https://doi.org/10.15388/vu.thesis.560
https://orcid.org/0000-0003-2097-4446

VILNIUS UNIVERSITY

Eglė Audronytė

Characteristics and Diagnostic Properties of Odour Identification, Odour Discrimination and Olfactory Memory in Early-Stage Alzheimer's Disease

DOCTORAL DISSERTATION

Medical and Health Sciences,
Medicine (M 001)

VILNIUS 2023

The dissertation was prepared between 2017 and 2022 at the Clinic of Neurology and Neurosurgery, Institute of Clinical Medicine, Vilnius University Faculty of Medicine.

Academic supervisor:
Prof. Dr. Gintaras Ferdinandas Kaubrys (Vilnius University, Medical and Health Sciences, Medicine – M 001).

Dissertation Defence Panel:
Chairman – Prof. Dr. Janina Tutkuvienė (Vilnius University, Medical and Health sciences, Medicine – M 001).

Members:
Doc. Dr. Nataša Giedraitienė (Vilnius University, Medical and Health sciences, Medicine – M 001),
Prof. Dr. Katrin Gross-Paju (Tallinn University of Technology, Medical and Health sciences, Medicine – M 001),
Prof. Dr. Eugenijus Lesinskas (Vilnius University, Medical and Health sciences, Medicine – M 001),
Prof. Dr. Antanas Vaitkus (Lithuanian University of Health Sciences, Medical and Health sciences, Medicine – M 001).

The doctoral dissertation will be defended at a public meeting of the Dissertation Defence Panel at 12:00 on November 30, 2023 in the Conference Hall of Vilnius University hospital Santaros Klinikos.
Address: Santariškių str. 2, LT-08661, Vilnius, Lithuania.

The text of this dissertation can be accessed at the library of Vilnius University, as well as on the website of Vilnius University:
www.vu.lt/lt/naujienos/ivykiu-kalendorius

https://doi.org/10.15388/vu.thesis.560
https://orcid.org/0000-0003-2097-4446

VILNIAUS UNIVERSITETAS

Eglė Audronytė

Kvapų identifikacijos, diskriminacijos bei olfaktorinės atminties sutrikimai ir jų diagnostinė reikšmė ankstyvos Alzheimerio ligos metu

DAKTARO DISERTACIJA

Medicinos ir sveikatos mokslai,
Medicina (M 001)

VILNIUS 2023

Disertacija rengta 2017–2022 metais Vilniaus universiteto Medicinos fakulteto Klinikinės medicinos instituto Neurologijos ir neurochirurgijos klinikoje.

Mokslinis vadovas:
Prof. dr. Gintaras Ferdinandas Kaubrys (Vilniaus universitetas, medicinos ir sveikatos mokslai, medicina – M 001).

Gynimo taryba:
Pirmininkė – prof. dr. Janina Tutkuvienė (Vilniaus universitetas, medicinos ir sveikatos mokslai, medicina – M 001).

Nariai:
Doc. dr. Nataša Giedraitienė (Vilniaus universitetas, medicinos ir sveikatos mokslai, medicina – M 001),
Prof. dr. Katrin Gross-Paju (Talino technikos universitetas, medicinos ir sveikatos mokslai, medicina – M 001),
Prof. dr. Eugenijus Lesinskas (Vilniaus universitetas, medicinos ir sveikatos mokslai, medicina – M 001),
Prof. dr. Antanas Vaitkus (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, medicina – M 001).

Disertacija ginama viešame Gynimo tarybos posėdyje 2023 m. lapkričio 30 d., 12:00 val. Vilniaus universiteto ligoninės Santaros klinikų konferencijų salėje. Adresas: Santariškių g. 2, LT-08661, Vilnius, Lietuva.

Disertaciją galima peržiūrėti Vilniaus universiteto bibliotekoje ir VU interneto svetainėje adresu: *https://www.vu.lt/naujienos/ivykiu-kalendorius*

ABBREVIATIONS

AChEI	Acetylcholinesterase Inhibitor
AD	Alzheimer's Disease
ADAS-Cog	Alzheimer's Disease Assessment Scale-Cognitive Subscale
ADI	Alzheimer's Disease International
ANOVA	One-Way Analysis of Variance
ApoE4	Apolipoprotein E ε4
AUC	Area Under the Curve
Aβ	Amyloid-beta
CDR	Clinical Dementia Rating Scale
CI	Confidence interval
CSF	Cerebrospinal Fluid
FDG-PET	[18F]-fluorodeoxyglucose Positron Emission Tomography
fMRI	Functional Magnetic Resonance Imaging
GDS	Geriatric Depression Scale
HIS	Hachinski Ischemic Score
IQR	Interquartile Range
MCI	Mild Cognitive Impairment
MMSE	Mini-Mental State Examination
MRI	Magnetic resonance imaging
NIA/AA	National Institute on Aging-Alzheimer's Association
ROC	Receiver Operating Characteristic
SCD	Subjective Cognitive Decline
UPSIT	The University of Pennsylvania Smell Identification Test
VFT	Verbal Fluency Tests Combined Result

CONTENTS

LIST OF SCIENTIFIC PAPERS 8
PREAMBLE 9
1. INTRODUCION 10
1.1. Research Problem and Relevance of the Study 10
1.2. Research Aims 11
1.3. Research Objectives 11
1.4. Scientific Novelty 12
1.5. Practical Value of the Study 12
1.6. Defended Statements 13
2. LITERATURE REVIEW 14
2.1. Odour Identification 14
2.2. Odour Discrimination 15
2.3. Olfactory Memory 15
3. RESEARCH DESIGN AND METHODS 17
3.1. Ethical Approval 17
3.2. Participants 17
3.3. Assessment of Demographic and Cognitive Characteristics 18
3.4. Assessment of Odour Identification 19
3.5. Assessment of Odour Discrimination 19
3.6. Assessment of Olfactory Memory 20
3.7. Statistical Analysis 20
4. RESULTS 22
4.1. Demographic and Clinical Characteristics 22
4.2. Cognitive Characteristics 23
4.2.1. Global Cognitive Assessment 23
4.2.2. Verbal Memory Assessment 23
4.3. Odour Identification 25
4.3.1. Odour Identification Assessment 25
4.3.2. Diagnostic Characteristics of the Odour Identification Score 27

4.3.3. Identification of the Specific Odours 29
4.3.4. Diagnostic Characteristics of the Shortened Version of the Odour Identification Score 30
4.4. Odour Discrimination 32
4.4.1. Odour Discrimination Assessment 32
4.4.2. Diagnostic Characteristics of the Odour Discrimination Score .. 34
4.4.3. Discrimination of the Specific Odours 36
4.4.4. Diagnostic Characteristics of the Shortened Version of the Odour Discrimination Score 37
4.5. Olfactory Memory 39
5. DISCUSSION 42
6. CONCLUSIONS 47
7. FUTURE PERSPECTIVES/PRACTICAL RECOMMENDATIONS 48
SUMMARY IN LITHUANIAN 49
REFERENCES 82
COPIES OF PUBLICATIONS 90
LIST OF PUBLICATIONS NOT INCLUDED IN THE THESIS 131
LIST OF PRESENTATIONS 132
ANNEX 133

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. **Audronyte E**, Pakulaite-Kazliene G, Sutnikiene V, Kaubrys G. *Properties of odor identification testing in screening for early-stage Alzheimer's disease*. Sci Rep. 2023;13(1):6075. Published 2023 Apr 13.
doi:10.1038/s41598-023-32878-w

II. **Audronyte E**, Pakulaite-Kazliene G, Sutnikiene V, Kaubrys G. *Odor Discrimination as a Marker of Early Alzheimer's Disease*. J Alzheimers Dis. 2023;94(3):1169-1178. Published 2023 Aug 1.
doi:10.3233/JAD-230077

III. **Audronyte E**, Sutnikiene V, Pakulaite-Kazliene G, Kaubrys G. *Brief Test of Olfactory Dysfunction Based on Diagnostic Features of Specific Odors in Early-Stage Alzheimer Disease*. Med Sci Monit. 2023;29:e940363. Published 2023 May 27.
doi:10.12659/MSM.940363

IV. **Audronyte E**, Sutnikiene V, Pakulaite-Kazliene G, Kaubrys G. *Olfactory memory in mild cognitive impairment and Alzheimer's disease*. Front. Neurol. 2023;14:1165594. Published 2023 June 02.
doi: 10.3389/fneur.2023.1165594

PREAMBLE

The doctoral dissertation is submitted for defence as a set of research articles and some parts have been quoted verbatim from the previously published articles listed at the end of the book.

The dissertation consists of several parts, which are all connected.

First, we analysed odour identification in patients with early-stage Alzheimer's disease and diagnostic properties of odour identification testing for diagnosing early-stage Alzheimer's disease. Then we analysed odour discrimination in patients with early-stage Alzheimer's disease and diagnostic properties of odour discrimination testing for diagnosing early-stage Alzheimer's disease. After that we analysed the performance of patients with early-stage Alzheimer's disease in identifying and discriminating specific odorants and created shortened versions of the tests. We also analysed diagnostic properties of these shortened versions of odour identification and odour discrimination tests for diagnosing early-stage Alzheimer's disease. Finally, we analysed olfactory memory in patients with early-stage Alzheimer's disease and explored the differences between olfactory and verbal memory deficits.

1. INTRODUCION

1.1. Research Problem and Relevance of the Study

Dementia is a leading cause of disability and dependency globally (1). In 2019, more than 55 million persons were estimated to have dementia worldwide (1). The prevalence of dementia is continuously increasing and is estimated to reach 139 million cases by 2050 (1,2). Consequently, the social and economic impacts of the disease are becoming major issues, making it a global healthcare priority (1,2). Despite this, most patients remain undiagnosed, with Alzheimer's Disease International (ADI) estimating 75% of undiagnosed dementia cases worldwide (2). Therefore, identifying affordable and widely accessible measures to achieve accurate diagnosis is increasingly important.

Alzheimer's disease (AD) is the most common cause of dementia and is estimated to cause 60–70% of all cases (3). Despite recent advances in neuroimaging, cerebrospinal fluid (CSF), and blood biomarkers, diagnosing early-stage AD remains challenging (4-6). Currently available biomarkers for diagnosing AD measure brain amyloid-beta (Aβ) protein deposition and neuronal degeneration and require CSF analysis or advanced neuroimaging techniques (4,5). Thus, the routine use of these biomarkers is limited because of the cost and invasive nature of the tests. To select patients who would benefit from these testing methods, identifying markers that would be easily accessible is necessary for the screening of wide populations.

These screening markers should not only reliably identify patients with AD but should also be able to do so in the early stages of the disease. In 2022, most medications used in clinical trials for AD were disease-modifying therapies (83.2%), predominantly aimed at patients with early-stage AD (7). As these medications enter clinical practice, there is a growing need for reliable and sensitive markers of early-stage AD that would also be accessible in community settings.

Olfactory testing was proposed as a simple, non-invasive and affordable marker that could be used for this purpose. Olfactory dysfunction in AD patients has been studied for nearly 50 years at this point (8). Numerous studies have confirmed that it is a common symptom, present in up to 90% of patients with AD (9,10). It is not only a common symptom, but also a very early sign of AD. Olfactory impairment is consistently found in patients with mild cognitive impairment (MCI) (11-13). It is also present in patients with subjective cognitive decline (SCD) and is thought to precede cognitive

symptoms for several years (14,15). The pathological evidence supports these clinical findings. Structures involved in the processing of olfactory information (especially entorhinal and transentorhinal areas) are affected by AD pathology early in the course of the disease (16,17). Functional magnetic resonance imaging (fMRI) and [18F]-fluorodeoxyglucose positron emission tomography (FDG-PET) also confirmed structural and functional abnormalities of olfaction-related regions in AD patients as early as SCD (18-21).

Despite these findings, olfactory testing is rarely used in clinical practice. In order to introduce olfactory testing into everyday practice, more data are needed regarding specific olfactory tests and their diagnostic properties.

This doctoral dissertation is based on four published research articles. Its overall aim is to evaluate olfactory impairments in early-stage AD, determine their diagnostic properties in identifying patients with early-stage AD and explore the differences between olfactory and verbal memory deficits in AD patients. **Paper I** analyses odour identification in patients with early-stage AD and diagnostic properties of odour identification testing for diagnosing early-stage AD. **Paper II** analyses odour discrimination in patients with early-stage AD and diagnostic properties of odour discrimination testing for diagnosing early-stage AD. **Paper III** analyses the performance of patients with early-stage AD in identifying and discriminating specific odorants and diagnostic properties of shortened versions of odour identification and odour discrimination tests for diagnosing early-stage AD. **Paper IV** analyses olfactory memory in patients with early-stage Alzheimer's disease and explores the differences between olfactory and verbal memory deficits.

1.2. Research Aims

The aim of this thesis was to evaluate olfactory impairments in early-stage AD, determine their diagnostic properties for identifying patients with early-stage AD and explore the differences between olfactory and cognitive deficits.

1.3. Research Objectives

1. To evaluate odour identification and its diagnostic properties in early-stage AD (**paper I**).
2. To evaluate odour discrimination and its diagnostic properties in early-stage AD (**paper II**).

3. To compare olfactory impairments with cognitive impairments and explore the additional value of olfactory testing in diagnosing early-stage AD (**papers I, II**).
4. To evaluate identification and discrimination of specific odorants in early-stage AD, create shortened versions of the olfactory tests and explore their diagnostic properties (**paper III**).
5. To evaluate olfactory memory and compare patterns of olfactory and verbal memory impairments in patients with early-stage AD (**paper IV**).

1.4. Scientific Novelty

Generally accepted criteria for MCI due to AD (4) were used in the current study, addressing the inconsistencies remaining due to variable definitions of MCI subtypes or lack of subtyping of MCI in the previous research on odour identification in patients with AD.

Odour discrimination and olfactory memory were tested in addition to odour identification, addressing the lack of data regarding these functions in patients with AD.

Finally, the Sniffin' Sticks test was used in the current study instead of the more commonly used the University of Pennsylvania Smell Identification Test (UPSIT). Gathering data on different olfactory tests is very important because the cost of the most commonly used tests could be a factor limiting their wider application. The UPSIT and all other tests derived from it are single-use "scratch-and-sniff" tests that must be purchased separately for each patient (22,23). The Sniffin' Sticks test was designed to be used repetitively over a period of several months (24).

1.5. Practical Value of the Study

Diagnosing early-stage AD remains challenging. Introduction of olfactory testing into clinical practice could serve as an affordable and accessible method of improving the diagnostic process.

Despite extensive research on olfactory impairments in AD, olfactory testing is rarely used in clinical practice at the time. The current study addresses the issues limiting wider application of olfactory testing such as lack of generally accepted AD diagnostic criteria in the previous research and insufficient data on odour discrimination and olfactory memory. Moreover, shortened versions of olfactory tests were created in order to improve the applicability of olfactory testing in everyday practice.

1.6. Defended Statements

1. Odour identification is impaired in early-stage AD and odour identification testing has good diagnostic properties for differentiating patients with early-stage AD from healthy subjects (**paper I**).
2. Odour discrimination is impaired in early-stage AD and odour discrimination testing has good diagnostic properties for differentiating patients with early-stage AD from healthy subjects (**paper II**).
3. Odour identification and odour discrimination scores correlate with results of cognitive testing and provide additional value in diagnosing early-stage AD (**papers I and II**).
4. Shortened versions of odour identification and odour discrimination tests that are created based on performance of patients with early-stage AD have good diagnostic properties for differentiating patients with early-stage AD from healthy subjects (**paper III**).
5. Olfactory memory is impaired in patients with early-stage AD but demonstrates a different pattern of impairment than verbal memory (**paper IV**).

2. LITERATURE REVIEW

2.1. Odour Identification

Odour identification tests have been the most studied olfactory assessment methods in patients with AD. Impairment of odour identification is found in the earliest stages of AD (14,15). Moreover, results from longitudinal studies suggest that impairment of odour identification could be used to predict future cognitive decline during follow-up. In longitudinal studies, odour identification impairment was found to be associated with an increased risk of MCI in healthy individuals (25-28) as well as with an increased risk of conversion to dementia in patients with MCI (29-32). In contrast, intact odour identification abilities were found to be reliable in identifying individuals who rarely transition to dementia in the future (33).

Despite that, odour identification testing is rarely used in clinical practice. There are several reasons for that. First of all, some uncertainties remain regarding changes observed during the early stages of the disease. Studies with MCI and SCD patients often use variable definitions of subtypes or do not subtype cognitive impairments at all (12, 14). This complicates the interpretation of the results with regard to AD specifically and makes it difficult to apply them in clinical practice, as it is likely that a heterogeneous mix of MCI and SCD patients with varying neurological conditions was analysed together (12).

Another possible limitation of olfactory testing in clinical practice is the relatively long duration of assessment. The University of Pennsylvania Smell Identification Test (UPSIT) is the most commonly used odour identification test and consists of 40 odours (22,23). The Sniffin' Sticks Test, which is particularly popular in Europe, consists of 16 odours (22,24). However, intervals of at least 30 seconds are recommended between the presentation of odours to prevent olfactory desensitization (24). Various shortened versions of the UPSIT, such as the Brief Smell Identification Test, Quick Smell Identification Test, and Pocket Smell Test, were created in order to provide a more convenient method for screening patients (22,34). Some of these shortened versions have even been tested in patients with AD and have shown encouraging results (35,36). Attempts have also been made to select UPSIT items that would be most specific for detecting AD (37-39). A shortened version of the Sniffin' Sticks odour identification test was also created (40). However, it was not tested in patients with AD.

Despite these limitations, results from previous studies indicate that odour identification testing is a promising method for improving the accuracy

of an early-stage AD diagnosis and could be introduced into clinical practice if more data are obtained using generally accepted AD diagnostic criteria and assessment methods that are convenient and accessible in clinical practice.

2.2. Odour Discrimination

The odour discrimination ability of the AD patients has rarely been analysed in previous research, even though both odour identification and odour discrimination tasks are considered to reflect higher processing of odours, that is impaired in the case of AD (41).

The findings from previous studies indicate that odour discrimination is a significant and even more reliable predictor of future cognitive decline, than odour identification (42). However, other authors did not find odour discrimination to be superior to odour identification, even though both of them performed better then odour threshold in differentiating patients with AD and patients with MCI from cognitively normal participants (43).

More research is needed on the subject of odour discrimination, as odour identification tasks have several shortcomings. First of all, performance on odour identification tests is heavily influenced by patients' personal and cultural experiences and familiarity with different odours, making it impossible to use the same odour identification tests across different populations without adaptations (44). Secondly, odour identification is known to be influenced by a subject's language abilities, making it difficult for researchers to interpret the results (45,46). Odour discrimination, although not completely independent of these factors, does not experience these limitations to the same extent as odour identification.

Thus, odour discrimination is also a promising method of diagnosing early-stage AD with potentially even better applicability than odour identification.

2.3. Olfactory Memory

Data regarding olfactory memory in AD patients are especially sparse. Animal studies have yielded promising results. Olfactory memory deficits have been observed in mouse models of AD (47,48). Olfactory memory impairment and altered functioning of olfactory network was also found in apolipoprotein E ε4 (ApoE4) knock-in mice (49).

Studies on cognitively unimpaired human subjects at higher risk of AD (ApoE4 carriers) have confirmed these findings. ApoE4 carriers have

impaired olfactory memory abilities (50,51) and altered activation on fMRI during olfactory memory tasks (52).

However, few studies have been conducted in patients with AD. Furthermore, the results are inconsistent, with some authors finding olfactory memory to be affected in patients with AD (53,54), while others found only odour identification to be impaired, with no deficits in olfactory memory (55).

The lack of research on olfactory memory probably stems from the difficulties in assessing this function. Typically, odour familiarity ratings or various odour recognition tasks are employed for this purpose (53,56,57). However, there are no universally accepted methods for assessing olfactory memory, with some authors even measuring the verbal recall of previously presented odours (58).

Research on olfactory memory in AD patients is not only important as a way to find a marker that could improve diagnosis of early-stage AD. Since the anatomical structures involved in olfactory and verbal memory processes differ, collecting more data regarding specific patterns of olfactory and verbal memory impairment in patients with AD would help gain more insight into the progression of AD. As the pathogenesis of AD remains largely unknown, despite extensive research on the subject, a deeper understanding of AD symptoms and their progression would help gain more insight into the processes of the disease.

3. RESEARCH DESIGN AND METHODS

3.1. Ethical Approval

The study was approved by the Vilnius Regional Bioethics Committee (approval number: 2021/6-1355-830).

The study was conducted according to the principles of the Declaration of Helsinki.

All participants were informed of the study procedures, agreed to participate in the study and provided written informed consent by signing relevant written informed consent forms.

3.2. Participants

Ninety participants were enrolled in the study: 30 patients diagnosed with mild dementia due to AD (MD-AD), 30 patients diagnosed with MCI due to AD (MCI-AD), and 30 elderly subjects with normal cognition (CN).

The patients with MD-AD met the National Institute on Aging-Alzheimer's Association (NIA/AA) criteria for probable AD (McKhann et al, 2011 (5)) and had a Clinical Dementia Rating (CDR) of 1. All the patients were recruited from the memory clinic of Vilnius University Hospital Santaros Klinikos. Probable AD was diagnosed by a specialist based on core clinical criteria with increased level of certainty, as all the patients had documented progressive cognitive decline (5). Biomarker probability of AD aetiology was intermediate, as all the patients had evidence of neuronal injury based on structural magnetic resonance imaging (MRI) that was performed as a standard clinical practice when diagnosing AD based on regulations by the Ministry of Health of The Republic of Lithuania (5). Biomarkers of brain amyloid-beta (Aβ) protein deposition were not available.

