined as non-demented cases with Aβ plaques and non-AD cases as those non-demented individuals who do not have Aβ plaques. The presence or absence of NFT pathology, which can also occur in non-AD tauopathies, is not considered in this classification [20].

Here, we studied 766 autopsy brains for AD-related lesions correlated this with the presence or absence of dementia. Based on this, these cases were classified as symptomatic AD, pre-AD, or non-AD controls.

Materials and methods

Neuropathology

A total 766 autopsy cases were studied (Tables 1, 2). At the time of autopsy, brains were fixed in a 4 % aqueous solution of formaldehyde. Following fixation, the medial temporal lobe (MTL) and tissue from the occipital cortex containing the primary visual field were embedded in paraffin. Paraffin sections were cut at 12 μm thickness. Histopathological diagnoses of AD were made based on the analysis of sections of MTL and occipital cortex stained with the Gallyas and Campbell-Switzer silver techniques, and/or on sections immunoreacted with anti-abnormal τ-protein (anti-PHF-τ; AT-8; Innogenetics, Belgium, 1/1000) and anti-$Aβ_{17–24}$ (4G8; 1/5000, formic acid pretreatment, Sigma-Aldrich, St. Louis, USA). NFT staging and the assignment of Consortium to Establish a Registry for Alzheimer's Disease (CERAD) scores for neuritic plaque density were performed on Gallyas-stained and/or anti-PHF-τ-immunoreacted sections [1, 7, 8, 31]. The distribution of amyloid plaques in the MTL (Aβ phase (MTL)) was assessed according to previously published criteria [44]. Basically, the distribution of Aβ plaques was assessed semiquantitatively to determine the severity of Aβ plaque pathology [43]. Aβ phase (MTL), Braak-NFT stages, and CERAD scores for neuritic plaques were used to determine the degree of AD pathology according to the NIA-AA guidelines [20]. CAA was detected by anti-$Aβ_{17–24}$ immunoreactions and staged in 207 cases according to the following previously published scheme [41]: neocortical CAA (stage 1), additional allocortical and cerebellar CAA (stage 2), and subcortical CAA (brain stem, basal ganglia) (stage 3). GVD was observed in 161 cases using anti-pTDP43 immunostaining (pS409/410-2, Cosmo Bio Co., Ltd, Tokyo, Japan, 1/10,000, microwave pretreatment) and staged according to published criteria [40]: Stage 1 represents GVD restricted to the CA1 region in the Ammon's horn, stage 2 shows additional GVD in CA4 and/or the entorhinal region, stage 3 shows extension into the temporal neocortex, stage 4 to the amygdala and/or the hypothalamus, and finally, stage 5 GVD also involves midbrain nuclei, the cingulate gyrus, and/or frontal cortex. For CAA and GVD staging, additional paraffin sections of the superior frontal gyrus, cingulate gyrus, amygdala, basal ganglia, hypothalamus, midbrain, and/or pons were immunostained for Aβ or pTDP43. These additional sections were analyzed in those cases in which CAA or GVD was observed in MTL and occipital lobe sections.

Clinical dementia rating (CDR) scores were retrospectively obtained in 282 cases by screening the clinical records as reported previously [43]. Cases with CDR scores of 0.5 and higher were considered demented or cognitively impaired. In

Table 1 Description of the case collective

	N	Mean age in years (range)	Male/ female	Mean Aβ phase-MTL (range)	Mean NFT stage (range)	Mean CERAD score (range)
All cases	766	74.75 (20–104)	420/346	1.81 (0–4)	2.3 (0–6)	0.77 (0–3)
AD	114	81.52 (56–101)	44/70	3.74 (1–4)	4.96 (3–6)	2.44 (0–3)
Pre-AD	404	75.98 (48–104)	230/174	2.38 (1–4)	2.15 (0–6)	0.54 (0–3)
Non-AD	248	69.64 (20–98)	146/102	0 (0)	1.31 (0–4)	0 (0)

Entire sample subclassified into AD, pre-AD, and non-AD cases

Table 2 List of cases used for biochemical analysis

Case no.	Diagnosis	Age	Gender	Aβ phase (MTL)	Braak-NFT-stage	CERAD-plaque-score
1	no AD	60	M	0	0	0
2	no AD	66	M	0	1	0
3	no AD	69	F	0	1	0
4	no AD	71	F	0	1	0
5	pre-AD	73	F	3	1	0
6	pre-AD	84	F	3	2	0
7	pre-AD	71	M	3	2	1
8	pre-AD	77	F	3	2	0
9	pre-AD	78	F	3	2	0
10	AD	79	F	3	4	2
11	AD	78	M	4	4	1
12	AD	62	F	4	6	3
13	AD	91	F	3	4	1
14	AD	84	M	4	6	3
15	AD	64	F	4	6	3

Control and AD cases not included in Fig. 2 were previously included in a study where these results are depicted [35]

M male, *F* female

484 cases, the clinical records provided information about the presence or absence of dementia according to DSM-IV but did not provide sufficient information to determine the CDR score. Demented cases with AD pathology according to recommended criteria for the neuropathological diagnosis of AD [20] and lacking evidence of other forms of dementia were classified as clinical AD cases. When applying the NIA-AA criteria, controls were defined by the absence of any Aβ plaques. Non-demented cases with Aβ plaques were neuropathologically categorized as pre-AD cases.

All autopsy brains were collected from individuals who died in university or municipal hospitals in Germany (Bonn, Frankfurt/Main, Mainz, Offenbach/Main, Ulm), USA (Little Rock, AR), the United Kingdom (Newcastle upon Tyne), or Austria (Vienna) in accordance with local ethical committee guidelines and the laws governing the use of human tissue.

Biochemical analysis

To characterize the role of soluble/dispersible Aβ in the course of clinical and preclinical AD, fresh-frozen human brain tissue from 6 AD, 5 pre-AD, and 4 control cases obtained at autopsy was used for the determination of native Aβ aggregates in the AD and control brain (Tables 1, 2).

Protein extraction from fresh-frozen human occipital (Brodmann areas 17, 18, 19) and temporal cortex (Brodmann areas 35 and 36) was carried out in 2 ml of a 0.32 M sucrose solution with protease and phosphatase inhibitor cocktail (PhosSTOP, Roche, Mannheim, Germany). The tissue was homogenized as previously described [34]. The homogenate was kept on ice for 30 min and the supernatant was clarified by centrifugation for 30 min at 14,000*g*. To avoid separation of high-molecular-weight proteins into the membrane-associated and plaque-associated fractions, centrifugation speeds higher than 14,000*g* were not used. The resultant supernatant, containing soluble and dispersed proteins as previously shown [34], was aliquoted into an appropriate volume and stored at −80 °C until used. Protein amounts were determined using a BCA Protein Assay (Bio-Rad, Hercules, CA, USA).

For BN-PAGE of the supernatant containing soluble and dispersed proteins, 50 μg of total protein was prepared with 4X NativePAGE sample buffer (Invitrogen) and subjected to native PAGE 4–16 % Bis–Tris gel electrophoresis according to the manufacturer's protocol (Invitrogen). Native-Mark unstained protein standards (Invitrogen) were used as molecular weight markers. The gel was equilibrated in transfer buffer containing 0.2 % SDS for 10 min. After protein transfer onto nitrocellulose membranes (Bio-Rad), the membranes were boiled in PBS buffer in a microwave oven for 6 min followed by blocking with non-fat dry milk (Roth, Karlsruhe, Germany) for 1 h at room temperature.

For immunodetection of the blotted proteins, the membranes were incubated 24 h at 4 °C with the anti-$A\beta_{42}$ antibody MBC42 (1/500 [48]). This antibody was used because we have previously shown that it detects $A\beta_{42}$ in BN-PAGE protein analysis without cross-reaction with amyloid precursor protein (APP) [35]. Since soluble/dispersible $A\beta_{40}$ could not be detected in an earlier study after

BN-PAGE analysis of AD brain homogenates [35], we focused in the present study on $A\beta_{42}$ aggregates. After rinsing steps, the corresponding secondary antibodies were applied for 2 h at RT. Blots were developed with an ECL detection system (Supersignal Pico Western system, ThermoScientific-Pierce, Waltham, MA, USA) and illuminated in ECL Hyperfilm (GE Healthcare, Buckinghamshire, UK).

Statistical analysis

Comparisons between non-AD, pre-AD, and AD cases were assessed for statistical significance using the Mann–Whitney U test. p values were corrected for multiple testing. SPSS Statistics 19 software (SPSS, Chicago, IL, USA) was used to perform the statistical analysis.

Results

Distribution of Aβ plaques, NFTs, neuritic plaques, CAA, and GVD in clinical AD, pre-AD, and non-AD control cases

Mean ages, Aβ phases, NFT stages, and CERAD scores of AD, pre-AD, and non-AD cases are provided in Table 1. AD cases in general had more widely distributed Aβ plaques, NFTs, neuritic plaques, CAA, and GVD—as represented by the respective phases, stages, and scores—than did pre-AD or control cases ($p < 0.002$). Non-AD cases did not exhibit Aβ plaques and neuritic plaques but some cases did show early stages of NFT and GVD pathology.

In detail, Aβ plaques were not found, by definition, in non-AD cases. In pre-AD cases, Aβ phases (MTL) 1–3 were predominantly found, whereas nearly all AD cases had Aβ phase (MTL) 4, i.e., end-stage Aβ pathology (Fig. 1a). Thus, the distribution of Aβ plaques as represented by phases of Aβ deposition in the MTL [44] was higher in AD than in pre-AD cases ($p < 0.002$). The Aβ pathology in pre-AD cases was also more severe than that in non-AD controls ($p < 0.002$) inasmuch as, by definition, all pre-AD and AD cases had Aβ plaques and non-AD did not.

The distribution of NFTs as represented by NFT stage [8] was higher in AD than in pre-AD cases ($p < 0.002$; Fig. 1b). Similarly, pre-AD cases exhibited more widely distributed NFT pathology than did non-AD cases ($p < 0.002$). However, most non-AD cases exhibited early NFT stages, indicating that there are a significant number of cases without Aβ plaques, classified as non-AD, that already have NFT pathology. All AD cases, 95.4 % of the

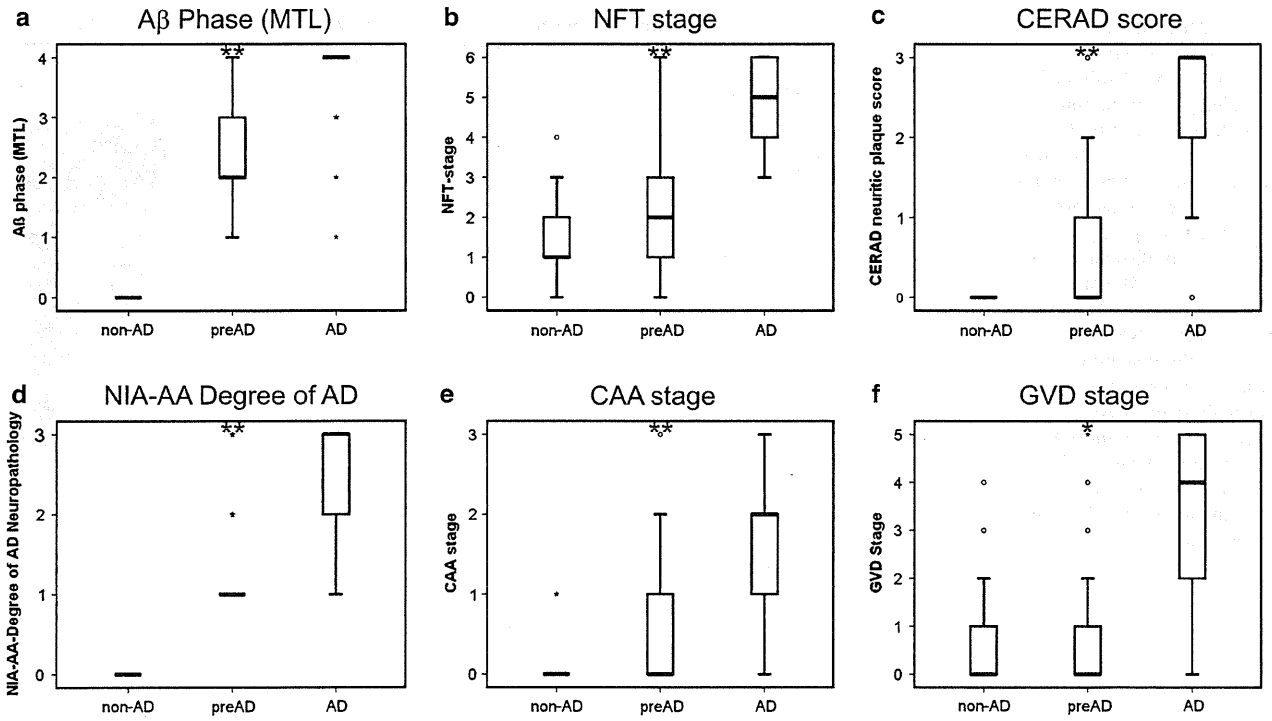

Fig. 1 Box plot diagrams indicating the distribution of Aβ phases within the MTL (**a**), NFT stages (**b**), CERAD scores for neuritic plaque pathology (**c**), NIA-AA degrees of AD pathology (**d**), CAA stages (**e**), and GVD stages (**f**) for clinical (AD) and preclinical (pre-AD) AD cases and for controls with no AD, according to the current guidelines for clinical and neuropathological diagnosis of AD [20, 30, 39]. Mann–Whitney U test corrected for multiple testing: $^{*}p < 0.05$, $^{**}p < 0.01$

pre-AD cases, and 80.5 % of non-AD cases in our sample exhibited NFTs of at least NFT stage I.