Patients with MCI-AD met the NIA/AA criteria for MCI due to AD (Albert et al, 2011 (4)) and had a CDR of 0.5. All the patients were recruited from the memory clinic of Vilnius University Hospital Santaros Klinikos. MCI due to AD was diagnosed by a specialist when clinical and cognitive criteria were established and aetiology of MCI was consistent with AD pathophysiological process based on exclusion of vascular, traumatic, medical causes of cognitive decline and documented longitudinal decline in cognition (4). Biomarker probability of AD aetiology was intermediate, as all the patients had evidence of neuronal injury based on structural MRI that was performed as a standard clinical practice when diagnosing AD based on

regulations by the Ministry of Health of The Republic of Lithuania (4). Biomarkers of brain amyloid-beta (Aβ) protein deposition were not available.

Elderly subjects with CN had no cognitive complaints, a CDR of 0, and no neurological disorders.

Patients were only enrolled in the study if they were treatment-naïve or were taking a stable dose of an acetylcholinesterase inhibitor (AChEI) for at least 3 months.

Exclusion criteria based on possible effects on cognitive functioning were central nervous system disorders other than MCI-AD or MD-AD, a Hachinski Ischemic Score (HIS) ≥ 4, indicating possible significant cerebrovascular disease, previous significant head trauma, psychiatric conditions such as psychosis, substance abuse, significant depression (Geriatric Depression Scale (GDS) score > 9), and use of psychoactive medications. Exclusion criteria based on possible effects on olfaction were smoking, nasal trauma or surgery, significant exposure to volatile substances, and recent viral infections.

3.3. Assessment of Demographic and Cognitive Characteristics

Demographic information (age, sex, duration of AD symptoms, and medical history) was obtained from each participant.

For the assessment of global cognitive functioning, the Mini-Mental State Examination (MMSE) was performed (scores ranging from 0 to 30 with 0 representing the worst [all the tasks performed incorrectly] and 30 indicating the best [all the tasks performed correctly] performance) (59).

Cognitive and functional performance was additionally evaluated using the CDR scale (scores ranging from 0 to 3 with 0 representing the best [no impairment] and 3 indicating the worst [severe impairment] performance) (60).

Further evaluation was performed using the Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog, scores ranging from 0 to 70 with 0 representing the best and 70 indicating the worst performance) with additional delayed recall and number cancellation tasks (ADAS-Cog 13, scores ranging from 0 to 85 with 0 representing the best and 85 indicating the worst performance) (59). The delayed recall was scored from 0 to 10 (the number of words not recalled). The number cancellation task was scored from 0 to 5 (0 representing the best [≥30 correct responses] and 5 representing the worst [0-5 correct responses] performance). Additionally, delayed verbal recall was evaluated after 30 minutes.

Phonemic (PAS) and categorical (animals) verbal fluency were also evaluated.

3.4. Assessment of Odour Identification

The Sniffin' Sticks odour identification test was performed (Burghart®, Wedel, Germany).

The Sniffin' Sticks odour identification test consists of 16 odours: orange, leather, cinnamon, peppermint, banana, lemon, liquorice, turpentine, garlic, coffee, apple, clove, pineapple, rose, anise, and fish.

Each odour was presented using a felt tip pen. The cap was removed, and the odour was presented only once for 3–4 seconds with the tip of the pen placed approximately 2 cm in front of both nostrils. The participants were asked to choose which of the four items in the answering card best described the odour. They were prompted to choose an item, even if they were uncertain.

A time interval of 30 seconds was maintained between the odour presentations. The odour identification score is the number of correct responses out of sixteen (61).

The examiner used odourless gloves during the odour identification testing. The participants were asked not to drink or eat anything for at least 15 minutes prior to testing (61).

3.5. Assessment of Odour Discrimination

The Sniffin' Sticks odour discrimination test was performed (Burghart®, Wedel, Germany).

The Sniffin' Sticks odour discrimination test consists of 16 triplets of odours, presented using felt tip pens. The participant was asked to identify which item had a different odour from the other two in each triplet. The odours were presented in the order provided by the test instructions.

Each odour was presented only once, for 3–4 seconds with the tip of the pen placed approximately 2 cm in front of both nostrils. A time interval of 3 seconds was maintained between odours in the same triplet. A time interval of 30 seconds was maintained between the sets of triplets. The odour discrimination score is the number of correct responses out of 16 (61).

The examiner used odourless gloves, and the subjects wore a blindfold during the odour discrimination testing. The participants were asked not to drink or eat anything for at least 15 minutes prior to testing (61).

3.6. Assessment of Olfactory Memory

The olfactory memory assessment task was designed using odours from the Sniffin' Sticks odour identification test (Burghart®, Wedel, Germany).

In the encoding phase of the olfactory memory task, five odours were randomly assigned to each participant (target odours). Each of these five odours was presented for 3 seconds with the tip of the pen placed approximately 2 cm in front of both nostrils. The participants were instructed to memorize the odours without verbal clues.

Immediate olfactory recognition memory was assessed immediately after the encoding phase. Five new odours were randomly assigned to each participant (distractors). Distractors were presented with the target odours in a randomized manner. Each of the 10 odours was presented for 3 seconds with the tip of the pen placed approximately 2 cm in front of both nostrils. Participants were instructed to choose whether the odour was new or presented previously (target odour). The immediate odour recognition score was the number of correct answers (0–10).

Delayed olfactory recognition memory was tested 30 minutes after the encoding phase. Five new odours were randomly assigned to each participant (second group of distractors). Distractors were presented with the target odours in a randomized manner. Each of the 10 odours was presented for 3 seconds with the tip of the pen placed approximately 2 cm in front of both nostrils. Participants were instructed to choose whether the odour was new or presented previously (target odour). The delayed odour recognition score was the number of correct answers (0–10).

A time interval of 30 seconds was kept between odours.

All participants were instructed not to drink or eat anything for at least 15 min prior to testing. The examiner wore odourless gloves and the participants wore a blindfold.

3.7. Statistical Analysis

Statistical analysis was performed with IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA).

The Shapiro–Wilk test was used to determine the normality of data distribution.

Differences in categorical variables between groups were analysed using two-tailed chi-square and Fisher's exact tests.

Differences between two groups of non-normally distributed numerical variables were analysed using the Mann–Whitney U test.

Comparisons between three groups of numerical variables were performed using one-way analysis of variance (ANOVA) for normally distributed variables and the Kruskal–Wallis test for non-normally distributed variables.

The Spearman rank correlation coefficient was used to determine correlation between variables.

Linear regression was used to analyse prediction of continuous dependent variables, and multinomial logistic regression was used to analyse prediction of categorical variables.

The performance of the diagnostic tests was evaluated by receiver operating characteristic (ROC) curve analysis.

Statistical significance was set at a p-value of < 0.05. In the case of multiple comparisons using Fisher's exact test, Bonferroni correction was applied, and a p-value < 0.016 was considered significant.

4. RESULTS

4.1. Demographic and Clinical Characteristics

The demographic and clinical characteristics of the participants are presented in **Table 1**.

The participants in the three groups showed no significant difference in sex distribution (chi-square test, $p > 0.05$). There were also no significant differences according to education, depressive symptoms (GDS results), and HIS (for all, Kruskal–Wallis $p > 0.05$).

Participants in the MD-AD group were significantly older than those in the MCI-AD and CN groups, however, the MCI-AD and CN groups did not differ significantly in age (Kruskal–Wallis $p < 0.05$; post-hoc analysis revealed significant differences between the CN and MD-AD, MCI-AD and MD-AD groups, and no significant difference between the CN and MCI-AD groups).

As expected, patients with MD-AD had a significantly longer duration of AD symptoms than those with MCI-AD (Mann–Whitney U test, $p < 0.001$). There were significantly more patients taking AChEIs in the MD-AD group than in the MCI-AD group (two-tailed chi-square test, $p < 0.05$).

Table 1. Demographic and clinical characteristics of the participants

	CN (N=30)	MCI-AD (N=30)	MD-AD (N=30)
Male, n (%) *	13 (43.33%)	13 (43.33%)	12 (40%)
Age (years) **	74 [68.75–76]	72 [67.75–77.25]	78 [75–79.25]
Years of education *	15 [13.5–16]	16 [14–16]	16 [13–16]
HIS *	1 [0–1]	1 [0–1]	1 [1–1.25]
GDS *	5.5 [4–6.25]	5.5 [4–6]	5 [4–6.25]
Duration of AD symptoms (in years) ***	N/A	3 [2–3]	4 [3–5]
Use of AChEI, n (%) ***	N/A	3 (10%)	14 (46.7%)

Abbreviations: CN, elderly subjects with normal cognition; MCI-AD, mild cognitive impairment due to Alzheimer's disease; MD-AD, mild dementia due to Alzheimer's disease; HIS, Hachinski Ischemic Score; GDS, geriatric depression scale; AChEI, acetylcholinesterase inhibitor.

Data are presented as median and interquartile range unless otherwise specified.

* Groups do not differ significantly.

** MD-AD group differs significantly from CN and MCI-AD groups. CN and MCI-AD groups do not differ significantly from each other.

*** MD-AD group differs significantly from MCI-AD group.

4.2. Cognitive Characteristics

4.2.1. Global Cognitive Assessment

The performance on the MMSE, CDR, ADAS-Cog, ADAS-Cog 13 and verbal fluency tasks (combined verbal fluency score, VFT) was significantly different between all three groups (for all, Kruskal–Wallis $p < 0.05$, post-hoc analysis revealed significant differences among the three groups). The results of cognitive tests are presented in **Table 2**.

Table 2. Cognitive assessment of the participants

	CN (N=30)	MCI-AD (N=30)	MD-AD (N=30)
CDR sum of boxes *	0 [0–0]	2 [1.5–2.5]	5 [4.5–5.5]
MMSE *	29 [29–30]	26 [25–26]	22 [21–23]
ADAS-Cog *	5.33 [4.59–7]	11.33 [9.17–13.75]	17.67 [15.17–20.33]
ADAS-Cog 13 *	10.83 [7.92–12.5]	20.84 [18.58–23]	29.34 [26.67–32.33]
VFT *	57.5 [43–63]	41 [35–50.75]	29.5 [21–39]

Abbreviations: CN, elderly subjects with normal cognition; MCI-AD, mild cognitive impairment due to Alzheimer's disease; MD-AD, mild dementia due to Alzheimer's disease; CDR, clinical dementia rating; MMSE, Mini Mental State Examination; ADAS-Cog, Alzheimer's Disease Assessment Scale–Cognitive Subscale; ADAS-Cog 13, ADAS-Cog with additional delayed recall and number cancellation tasks; VFT, combined verbal fluency score.

Data are presented as median and interquartile range.

* All three groups differ significantly.

4.2.2. Verbal Memory Assessment

Immediate verbal recall (third trial on the ADAS-Cog word recall task), delayed verbal recall after 5 minutes, and delayed verbal recall after 30 minutes all differed significantly between the CN and MCI-AD groups, and the CN and MD-AD groups, but did not differ significantly between the MCI-AD and MD-AD groups (in all three cases, Kruskal–Wallis test $p < 0.05$; post hoc analysis revealed significant differences between the CN and MCI-AD groups, and CN and MD-AD groups [$p < 0.05$], and no significant difference between the MCI-AD and MD-AD groups [$p > 0.05$]). The results of the verbal memory tasks are shown in **Fig. 1**. Detailed results of verbal memory tasks can be found in **paper IV**.

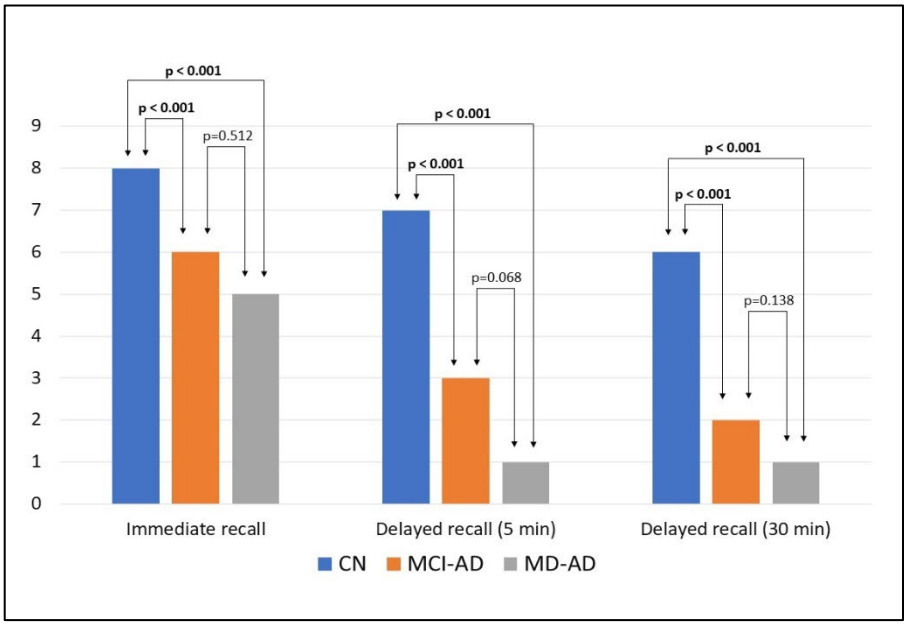


Fig. 1. Results of verbal recall memory tasks in three groups of participants. Bars represent medians

Abbreviations: CN, elderly subjects with normal cognition; MCI-AD, mild cognitive impairment due to Alzheimer's disease; MD-AD, mild dementia due to Alzheimer's disease.

The results of the verbal memory tasks were compared between patients with AD (MCI-AD and MD-AD participants) taking AChEIs and treatment-naïve patients.

Patients on AChEIs did not differ from treatment-naïve patients in immediate verbal recall, delayed verbal recall after 5 minutes, or delayed verbal recall after 30 minutes tasks (Mann–Whitney U test, $p > 0.05$ in all cases). However, the duration of AD symptoms was significantly longer in patients taking AChEIs than in those who were not (Mann–Whitney U test, $p < 0.001$).

Upon analysing the separate groups, the results remained the same. In both MCI-AD and MD-AD groups there were no significant differences between patients on AChEIs and patients not taking them in immediate verbal recall, delayed verbal recall after 5 minutes, and delayed verbal recall after 30 minutes (Mann–Whitney U test, $p > 0.05$ in all cases). The difference in the duration of AD symptoms between patients on treatment and untreated participants remained significant both in the MCI-AD, and in the MD-AD groups (Mann–Whitney U test, $p < 0.05$ in both cases).

Detailed results of verbal memory tasks can be found in **paper IV**.

4.3. Odour Identification

4.3.1. Odour Identification Assessment

One-way ANOVA revealed significant differences in odour identification scores among all three groups (mean and standard deviation: CN, 12.77±1.43; MCI-AD, 9.3±2.23; MD-AD, 7.0±2.13; $p < 0.001$, the post-hoc analysis revealed significant differences among all three groups). The results of odour identification testing are shown in **Fig. 2**.

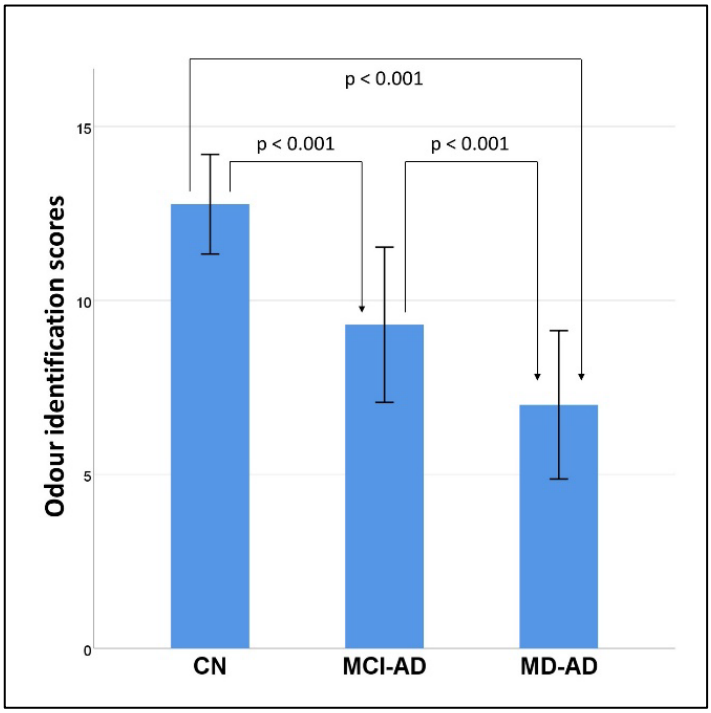


Fig. 2. Results of odour identification task in three groups of participants. Bars represent means and error bars represent standard deviations

Abbreviations: CN, elderly subjects with normal cognition; MCI-AD, mild cognitive impairment due to Alzheimer's disease; MD-AD, mild dementia due to Alzheimer's disease.

There was a significant, although weak, correlation between odour identification scores and age when the results of all participants were analysed (Spearman's rho: –0.334; $p = 0.001$). The correlation between odour identification and age remained significant in the CN group (Spearman's rho:

–0.365; p = 0.047) but not in the MCI-AD and MD-AD groups when analysed separately.

In the sample of all participants, odour identification scores were strongly correlated with the results of the cognitive tests: MMSE (Spearman's rho: 0.735; $p < 0.001$), ADAS-Cog 13 (Spearman's rho: –0.771; $p < 0.001$), CDR Sum of Boxes (Spearman's rho: -0.775; $p < 0.001$), and verbal fluency tests combined results [VFT = fluency PAS + fluency animals] (Spearman's rho: 0.720; $p < 0.001$). The relationship between odour identification scores and the ADAS-Cog 13 results is shown in **Fig. 3**.

Multiple linear regression models with age, sex, education, and cognitive test scores (MMSE, ADAS-Cog-13, CDR Sum of Boxes, and VFT) as independent variables were tested to determine whether they significantly predicted odour identification scores. The overall regression was statistically significant in all four models: model with MMSE ($R^2 = 0.525$, $F = 23.452$, $p < 0.001$), model with ADAS-Cog 13 ($R^2 = 0.560$, $F = 27.079$, $p < 0.001$), model with CDR Sum of Boxes ($R^2 = 0.569$, $F = 28.068$, $p < 0.001$), and model with VFT ($R^2 = 0.464$, $F = 18.403$, $p < 0.001$). However, only cognitive test scores significantly predicted odour identification scores in each case (MMSE: $\beta = 0.702$, $p < 0.001$; ADAS-Cog 13: $\beta = –0.735$, $p < 0.001$; CDR Sum of Boxes: $\beta = –0.735$, $p < 0.001$; VFT: $\beta = 0.719$; $p < 0.001$). None of the other predictors (age, sex, and education) significantly predicted odour identification scores in any of the models ($p > 0.05$).

Detailed analysis of correlations between odour identification scores and results of cognitive assessment in separate groups can be found in **paper I**.

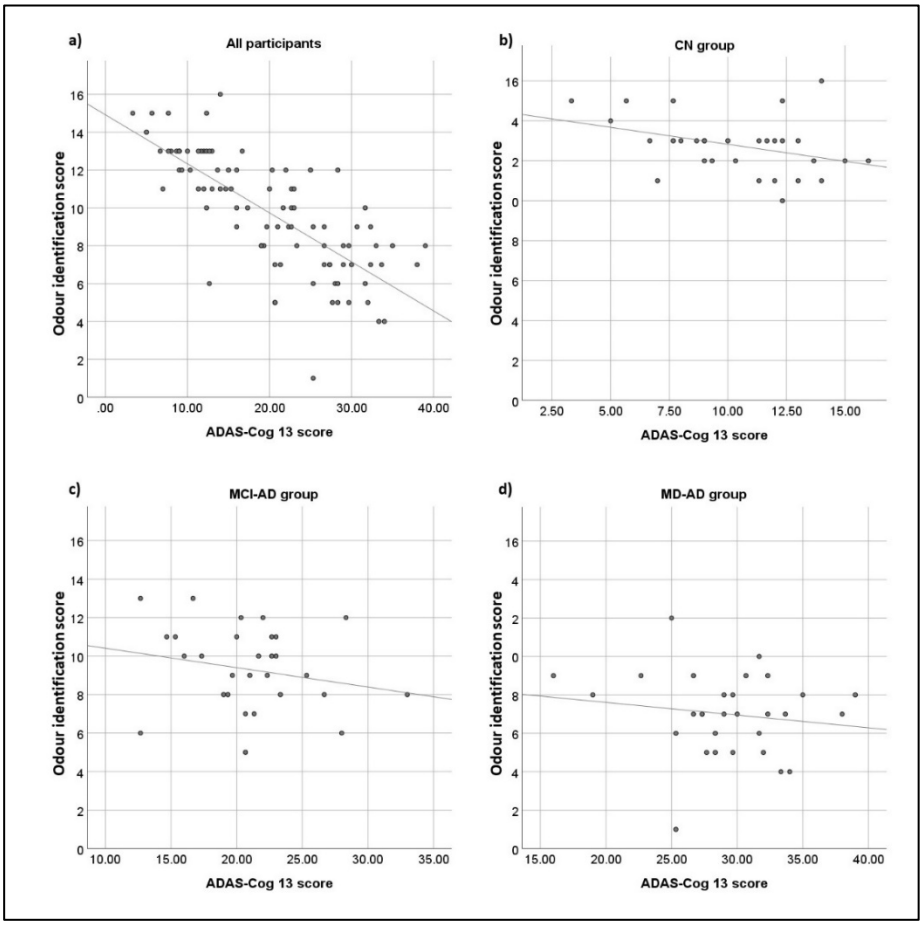


Fig. 3. Relationship between odour identification scores and ADAS-Cog 13 results

Abbreviations: CN, elderly subjects with normal cognition; MCI-AD, mild cognitive impairment due to Alzheimer's disease; MD-AD, mild dementia due to Alzheimer's disease; ADAS-Cog 13, Alzheimer's Disease Assessment Scale-Cognitive Subscale with additional delayed recall and number cancellation tasks.

4.3.2. Diagnostic Characteristics of the Odour Identification Score

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the performance of the odour identification score in differentiating the CN group from AD (MCI-AD or MD-AD), MCI-AD, and MD-AD patients and MCI-AD patients from MD-AD patients. The ROC curves with areas under the curve (AUC) are shown in **Fig. 4**.

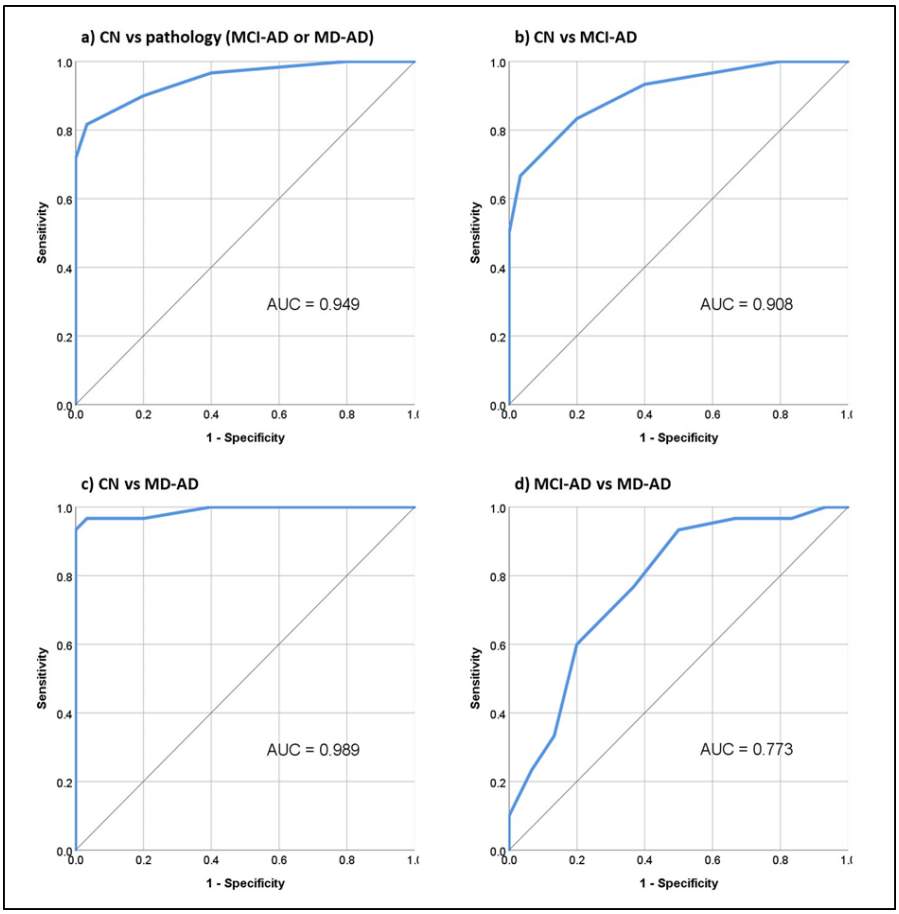


Fig. 4. Performance of odour identification score in differentiating between groups of participants

Abbreviations: CN, elderly subjects with normal cognition; MCI-AD, mild cognitive impairment due to Alzheimer's disease; MD-AD, mild dementia due to Alzheimer's disease; AUC, area under curve.

A cut-off score of ≤11 correct responses indicating AD was chosen.

Using this cut-off score for differentiating AD patients (MCI-AD or MD-AD) from CN participants, odour identification had sensitivity and specificity of 90% (95% confidence interval [CI]: 79.49–96.24%) and 80% (95% CI: 61.43–92.29%), respectively. In addition, the negative and positive predictive values were 80% (95% CI: 64.71–89.72%) and 90% (95% CI: 81.41–94.87%), respectively. The overall diagnostic accuracy was 86.67% (95% CI: 77.87–92.92%).

The diagnostic characteristics remained good when differentiating MCI-AD patients from CN participants. Using the same cut-off score of ≤11,

odour identification had a sensitivity and specificity of 83.33% (95% CI 65.28–94.36%) and 80% (95% CI: 61.43–92.29%), respectively. In addition, the negative and positive predictive values were 82.76% (95% CI: 67.89–91.59%) and 80.65% (95% CI: 66.68–89.66%), respectively. The overall diagnostic accuracy was 81.67% (95% CI: 69.56–90.48%).

Multinomial logistic regression was performed to analyse the relationship between predictor variables and membership in the three groups (CN, MCI-AD, and MD-AD).

First, a model using age, education, sex, and ADAS-Cog 13 scores as predictor variables was tested. The fit between the model containing only the intercept and the data improved with the addition of predictor variables (X^2 = 139.656, $p < 0.001$; Nagelkerke $R^2 = 0.887$). Pearson's X^2 and Deviance X^2 tests indicated that the model exhibited a good fit for the data ($p > 0.05$). The overall percentage of correctly classified cases using this model was 82.2% (93.3% CN, 70% MCI-AD, and 83.3% MD-AD cases correctly classified), with the ADAS-Cog 13 score as the strongest and most significant predictor ($X^2 = 122.652$, $p < 0.001$).

The odour identification scores were included in the model. The model with age, education, sex, ADAS-Cog 13 scores, and odour identification scores also showed a significant improvement in fit over a null model ($X^2 = 150.677$, $p < 0.001$; Nagelkerke $R^2 = 0.914$). Pearson's X^2 and Deviance X^2 tests indicated that the model exhibited a good fit for the data ($p > 0.05$). The overall percentage of correctly classified cases using this model was 87.8% (100% CN, 83.3% MCI-AD, and 80% MD-AD cases correctly classified), with ADAS-Cog 13 scores and olfactory identification scores both being strong and significant predictors ($X^2 = 52.635$ and 11.022, respectively; $p < 0.001$ and 0.004, respectively).

4.3.3. Identification of the Specific Odours

Five odours (leather, lemon, liquorice, apple, and pineapple) were excluded from further analysis because of poor identification (<70% correct responses) in the CN group.

The identification scores for the remaining odours are presented in **Table 3**.

Table 3. Identification of the specific Odours

Odour	CN correct responses (%)	MCI-AD correct responses (%)	MD-AD correct responses (%)
Clove [a, b]	30 (100)	19 (63.33) *	18 (60) *

Odour	CN correct responses (%)	MCI-AD correct responses (%)	MD-AD correct responses (%)
Fish	29 (96.67)	28 (93.33)	20 (66.67) *
Orange	29 (96.67)	23 (76.67)	19 (63.33) *
Garlic [b]	29 (96.67)	22 (73.33)	17 (56.67) *
Coffee	28 (93.33)	24 (80)	19 (63.33) *
Cinnamon [b]	28 (93.33)	19 (63.33) *	10 (33.33) *
Peppermint	27 (90)	24 (80)	16 (53.33) *
Rose	27 (90)	22 (73.33)	15 (50) *
Banana [b]	26 (86.67)	16 (53.33) *	7 (23.33) *
Anis	25 (83.33)	19 (63.33)	17 (56.67)
Turpentine	22 (73.33)	12 (40)	16 (53.33)

Abbreviations: CN, elderly subjects with normal cognition; MCI-AD, mild cognitive impairment due to Alzheimer's disease; MD-AD, mild dementia due to Alzheimer's disease.

* Significantly different from the NC group ($p < 0.016$).

[a] The odour that differed the most ($p < 0.001$) between the MCI-AD and NC groups.

[b] The odours that differed the most ($p < 0.001$) between the MD-AD and NC groups.

Nine of the remaining 11 odours (all except anis and turpentine) had significantly worse identification scores in the MD-AD group than in the CN group (Fisher's exact test, $p < 0.016$). Three odours (clove, garlic, and banana) had significantly worse identification scores in the MCI-AD group than in the CN group (Fisher's exact test, $p < 0.016$). The identification scores of all 11 odours did not differ significantly between the MCI-AD and MD-AD groups (Fisher's exact test, $p > 0.016$).

4.3.4. Diagnostic Characteristics of the Shortened Version of the Odour Identification Score

Four odours that had the greatest differences in identification scores between the MD-AD and CN groups were selected (Fisher's exact test, $p < 0.001$). The identification score for these four odours (clove, garlic, cinnamon, and banana) was calculated. ROC curve analysis was performed to evaluate the performance of the four-odour identification score in differentiating the CN participants from patients with AD (MCI-AD or MD-AD), MCI-AD, and MD-AD and the patients with MCI-AD from those with MD-AD. The ROC curves with the area under the curve (AUC) are shown in **Fig 5**.

A cut-off score of ≤3 for detecting AD was chosen.

Using this cut-off score for differentiating patients with AD (MCI-AD or MD-AD) from CN participants, the four-odour identification score had a sensitivity and specificity of 91.67% (95% CI: 81.61%–97.24%) and 76.67% (95% CI: 57.72%–90.07%), respectively. The negative and positive predictive

values were 82.14% (95% CI: 66.01%–91.59%) and 88.71% (95% CI: 80.35%–93.79%), respectively. The overall diagnostic accuracy was 86.67% (95% CI: 77.87%–92.92%).

The diagnostic characteristics remained good when differentiating patients with MCI-AD from CN participants. Using the same cut-off score of ≤3, the four-odour identification score had a sensitivity and specificity of 86.67% (95% CI: 69.28%–96.24%) and 76.67% (95% CI: 57.72%–90.07%), respectively. The negative and positive predictive values were 85.19% (95% CI: 69.33%–93.60%) and 78.79% (95% CI: 65.67%–87.82%), respectively. The overall diagnostic accuracy was 81.67% (95% CI: 69.56%–90.48%).

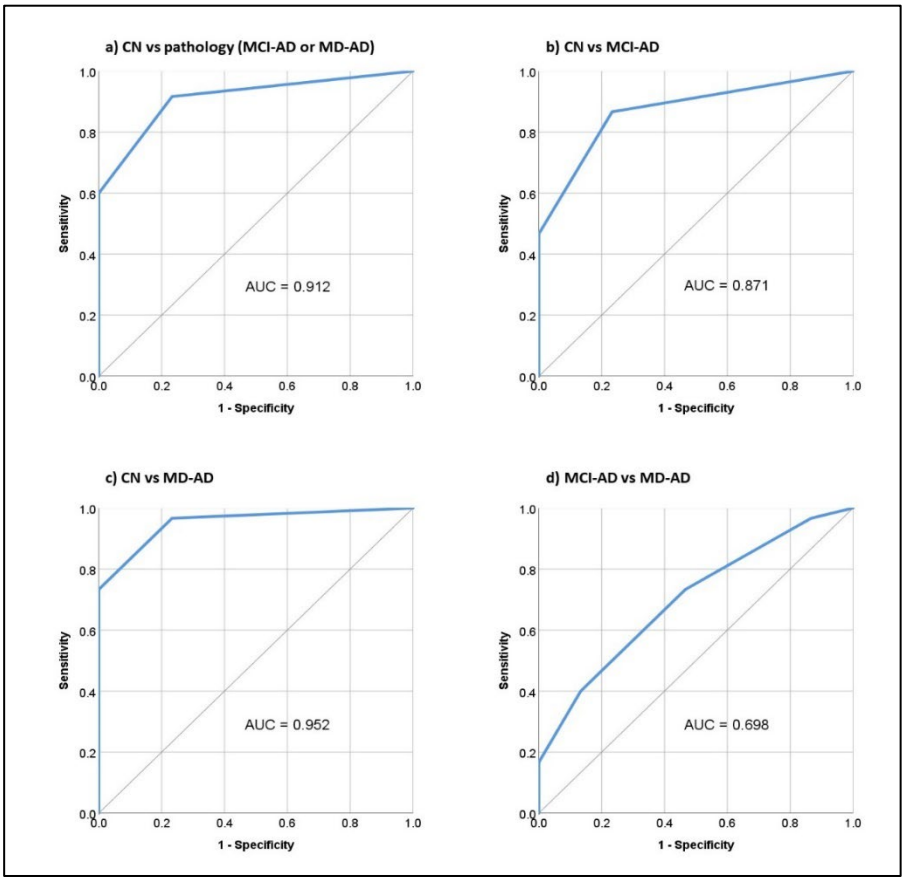


Fig. 5. Performance of the four-odour identification score in differentiating between groups of participants

Abbreviations: CN, elderly subjects with normal cognition; MCI-AD, mild cognitive impairment due to Alzheimer's disease; MD-AD, mild dementia due to Alzheimer's disease; AUC, area under curve.

4.4. Odour Discrimination

4.4.1. Odour Discrimination Assessment

The odour discrimination scores differed significantly among the three groups (medians and interquartile ranges (IQR): CN 12.5 (3), MCI-AD 9 (3), MD-AD 6 (2); Kruskal–Wallis $p < 0.05$, post hoc analysis revealed significant differences between all three groups). The results of odour discrimination testing are shown in **Fig. 6**.

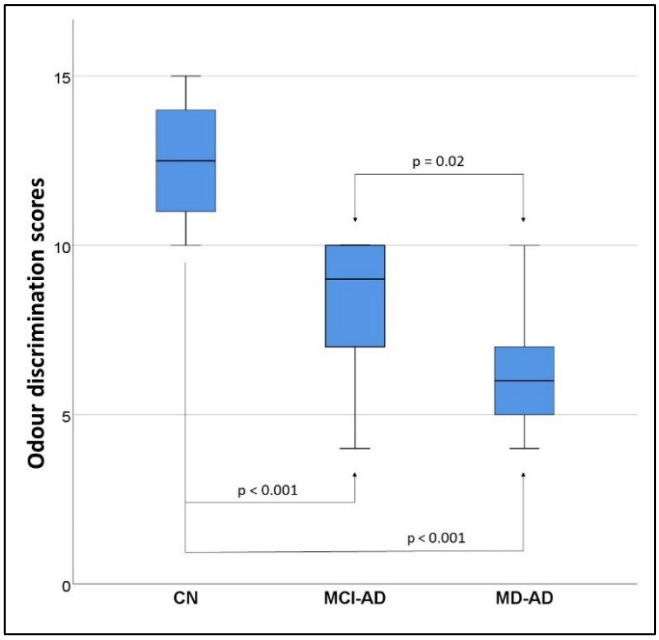


Fig. 6. Results of odour discrimination task in three groups of participants. Lines represent medians, boxes represent Interquartile ranges, and error bars represent minimum and maximum scores

Abbreviations: CN, elderly subjects with normal cognition; MCI-AD, mild cognitive impairment due to Alzheimer's disease; MD-AD, mild dementia due to Alzheimer's disease.

The odour discrimination scores correlated significantly, although very weakly, with age when analysing the entire sample (Spearman's Rho -0.28; $p = 0.008$). There were no significant correlations between odour discrimination ability and age in the separate groups.

In the sample of all participants, the odour discrimination scores strongly correlated with the results of the MMSE and ADAS-Cog 13

(Spearman's rho 0.78 and -0.77, respectively; $p < 0.001$). The odour discrimination scores also showed a strong correlation with the CDR sum of boxes (Spearman's rho = -0.82; $p < 0.001$). The correlation between the odour discrimination scores and verbal fluency tests was also significant but at a moderate level (Spearman's rho for PAS fluency = 0.64, for animal fluency = 0.64; $p < 0.001$ for both). The relationship between odour discrimination scores and the ADAS-Cog results is shown in **Fig. 7**.

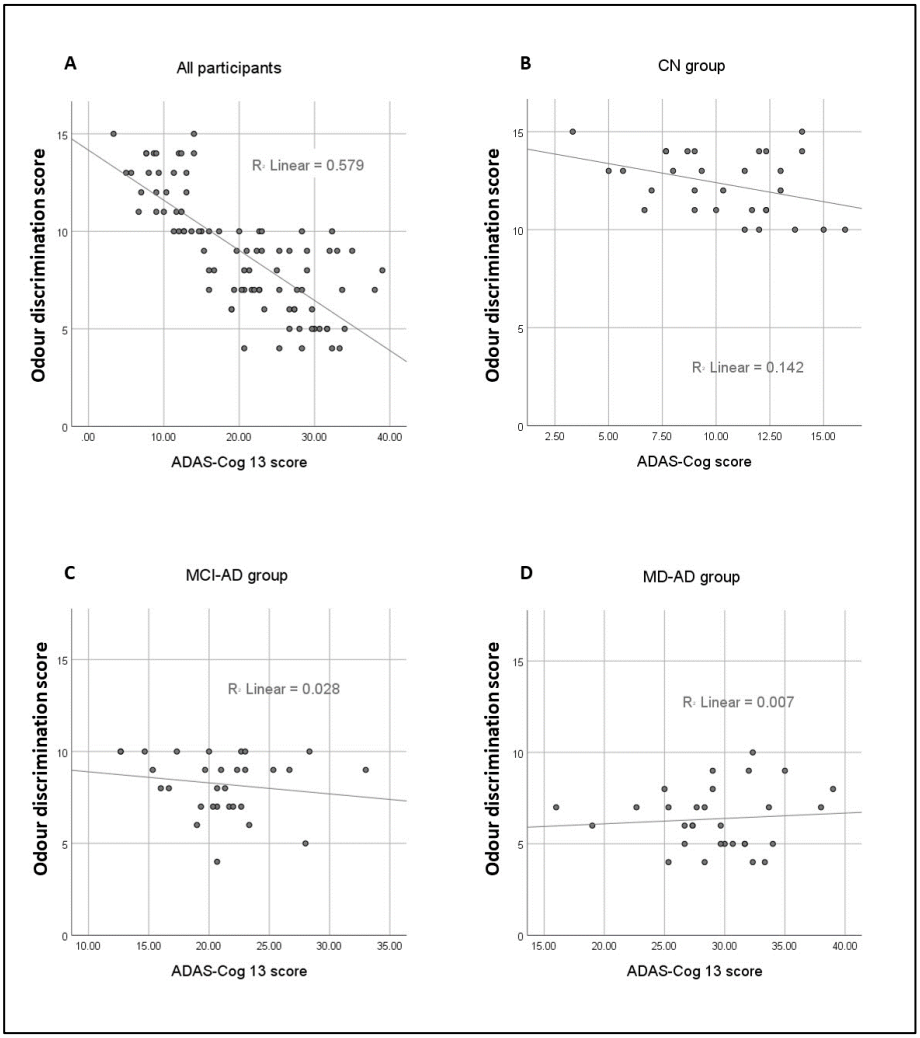


Fig. 7. Relationship between odour discrimination scores and ADAS-Cog 13 results

Abbreviations: CN, elderly subjects with normal cognition; MCI-AD, mild cognitive impairment due to Alzheimer's disease; MD-AD, mild dementia due to Alzheimer's disease; ADAS-Cog, Alzheimer's Disease Assessment Scale-Cognitive Subscale.

Multiple linear regression models, including age, sex, education, and cognitive test scores (MMSE, ADAS-Cog-13, CDR Sum of Boxes, and VFT) as independent variables, were tested to determine whether they significantly predicted olfactory discrimination scores. The overall regression was statistically significant for all four models: model with MMSE $R^2 = 0.62$, $F = 35.05$, $p < 0.001$; model with ADAS-Cog 13 $R^2 = 0.58$, $F = 29.43$, $p < 0.001$; model with CDR Sum of Boxes $R^2 = 0.63$, $F = 36.25$, $p < 0.001$; model with VFT $R^2 = 0.46$, $F = 17.72$, $p < 0.001$. However, only cognitive test scores significantly predicted olfactory discrimination scores in each case (β for MMSE = 0.79, $p < 0.001$; for ADAS-Cog 13 = -0.77, $p < 0.001$; for CDR Sum of Boxes = -0.8, $p < 0.001$; for VFT = 0.73; $p < 0.001$). None of the other predictors (age, sex, and education) significantly predicted the olfactory discrimination scores in any of the models ($p > 0.05$).

Detailed analysis of correlations between odour identification scores and results of cognitive assessment in separate groups can be found in **paper II**.

4.4.2. Diagnostic Characteristics of the Odour Discrimination Score

ROC analysis was performed to evaluate the performance of the odour discrimination score in differentiating the CN group from AD patients (MCI-AD or MD-AD), the CN group from MCI-AD patients, the CN group from MD-AD patients, and MCI-AD patients from MD-AD patients. The ROC curves with areas under the curve (AUC) are shown in **Fig. 8**.

A cut-off score of ≤10 correct responses was chosen for differentiating patients with AD (MCI-AD or MD-AD) from subjects with CN; the score had a sensitivity of 100% (95% CI, 94.04% to 100.00%) and specificity of 83.33% (95% CI, 65.28% to 94.36%). The negative predictive value was 100%, and the positive predictive value was 92.31% (95% CI, 84.35%–96.39%). The overall diagnostic accuracy was 94.44% (95% CI, 87.51% to 98.17%).

The diagnostic characteristics remained good when differentiating MCI-AD patients from subjects with CN. The same cut-off score of ≤10 had a sensitivity of 100% (95% CI, 88.43% to 100.00%) and specificity of 83.33% (95% CI, 65.28% to 94.36%). The negative predictive value was 100% and the positive predictive value was 85.71% (95% CI, 72.94% to 93.03%). The overall diagnostic accuracy was 91.67% (95% CI, 81.61% to 97.24%).