Neuritic plaque pathology as determined by the CERAD score [31] showed (by definition) no neuritic plaques in non-AD controls, displayed a moderate frequency of neuritic plaques in pre-AD cases, and showed high frequencies of neuritic plaques in demented individuals with AD (Fig. 1c). Consequently, CERAD scores were higher in AD than in pre-AD cases ($p < 0.002$) and higher in pre-AD than in non-AD cases ($p < 0.002$). Neuritic plaques were observed in 97.3 % of the AD and in 28.4 % of the pre-AD cases.

The NIA-AA degree of AD pathology, a parameter that combines Aβ phase, NFT stage, and CERAD score, likewise distinguished AD, pre-AD, and non-AD control cases (Fig. 1d). NIA-AA degrees of AD pathology were high or intermediate in clinically symptomatic AD cases but low in pre-AD cases ($p < 0.002$). Non-AD control cases had, by definition, no AD pathology. Accordingly, the degree of AD pathology in pre-AD cases was higher than in non-AD controls ($p < 0.002$).

CAA was seen in 5.5 % of non-AD controls, thereby indicating that CAA can precede plaque pathology in a small number of cases (Fig. 1e). About 37.1 % of pre-AD cases had CAA mainly restricted to neocortical areas (CAA stage 1), i.e., CAA was more frequently seen in pre-AD than in non-AD control cases ($p < 0.002$), whereas AD cases had more widely distributed CAA-affected vessels than did pre-AD cases, as indicated by more advanced CAA stages ($p < 0.002$) [41]. CAA was observed in 92.6 % of AD cases.

GVD was found more widely distributed in clinical AD than in pre-AD, as represented by GVD stage ($p < 0.002$; Fig. 1f) [40]. Pre-AD as well as non-AD control cases exhibited early stages of GVD. Although pre-AD and non-AD cases did not vary significantly with respect to GVD stage ($p = 0.075$), there was a trend toward higher GVD stages in pre-AD cases compared with non-AD controls. GVD was found in 96.3 % of AD, in 46.2 % of pre-AD, and in 28.6 % of the non-AD control cases.

Soluble and dispersible Aβ in AD, pre-AD, and non-AD cases

Analysis of the soluble/dispersible fraction of brain homogenates by BN-PAGE showed large amounts of soluble/dispersible, high-molecular-weight Aβ in the neocortex of AD cases (Fig. 2). Here, a strongly labeled smear of $Aβ_{42}$-positive material between ~100 and 1236 kDa was observed. In pre-AD and non-AD cases, such a smear was not present. Pre-AD cases exhibited various patterns of

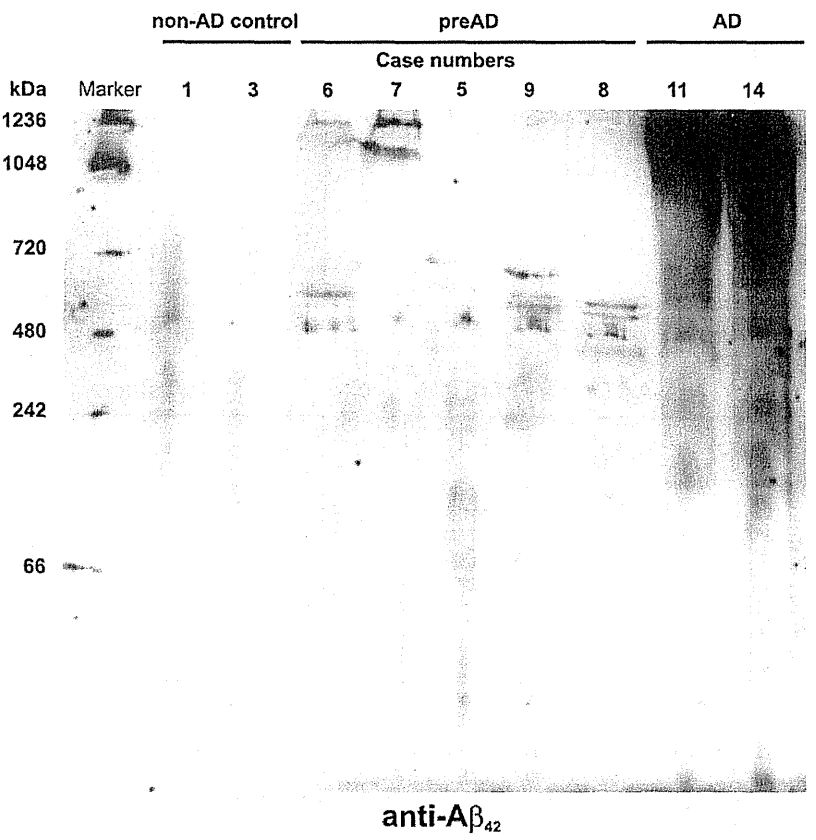

Fig. 2 BN-PAGE and subsequent Western blot analysis of soluble/dispersible Aβ aggregates from human brain homogenates of AD, pre-AD, and non-AD control cases. AD cases show significant high-molecular-weight smears of $Aβ_{42}$-positive aggregates, whereas only distinct oligomer bands between 480 and >1200 kDa were observed in pre-AD cases. Non-AD cases did not exhibit significant $Aβ_{42}$-positive material. Low-molecular-weight Aβ aggregates with a molecular weight lower than 100 kDa were not observed in any cases. Case numbers related to Table 2 are provided

well-defined, mild-to-moderately stained oligomer bands at ~480, ~580, ~600, ~680, ~1100, and ~1200 kDa. The ~1100 and ~1200 kDa bands were observed in only two pre-AD cases (Fig. 2). In non-AD, control cases such bands were not found (Fig. 2).

Discussion

This comparison between AD, pre-AD, and non-AD cases, classified according to the current clinical and neuropathological criteria [20, 30, 39], confirms that clinical AD cases have the most widely distributed AD-related pathologies, namely Aβ plaques, NFTs, neuritic plaques, CAA, and GVD, whereas pre-AD cases have less. Although cases classified as having no AD exhibited fewer NFTs than pre-AD cases and had no plaques, some of them did display very early stages of NFT, GVD pathology, and occasionally of CAA. Since the expansion of GVD pathology is associated with AD but not with other tauopathies [40], we hypothesize that coexisting NFT and GVD pathology in cases classified as non-AD cases may

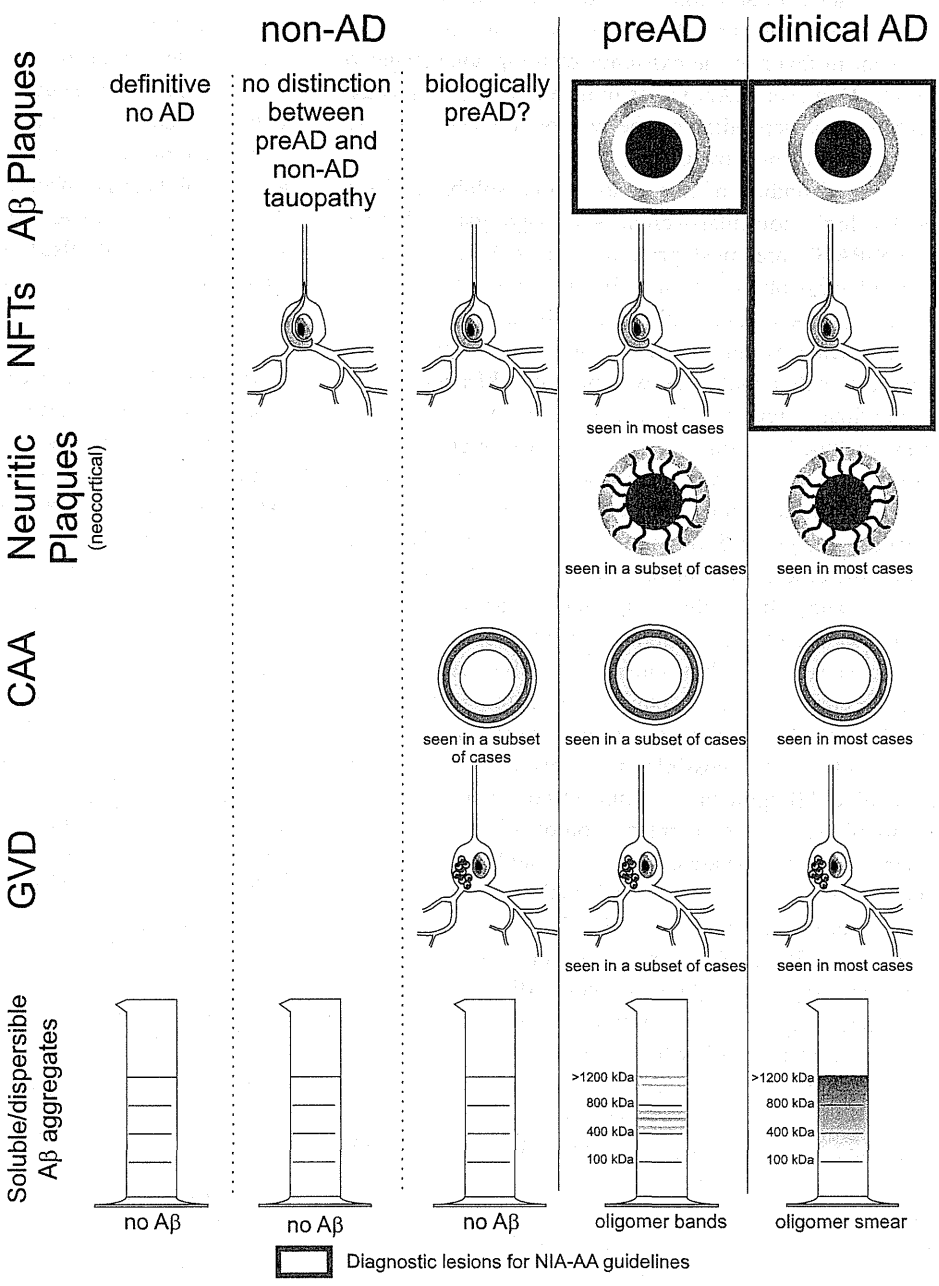

Fig. 3 Schematic representation of AD-related pathological features in non-AD, pre-AD, and AD cases. Obligatory pathologies for AD diagnosis, according to the NIA-AA guidelines [20], are indicated by *boxes*. The presence of CAA, neuritic plaques, and GVD pathology is not obligatory under NIA-AA guidelines, but these features are frequently seen in AD cases. Non-AD cases according to the current NIA-AA criteria were subclassified into possible pre-amyloid plaque stage cases, i.e., biologically pre-AD cases, cases with τ pathology without clear distinction between AD and non-AD changes, and cases with no AD-related lesions at all

also represent early AD-related lesions. That NFT pathology as well as pretangle τ pathology precedes Aβ deposition has already been described in detail [5, 9, 10], but this point is controversial because τ pathology without Aβ deposits could not be definitively distinguished from early lesions of non-AD tauopathies. With GVD as a second AD-specific lesion, in addition to NFTs, in a subset of these cases, it becomes evident that AD pathology indeed starts before the detection of Aβ plaques (Fig. 3). Accordingly, a major conclusion of this study is that cases currently classified as non-AD because of the absence of Aβ plaques may, in fact, represent incipient pre-AD when NFTs and GVD are seen. In addition, CAA can occur in some cases in the absence of parenchymal Aβ, and this is a further argument in favor of the existence of pre-plaque stages of pre-AD. However, CAA varies in its expression in AD and pre-AD cases, depending on the type of AD, and some AD cases do not even show CAA [2, 4, 42].

A second finding of this study is that soluble and dispersible high-molecular-weight $A\beta_{42}$ aggregates obtained by BN-PAGE are most prominent in AD cases, with a smear of $A\beta_{42}$ oligomers of ~100 kDa up to >1200 kDa, whereas pre-AD cases exhibited only distinct bands of high-molecular-weight soluble and dispersible $A\beta_{42}$ aggregates with a molecular weight of 480 kDa and higher. As previously reported for the AD and non-AD control cases included in this study [35], Aβ oligomers with molecular weights lower than 100 kDa are not found in AD, pre-AD, and non-AD cases. The pattern of high-molecular-weight Aβ oligomer bands in pre-AD varied among the cases. This suggests that increasing amounts of soluble and dispersible high-molecular-weight $A\beta_{42}$ aggregates and changes in the pattern of oligomer sizes to a broader spectrum of high-molecular-weight Aβ oligomer types in cortical neuropil play a role in the conversion of pre-AD into AD, which is a critical event in the pathogenesis of AD. A possible mechanism for soluble and dispersible Aβ aggregates to impact conversion from pre-AD to clinical AD is the interaction of Aβ oligomers with synapses, as demonstrated by other authors in in vitro studies [12, 25, 28, 38, 45] as well as the known capacity of Aβ to exacerbate τ pathology [17, 27, 32].

We used BN-PAGE for the detection of soluble and dispersible $A\beta_{42}$ aggregates to avoid artificial dissociation of Aβ aggregates caused by treatment with sodium dodecyl sulfate (SDS) [35, 46], which is required for SDS–PAGE protein analysis. Moreover, in BN-PAGE analysis, high-molecular-weight Aβ aggregates were found to be the predominant type of Aβ aggregates in the soluble/dispersible fraction, whereas low-molecular-weight Aβ oligomers, such as dimers or Aβ*56, were not observed in detectable amounts. We know that BN-PAGE is less sensitive for the detection of Aβ in comparison with SDS–PAGE, especially because $A\beta_{40}$ aggregates are not seen using BN-PAGE in human brain homogenates but are observed using SDS–PAGE [35]. These results have been discussed previously in detail [35]. Here, we did not focus on total Aβ levels or $A\beta_{40}$ in the brain, which correlate with Aβ plaques as shown previously for the human brain and for mouse models of AD [11, 21]. The BN-PAGE analysis of $A\beta_{42}$ aggregates shown here provides additional information about changes in the spectrum and quantity of soluble and dispersed $A\beta_{42}$ oligomers, protofibrils, and fibrils in the cortical neuropil of AD cases in comparison with pre-AD and non-AD cases.