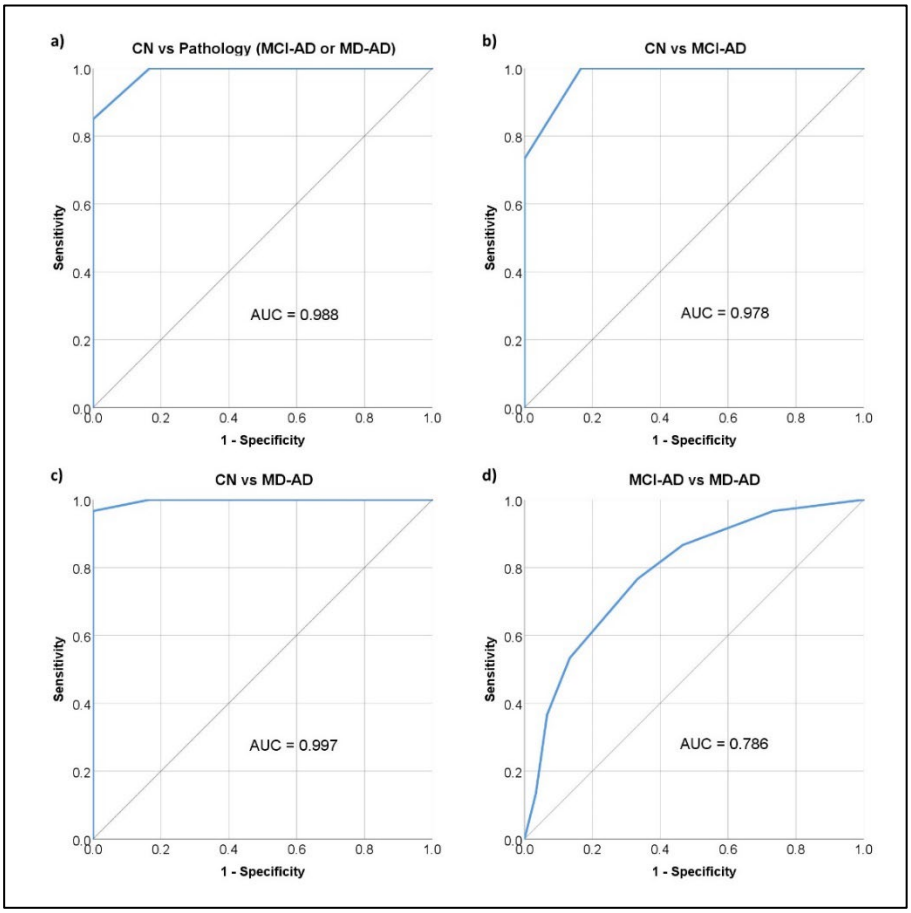


Fig. 8. Performance of the odour discrimination score in differentiating between groups of participants

Abbreviations: CN, elderly subjects with normal cognition; MCI-AD, mild cognitive impairment due to Alzheimer's disease; MD-AD, mild dementia due to Alzheimer's disease; AUC, area under curve.

Multinomial logistic regression was performed to analyse the relationship between the predictor variables and membership in the three groups (CN, MCI-AD, and MD-AD). First, a model with age, education, sex, and ADAS-Cog 13 scores as predictor variables was tested. The fit between the model containing only the intercept and the data improved with the addition of predictor variables ($X^2 = 139.66$, $p < 0.001$; Nagelkerke $R^2 = 0.89$). Pearson's X^2 and deviance X^2 tests indicated that the model exhibited a good fit to the data ($p > 0.05$). The overall percentage of correctly classified cases using this model was 82.2% (CN, 93.3%; MCI-AD, 70%; MD-AD, 83.3%),

with the ADAS-Cog 13 score as the strongest and most significant predictor ($X^2 = 122.65$, $p < 0.001$).

The odour discrimination scores were included in the model. The model with age, education, sex, ADAS-Cog 13 scores, and odour discrimination scores also showed a significant improvement in fit over a null model ($X^2 = 158.11$, $p < 0.001$; Nagelkerke $R^2 = 0.93$). Pearson's X^2 and Deviance X^2 tests indicated that the model exhibited a good fit to the data ($p > 0.05$). The overall percentage of correctly classified cases using this model was 92.2% (CN, 96.7%; MCI-AD, 86.7%; MD-AD, 93.3%), with the ADAS-Cog 13 and odour discrimination scores both being strong and significant predictors ($X^2 = 28.01$ and 18.45, respectively, $p < 0.001$ for both).

4.4.3. Discrimination of the Specific Odours

Five triplets were excluded from further analysis because of poor discrimination (<70% of correct responses) in the CN group.

The discrimination scores of the remaining odours are presented in **Table 4**.

Odour discrimination scores in nine of the remaining 11 triplets were significantly worse in the MD-AD group than in the CN group (Fisher's exact test, $p < 0.016$), while odour discrimination scores in eight triplets were significantly worse in the MCI-AD group than in the CN group (Fisher's exact test, $p < 0.016$). Odour discrimination scores in the triplet containing 2-phenylethanol as the target odour and isoamyl acetate as the non-target odour differed significantly between the MCI-AD and MD-AD groups (Fisher's exact test, $p < 0.001$). Odour discrimination scores in all the remaining ten triplets did not differ significantly between the MCI-AD and MD-AD groups (Fisher's exact test, $p > 0.016$).

Table 4. Discrimination of the specific Odours

Target odour	Non-target odour	CN correct responses (%)	MCI-AD correct responses (%)	MD-AD correct responses (%)
2-Phenylethanol [a]	Isoamyl acetate [b]	29 (96.67)	20 (66.67) *	6 (20) *
(+)-Limonene	(+)-Fenchone [a, b]	29 (96.67)	13 (43.33) *	15 (50) *
Pyridine	(-)-Limonene [b]	28 (93.33)	21 (70)	13 (43.33) *
Octyl acetate	Cinnamaldehyde [b]	28 (93.33)	19 (63.33) *	11 (36.67) *
2-Phenylethanol	(+)-Menthol [a, b]	28 (93.33)	16 (53.33) *	12 (40) *
1-Butanol	(+)-Fenchone [b]	27 (90)	21 (70)	11 (36.67) *
Eucalyptol	α-Ionone [b]	27 (90)	17 (56.67) *	10 (33.33) *
(-)-Limonene	Citronellal [b]	25 (83.33)	14 (46.67) *	9 (30) *

Target odour	Non-target odour	CN correct responses (%)	MCI-AD correct responses (%)	MD-AD correct responses (%)
Anethole	Eugenol [a, b]	23 (76.67)	10 (33.33) *	11 (36.67) *
Isoamyl acetate	Anethole	21 (70)	22 (73.33)	17 (56.67)
Citronellal	Linalool	21 (70)	10 (33.33) *	16 (53.33)

Abbreviations: CN, elderly subjects with normal cognition; AD, Alzheimer's disease; MCI-AD, mild cognitive impairment due to Alzheimer's disease; MD-AD, mild dementia due to Alzheimer's disease.

* Significantly different from the NC group ($p < 0.016$).

[a] The triplets that differed the most ($p < 0.001$) between the MCI-AD and NC groups.

[b] The triplets that differed the most ($p < 0.001$) between the MD-AD and NC groups

4.4.4. Diagnostic Characteristics of the Shortened Version of the Odour Discrimination Score

The three triplets that differed the most between the MCI-AD and CN groups and the triplet with 2-phenylethanol as the target odour and isoamyl acetate as the non-target odour that differed significantly between the MCI-AD and MD-AD groups were selected for the four-odour discrimination score.

ROC curve analysis was performed to evaluate the performance of the four-odour discrimination score in differentiating the NC participants from patients with AD (MCI-AD or MD-AD), MCI-AD, and MD-AD and the patients with MCI-AD from those with MD-AD. The ROC curves with AUC are shown in **Fig 9**.

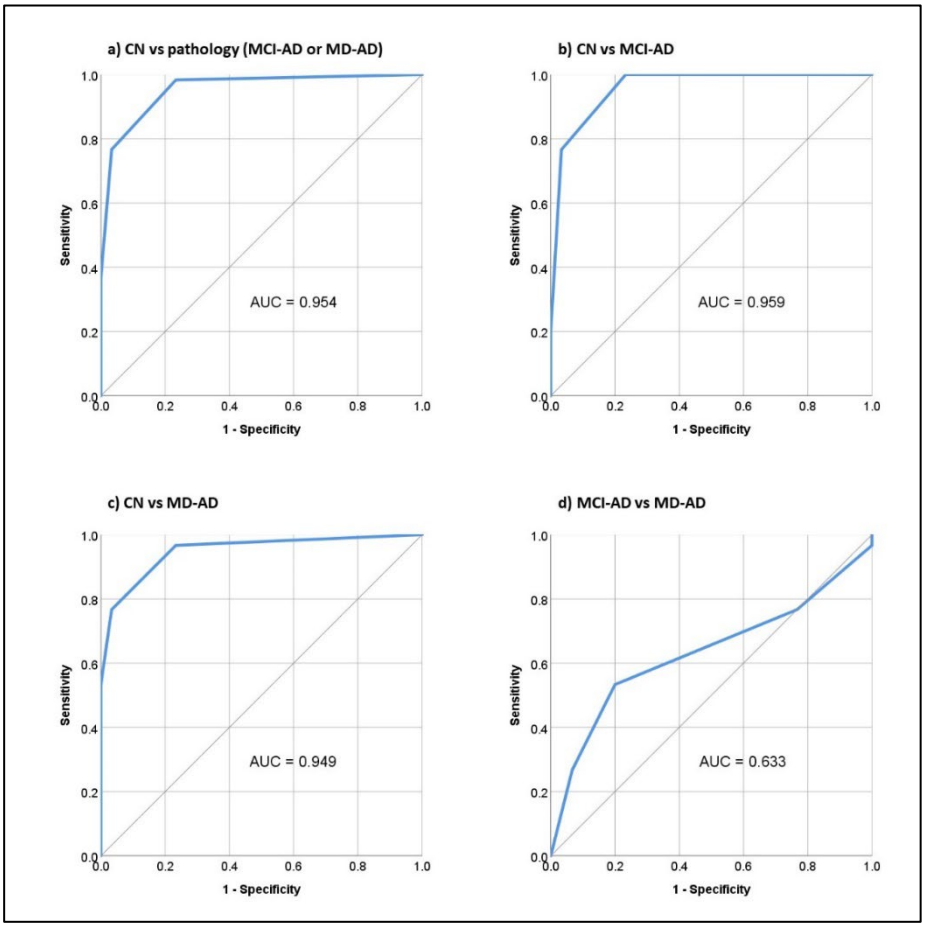


Fig 9. Performance of the four-odour discrimination score in differentiating between groups of participants

Abbreviations: CN, elderly subjects with normal cognition; MCI-AD, mild cognitive impairment due to Alzheimer's disease; MD-AD, mild dementia due to Alzheimer's disease; AUC, area under curve.

A cut-off score of ≤3 for detecting AD was chosen.

Using this cut-off score for differentiating patients with AD (MCI-AD or MD-AD) from CN participants, the four-odour discrimination score had a sensitivity and specificity of 98.33% (95% CI: 91.06%–99.96%) and 76.67% (95% CI: 57.72%–90.07%), respectively. The negative and positive predictive values were 95.83% (95% CI: 76.53%–99.39%) and 89.39% (95% CI: 81.49%–94.16%), respectively. The overall diagnostic accuracy was 91.11% (95% CI: 83.23%–96.08%).

The diagnostic characteristics remained good when differentiating patients with MCI-AD from CN participants. Using the same cut-off score of

≤3, the four-odour discrimination score had a sensitivity and specificity of 100% (95% CI: 88.43%–100%) and 76.67% (95% CI: 57.72%–90.07%), respectively. The negative and positive predictive values were 100% and 81.08% (95% CI: 69.14%–89.13%), respectively. The overall diagnostic accuracy was 88.33% (95% CI: 77.43%–95.18%).

4.5. Olfactory Memory

Olfactory immediate recognition memory and olfactory delayed recognition memory were significantly worse in patients with MD-AD than in those with MCI-AD or CN. The MCI-AD and CN groups did not differ significantly from each other (in both cases, Kruskal–Wallis test, $p < 0.05$; post hoc analysis revealed significant differences between the MD-AD and MCI-AD groups and the MD-AD and CN groups [$p < 0.05$], and no significant difference between the MCI-AD and CN groups [$p > 0.05$]). The results of the olfactory memory tasks are shown in **Fig. 10**. Detailed results of olfactory memory tasks can be found in **paper IV**.

In patients with AD (MCI-AD and MD-AD groups), olfactory immediate recognition memory scores were significantly correlated with the duration of AD symptoms (Spearman's rho = -0.366, $p < 0.05$), CDR sum of boxes (Spearman's rho = -0.328, $p < 0.05$), and VFT (Spearman's rho = 0.355, $p < 0.05$). Olfactory delayed recognition memory scores correlated significantly with the duration of AD symptoms (Spearman's rho = -0.360, $p < 0.05$), CDR sum of boxes (Spearman's rho = -0.317, $p < 0.05$), VFT (Spearman's rho = 0.303, $p < 0.05$), and delayed verbal recall after 5 minutes (Spearman's rho = 0.258, $p < 0.05$). Neither olfactory immediate recognition memory scores, nor olfactory delayed recognition memory scores correlated significantly with age (Spearman's rho = -0.194, p=0.138 and Spearman's rho = -0.226, p=0.082, respectively).

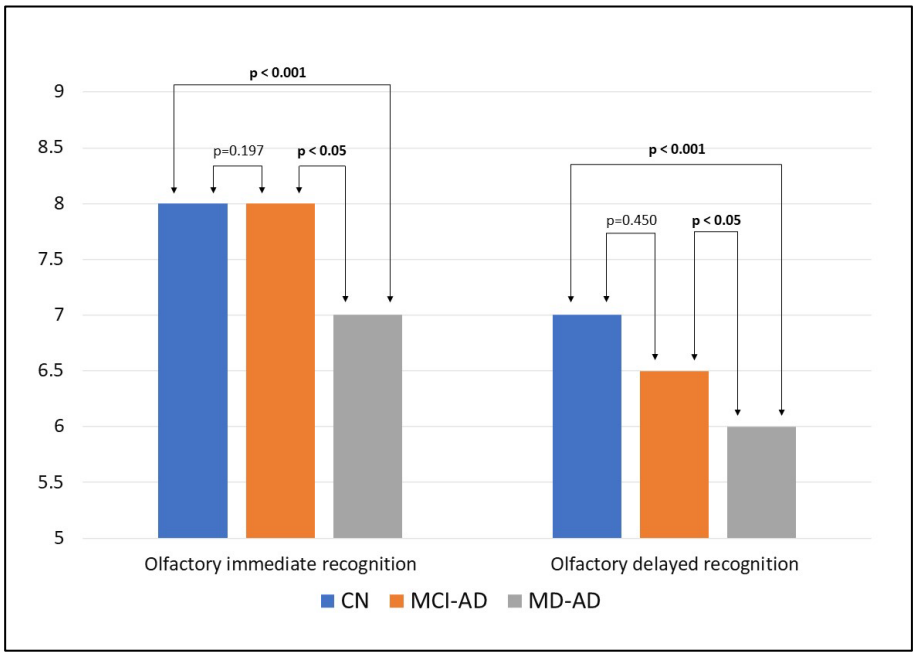


Fig. 10. Results of olfactory recognition memory tasks in three groups of participants. Bars represent medians

Abbreviations: CN, elderly subjects with normal cognition; MCI-AD, mild cognitive impairment due to Alzheimer's disease; MD-AD, mild dementia due to Alzheimer's disease.

Multiple linear regression models were created to determine which variables that correlated with olfactory memory scores in AD patients could significantly predict them. Additionally, age, sex, and GDS scores were included in the models as factors known to influence memory and olfaction in the general population.

In the model with age, sex, GDS score, duration of AD symptoms, CDR sum of boxes, and VFT as independent variables and olfactory immediate recognition as the dependent variable, the overall regression was significant (R^2 = 0.247, F = 2.904, p=0.016). The strongest predictor was duration of AD symptoms (β = -0.261, p=0.066). In a stepwise regression model, duration of AD symptoms (β = -0.290, p=0.021) and VFT (β = 0.306, p=0.015) remained the only significant predictors of olfactory immediate recognition memory score.

In the model with age, sex, GDS score, duration of AD symptoms, CDR sum of boxes, VFT, and delayed verbal recall after 5 minutes as independent variables and olfactory delayed recognition as dependent variable, the overall regression was also significant (R^2 = 0.232, F = 2.249,

p=0.045). The strongest predictor was duration of AD symptoms as well (β = -0.302, p=0.043). In a stepwise regression model duration of AD symptoms (β = -0.291, p=0.022) and VFT (β = 0.268, p=0.035) remained the only significant predictors of olfactory delayed recognition memory score.

The results of the olfactory memory tasks were compared between patients with AD (MCI-AD and MD-AD participants) taking AChEIs and treatment-naïve patients. Patients on AChEIs had significantly worse results than treatment-naïve patients in both olfactory immediate recognition (median and IQR 6 (6–7) and 7 (6–8), respectively; Mann–Whitney U test, p=0.024) and olfactory delayed recognition (median and IQR 6 (6–6) and 6 (5–6), respectively; Mann–Whitney U test, p=0.025) tasks.

Analysis of the separate groups revealed that these differences were no longer significant at the group level. In the MCI-AD group, there were no significant differences between patients on AChEIs and patients not taking them, neither in olfactory immediate recognition (median and IQR 6 (6–6) and 8 (6–8) respectively; Mann–Whitney U test, p=0.600), nor in olfactory delayed recognition (median and IQR 6 (5–6) and 7 (6–8), respectively; Mann–Whitney U test, p=0.387).

In the MD-AD group, there were also no significant differences between patients on AChEIs and those not taking them, neither in olfactory immediate recognition (median and IQR 6 (5.75–7) and 7 (6–7), respectively; Mann–Whitney U test, p=0.208), nor in olfactory delayed recognition (median and IQR 5.5 (5–6) and 6 (5–7), respectively; Mann–Whitney U test, p=0.313).

Diagnostic properties of olfactory memory scores were not tested as MCI-AD and CN groups did not differ significantly from each other.

5. DISCUSSION

In the current study, we found that both odour identification and odour discrimination were significantly impaired in the prodromal stage of AD (MCI-AD patients), and the impairment was even more severe in the later stages of the disease (mild dementia patients). This confirms the findings of previous studies, where olfactory impairment was also found to be present in the earliest stages of AD and further worsened during disease progression (11,15).

The performance of the subjects in the current study was consistent with that reported in previous research. According to normative data, the scores of healthy elderly subjects on odour identification range from 12.06±2.31 to 13.0±0,92 and on odour discrimination – from 10.66±2.5 to 13.80±0.77 (62,63). The results of the CN group in the current study were within this range. The performance of patients with early AD was also similar to that reported in previous studies. The performance of patients with MCI in odour identification varied from 9.3±4.0 to 10.2±2.5 and in odour discrimination – from 7.9±3.2 to 10.3±2.6 in previous studies (13,15,64-66). The performance of patients with AD in odour identification varied from 6.7±2.3 to 7.8±3.4 and in odour discrimination – from 5.6±3.8 to 9.6±2.3 in previous studies (13,15,64-66). Collectively, the results from the current study and previous research suggest that olfactory impairment is fairly consistent across various samples of patients with AD. Therefore, olfactory testing can be applied to various populations.

Odour identification and odour discrimination scores were strongly and significantly correlated with the results of the cognitive assessment. Linear regression analysis further demonstrated a significant relationship between these variables. However, age, sex, and education did not significantly predict odour identification and odour discrimination scores in the linear regression models. These findings are in accordance with structural and functional changes in the olfactory system, which have been demonstrated in previous studies, and further prove that olfactory impairment is associated with processes of AD itself and cannot be explained by other factors that have been proven to influence olfaction in the general population, such as age and sex (16-21).

In the current study, odour identification and odour discrimination both demonstrated excellent capabilities in differentiating patients with early AD (MCI-AD or MD-AD) from healthy controls (AUC = 0.949 and 0.988 respectively) and in differentiating patients with prodromal AD (MCI-AD)

from healthy controls (AUC = 0.908 and 0.978 respectively). The diagnostic qualities of odour identification were tested in previous research and yielded similar results (13-15,25-33,64,66). However, information on the diagnostic qualities of olfactory discrimination tests is rather sparse. In some studies odour discrimination was found to be a significant and more reliable predictor of future cognitive decline, than odour identification (42). However, other authors did not find odour discrimination to be superior to odour identification, even though both of them performed better then odour threshold in differentiating patients with AD and patients with MCI from cognitively normal participants (43). The results of our study support further research on odour discrimination in AD as it demonstrates good diagnostic qualities and is known to be less dependent on personal and cultural experiences, familiarity with different odours and language abilities of the subjects than odour identification (44-46).

The diagnostic characteristics of odour identification and odour discrimination were not as good when differentiating between the different stages of AD in the current study (MCI-AD vs. MD-AD, AUC = 0.773 and 0.786 respectively). Similar results were found in previous studies, where odour identification also had better qualities in differentiating healthy participants from patients with AD than in differentiating between different stages of AD (65). This suggests that olfactory changes occur early in the course of the disease and are pronounced even in the prodromal stage of AD, thus making olfactory testing very suitable for diagnosing early AD and less reliable for monitoring disease progression.

This is also supported by the results of multinomial regression models. Inclusion of olfactory measures (odour identification or odour discrimination) into multinomial regression models containing demographic and cognitive data as predictive variables resulted in improved overall classification of the subjects. Correct classification of MCI-AD cases improved the most, once again confirming the additional value of olfactory testing in diagnosing AD at the early stages.

It is an important finding, since diagnosing early AD is the most challenging task in clinical practice, especially in primary care settings. More than half (51%) of primary care physicians surveyed by the Alzheimer's Association said they were uncomfortable diagnosing MCI due to AD (67). Currently available biomarkers are not able to solve this issue, as they are not easily accessible in community settings. Lack of specialists and facilities to perform diagnostic testing was the most commonly cited challenge by primary care physicians in the United States of America, when diagnosing mild cognitive impairment (MCI) due to AD (67). Objective olfactory testing may

be very useful for improving diagnostic certainty of diagnosing early-stage AD. In particular, 72% of primary care physicians stated that they find it challenging to differentiate MCI from normal aging (67). As odour identification and odour discrimination proved to have excellent characteristics in differentiating MCI-AD patients from healthy controls, they could be very helpful with this task.

In order to improve applicability of olfactory testing in the clinical practice we further analysed identification and discrimination of specific odorants and created shortened versions of olfactory tests that would be less time consuming and more convenient.

The identification scores of clove, garlic, cinnamon, and banana odours differed the most between the patients with AD and healthy participants in the current study. Although the results of previous studies are not completely uniform, they also indicate that clove (37,38), banana (68,69), and garlic (69-71) are among the most sensitive odours for testing patients with AD. However, cinnamon was not found to be sensitive for detecting olfactory dysfunction in patients with AD in previous studies. Furthermore, it was not included in the sets of 10 odours that were determined to be the most suitable for testing patients with AD (37,38).