In summary, the findings presented here show that the distinction between AD, pre-AD, and non-AD control cases according to current recommendations provides a valuable tool for identifying pre-AD cases, which are neuropathologically characterized by early stages in the distribution of AD-related pathologies. Moreover, some cases classified as non-AD controls because of an absence of Aβ plaques actually did show early stages of AD-related NFT and GVD pathology, and such cases could also be considered as pre-AD cases from a biological point of view (Fig. 3). The clinically important conversion from pre-AD to AD is not only accompanied by more widespread Aβ plaque deposition, NFT pathology, neuritic plaques, CAA, and GVD, but also by the appearance of large amounts of various soluble/dispersible high-molecular-weight Aβ aggregates in the neuropil of AD cases compared to pre-AD cases and non-AD controls. This indicates that quantitative and qualitative changes in the aggregation status of soluble and dispersed types of Aβ aggregates may play an important role in the conversion from non-demented pre-AD to clinical AD (Fig. 3).

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Conflict of interest DRT received a consultant honorarium from Simon-Kucher and Partners (Germany), and GE Healthcare (UK) and collaborated with Novartis Pharma Basel (Switzerland). CAFvA received honoraria for serving on the scientific advisory board of Nutricia GmbH and has received funding for travel and speaker honoraria from Sanofi-Aventis, Novartis, Pfizer, Eisai, and Nutricia GmbH, and has received research support from Heel GmbH.

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EXTRA
Dementia
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Original Research Article

Symptoms of Early Dementia-11 Questionnaire (SED-11Q): A Brief Informant-Operated Screening for Dementia

Yohko Maki[a, b] Tomoharu Yamaguchi[a, c] Haruyasu Yamaguchi[a]

[a]Gunma University School of Health Sciences, [b]Geriatrics Research Institute and Hospital, and [c]Department of Rehabilitation, Gunma University of Health and Welfare, Maebashi, Japan

Key Words

Dementia screening · Dementia · Alzheimer's disease · Activities of daily living · Cognitive deficits · Early detection · Mild cognitive impairment · Non-Alzheimer's disease

Abstract

The aim of this study was to develop a brief informant-based questionnaire, namely the Symptoms of Early Dementia-11 Questionnaire (SED-11Q), for the screening of early dementia. 459 elderly individuals participated, including 39 with mild cognitive impairment in the Clinical Dementia Rating scale (CDR) 0.5, 233 with mild dementia in CDR 1, 106 with moderate dementia in CDR 2, and 81 normal controls in CDR 0. Informants were required to fill out a 13-item questionnaire. Two items were excluded after analyzing sensitivities and specificities. The final version of the SED-11Q assesses memory, daily functioning, social communication, and personality changes. Receiver operator characteristic curves assessed the utility to discriminate between CDR 0 (no dementia) and CDR 1 (mild dementia). The statistically optimal cutoff value of 2/3, which indicated a sensitivity of 0.84 and a specificity of 0.90, can be applied in the clinical setting. In the community setting, a cutoff value of 3/4, which indicated a sensitivity of 0.76 and a specificity of 0.96, is recommended to avoid false positives. The SED-11Q reliably differentiated nondemented from demented individuals when completed by an informant, and thus is practical as a rapid screening tool in general practice, as well as in the community setting, to decide whether to seek further diagnostic confirmation.

Introduction

There is widespread underascertainment of Alzheimer's disease (AD) and other dementias [1], and a combination of pharmacological and nonpharmacological treatments could slow disease progression and maintain individuals at their highest level of functioning

Haruyasu Yamaguchi
3-39-15 Showa-machi
Maebashi 371-8514 (Japan)
E-Mail yamaguti@health.gunma-u.ac.jp

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when they are at the early stages of disease [2]. For early detection of dementia, a rapid screening test in clinical settings would be extremely useful, as it could help general practitioners with time constraints to decide whether or not to proceed with more in-depth clinical evaluation. Such a screening test is also useful for community health promotion to detect undiagnosed individuals.

An ideal practical screening test should be easy to administer within tight time constraints but must also be accurate enough to detect dementia. There are two general methods to screen for dementia: patient performance-based testing and informant interviews.

Regarding patient performance-based testing, the most widely used brief test is the Mini-Mental State Examination (MMSE) [3]. However, this test is time-consuming to administer in a clinical setting with time constraints. Furthermore, the ceiling effect makes the MMSE insensitive to the early stages of dementia [4], especially for highly educated individuals [5, 6]. In contrast, a brief test such as the MMSE may falsely identify those with low education or poor cognitive functioning as demented. Other brief tests have also been developed. However, because of the requirement of brevity, most of them are limited to a single cognitive domain. For instance, the tests that are weighted towards memory such as the Short Blessed Test [7] and the Memory Impairment Screen [8] may not be sensitive enough for detecting nonamnestic dementias. Another problem is the psychological burden imposed by cognitive tests, as cognitive tests for dementia are themselves stressful [1].

In the community-based setting, performance-based tests may be impractical for detecting underdiagnosed dementia. A lack of self-awareness of cognitive decline is a characteristic of dementia [9, 10], and demented individuals tend to deny their cognitive decline and refuse the test. Ethical issues are also important; using screening tests with low specificity could lead to a misdiagnosis of dementia in elderly individuals, which could cause unnecessary anxiety. The detection of dementia should be conducted without unduly alarming the patient. In this respect, informant-based assessments are preferable.

When evaluating informant-based assessments, the Clinical Dementia Rating scale (CDR) [11] is widely used. CDR meets the requirement of accuracy, but it is not an easily administered screening tool. It is a semistructured interview that has to be carried out by trained practitioners and takes at least 30 min.

In the present study, we introduce a brief informant-based screening questionnaire to identify dementia in both clinical and community-based settings, namely the Symptoms of Early Dementia-11 Questionnaire (SED-11Q). This questionnaire is easily administered and is both patient and informant friendly. Questions addressing early signs of dementia were selected on the basis of clinical experience. The SED-11Q aims to investigate the state of ordinary daily activities often performed by an elderly individual living independently. The questions it asks are not only easy to answer, but are also informative. Quantifying difficulties in daily living may provide more sensitive information about early functional changes rather than questions about cognitive function in a single domain. This is because functional integrity is a key differentiating feature of dementia, and a decline in multifaceted cognitive domains directly leads to functional impairments. In addition, as deficits caused by dementia are manifested in various aspects, the SED-11Q also includes questions on social interaction and personality.