In previous studies, identification of the rose odour was most consistently found to be impaired in patients with AD (37,39,70-73). In our study, the identification of the rose odour was also significantly impaired in the MD-AD group; however, it was not among the odours that differed the most between MD-AD and NC groups. Thus, it was not included in the shortened version of the odour identification score.

The shortened odour identification score, consisting of four odours (clove, garlic, cinnamon, and banana), had good diagnostic qualities for detecting AD and even MCI due to AD in the current study. Previous studies had determined that the three-item Pocket Smell Test had acceptable, albeit slightly worse diagnostic accuracy (35,74-76). However, in previous studies, standard tests containing lemon/lilac/smoke or apple/gas/rose odorants were used, and these combinations were not chosen specifically for patients with AD. Considering these results of previous studies, our findings indicate that short versions (3–4 items) of the odour identification test have good diagnostic properties and could be useful in diagnosing early-stage AD, particularly if specific items that are the most sensitive for predicting AD are chosen.

Odour discrimination demonstrated a different pattern of impairment than odour identification. In the case of odour identification, the identification scores for most odours were significantly worse in patients with MD-AD than in the healthy participants. However, the identification scores of only three

odours were significantly worse in patients with MCI-AD than in the healthy participants. In contrast, the scores of the odour discrimination task for most odours were significantly worse in patients with MCI-AD than in the healthy participants, indicating a more pronounced impairment in odour discrimination during the early stage of the disease. Accordingly, the shortened version of the odour discrimination task had better diagnostic qualities for prodromal AD than the shortened version of odour identification task. Early changes in the performance of the odour discrimination task might be related to the different nature of the tests. Both odour identification and discrimination reflect higher processing of odours; however, olfactory short-term memory is involved to a greater extent in odour discrimination tasks (77).

Both of the shortened olfactory tasks had poor abilities to differentiate between the different stages of AD, similar to standard versions of the tests (MCI-MD vs. MD-AD; ROC AUC = 0.698 for the four-odour identification score and ROC AUC = 0.633 for the four-odour discrimination score).

Finally, olfactory memory was tested in the current study. We found olfactory recognition memory (immediate, as well as delayed) to be impaired in patients with mild dementia, however, performance of MCI-AD participants did not differ significantly from cognitively normal participants.

Data on olfactory memory in patients with early-stage AD are limited. In a study performed in 2008, authors found no olfactory memory deficits in prodromal AD (MCI patients) (55). However, in a more recent work, researchers revealed olfactory memory to be impaired in MCI, as well as in patients with subjective cognitive decline (SCD) (53).

In the current study olfactory recognition memory (immediate, as well as delayed) scores correlated with the duration of the symptoms in AD patients. Furthermore, multiple linear regression analysis showed that duration of AD symptoms was a strong predictor of olfactory recognition memory (immediate, as well as delayed) scores. Thus, we can conclude that olfactory memory is a symptom of AD and that deficits in olfactory memory progress during the course of the disease. However, these changes are not already pronounced during the prodromal stage of the disease (MCI due to AD).

Even though changes of olfactory memory were not significant in patients with MCI due to AD and therefore could not be applied to diagnosing early-stage AD, data regarding olfactory memory in AD patients provide an insight into differences between olfactory and verbal memory.

First, verbal recall memory (immediate, as well as delayed) is an earlier symptom of AD. Unlike olfactory memory, it was already significantly impaired in MCI-AD patients. Second, the response to treatment with AChEIs

was different. In the current study, treatment-naive patients showed better olfactory memory results than patients taking AChEIs. However, the duration of AD symptoms was significantly shorter in treatment-naive patients. Longer disease duration accounts for the more pronounced olfactory memory impairment in patients taking AChEIs. It is interesting to note that verbal memory did not differ significantly between patients taking AChEIs and those who were not, despite the longer duration of the disease. Thus, we can conclude that AChEI treatment had a significant effect on verbal memory and was able to compensate for the longer progression of the disease; however, the same effect was not observed for olfactory memory. Thus, not only do olfactory and verbal memory deficits manifest in different patterns in patients with AD, but the response to cholinergic stimulation is also distinct.

Even today, the specific brain regions responsible for human olfactory memory are poorly understood; however, multiple structures are known to be involved in this process, and this network differs significantly from verbal memory (78-80). The results of our study also highlighted the differences between these two types of memory.

The present study has several limitations. First, the cross-sectional design limits the accuracy of the conclusions regarding the progression of changes during the course of AD. Second, CSF analysis and PET were not used, and including these modalities would have helped us analyse the relationship between olfactory changes and brain Aβ deposition and neuronal degeneration. Moreover, CSF and PET biomarkers would help in confirming the diagnosis. In the current study, all the participants met criteria for probable AD or MCI consistent with AD pathophysiological process (4,5). However, CSF and PET biomarkers would be helpful in increasing the level of certainty. Third, although the differences were significant in our small sample, studies involving larger sample sizes would help confirm these findings.

6. CONCLUSIONS

1. Odour identification is impaired in early-stage AD and odour identification testing has good diagnostic properties for differentiating patients with early-stage AD from healthy subjects.
2. Odour discrimination is impaired in early-stage AD and odour discrimination testing has good diagnostic properties for differentiating patients with early-stage AD from healthy subjects.
3. Odour identification and odour discrimination scores correlate with results of cognitive testing and provide additional value in diagnosing early-stage AD.
4. Shortened versions of odour identification and odour discrimination tests that are created based on performance of patients with early-stage AD have good diagnostic properties for differentiating patients with early-stage AD from healthy subjects.
5. Olfactory memory is impaired in patients with early-stage AD but demonstrates a different pattern of impairment than verbal memory.

7. FUTURE PERSPECTIVES/PRACTICAL RECOMMENDATIONS

This doctoral dissertation confirms the early occurrence of olfactory deficits in patients with AD. Both odour identification and odour discrimination testing proved to have good diagnostic properties for diagnosing early-stage AD, therefore their inclusion into routine clinical practice should be encouraged. Olfactory testing could serve as a reliable, non-invasive, and affordable marker that would improve the diagnostic process, especially in primary care.

Further studies with shortened versions of odour identification and odour discrimination tests are needed in order to confirm the encouraging findings of the current study.

In addition, future studies should include CSF and PET biomarkers in order to ensure the highest level of certainty regarding the diagnosis and analyse relationship between these biomarkers and olfactory changes.

SUMMARY IN LITHUANIAN

SANTRUMPOS

AChEI	Acetilcholinesterazės inhibitoriai
ADAS-Cog	Alzheimerio ligos vertinimo skalės kognityvinė subskalė
ADI	*Alzheimer's Disease International* organizacija
AL	Alzheimerio liga
ANOVA	Dispersinė analizė
AUC	Plotas po ROC kreive
Aβ	Beta amiloidas
CDR	Klinikinė demencijos vertinimo skalė
FDG-PET	[18F]-fluorodeoksigliukozės pozitronų emisijos tomografija
fMRT	Funkcinė magnetinio rezonanso tomografija
GDS	Geriatrinė depresijos skalė
HII	Hačinskio išemijos indeksas
IQR	Tarpkvartilinis plotis
LKS	Lengvas kognityvinis sutrikimas
MMSE	Protinės būklės mini tyrimas
MRT	Magnetinio rezonanso tomografija
NIA/AA	*National Institute on Aging-Alzheimer's Association*
PI	Patikimumo intervalas
ROC kreivė	angl. *Receiver operating characteristic* kreivė
SKP	Subjektyvus kognityvinis pablogėjimas
UPSIT	Pensilvanijos universiteto kvapų identifikacijos testas
VFT	Bendras žodinio sklandumo testų rezultatas

MOKSLINIŲ PUBLIKACIJŲ SĄRAŠAS

Ši daktaro disertacija parengta pagal keturias mokslines publikacijas. Mokslinės publikacijos tolesniame tekste žymimos nurodytais romėniškaisiais skaitmenimis:

I. **Audronyte E**, Pakulaite-Kazliene G, Sutnikiene V, Kaubrys G. Properties of odor identification testing in screening for early-stage Alzheimer's disease. Sci Rep. 2023;13(1):6075. Published 2023 Apr 13. doi:10.1038/s41598-023-32878-w
II. **Audronyte E**, Pakulaite-Kazliene G, Sutnikiene V, Kaubrys G. Odor Discrimination as a Marker of Early Alzheimer's Disease. J Alzheimers Dis. 2023;94(3):1169-1178. Published 2023 Aug 1. doi:10.3233/JAD-230077
III. **Audronyte E**, Sutnikiene V, Pakulaite-Kazliene G, Kaubrys G. Brief Test of Olfactory Dysfunction Based on Diagnostic Features of Specific Odors in Early-Stage Alzheimer Disease. Med Sci Monit. 2023;29:e940363. Published 2023 May 27. doi:10.12659/MSM.940363
IV. **Audronyte E**, Sutnikiene V, Pakulaite-Kazliene G, Kaubrys G. Olfactory memory in mild cognitive impairment and Alzheimer's disease. Front. Neurol. 2023;14:1165594. Published 2023 June 02. doi: 10.3389/fneur.2023.1165594

PREAMBULĖ

Ši daktaro disertacija teikiama ginti kaip mokslinių straipsnių rinkinys. Kai kurios jos dalys pažodžiui cituojamos iš anksčiau publikuotų straipsnių, išvardytų knygos pabaigoje.

Disertaciją sudaro kelios tarpusavyje susijusios dalys.

Pirmiausia įvertinti ankstyvųjų stadijų Alzheimerio liga sergančių pacientų kvapų identifikacijos (atpažinimo) sugebėjimai ir kvapų identifikacijos testų diagnostinės savybės Alzheimerio ligos ankstyvųjų stadijų diagnozei nustatyti. Paskui įvertinti ankstyvųjų stadijų Alzheimerio liga sergančių pacientų kvapų diskriminacijos (atskyrimo) sugebėjimai ir kvapų diskriminacijos testų diagnostinės savybės Alzheimerio ligos ankstyvųjų stadijų diagnozei nustatyti. Tada buvo įvertintas ankstyvųjų stadijų Alzheimerio liga sergančių pacientų sugebėjimas identifikuoti ir diskriminuoti specifinius kvapus ir sukurti sutrumpinti kvapų identifikacijos ir kvapų diskriminacijos testų variantai. Taip pat buvo įvertintos šių sutrumpintų testų variantų diagnostinės savybės Alzheimerio ligos ankstyvųjų stadijų diagnozei nustatyti. Galiausiai įvertinta ankstyvųjų stadijų Alzheimerio liga sergančių pacientų olfaktorinė (kvapų) atmintis bei išanalizuoti olfaktorinės atminties ir verbalinės atminties skirtumai.

1. ĮVADAS

1.1. Tiriamoji problema ir jos aktualumas

Demencija yra dažniausia negalios ir savarankiškumo netekimo priežastis pasaulyje, o jos paplitimas vis didėja (1). Skaičiavimų duomenimis, 2019 m. demencijos sindromą turėjo daugiau kaip 55 mln. asmenų pasaulyje, o iki 2050 m. šis skaičius, manoma, pasieks 139 mln. (1, 2). Taigi socialinė ir ekonominė demencijos sukeliama našta yra svarbi problema ir demencija tampa prioritetine sveikatos priežiūros sritimi visame pasaulyje (1, 2). Nepaisant to, daugumai pacientų demencija net nėra diagnozuojama. *Alzheimer's Disease International* (ADI) organizacijos duomenimis, 75 % demencijos atvejų pasaulyje lieka nenustatyta (2). Norint užtikrinti tinkamą dėmesį šiai vis aktualesnei problemai, pirmiausia svarbu rasti prieinamų ir plačiai pritaikomų priemonių, kurios galėtų pagerinti diagnostikos procesą.

Alzheimerio liga (AL) yra dažniausia demencijos priežastis, sukelianti 60–70 % visų demencijos atvejų (3). Nepaisant pažangos radiologinių, smegenų skysčio ir kraujo biožymenų srityje, diagnozuoti AL ankstyvosiose stadijose išlieka sudėtinga (4–6). Šiuo metu AL diagnostikoje taikomi biožymenys vertina beta amiloido (Aβ) baltymo atsidėjimą galvos smegenyse ir neuronų degeneraciją, tačiau, norint atlikti šiuos tyrimus, reikalinga smegenų skysčio analizė arba pažangūs vaizdinimo metodai (4, 5). Taigi platų šių biožymenų taikymą klinikinėje praktikoje riboja didelė jų kaina ir invazinis tyrimų pobūdis. Norint atrinkti pacientus, kuriems šie tyrimo metodai būtų naudingiausi, reikia nustatyti žymenis, kurie būtų lengvai prieinami ir galėtų būti plačiai taikomi klinikinėje praktikoje.

Šie žymenys turėtų ne tik patikimai identifikuoti sergančiuosius AL, bet ir sugebėti tai padaryti ankstyvosiose ligos stadijose. 2022 m. dauguma klinikiniuose AL tyrimuose tirtų vaistų buvo ligą modifikuojantys medikamentai (83,2 %), kurie daugiausia yra skirti pacientams, sergantiems ankstyvosios stadijos AL (7). Šiuos vaistus pradedant taikyti klinikinėje praktikoje, vis didėja patikimų ir jautrių ankstyvosios AL stadijos žymenų, kurie būtų prieinami ir kasdienėje praktikoje, poreikis.

Kaip vienas iš galimų neinvazinių ir prieinamų žymenų buvo pasiūlytas uoslės funkcijų ištyrimas. Jau beveik prieš 50 metų buvo pastebėta, kad AL sergančių pacientų uoslė yra sutrikusi (8). Nuo to laiko atlikta daug tyrimų ir nustatyta, kad uoslės sutrikimai yra dažnas simptomas, pasireiškiantis iki 90 % sergančiųjų AL (9, 10). Labai svarbu, kad uoslės sutrikimai – ne tik dažnas, bet ir ankstyvas AL požymis, kuris yra matomas jau lengvo kognityvinio sutrikimo (LKS) ir subjektyvaus kognityvinio

pablogėjimo (SKP) metu (11–15). Manoma, kad uoslės pablogėjimas pasireiškia keletą metų prieš kognityvinių sutrikimų atsiradimą (14, 15). Patologiniai tyrimai patvirtina šiuos klinikinius radinius. Pokyčių struktūrose, susijusiose su uoslės informacijos apdorojimu (ypač entorininėse ir transentorininėse srityse), būna ankstyvųjų AL stadijų metu (16, 17). Tyrimai, pasitelkus funkcinę magnetinio rezonanso tomografiją (fMRT) ir [18F]-fluordeoksigliukozės pozitronų emisijos tomografiją (FDG-PET), taip pat patvirtina struktūrinius ir funkcinius su uosle susijusių sričių pakitimus pacientams, sergantiems ankstyvųjų stadijų AL (jau SKP metu) (18–21).

Nepaisant šių duomenų, klinikinėje praktikoje uoslės tyrimai retai atliekami. Norint į kasdienę AL diagnostikos praktiką įtraukti uoslės funkcijų ištyrimą, reikia daugiau duomenų apie skirtingus uoslės vertinimo metodus ir jų diagnostines savybes.

Ši daktaro disertacija parengta remiantis keturiais publikuotais moksliniais straipsniais. Bendras jos tikslas – įvertinti ankstyvųjų stadijų AL sergančių pacientų uoslės sutrikimus, nustatyti jų diagnostines savybes bei ištirti olfaktorinės ir verbalinės atminties sutrikimų skirtumus ankstyvųjų AL stadijų metu. **I straipsnyje** analizuojama pacientų, sergančių ankstyvųjų stadijų AL, kvapų identifikacija ir kvapų identifikacijos testų diagnostinės savybės nustatant ankstyvąsias AL stadijas. **II straipsnyje** analizuojama ankstyvųjų stadijų AL sergančių pacientų kvapų diskriminacija ir kvapų diskriminacijos testų diagnostinės savybės nustatant ankstyvąsias AL stadijas. **III straipsnyje** analizuojami pacientų, sergančių ankstyvųjų stadijų AL, gebėjimai identifikuoti ir diskriminuoti konkrečius kvapus bei sutrumpintų kvapų identifikacijos ir kvapų diskriminacijos testų diagnostinės savybės nustatant ankstyvąsias AL stadijas. **IV straipsnyje** vertinama ankstyvųjų stadijų AL sergančių pacientų olfaktorinė atmintis ir analizuojami olfaktorinės ir verbalinės atminties sutrikimų skirtumai.

1.2. Tyrimo tikslas

Įvertinti uoslės sutrikimus, pasireiškiančius ankstyvosiose AL stadijose, nustatyti uoslės testų diagnostines savybes diagnozuojant ankstyvąsias AL stadijas bei išanalizuoti uoslės ir kognityvinių funkcijų sutrikimų skirtumus ankstyvųjų AL stadijų metu.

1.3. Tyrimo uždaviniai

1. Įvertinti kvapų identifikacijos sutrikimus ir jų diagnostinę vertę ankstyvosiose Alzheimerio ligos stadijose (lengvo kognityvinio

sutrikimo dėl Alzheimerio ligos ir lengvos demencijos dėl Alzheimerio ligos metu) **(I straipsnis)**.

2. Įvertinti kvapų diskriminacijos sutrikimus ir jų diagnostinę vertę ankstyvosiose Alzheimerio ligos stadijose (lengvo kognityvinio sutrikimo dėl Alzheimerio ligos ir lengvos demencijos dėl Alzheimerio ligos metu) **(II straipsnis)**.
3. Palyginti kvapų identifikacijos ir kvapų diskriminacijos sutrikimus su kognityvinių testų rezultatais ankstyvosios Alzheimerio ligos metu **(I, II straipsnis)**.
4. Įvertinti specifinių kvapų identifikacijos ir diskriminacijos sutrikimus ankstyvosiose Alzheimerio ligos stadijose, sukurti sutrumpintus kvapų identifikacijos ir kvapų diskriminacijos testų variantus, pritaikytus ankstyvosios Alzheimerio ligos stadijos diagnostikai bei įvertinti jų diagnostines savybes **(III straipsnis)**.
5. Įvertinti olfaktorinės atminties sutrikimus bei palyginti juos su verbalinės atminties sutrikimais ankstyvosiose Alzheimerio ligos stadijose **(IV straipsnis)**.

1.4. Mokslinis naujumas

Ankstesniuose tyrimuose taikyti skirtingi LKS diagnostiniai kriterijai arba LKS apskritai nebuvo skirstomas į atskirus tipus, todėl gautus duomenis sunku pritaikyti AL diagnostikai klinikinėje praktikoje. Šiame tyrime buvo taikyti visuotinai pripažinti AL sukelto LKS diagnostiniai kriterijai (4).

Šiuo metu trūksta duomenų apie kvapų diskriminaciją bei olfaktorinę atmintį ankstyvųjų AL stadijų metu, todėl tyrimo metu, be kvapų identifikacijos, buvo vertinamos ir šios funkcijos.

Galiausiai, šiame tyrime vietoj dažniausiai ankstesniuose tyrimuose naudoto Pensilvanijos universiteto kvapų identifikacijos testo (UPSIT) buvo taikytas „Sniffin' Sticks“ testas. Duomenys apie skirtingus uoslės testus yra labai svarbūs, nes dažniausiai naudojamų testų kaina gali būti veiksnys, ribojantis jų platesnį taikymą. UPSIT ir visi jo pagrindu sukurti testai yra vienkartiniai, todėl kiekvienam pacientui juos reikia pirkti atskirai (22, 23). „Sniffin' Sticks“ testas yra daugkartinio naudojimo ir gali būti pakartotinai atliekamas kelis mėnesius (24).

1.5. Praktinė tyrimo vertė

AL diagnozės nustatymas ankstyvosiose ligos stadijose išlieka sudėtingas. Uoslės testai galėtų būti naudingi kaip lengvai prieinamas ir paprastai pritaikomas metodas, palengvinantis nustatyti diagnozę.

Nepaisant daugelio atliktų tyrimų, analizavusių uoslės sutrikimus sergant AL, šiuo metu uoslės vertinimas klinikinėje praktikoje vis dar retai atliekamas. Šiame tyrime sprendžiamos problemos, ribojančios platesnį uoslės sutrikimų vertinimo pritaikymą. Pavyzdžiui, ankstesniuose tyrimuose dažnai nebuvo taikomi visuotinai pripažinti AL diagnostikos kriterijai bei nebuvo vertinamos tokios uoslės funkcijos kaip kvapų diskriminacija ir olfaktorinė atmintis. Be to, šiame tyrime buvo sukurtos sutrumpintos uoslės testų versijos, nes norėta palengvinti uoslės testų pritaikymą kasdienėje praktikoje.

1.6. Ginamieji teiginiai

1. Kvapų identifikacija yra sutrikusi ankstyvosiose AL stadijose, o kvapų identifikacijos testai pasižymi geromis diagnostinėmis savybėmis ankstyvosioms AL stadijoms nustatyti (**I straipsnis**)
2. Kvapų diskriminacija yra sutrikusi ankstyvosiose AL stadijose, o kvapų diskriminacijos testai pasižymi geromis diagnostinėmis savybėmis ankstyvosioms AL stadijoms nustatyti (**II straipsnis**)
3. Kvapų identifikacijos ir kvapų diskriminacijos testų rezultatai koreliuoja su kognityvinių funkcijų įvertinimo duomenimis ir suteikia papildomos diagnostinės vertės ankstyvosioms AL stadijoms nustatyti (**I, II straipsniai**)
4. Sutrumpinti kvapų identifikacijos ir kvapų diskriminacijos testų variantai pasižymi geromis diagnostinėmis savybėmis ankstyvosioms AL stadijoms nustatyti (**III straipsnis**)
5. Olfaktorinė atmintis yra sutrikusi ankstyvosiose AL stadijose, tačiau šie sutrikimai skiriasi nuo matomų verbalinės atminties sutrikimų (**IV straipsnis**)

2. METODAI

2.1. Leidimas atlikti mokslinį darbą

Gautas Vilniaus regioninio biomedicininių tyrimų etikos komiteto leidimas (Nr. 2021/6-1355-830).

Tyrimas buvo atliekamas remiantis Helsinkio deklaracijos nuostatomis.

Visi tiriamieji buvo supažindinti su tyrimo procedūromis ir sutiko dalyvauti tyrime, savo sutikimą patvirtino pasirašydami atitinkamas informuoto asmens sutikimo formas.

2.2. Tiriamieji

Į tyrimą buvo įtraukta devyniasdešimt tiriamųjų: 30 pacientų, kuriems diagnozuota lengva demencija dėl AL (MD-AD grupė), 30 pacientų, kuriems diagnozuotas LKS dėl AL (MCI-AD grupė), ir 30 pažintinių funkcijų sutrikimų neturinčių pagyvenusių asmenų (CN grupė).

MD-AD grupės tiriamiesiems lengva demencija dėl AL buvo diagnozuota remiantis *National Institute on Aging-Alzheimer's Association* (NIA/AA) tikėtinos AL kriterijais (McKhann et al., 2011 (5)), o jų klinikinės demencijos vertinimo skalės (*Clinical dementia Rating*, CDR) įvertis buvo 1. Visi MD-AD grupės tiriamieji buvo konsultuoti Vilniaus universiteto ligoninės Santaros klinikų Alzheimerio ligos kabinete, tikėtinos AL diagnozė nustatyta gydytojo specialisto, remiantis bendraisiais klinikiniais kriterijais su didesnio patikimumo lygiu (visiems pacientams buvo dokumentuotas progresuojantis pažintinių funkcijų blogėjimas) (5). Remiantis biožymenų įvertinimu, AL tikimybė buvo vidutinė, kadangi visi pacientai turėjo neuronų degeneracijos požymių, remiantis magnetinio rezonanso tomografijos (MRT) įvertinimu, tačiau nebuvo įvertintas beta amiloido (Aβ) baltymo atsidėjimas galvos smegenyse.

MCI-AD grupės tiriamiesiems LKS dėl AL buvo diagnozuotas remiantis NIA/AA LKS dėl AL kriterijais (Albert et al., 2011 (4)), o jų CDR įvertis buvo 0,5. Visi MCI-AD grupės tiriamieji buvo konsultuoti Vilniaus universiteto ligoninės Santaros klinikų Alzheimerio ligos kabinete, LKS dėl AL diagnozė nustatyta gydytojo specialisto, patvirtinus klinikinius ir pažintinių funkcijų kriterijus bei LKS etiologijai esant suderinamai su AL patofiziologiniais procesais, remiantis dokumentuotu progresuojančiu pažintinių funkcijų blogėjimu bei kitų galimų pažintinių funkcijų pablogėjimo priežasčių atmetimu (kraujagyslinės patologijos, trauminių pažeidimų ir kt.) (4). Remiantis biožymenų įvertinimu, AL tikimybė buvo vidutinė, kadangi visi pacientai turėjo neuronų degeneracijos požymių, remiantis magnetinio rezonanso tomografijos (MRT) įvertinimu, tačiau nebuvo įvertintas beta amiloido (Aβ) baltymo atsidėjimas galvos smegenyse.

CN grupės tiriamieji neturėjo nusiskundimų dėl pažintinių funkcijų sutrikimo, o jų CDR įvertis buvo 0.

Į tyrimą buvo įtraukti tik tie pacientai, kurie nevartojo acetilcholinesterazės inhibitorių (AChEI) arba bent tris mėnesius vartojo stabilią AChEI dozę.

Neįtraukimo kriterijai, remiantis galimu poveikiu pažintinėms funkcijoms, buvo kitos centrinės nervų sistemos ligos, išskyrus LKS dėl AL arba AL, galimai reikšminga cerebrovaskulinė patologija (Hačinskio išemijos indeksas (HII) ≥ 4), sunki galvos smegenų trauma anamnezėje, psichiatrinės būklės, tokios kaip psichozė, piktnaudžiavimas narkotinėmis medžiagomis bei alkoholiu, reikšminga depresija (Geriatrinės depresijos skalės (GDS) įvertis >9) ir psichoaktyviųjų vaistų vartojimas.

Neįtraukimo kriterijai dėl galimo poveikio uoslei buvo rūkymas, nosies traumos ar operacijos, reikšmingas lakiųjų medžiagų poveikis ir neseniai persirgtos virusinės infekcijos.

2.3. Demografinių ir kognityvinių rodiklių vertinimas

Įvertinti kiekvieno tiriamojo demografiniai rodikliai (amžius, lytis, AL simptomų trukmė, gretutinės ligos ir vartojami medikamentai).

Bendram kognityvinių funkcijų vertinimui buvo atliktas mini protinės būklės tyrimas (MMSE) (įvertis nuo 0 iki 30 balų, kai 0 yra blogiausias [visos užduotys atliktos neteisingai], o 30 – geriausias [visos užduotys atliktos teisingai] rezultatas) (59).

Kognityviniai ir funkciniai gebėjimai buvo papildomai įvertinti pagal CDR skalę (bendras įvertis nuo 0 iki 3, kai 0 yra geriausias [jokių sutrikimų], o 3 – blogiausias [sunki demencija] įvertis) (60).

Taip pat buvo atliktas vertinimas pagal Alzheimerio ligos vertinimo skalės kognityvinę subskalę (ADAS-Cog, įvertis nuo 0 iki 70 balų, kai 0 yra geriausias [visos užduotys atliktos teisingai], o 70 – blogiausias [visos užduotys atliktos neteisingai] rezultatas) su papildomomis atidėto atsiminimo ir skaičių išbraukimo užduotimis (ADAS-Cog 13, įvertis nuo 0 iki 85 balų, kai 0 yra geriausias [visos užduotys atliktos teisingai], o 85 – blogiausias [visos užduotys atliktos neteisingai] rezultatas) (59). Atidėtas atsiminimas buvo vertinamas nuo 0 iki 10 balų (neprisimintų žodžių skaičius). Skaičių išbraukimo užduotis buvo vertinama nuo 0 iki 5 balų (kai 0 balų yra geriausias [≥30 teisingų atsakymų], o 5 balai – blogiausias [0–5 teisingi atsakymai] rezultatas). Atidėtas atsiminimas pakartotinai įvertintas po 30 minučių.

Taip pat buvo įvertintas foneminis (PAS) ir kategorinis (gyvūnai) žodinis sklandumas.

2.4. Kvapų identifikacijos vertinimas

Atliktas „Sniffin' Sticks“ kvapų identifikacijos testas (Burghart®, Vokietija).

„Sniffin' Sticks“ kvapų identifikacijos testą sudaro 16 kvapų: apelsinų, odos, cinamono, pipirmėčių, bananų, citrinų, saldymedžio, terpentino, česnakų, kavos, obuolių, gvazdikėlių, ananasų, rožių, anyžių ir žuvies.

Kiekvienas kvapas pateikiamas flomasteriuose. Dangtelis nuimamas, o kvapas pateikiamas tik vieną kartą 3–4 sekundes, flomasterio galiuką laikant maždaug 2 cm atstumu nuo abiejų šnervių. Tiriamųjų prašoma pasirinkti, kuris iš keturių atsakymų kortelėje nurodytų žodžių geriausiai apibūdina kvapą. Tiriamųjų prašoma pasirinkti atsakymą, net jei jie abejoja.

Tarp skirtingų kvapų pateikimo daroma 30 sekundžių pertrauka. Kvapų identifikacijos testo rezultatas – teisingų atsakymų skaičius iš šešiolikos pateiktų kvapų (61).

Atlikdamas kvapų identifikacijos testą, tyrėjas mūvi bekvapes pirštines. Tiriamųjų prašoma nieko negerti ir nevalgyti bent 15 minučių prieš testavimą (61).

2.5. Kvapų diskriminacijos vertinimas

Atliktas „Sniffin' Sticks“ kvapų diskriminacijos testas (Burghart®, Vokietija).

„Sniffin' Sticks“ kvapų diskriminacijos testą sudaro 16 kvapų trejetų. Kiekvienas kvapas pateikiamas flomasteriuose. Tiriamųjų prašoma pasirinkti, kuris kvapas kiekviename trejete skiriasi nuo kitų dviejų kvapų. Kvapai pateikiami testo instrukcijose nurodyta tvarka.

Kiekvienas kvapas pateikiamas tik vieną kartą, 3–4 sekundes, flomasterio galiuką laikant maždaug 2 cm atstumu nuo abiejų šnervių. Tarp to paties trejeto kvapų daroma 3 sekundžių pertrauka. Tarp atskirų trejetų daroma 30 sekundžių pertrauka. Kvapų diskriminacijos testo rezultatas – teisingų atsakymų skaičius iš šešiolikos pateiktų trejetų (61).

Atlikdamas kvapų diskriminacijos testą, tyrėjas mūvi bekvapes pirštines, o tiriamieji dėvi akių raištį. Tiriamųjų prašoma nieko negerti ir nevalgyti bent 15 minučių prieš testavimą (61).

2.6. Olfaktorinės atminties vertinimas

Olfaktorinės atminties vertinimo užduočiai buvo naudojami kvapai iš „Sniffin' Sticks“ kvapų identifikacijos testo (Burghart®, Wedel, Vokietija).

Per olfaktorinės atminties vertinimo užduoties kodavimo etapą kiekvienam tiriamajam atsitiktine tvarka buvo priskirti penki kvapai (tiksliniai kvapai). Kiekvienas iš šių penkių kvapų buvo pateikiamas 3 sekundes, flomasterio galiuką laikant maždaug 2 cm atstumu nuo abiejų šnervių. Tiriamiesiems buvo nurodyta įsiminti kvapus be žodinių užuominų.

Iš karto po kodavimo etapo buvo vertinama betarpiška olfaktorinė atmintis. Kiekvienam tiriamajam atsitiktine tvarka buvo priskirti penki nauji kvapai (distraktoriai). Distraktoriai ir tiksliniai kvapai buvo pateikiami atsitiktine tvarka. Kiekvienas iš 10 kvapų buvo pateikiamas 3 sekundes, flomasterio galiuką laikant maždaug 2 cm atstumu nuo abiejų šnervių. Tiriamųjų buvo prašoma pasirinkti, ar kvapas yra naujas, ar jau buvo pateiktas anksčiau (tikslinis kvapas). Betarpiškos olfaktorinės atminties įvertinimo rezultatas buvo teisingų atsakymų skaičius (0–10).

Atidėta olfaktorinė atmintis buvo vertinama praėjus 30 minučių po kodavimo etapo. Kiekvienam tiriamajam atsitiktine tvarka buvo paskirti penki nauji kvapai (antroji distraktorių grupė). Distraktoriai ir tiksliniai kvapai buvo pateikiami atsitiktine tvarka. Kiekvienas iš 10 kvapų buvo pateikiamas 3 sekundes, flomasterio galiuką laikant maždaug 2 cm atstumu nuo abiejų šnervių. Tiriamųjų buvo prašoma pasirinkti, ar kvapas yra naujas, ar jau buvo pateiktas anksčiau (tikslinis kvapas). Atidėtos olfaktorinės atminties įvertinimo rezultatas buvo teisingų atsakymų skaičius (0–10).

Tarp skirtingų kvapų pateikimo buvo daroma 30 sekundžių pertrauka.

Testavimo metu tyrėjas mūvėjo bekvapes pirštines, o tiriamieji dėvėjo akių raištį. Tiriamųjų buvo prašoma nieko negerti ir nevalgyti bent 15 minučių prieš testavimą (61).

2.7. Statistinė duomenų analizė

Statistinė duomenų analizė atlikta naudojant IBM SPSS Statistics 26.0 versiją (IBM Corp., Armonk, NY, JAV).

Duomenų pasiskirstymo normalumui nustatyti naudotas Šapiro ir Vilko testas.

Kuo skiriasi grupių kategoriniai kintamieji, buvo analizuojama naudojant chi kvadrato ir Fišerio tiksliuosius testus.

Skirtumai tarp dviejų grupių nenormaliai pasiskirsčiusių kiekybinių kintamųjų buvo analizuojami pagal Mano ir Vitnio U testą.

Trijų grupių kiekybinių kintamųjų skirtumai analizuoti taikant dispersinę analizę (ANOVA), jei kintamieji buvo normaliai pasiskirstę, ir Kruskalo ir Voliso testą, jeigu kintamieji nebuvo normaliai pasiskirstę.

Kintamųjų koreliacijai vertinti naudotas Spirmeno ranginės koreliacijos koeficientas.

Intervalinių kintamųjų prognozavimui analizuoti naudota tiesinė regresija, o kategorinių kintamųjų prognozavimui analizuoti – daugialypė logistinė regresija.

Diagnostinės testų savybės buvo įvertintos atliekant ROC (angl. *Receiver operating characteristic*) kreivių analizę.

Skirtumai laikyti statistiškai reikšmingais, kai p reikšmė buvo <0,05. Daugkartinių palyginimų atveju, naudojant Fišerio tikslųjį testą, taikyta Bonferoni korekcija ir skirtumai laikyti statistiškai reikšmingais, kai p reikšmė buvo <0,016.

3. REZULTATAI

3.1. Demografiniai ir klinikiniai rodikliai

Tiriamųjų demografiniai ir klinikiniai rodikliai pateikiami **1 lentelėje**.

Tiriamųjų pasiskirstymas pagal lytį trijose tiriamųjų grupėse reikšmingai nesiskyrė (chi kvadrato testas, $p > 0,05$). Taip pat nebuvo reikšmingų skirtumų pagal išsilavinimą, depresijos simptomus (GDS rezultatai) ir HII (visais atvejais Kruskalo ir Voliso testas, $p > 0,05$).

MD-AD grupės tiriamieji buvo reikšmingai vyresni už MCI-AD ir CN grupių dalyvius, tačiau MCI-AD ir CN grupės reikšmingai pagal tiriamųjų amžių nesiskyrė (Kruskalo ir Voliso testas, $p < 0,05$; *post-hoc* analizės duomenimis, skirtumai tarp CN ir MD-AD bei MCI-AD ir MD-AD grupių buvo statistiškai reikšmingi, o skirtumas tarp CN ir MCI-AD grupių statistiškai reikšmingas nebuvo).

Kaip ir tikėtasi, MD-AD grupės tiriamųjų AL simptomų trukmė buvo reikšmingai ilgesnė nei MCI-AD grupės tiriamųjų (Mano ir Vitnio U testas, $p < 0,001$). AChEI vartojančių pacientų MD-AD grupėje buvo reikšmingai daugiau nei MCI-AD grupėje (chi kvadrato testas, $p < 0,05$).

1 lentelė. Demografiniai ir klinikiniai rodikliai

	CN grupė (N=30)	MCI-AD grupė (N=30)	MD-AD grupė (N=30)
Vyrai, n (%) *	13 (43,33%)	13 (43,33%)	12 (40%)
Amžius (metais) **	74 [68,75–76]	72 [67,75–77,25]	78 [75–79,25]
Išsilavinimas (metais) *	15 [13,5–16]	16 [14–16]	16 [13–16]
HII *	1 [0–1]	1 [0–1]	1 [1–1,25]
GDS *	5,5 [4–6,25]	5,5 [4–6]	5 [4–6,25]
AL simptomų trukmė (metais) ***	N/A	3 [2–3]	4 [3–5]
AChEI vartojimas, n (%) ***	N/A	3 (10%)	14 (46,7%)

Sutrumpinimai: CN grupė – pažintinių funkcijų sutrikimų neturinčių pagyvenusių asmenų grupė; MCI-AD grupė – pacientų, kuriems diagnozuotas lengvas kognityvinis sutrikimas dėl Alzheimerio ligos, grupė; MD-AD grupė – pacientų, kuriems diagnozuota lengva demencija dėl Alzheimerio ligos, grupė; HII – Hačinskio išemijos indeksas; GDS – geriatrinė depresijos skalė; AChEI – acetilcholinesterazės inhibitoriai.

Duomenys pateikti kaip mediana ir tarpkvartilinis plotis, jeigu nenurodyta kitaip.

* Grupės reikšmingai nesiskiria.

** MD-AD grupė reikšmingai skiriasi nuo CN ir MCI-AD grupių. CN ir MCI-AD grupės tarpusavyje reikšmingai nesiskiria.

*** MD-AD grupė reikšmingai skiriasi nuo MCI-AD grupės.

3.2. Kognityviniai rodikliai

3.2.1. Bendrasis kognityvinių funkcijų įvertinimas

Visos trys tiriamųjų grupės reikšmingai skyrėsi pagal MMSE, CDR, ADAS-Cog, ADAS-Cog 13 ir žodinio sklandumo testų rezultatus (visais atvejais Kruskalo ir Voliso testas, $p < 0,05$; *post-hoc* analizės duomenimis, visos trys grupės reikšmingai skyrėsi tarpusavyje). Kognityvinių testų rezultatai pateikiami **2 lentelėje**.

2 lentelė. Kognityvinių testų rezultatai

	CN grupė (N=30)	MCI-AD grupė (N=30)	MD-AD grupė (N=30)
CDR įvertinimų suma *	0 [0–0]	2 [1,5–2,5]	5 [4,5–5,5]
MMSE *	29 [29–30]	26 [25–26]	22 [21–23]

	CN grupė (N=30)	MCI-AD grupė (N=30)	MD-AD grupė (N=30)
ADAS-Cog *	5,33 [4,59–7]	11,33 [9,17–13,75]	17,67 [15,17–20,33]
ADAS-Cog 13 *	10,83 [7,92–12,5]	20,84 [18,58–23]	29,34 [26,67–32,33]
VFT *	57,5 [43–63]	41 [35–50,75]	29,5 [21–39]

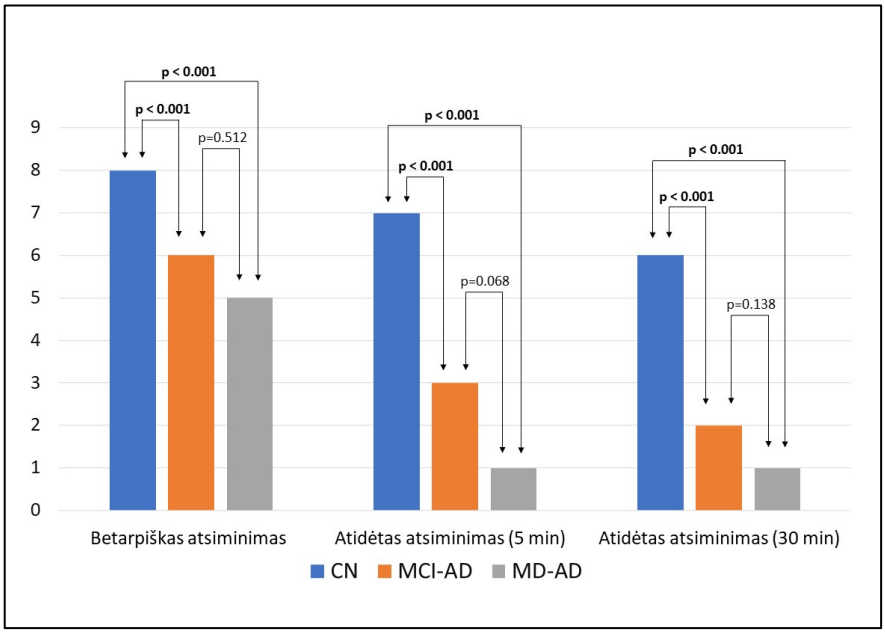
Sutrumpinimai: CN grupė – pažintinių funkcijų sutrikimų neturinčių pagyvenusių asmenų grupė; MCI-AD grupė – pacientų, kuriems diagnozuotas lengvas kognityvinis sutrikimas dėl Alzheimerio ligos, grupė; MD-AD grupė – pacientų, kuriems diagnozuota lengva demencija dėl Alzheimerio ligos, grupė; CDR – klinikinė demencijos vertinimo skalė; MMSE – mini protinės būklės tyrimas; ADAS-Cog – Alzheimerio ligos vertinimo skalės kognityvinė subskalė; ADAS-Cog 13 – Alzheimerio ligos vertinimo skalės kognityvinė subskalė su papildomomis atidėto atsiminimo ir skaičių išbraukimo užduotimis; VFT – bendras žodinio sklandumo testų rezultatas.

Duomenys pateikti kaip mediana ir tarpkvartilinis plotis.

* Visos trys grupės reikšmingai skiriasi.

3.2.2. Verbalinės atminties vertinimas

Betarpiškas atsiminimas (trečiasis ADAS-Cog betarpiško atsiminimo užduoties bandymas), atidėtas atsiminimas po 5 minučių ir atidėtas atsiminimas po 30 minučių reikšmingai skyrėsi tarp CN ir MCI-AD grupių bei CN ir MD-AD grupių, tačiau reikšmingai nesiskyrė tarp MCI-AD ir MD-AD grupių (visais atvejais Kruskalo ir Voliso testas, $p < 0,05$; *post hoc* analizės duomenimis, CN ir MCI-AD grupės bei CN ir MD-AD grupės skyrėsi reikšmingai [$p < 0,05$], o tarp MCI-AD ir MD-AD grupių reikšmingo skirtumo nebuvo [$p > 0,05$]). Verbalinės atminties užduočių rezultatai pateikiami **1 pav.** Išsamūs verbalinės atminties užduočių rezultatai pateikiami **IV straipsnyje**.



1 pav. Verbalinės atminties įvertinimo rezultatai (stulpeliai nurodo medianas).

Sutrumpinimai: CN grupė – pažintinių funkcijų sutrikimų neturinčių pagyvenusių asmenų grupė; MCI-AD grupė – pacientų, kuriems diagnozuotas lengvas kognityvinis sutrikimas dėl Alzheimerio ligos, grupė; MD-AD grupė – pacientų, kuriems diagnozuota lengva demencija dėl Alzheimerio ligos, grupė.

Verbalinės atminties užduočių rezultatai buvo palyginti tarp Alzheimerio liga sergančių pacientų (MCI-AD ir MD-AD grupių tiriamųjų), kurie vartojo AChEI, ir Alzheimerio liga sergančių pacientų, kuriems gydymo nebuvo skiriama.