Methods

459 elderly individuals participated, including 39 with mild cognitive impairment (MCI) in CDR 0.5, 233 with mild dementia in CDR 1, 106 with moderate dementia in CDR 2, and 81 normal controls in CDR 0. The demented individuals were outpatients, and of the 81 normal controls, 64 were community dwellers and 17 were outpatients (table 1). The subjects were

Table 1. Demographic data

	CDR 0 (n = 81)	CDR 0.5 (n = 39)	CDR 1 (n = 233)	CDR 2 (n = 106)
Age, years	71.7±6.0	78.7±6.9	79.6±9.7	79.5±13.2
Gender (male/female)	74/7	18/21	82/151	29/77
MMSE	28.5±2.1	26.4±2.2	21.1±4.2	16.1±4.2
SED-11Q	1.00±1.29	3.21±2.14	5.71±2.78	7.25±2.88
Causative diseases				
AD			5.76±2.76 (127)	6.95±2.79 (40)
Others			5.66±2.81 (106)	7.44±2.93 (66)

With the exception of gender (number of patients) values represent mean ± SD. Values in parentheses represent the number of patients. The ages were significantly different between the groups ($p < 0.001$). CDR 0 was detected significantly more frequently in the younger groups. There was no difference between the affected groups of CDR 0.5, CDR 1, and CDR 2.

diagnosed based on criteria for dementia diseases such as NINCDS-ADRDA for AD [12], the third report of the Dementia with Lewy Bodies Consortium for Lewy body dementia [13], criteria by Neary et al. [14] for frontotemporal lobar degeneration, criteria by the NINDS-AIREN International Workshop for vascular dementia [15], and MCI by the report of the International Working Group on Mild Cognitive Impairment [16]. CDR 0.5 was regarded as MCI, although a different classification was proposed, whereby CDR 0.5 encompasses both mild and earlier dementia [17] or it corresponds to very mild dementia [18]. Depression was an exclusive criterion for normal controls in CDR 0 and subjects with MCI in CDR 0.5. The ethics board of the Gunma University School of Health Sciences approved all procedures (No. 21–27), and written informed consent was obtained from the participants.

Originally, the questionnaire consisted of 13 questions, which are shown in table 2. In the clinical and community setting, the informants were required to fill out the questionnaire including the 13 items. Based on the results of the current study, we decided to exclude 2 items: item 2 (misplacing) and item 13 (delusions). The format of the SED-11Q is shown in figure 1. In the present study, informants were limited to family members, and nonfamily caregivers were excluded. Informants had normal cognitive abilities without psychiatric diseases, delirium, or verbal incomprehension including aphasia.

The patients were also tested using the MMSE. All analyses were conducted using the Japanese version of SPSS for Windows version 19.0 (IBM Corp., New York, N.Y., USA). Significance was set at $p < 0.05$.

Results

Demographic data are shown in table 1. The ratio of positive answers in subitems in the quotient is shown in figure 2. In CDR 0, 51% checked item 2, whereas no one checked items 7 and 13. In CDR 1, more than 50% checked items 1, 2, 3, 6, 8, 9, 10, and 11. Sensitivities and specificities of the subitems in CDR 0 and CDR 1 are shown in table 2. Item 2 (misplacing) showed the lowest specificity, i.e. 0.49, out of the 13 items. Item 13 (delusions) showed the lowest sensitivity, i.e. 0.18, although the specificity was 1. Therefore, these 2 items were removed in the SED-11Q. Instead, a notification was added to recommend medical consultation whenever delusions or illusions were detected.

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Table 2. Sensitivity and specificity of the 13 subitems in the differentiation between CDR 0 and CDR 1

Item		Sensitivity	Specificity
1	Repetitive talking	0.81	0.79
2	Misplacing	0.85	0.49
3	Context understanding	0.52	0.99
4	Indifference about clothing	0.31	0.91
5	Cleaning up	0.35	0.91
6	Forgetting one of two items	0.60	0.90
7	Self-medication	0.47	1.00
8	Time consuming	0.62	0.88
9	Planning	0.52	0.98
10	Complex topics	0.64	0.93
11	Loss of interest	0.54	0.93
12	Irritable and suspicious	0.33	0.81
13	Delusions	0.18	1.00

Scores were as follows: 1.00 ± 1.29 (mean ± SD) in CDR 0, 3.21 ± 2.14 in CDR 0.5, 5.71 ± 2.78 in CDR 1, and 7.25 ± 2.88 in CDR 2. There was a significant difference among the CDR groups [analysis of variance $F(3, 455) = 106.264$, $p < 0.001$], and post hoc analysis with Bonferroni correction indicated the following significant differences: CDR 2 higher than CDR 1, CDR 1 higher than CDR 0.5, and CDR 0.5 higher than CDR 0 ($p < 0.001$). Modes of the scores were 0 in CDR 0, 2 in CDR 0.5, 5 in CDR 1, and 9/10 in CDR 2 (fig. 3).

Comparing CDR 0 and CDR 1 in the SED-11Q, the area under receiver operating characteristic (ROC) curve was 0.932 [$p < 0.001$, 95% confidence interval (CI): 0.903–0.961]. A cutoff value of 2/3 indicated a sensitivity of 0.841 (95% CI: 0.817–0.857), a specificity of 0.901 (95% CI: 0.830–0.947), a positive predictive value of 0.961 (95% CI: 0.933–0.979), and a negative predictive value of 0.664 (95% CI: 0.611–0.697). A cutoff value of 3/4 indicated a sensitivity of 0.764 (95% CI: 0.743–0.772), a specificity of 0.963 (95% CI: 0.903–0.987), a positive predictive value of 0.983 (95% CI: 0.957–0.994), and a negative predictive value of 0.586 (95% CI: 0.550–0.601) (fig. 4). The correlation coefficient of the SED-11Q and the MMSE was significant: $r = -0.424$ and $r < 0.001$.

Discussion

The SED-11Q reliably differentiated nondemented from demented individuals. The area under the ROC curve was 93%, suggesting good discrimination between the 2 groups. In the clinical setting with physicians and other medical staff, the statistically optimal cutoff value of 2/3, which indicates a sensitivity of 0.841 and a specificity of 0.901, can be applied. In the community setting, where community-dwelling elderly individuals are screened for detecting dementia, a cutoff value of 3/4, which indicates a sensitivity of 0.764 and a specificity of 0.963, is recommended because of high specificity and positive predictive values. Medical consultation is recommended whenever delusions or illusions are detected. In general, the SED-11Q was revealed to be practical as a rapid screening tool in general practice to decide whether or not to seek further diagnostic confirmation.

Consideration of Subitems

The 2 items that assessed memory function, item 1 (repetitive talking) and item 2 (misplacing), showed high sensitivities of 0.8 and more comparing CDR 0 to CDR 1. Amnestic disorder is one of the earliest signs of AD, and the reason for this could be the fact that the

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Date(MM/DD/YYYY) / /

Patient Name : Patient ID :

Respondent Name : Relationship

Respondent-completed / Interview by Name:

How has the patient's daily life been for the last month?
Please answer the following questions by circling the appropriate responses
(Exclude any difficulties caused by physical issues, e.g., pain).
Please ask for any help if needed.

He/she talks and asks about the same things repeatedly.	YES	NO	N/A Don't know
He/she has become unable to understand the context of facts.	YES	NO	N/A
He/she has become indifferent about clothing and other personal concerns.	YES	NO	N/A
He/she has begun to forget to turn off the faucet and/or close the door, and/or has become unable to clean up properly.	YES	NO	N/A
When doing two things at the same time, he/she forgets one of them.	YES	NO	N/A
He/she has become unable to take medication under proper management.	YES	NO	N/A
He/she has begun to take a longer time to do work (e.g., household chores), which could be done quickly before.	YES	NO	N/A
He/she has become unable to make a plan.	YES	NO	N/A
He/she cannot understand complex topics.	YES	NO	N/A
He/she has become less interested and willing, and stopped hobbies, etc.	YES	NO	N/A
He/she has become more irritable and suspicious than before.	YES	NO	N/A
TOTAL SED-11Q SCORE			

He/she has delusions, e.g., claims to have had valuables stolen.	YES	NO	N/A
He/she has illusions, e.g., sees something that isn't there.	YES	NO	N/A

If the answer is "yes" to either of these 2 questions, then a more comprehensive medical consultation is recommended.

Fig. 1. Format of the SED-11Q. The questionnaire represents an informant-based screening and should not be used as a patient-completed screening questionnaire. The total SED-11Q score is the sum of the items marked 'Yes'.

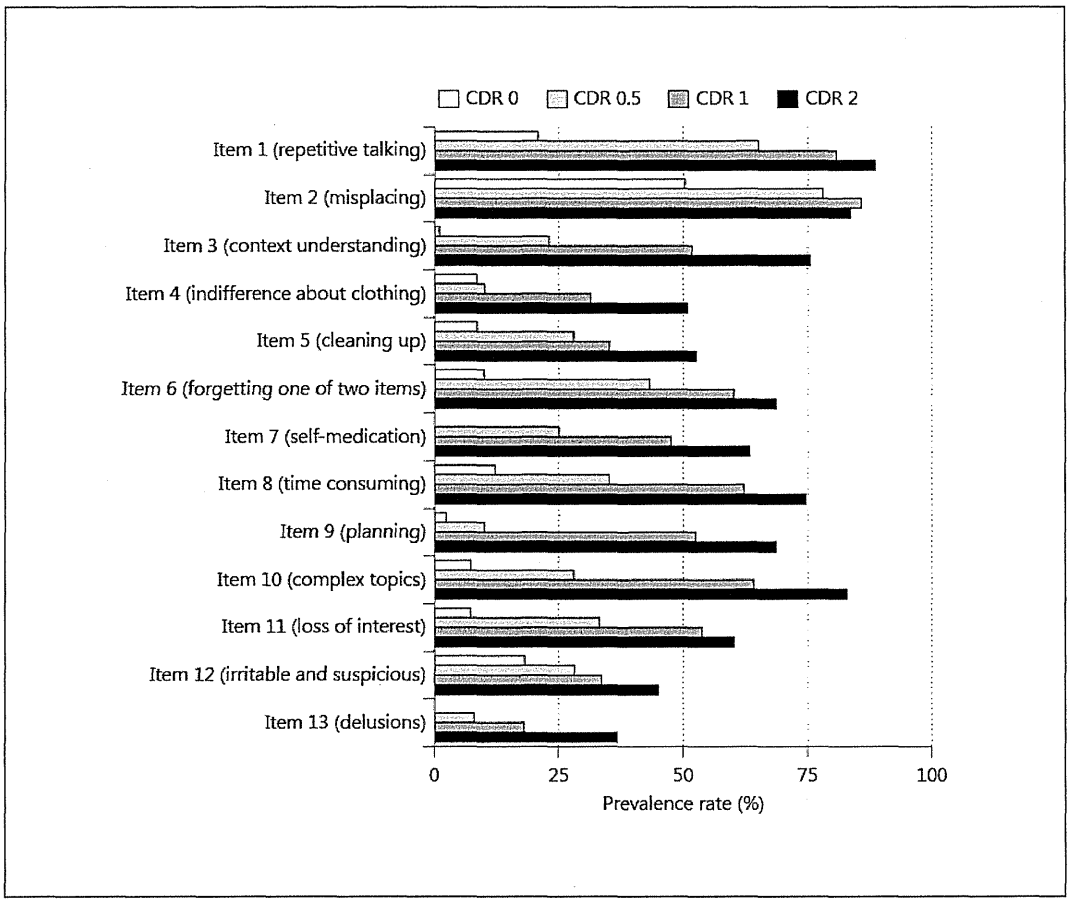

Fig. 2. The ratio of positive answers in the subitems (quotients) according to the CDR groups.

majority of the patients were those with AD in the present study. Item 2 (misplacing) was excluded from the SED-11Q because the specificity was 0.49, which suggested a danger of false positivity.

In CDR 1, deficits in instrumental activities of daily living (IADL) become obvious. In the present study, 4 items, item 3 (context understanding), item 6 (forgetting one of two items), item 8 (time-consuming), and item 9 (planning), showed sensitivities of 0.5 and more and specificities of 0.85 and more in the comparison between CDR 0 and CDR 1. It becomes difficult for the patients in CDR 1 to function independently, even if compensatory strategies are employed, and deficits can be noticed in cooperative activities and conversations in daily living.

The other 2 items regarding IADL, item 4 (indifference about clothing) and item 5 (cleaning up), showed sensitivities of less than 0.5, while specificities were 0.9 and more in the comparison between CDR 0 and CDR 1. It is possible that informants tend to pay little attention and overlook the deficits because of slow and gradual changes. These items remained in the list, considering a community-based use, as it could help elderly individuals if symptoms of dementia other than memory deficits are detected by using the questionnaire. Item 4 (indifference about clothing) could gradually lead to an apathetic attitude to life, which is one of the psychological behavioral symptoms of dementia and aggravates cognitive function.