Betarpiško atsiminimo, atidėto atsiminimo po 5 minučių ir atidėto atsiminimo po 30 minučių užduočių rezultatai tarp AChEI vartojančių ir nevartojančių pacientų nesiskyrė (visais atvejais Mano ir Vitnio U testas, $p > 0,05$). Tačiau AChEI vartojančių pacientų AL simptomų trukmė buvo reikšmingai ilgesnė nei AChEI nevartojančių (Mano ir Vitnio U testas, $p < 0,001$).

Išanalizavus atskirų tiriamųjų grupių duomenis, rezultatai išliko tokie patys. Tiek MCI-AD, tiek MD-AD tiriamųjų grupėse nebuvo reikšmingų skirtumų tarp pacientų, vartojančių AChEI, ir pacientų, kurie AChEI nevartoja, pagal betarpiško atsiminimo, atidėto atsiminimo po 5 minučių ir atidėto atsiminimo po 30 minučių užduočių rezultatus (visais atvejais Mano ir Vitnio U testas, $p > 0,05$). AL simptomų trukmės skirtumas tarp gydomų ir negydytų pacientų išliko reikšmingas tiek MCI-AD, tiek MD-AD grupėse (abiem atvejais Mano ir Vitnio U testas, $p < 0,05$).

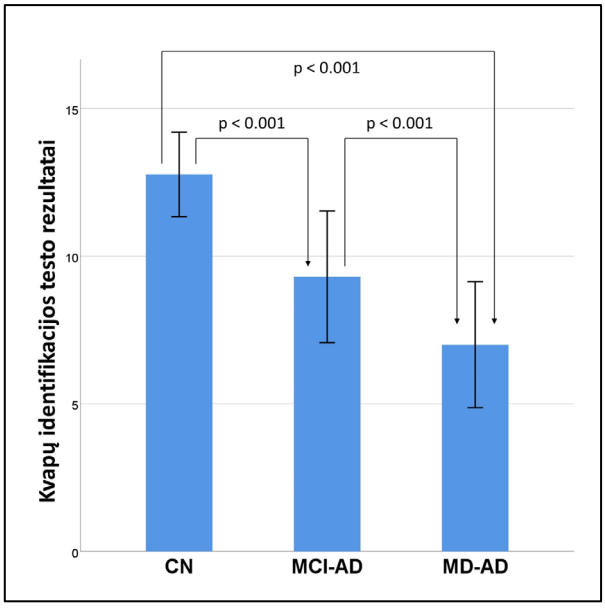
Išsamūs atskirų grupių verbalinės atminties užduočių rezultatai pateikiami **IV straipsnyje.**

3.3. Kvapų identifikacija

3.3.1. Kvapų identifikacijos įvertinimas

Dispersinės analizės (ANOVA) duomenimis, visų trijų grupių kvapų identifikacijos rezultatai reikšmingai skyrėsi (vidurkis ir standartinis nuokrypis grupėse: CN – 12,77±1,43; MCI-AD – 9,3±2,23; MD-AD – 7,0±2,13; $p < 0,001$, *post-hoc* analizės duomenimis, visos trys grupės reikšmingai skyrėsi). Kvapų identifikacijos testo rezultatai pateikiami **2 pav.**

Išanalizavus visų tiriamųjų duomenis, nustatytas reikšmingas, nors ir silpnas, kvapų identifikacijos testo rezultatų ir amžiaus (Spirmeno rho: -0,334; $p = 0,001$) ryšys. Analizuojant atskirų grupių duomenis, kvapų identifikacijos testo rezultatų ir amžiaus koreliacija išliko reikšminga CN grupėje (Spirmeno rho: -0,365; $p = 0,047$), bet ne MCI-AD ir MD-AD grupėse.

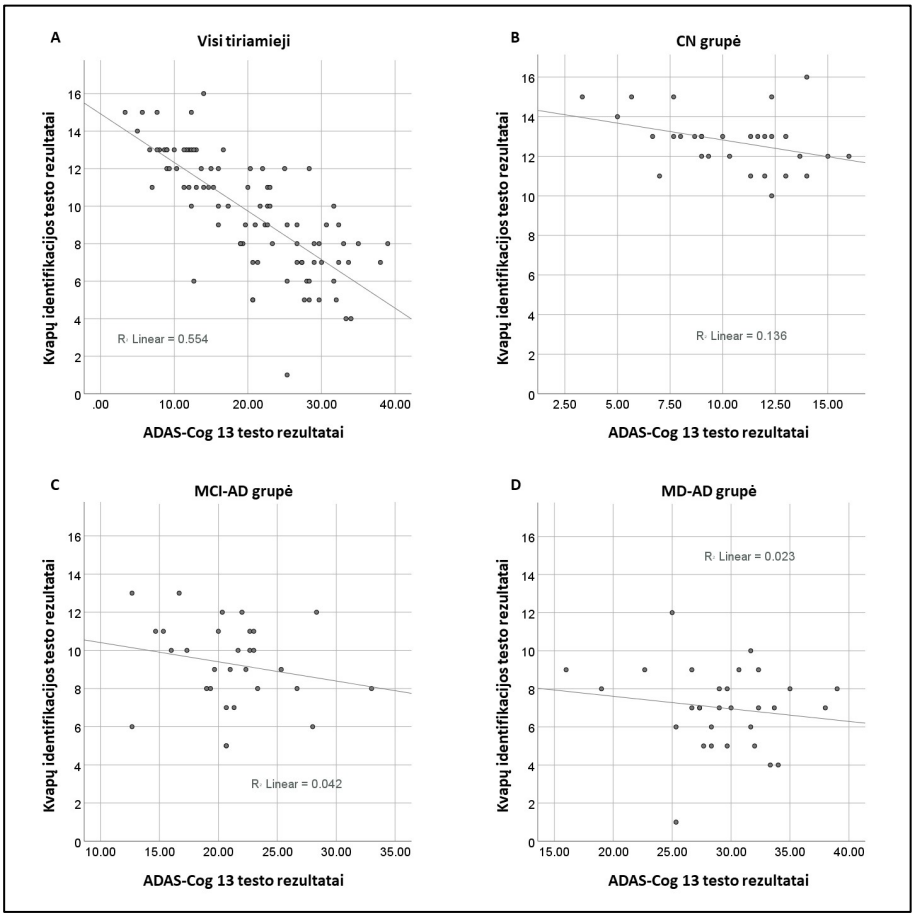


2 pav. Kvapų identifikacijos testo rezultatai (stulpeliai rodo vidurkius, o paklaidų stulpeliai – standartinius nuokrypius)

Sutrumpinimai: CN grupė – pažintinių funkcijų sutrikimų neturinčių pagyvenusių asmenų grupė; MCI-AD grupė – pacientų, kuriems diagnozuotas lengvas kognityvinis sutrikimas dėl Alzheimerio ligos, grupė; MD-AD grupė – pacientų, kuriems diagnozuota lengva demencija dėl Alzheimerio ligos, grupė.

Visų tiriamųjų kvapų identifikacijos testo rezultatai stipriai ir patikimai koreliavo su kognityvinių testų rezultatais: MMSE (Spirmeno rho: 0,735; $p < 0,001$), ADAS-Cog 13 (Spirmeno rho: -0,771; $p < 0,001$), CDR įvertinimų suma (Spirmeno rho: -0,775; $p < 0,001$) ir bendru žodinio sklandumo testų rezultatu [VFT = sklandumas PAS + sklandumas gyvūnai] (Spirmeno rho: 0,720; $p < 0,001$). Ryšys tarp kvapų identifikacijos testo rezultatų ir ADAS-Cog 13 rezultatų parodytas **3 pav.**

Išsami atskirų grupių kvapų identifikacijos testo ir kognityvinių testų rezultatų koreliacijų analizė pateikiama **I straipsnyje**.



3 pav. Kvapų identifikacijos ir ADAS-Cog 13 testų rezultatų priklausomybė

Sutrumpinimai: CN grupė – pažintinių funkcijų sutrikimų neturinčių pagyvenusių asmenų grupė; MCI-AD grupė – pacientų, kuriems diagnozuotas lengvas kognityvinis sutrikimas dėl Alzheimerio ligos, grupė; MD-AD grupė – pacientų, kuriems diagnozuota lengva demencija dėl Alzheimerio ligos, grupė; ADAS-Cog 13 – Alzheimerio ligos vertinimo skalės kognityvinė subskalė su papildomomis atidėto atsiminimo ir skaičių išbraukimo užduotimis.

Buvo įvertinti daugialypės tiesinės regresijos modeliai, kuriuose amžius, lytis, išsilavinimas ir kognityvinių testų rezultatai (MMSE, ADAS-Cog-13, CDR įvertinimų suma ir VFT) buvo nepriklausomi kintamieji, siekiant nustatyti, ar šie rodikliai reikšmingai prognozuoja kvapų identifikacijos testo rezultatus. Bendra regresija buvo statistiškai reikšminga visuose keturiuose modeliuose: modelyje su MMSE ($R^2 = 0,525$, $F = 23,452$, $p < 0,001$), modelyje su ADAS-Cog 13 ($R^2 = 0,560$, $F = 27,079$, $p < 0,001$), modelyje su CDR įvertinimų suma ($R^2 = 0,569$, $F = 28,068$, $p < 0,001$) ir modelyje su VFT ($R^2 = 0,464$, $F = 18,403$, $p < 0,001$). Tačiau kiekvienu atveju tik kognityvinių testų rezultatai reikšmingai prognozavo kvapų identifikacijos testo rezultatus (MMSE: $\beta = 0,702$, $p < 0,001$; ADAS-Cog 13: $\beta = -0,735$, $p < 0,001$; CDR įvertinimų suma: $\beta = -0,735$, $p < 0,001$; VFT: $\beta = 0,719$; $p < 0,001$). Nė vienas iš kitų nepriklausomų kintamųjų (amžius, lytis ir išsilavinimas) reikšmingai neprognozavo kvapų identifikacijos testo rezultatų nė viename modelyje ($p > 0,05$).

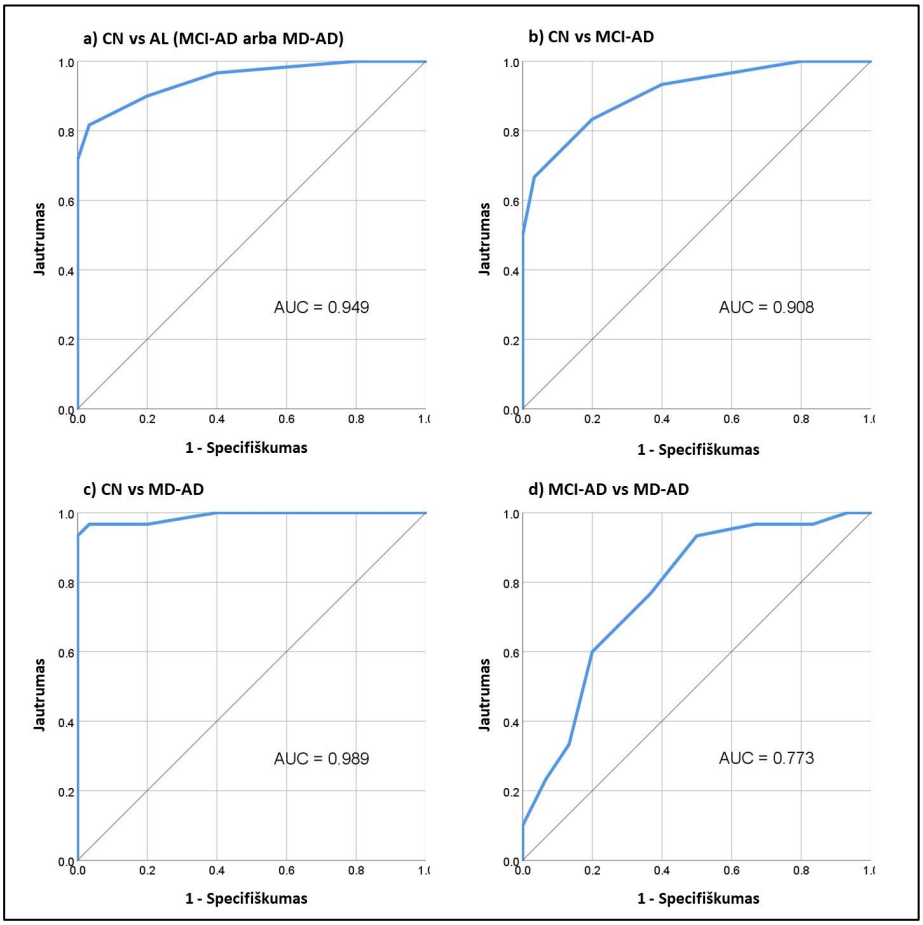
3.3.2. Kvapų identifikacijos testo diagnostinės savybės

Atlikta ROC (angl. *Receiver operating characteristic*) kreivių analizė, siekiant įvertinti kvapų identifikacijos testo galimybes atskirti CN grupės tiriamuosius nuo AL sergančių pacientų (MCI-AD ir MD-AD grupių tiriamieji), MCI-AD grupės tiriamųjų ir MD-AD grupės tiriamųjų, bei galimybes atskirti MCI-AD grupės tiriamuosius nuo MD-AD grupės tiriamųjų. ROC kreivės ir plotai po kreivėmis (AUC) vaizduojami **4 pav.**

Buvo pasirinkta ribinė vertė, kai ≤11 teisingų atsakymų rodė AL.

Naudojant šią ribinę vertę AL pacientams (MCI-AD arba MD-AD grupių tiriamiesiems) atskirti nuo CN tiriamųjų, kvapų identifikacijos testo jautrumas ir specifiškumas buvo atitinkamai 90 % (95 % patikimumo intervalas [PI]: 79,49–96,24 %) ir 80 % (95 % PI: 61,43–92,29 %). Neigiama ir teigiama prognostinė vertė buvo atitinkamai 80 % (95 % PI: 64,71–89,72 %) ir 90 % (95 % PI: 81,41–94,87 %). Bendras diagnostinis tikslumas buvo 86,67 % (95 % PI: 77,87–92,92 %).

Diagnostinės savybės išliko geros ir diferencijuojant MCI-AD grupės tiriamuosius nuo CN grupės tiriamųjų. Naudojant tą pačią ribinę vertę (≤11), kvapų identifikacijos testo jautrumas ir specifiškumas buvo atitinkamai 83,33 % (95 % PI 65,28–94,36 %) ir 80 % (95 % PI 61,43–92,29 %). Neigiama ir teigiama prognostinė vertė buvo atitinkamai 82,76 % (95 % PI: 67,89–91,59 %) ir 80,65 % (95 % PI: 66,68–89,66 %). Bendras diagnostinis tikslumas buvo 81,67 % (95 % PI: 69,56–90,48 %).



4 pav. Kvapų identifikacijos testo diagnostinės savybės diferencijuojant tiriamųjų grupes

Sutrumpinimai: CN grupė – pažintinių funkcijų sutrikimų neturinčių pagyvenusių asmenų grupė; MCI-AD grupė – pacientų, kuriems diagnozuotas lengvas kognityvinis sutrikimas dėl Alzheimerio ligos, grupė; MD-AD grupė – pacientų, kuriems diagnozuota lengva demencija dėl Alzheimerio ligos, grupė; AUC – plotas po ROC (angl. *Receiver operating characteristic*) kreive.

Siekiant įvertinti prognostinių kintamųjų ir priklausymo trims grupėms (CN, MCI-AD ir MD-AD) ryšį, buvo taikyta daugialypė logistinė regresija.

Pirmiausia buvo įvertintas modelis, kuriame prognostiniai kintamieji buvo amžius, išsilavinimas, lytis ir ADAS-Cog 13 testo rezultatai. Nulinio modelio atitiktis duomenims pagerėjo pridėjus prognostinių kintamųjų (X^2 = 139,656, $p < 0,001$; Nagelkerke $R^2 = 0,887$). Pirsono X^2 ir nuokrypio X^2 testai parodė, kad modelis gerai atitiko duomenis ($p > 0,05$). Bendras teisingai

suklasifikuotų atvejų procentas taikant šį modelį buvo 82,2 % (teisingai suklasifikuota 93,3 % CN, 70 % MCI-AD ir 83,3 % MD-AD atvejų), o ADAS-Cog 13 testo rezultatai buvo stipriausias ir reikšmingiausias prognostinis kintamasis ($X^2 = 122,652$, $p < 0,001$).

Į modelį buvo įtraukti kvapų identifikacijos testo rezultatai. Modelis su amžiumi, išsilavinimu, lytimi, ADAS-Cog 13 testo rezultatais ir kvapų identifikacijos testo rezultatais taip pat reikšmingai pagerino atitiktį duomenims, palyginti su nuliniu modeliu ($X^2 = 150,677$, $p < 0,001$; Nagelkerke $R^2 = 0,914$). Pirsono X^2 ir nuokrypio X^2 testai parodė, kad modelis gerai atitiko duomenis ($p > 0,05$). Bendras teisingai pagal šį modelį suklasifikuotų atvejų procentas buvo 87,8 % (teisingai suklasifikuota 100 % CN, 83,3 % MCI-AD ir 80 % MD-AD atvejų), o ADAS-Cog 13 testo rezultatai ir uoslės identifikacijos testo rezultatai abu buvo stiprūs ir reikšmingi prognostiniai kintamieji ($X^2 = 52,635$ ir 11,022 atitinkamai; $p < 0,001$ ir 0,004 atitinkamai).

3.3.3. Atskirų kvapų identifikacija

Penki kvapai (odos, citrinos, saldymedžio, obuolių ir ananasų) nebuvo įtraukti į tolesnę analizę, nes jie buvo blogai identifikuoti (<70 % teisingų atsakymų) CN grupėje.

Likusių kvapų identifikacijos rezultatai pateikiami **3 lentelėje**.

Devynių iš likusių 11 kvapų (visų, išskyrus anyžių ir terpentino) identifikacijos rezultatai MD-AD grupės buvo reikšmingai blogesni nei CN grupės (Fišerio tikslusis testas, $p < 0,016$). Trijų kvapų (gvazdikėlių, česnakų ir bananų) identifikacijos rezultatai MCI-AD grupės buvo reikšmingai blogesni nei CN grupės (Fišerio tikslusis testas, $p < 0,016$). MCI-AD ir MD-AD grupių visų 11 kvapų identifikacijos rezultatai reikšmingai nesiskyrė (Fišerio tikslusis testas, $p > 0,016$).

3 lentelė. Atskirų kvapų identifikacija

Kvapas	**CN teisingi atsakymai (%)**	**MCI-AD teisingi atsakymai (%)**	**MD-AD teisingi atsakymai (%)**
Gvazdikėliai [a, b]	30 (100)	19 (63,33) *	18 (60) *
Žuvis	29 (96,67)	28 (93,33)	20 (66,67) *
Apelsinas	29 (96,67)	23 (76,67)	19 (63,33) *
Česnakas [b]	29 (96,67)	22 (73,33)	17 (56,67) *
Kava	28 (93,33)	24 (80)	19 (63,33) *
Cinamonas [b]	28 (93,33)	19 (63,33) *	10 (33,33) *
Pipirmėtė	27 (90)	24 (80)	16 (53,33) *

Kvapas	CN teisingi atsakymai (%)	MCI-AD teisingi atsakymai (%)	MD-AD teisingi atsakymai (%)
Rožė	27 (90)	22 (73,33)	15 (50) *
Bananas [b]	26 (86,67)	16 (53,33) *	7 (23,33) *
Anyžiai	25 (83,33)	19 (63,33)	17 (56,67)
Terpentinas	22 (73,33)	12 (40)	16 (53,33)

Sutrumpinimai: CN grupė – pažintinių funkcijų sutrikimų neturinčių pagyvenusių asmenų grupė; MCI-AD grupė – pacientų, kuriems diagnozuotas lengvas kognityvinis sutrikimas dėl Alzheimerio ligos, grupė; MD-AD grupė – pacientų, kuriems diagnozuota lengva demencija dėl Alzheimerio ligos, grupė.

* Reikšmingai skyrėsi nuo CN grupės ($p < 0.016$).

[a] Kvapas, kuris labiausiai skyrėsi tarp MCI-AD ir CN grupių ($p < 0.001$)

[b] kvapai, kurie labiausiai skyrėsi tarp MD-AD ir CN grupių ($p < 0.001$)

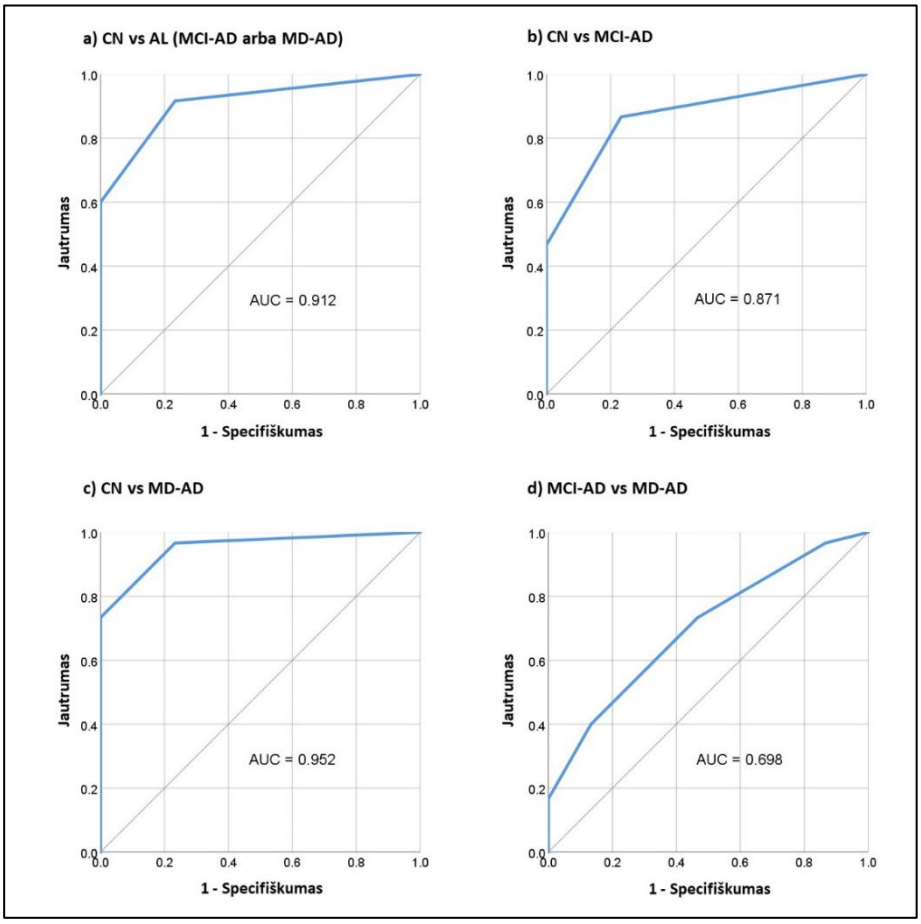
3.3.4. Sutrumpinto kvapų identifikacijos testo diagnostinės savybės

Buvo atrinkti keturi kvapai, kurių identifikacijos rezultatų skirtumai tarp MD-AD ir CN grupių buvo didžiausi (Fišerio tikslusis testas, $p < 0,001$). Apskaičiuotas šių keturių kvapų (gvazdikėlių, česnakų, cinamono ir bananų) identifikacijos rezultatas. Atlikta ROC kreivių analizė, siekiant įvertinti keturių kvapų identifikacijos testo galimybes atskirti CN grupės tiriamuosius nuo AL sergančių pacientų (MCI-AD ir MD-AD grupių tiriamieji), MCI-AD grupės tiriamųjų ir MD-AD grupės tiriamųjų, bei galimybes atskirti MCI-AD grupės tiriamuosius nuo MD-AD grupės tiriamųjų. ROC kreivės ir plotai po kreivėmis (AUC) pavaizduoti **5 pav**.