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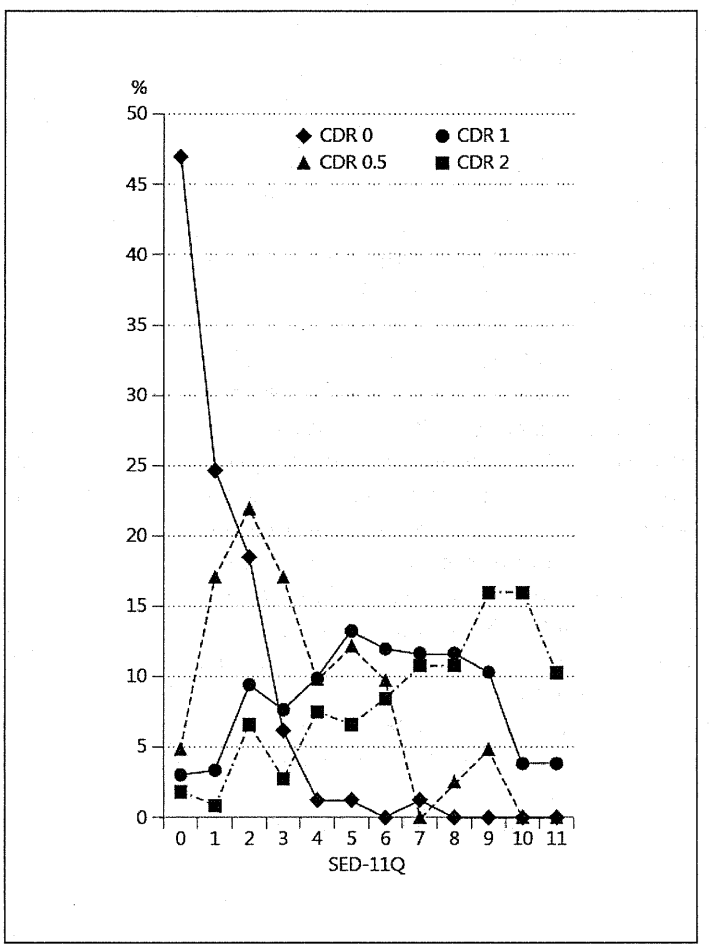

Fig. 3. Distribution according to the severity of dementia. The distribution is shown in the quotients. Modes of scores were 0 in CDR 0, 1 in CDR 0.5, 5 in CDR 1, and 9/10 in CDR 2.

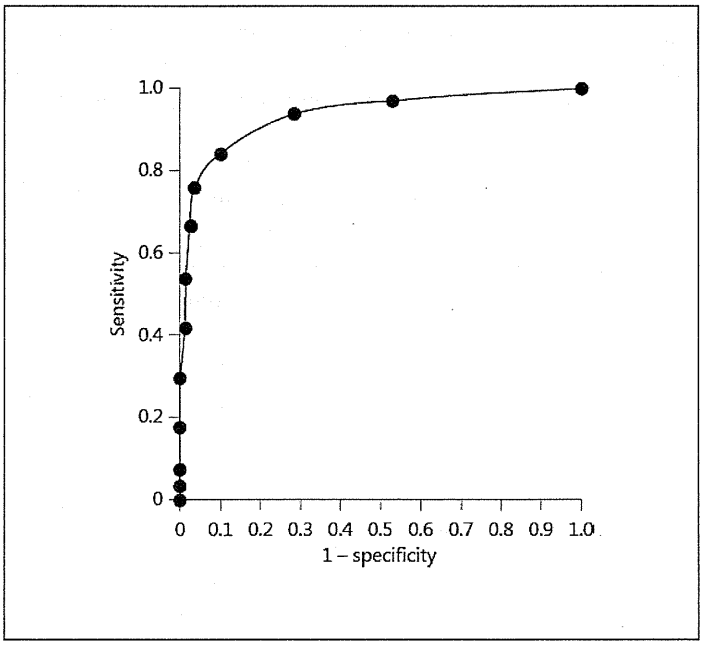

Fig. 4. The ROC curve of the SED-11Q in the differentiation between CDR 0 and CDR 1. The area under the curve is 0.932 ($p < 0.001$, 95% CI: 0.903–0.961). The statistically optimal cutoff value of 2/3 indicated a sensitivity of 0.841 and a specificity of 0.901.

Item 5 (cleaning up) is a typical behavior of demented individuals with attentional deficits and difficulties in executive function.

A relatively low sensitivity of 0.47 in item 7 (self-medication) is rather unexpected, because previous studies suggested that self-medication is one of the early signs of dementia [19–24]. Possible causes for this discrepancy could be that some patients may have developed long-term habits of medication, e.g., antihypertensive drugs, while others may not have taken any medications, as the questionnaire was filled out at the initial visit. A specificity of 100% suggested that self-medication could be a robust sign of dementia, and follow-up is needed to observe whether those already affected by dementia can acquire a habit of self-medication or not.

For item 10 (complex topics), cognitive decline is associated with difficulties in social interaction. Socially active lifestyles have a protective influence on mental decline: social isolation is associated with an increased risk of mental decline [25, 26], whereas a rich social network and interaction may protect against mental decline [27, 28]. It becomes difficult for patients to maintain socially active lives, and thus it is desirable that families and caregivers help the patients keep up social interaction.

Changes in interests are also characteristic of the early stages of dementia, as assessed by item 11 (loss of interest) in this questionnaire. Due to cognitive decline, patients are confronted with the loss of mental capacity. They have difficulty in doing what they could easily do and have enjoyed doing previously. Consequently, they lose their motivation and tend to be apathetic. Item 11 (loss of interest) is also related to social interaction; it becomes difficult to maintain social interaction even with those sharing the same hobbies. Families and caregivers should watch for changes because apathy and inaction are related to increased functional disability [29].

Personal changes are also observed in individuals with dementias other than AD, and the SED-11Q aims to detect these dementias. Item 12 asks about changes relating to irritability and suspicion, which are observed in frontotemporal lobar degeneration and are early signs of disease. Items 4 and 11 are associated with apathetic changes, which are more prominent in vascular dementia than in AD [30]. These 2 items could lead to depressive tendencies, which is a frequent symptom of Lewy body dementia [31].

Concerning item 13 (delusions), sensitivity was low, whereas specificity was 100%. Thus, it could be regarded as a determining factor in the diagnosis of dementia or other psychiatric diseases. In the SED-11Q, item 13 was excluded and notification was added to recommend medical consultation whenever delusions or illusions are detected.

Discriminative Ability of the SED-11Q

In the clinical setting, a statistically optimal cutoff value of 2/3 can be applied, whereas in the community setting, a cutoff value of 3/4 is recommended (fig. 4). In the clinical setting, medical staff can determine whether the informants are reliable or not, and they can interview the informants and patients if necessary, whereas in the community setting, such screening is often conducted without medical staff.

In general, during screening, false positives may be preferable to false negatives because failure of early detection could result in a lack of early intervention. However, considering the characteristics of dementia, false negatives are preferable in brief screening. Disease-modifying drugs have not been developed and cognitive and functional capacity would inevitably decline as the disease progresses. The US Preventive Services Task Force (USPSTF) warned that labeling an individual with dementia could potentially cause unnecessary anxiety, which is a risk factor for cognitive decline [32]. Therefore, physicians should perform a careful examination to make a definitive diagnosis, but cognitive tests for dementia are inherently potentially stressful. In this way, not only an incorrect diagnosis of dementia, but also the