Buvo pasirinkta ribinė vertė, kai ≤3 teisingų atsakymų rodė AL.

Naudojant šią ribinę vertę AL pacientams (MCI-AD arba MD-AD grupių tiriamiesiems) atskirti nuo CN tiriamųjų, keturių kvapų identifikacijos testo jautrumas ir specifiškumas buvo atitinkamai 91,67 % (95 % PI: 81,61 %–97,24 %) ir 76,67 % (95 % PI: 57,72 %–90,07 %). Neigiama ir teigiama prognostinė vertė buvo atitinkamai 82,14 % (95 % PI: 66,01 %–91,59 %) ir 88,71 % (95 % PI: 80,35 %–93,79 %). Bendras diagnostinis tikslumas buvo 86,67 % (95 % PI: 77,87 %–92,92 %).

Diagnostinės savybės išliko geros ir diferencijuojant MCI-AD grupės tiriamuosius nuo CN grupės tiriamųjų. Naudojant tą pačią ribinę vertę (≤3), keturių kvapų identifikacijos testo jautrumas ir specifiškumas buvo atitinkamai 86,67 % (95 % PI: 69,28 %–96,24 %) ir (95 % PI: 57,72 %–90,07 %). Neigiama ir teigiama prognostinė vertė buvo atitinkamai 85,19 % (95 % PI: 69,33 %–93,60 %) ir 78,79 % (95 % PI: 65,67 %–87,82 %). Bendras diagnostinis tikslumas buvo 81,67 % (95 % PI: 69,56 %–90,48 %).



5 pav. Keturių kvapų identifikacijos testo diagnostinės savybės diferencijuojant tiriamųjų grupes

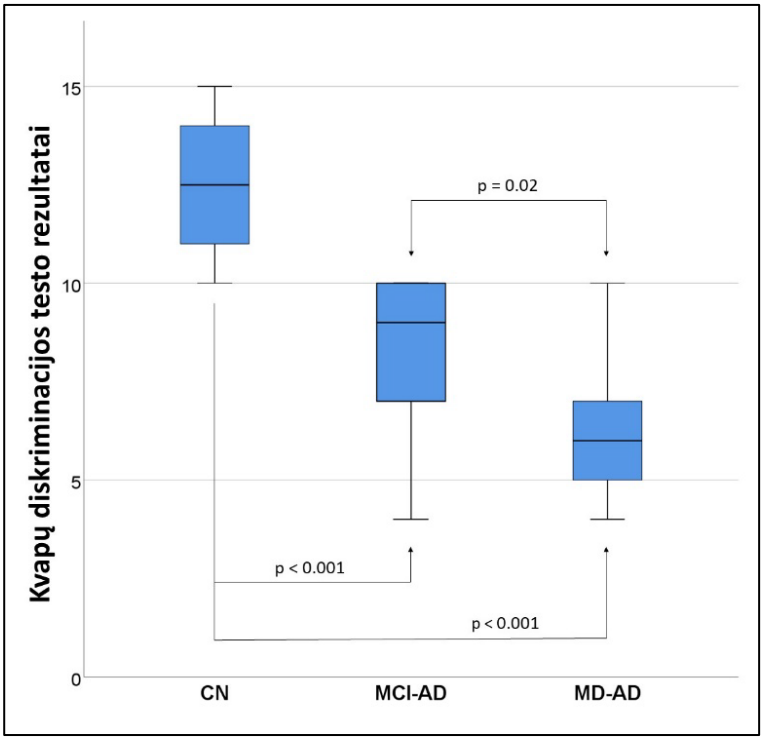
Sutrumpinimai: CN grupė – pažintinių funkcijų sutrikimų neturinčių pagyvenusių asmenų grupė; MCI-AD grupė – pacientų, kuriems diagnozuotas lengvas kognityvinis sutrikimas dėl Alzheimerio ligos, grupė; MD-AD grupė – pacientų, kuriems diagnozuota lengva demencija dėl Alzheimerio ligos, grupė; AUC – plotas po ROC (angl. *Receiver operating characteristic*) kreive.

3.4. Kvapų diskriminacija

3.4.1. Kvapų diskriminacijos vertinimas

Kvapų diskriminacijos testo rezultatai reikšmingai skyrėsi visų trijų grupių (medianos ir tarpkvartiliniai pločiai (IQR): CN 12,5 (3), MCI-AD 9 (3), MD-AD 6 (2); Kruskalo ir Voliso testas $p < 0,05$, *post hoc* analizė parodė

esant reikšmingų skirtumų tarp visų trijų grupių). Kvapų diskriminacijos testų rezultatai pateikiami 6 pav.



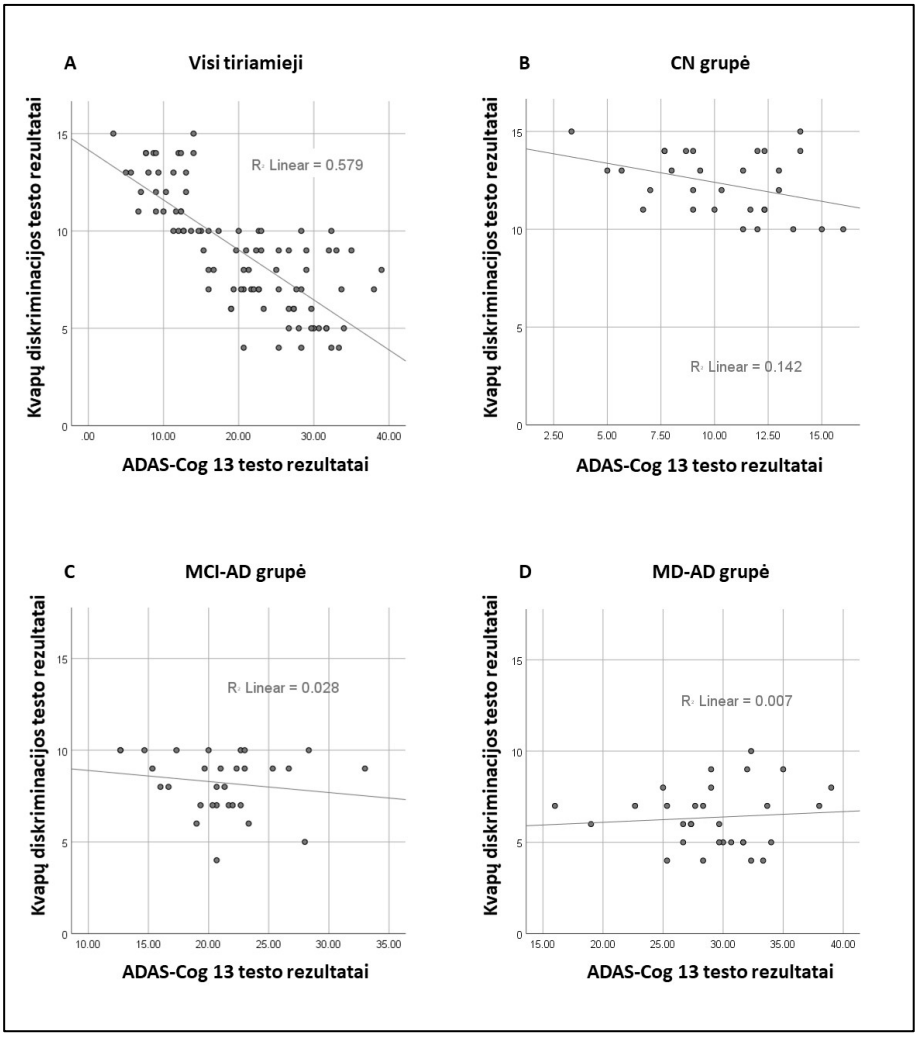
6 pav. Kvapų diskriminacijos testo rezultatai (linijos rodo medianas, dėžutės – tarpkvartilinius pločius, o paklaidų stulpeliai – mažiausius ir didžiausius įverčius)

Sutrumpinimai: CN grupė – pažintinių funkcijų sutrikimų neturinčių pagyvenusių asmenų grupė; MCI-AD grupė – pacientų, kuriems diagnozuotas lengvas kognityvinis sutrikimas dėl Alzheimerio ligos, grupė; MD-AD grupė – pacientų, kuriems diagnozuota lengva demencija dėl Alzheimerio ligos, grupė.

Išanalizavus visų tiriamųjų duomenis, nustatytas reikšmingas, nors ir silpnas, kvapų diskriminacijos testo rezultatų ir amžiaus ryšys (Spirmeno rho: -0,28; p = 0,008). Analizuojant atskirų grupių duomenis, reikšmingų koreliacijų tarp kvapų diskriminacijos testo rezultatų ir amžiaus nebuvo.

Visų tiriamųjų imties kvapų diskriminacijos testo rezultatai stipriai ir patikimai susiję su MMSE, ADAS-Cog 13 ir CDR rezultatais (Spirmeno rho atitinkamai 0,78, -0,77 ir -0,82; p < 0,001). Kvapų diskriminacijos testo rezultatų ir žodinio sklandumo testų įverčių koreliacija buvo vidutinio stiprumo, tačiau taip pat reikšminga (Spirmeno rho PAS sklandumui 0,64,

sklandumui gyvūnams 0,64; $p < 0,001$ abiem atvejais). Kvapų diskriminacijos testo rezultatų ir ADAS-Cog 13 rezultatų ryšys parodytas **7 pav**.



7 pav. Kvapų diskriminacijos ir ADAS-Cog 13 testų rezultatų ryšys

Sutrumpinimai: CN grupė – pažintinių funkcijų sutrikimų neturinčių pagyvenusių asmenų grupė; MCI-AD grupė – pacientų, kuriems diagnozuotas lengvas kognityvinis sutrikimas dėl Alzheimerio ligos, grupė; MD-AD grupė – pacientų, kuriems diagnozuota lengva demencija dėl Alzheimerio ligos, grupė; ADAS-Cog 13 – Alzheimerio ligos vertinimo skalės kognityvinė subskalė su papildomomis atidėto atsiminimo ir skaičių išbraukimo užduotimis.

Buvo įvertinti daugialypės tiesinės regresijos modeliai, kuriuose amžius, lytis, išsilavinimas ir kognityvinių testų rezultatai (MMSE, ADAS-

Cog-13, CDR įvertinimų suma ir VFT) buvo nepriklausomi kintamieji, siekta nustatyti, ar šie rodikliai reikšmingai prognozuoja kvapų diskriminacijos testo rezultatus. Bendra regresija buvo statistiškai reikšminga visuose keturiuose modeliuose: modelyje su MMSE ($R^2 = 0,62$, $F = 35,05$, $p < 0,001$), modelyje su ADAS-Cog 13 ($R^2 = 0,58$, $F = 29,43$, $p < 0,001$), modelyje su CDR įvertinimų suma ($R^2 = 0,63$, $F = 36,25$, $p < 0,001$) ir modelyje su VFT ($R^2 = 0,46$, $F = 17,72$, $p < 0,001$). Tačiau kiekvienu atveju tik kognityvinių testų rezultatai reikšmingai prognozavo kvapų diskriminacijos testo rezultatus (MMSE: $\beta = 0,79$, $p < 0,001$; ADAS-Cog 13: $\beta = -0,77$, $p < 0,001$; CDR įvertinimų suma: $\beta = -0,8$, $p < 0,001$; VFT: $\beta = 0,73$, $p < 0,001$). Nė vienas iš kitų nepriklausomų kintamųjų (amžius, lytis ir išsilavinimas) reikšmingai neprognozavo kvapų diskriminacijos testo rezultatų nė viename modelyje ($p > 0,05$).

Atskirų grupių išsami kvapų diskriminacijos testo ir kognityvinių testų rezultatų koreliacijų analizė pateikiama **II straipsnyje**.

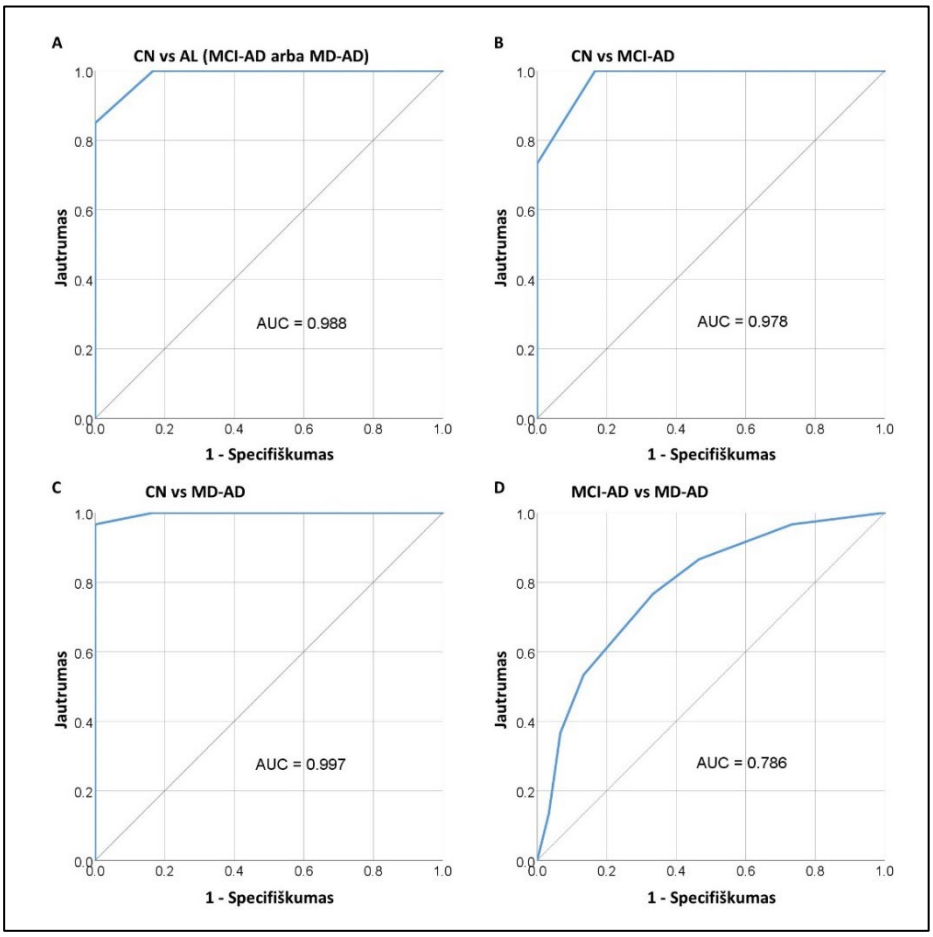
3.4.2. Kvapų diskriminacijos testo diagnostinės savybės

Atlikta ROC kreivių analizė, siekiant įvertinti kvapų diskriminacijos testo galimybes atskirti CN grupės tiriamuosius nuo AL sergančių pacientų (MCI-AD ir MD-AD grupių tiriamųjų), MCI-AD grupės tiriamųjų ir MD-AD grupės tiriamųjų, bei galimybes atskirti MCI-AD grupės tiriamuosius nuo MD-AD grupės tiriamųjų. ROC kreivės ir plotai po kreivėmis (AUC) vaizduojami **8 pav.**

Buvo pasirinkta ribinė vertė, kai ≤10 teisingų atsakymų rodė AL.

Naudojant šią ribinę vertę AL pacientams (MCI-AD arba MD-AD grupių tiriamiesiems) atskirti nuo CN tiriamųjų, kvapų diskriminacijos testo jautrumas ir specifiškumas buvo atitinkamai 100 % (95 % PI: 94,04–100 %) ir 83,3 % (95 % PI: 65,28–94,36 %). Neigiama ir teigiama prognostinė vertė buvo atitinkamai 100 % ir 92,31 % (95 % PI: 84,35–96,39 %). Bendras diagnostinis tikslumas buvo 94,44 % (95 % PI: 87,51–98,17 %).

Diagnostinės savybės išliko geros ir atskiriant MCI-AD grupės tiriamuosius nuo CN grupės tiriamųjų. Naudojant tą pačią ribinę vertę (≤10), kvapų diskriminacijos testo jautrumas ir specifiškumas buvo atitinkamai 100 % (95 % PI: 88,43–100 %) ir 83,3 % (95 % PI: 65,28–94,36 %). Neigiama ir teigiama prognostinė vertė buvo atitinkamai 100 % ir 85,71 % (95 % PI: 72,94–93,03 %). Bendras diagnostinis tikslumas buvo 91,67 % (95 % PI: 81,61–97,24 %).



8 pav. Kvapų diskriminacijos testo diagnostinės savybės diferencijuojant tiriamųjų grupes

Sutrumpinimai: CN grupė – pažintinių funkcijų sutrikimų neturinčių pagyvenusių asmenų grupė; MCI-AD grupė – pacientų, kuriems diagnozuotas lengvas kognityvinis sutrikimas dėl Alzheimerio ligos, grupė; MD-AD grupė – pacientų, kuriems diagnozuota lengva demencija dėl Alzheimerio ligos, grupė; AUC – plotas po ROC (ang. *Receiver operating characteristic*) kreive.

Siekiant įvertinti prognostinių kintamųjų ir priklausymo trims grupėms (CN, MCI-AD ir MD-AD) ryšį, buvo taikyta daugialypė logistinė regresija.

Pirmiausia buvo įvertintas modelis, kuriame prognostiniai kintamieji buvo amžius, išsilavinimas, lytis ir ADAS-Cog 13 testo rezultatai. Nulinio modelio atitiktis duomenims pagerėjo pridėjus prognostinius kintamuosius (X^2 = 139,656, p < 0,001; Nagelkerke R2 = 0,887). Pirsono X^2 ir nuokrypio X^2 testai parodė, kad modelis gerai atitiko duomenis (p > 0,05). Bendras

teisingai suklasifikuotų atvejų procentas taikant šį modelį buvo 82,2 % (teisingai suklasifikuota 93,3 % CN, 70 % MCI-AD ir 83,3 % MD-AD atvejų), o ADAS-Cog 13 testo rezultatai buvo stipriausias ir reikšmingiausias prognostinis kintamasis ($X^2 = 122,652$, $p < 0,001$).

Į modelį buvo įtraukti kvapų diskriminacijos testo rezultatai. Modelis, kurio prognostiniai kintamieji amžius, išsilavinimas, lytis, ADAS-Cog 13 testo rezultatai, ir kvapų diskriminacijos testo rezultatai taip pat reikšmingai pagerino atitiktį duomenims, palyginti su nuliniu modeliu ($X^2 = 158,11$, $p < 0,001$; Nagelkerke R2 = 0,93). Pirsono X^2 ir nuokrypio X^2 testai parodė, kad modelis gerai atitiko duomenis ($p > 0,05$). Bendras teisingai pagal šį modelį suklasifikuotų atvejų procentas buvo 92,2 % (teisingai suklasifikuota 96,7 % CN, 86,7 % MCI-AD ir 93,3 % MD-AD atvejų), o ADAS-Cog 13 testo rezultatai ir uoslės identifikacijos testo rezultatai abu buvo stiprūs ir reikšmingi prognostiniai kintamieji ($X^2 = 28,01$ ir 18,45 atitinkamai; $p < 0,001$ abiem atvejais).

3.4.3. Atskirų kvapų diskriminacija

Penki tripletai neįtraukti į tolesnę analizę, nes jie buvo blogai diskriminuojami (<70 % teisingų atsakymų) CN grupės tiriamųjų.

Likusių kvapų diskriminacijos rezultatai pateikiami **4 lentelėje**.

Devynių iš likusių 11 tripletų kvapų diskriminacijos rezultatai MD-AD grupėje buvo reikšmingai blogesni nei CN grupėje (Fišerio tikslusis testas, $p < 0,016$), o aštuonių tripletų kvapų diskriminacijos rezultatai jau MCI-AD grupėje buvo reikšmingai blogesni nei CN grupėje (Fišerio tikslusis testas, $p < 0,016$).

Reikšmingai skyrėsi MCI-AD ir MD-AD grupių kvapų diskriminacijos rezultatai triplete, kuriame tikslinis kvapas buvo 2-feniletanolis, o netikslinis kvapas – izoamilo acetatas (Fišerio tikslusis testas, $p < 0,001$). Visų likusių dešimties tripletų kvapų diskriminacijos balai MCI-AD ir MD-AD grupių reikšmingai nesiskyrė (Fišerio tikslusis testas, $p > 0,016$).

4 lentelė. Atskirų kvapų diskriminacija

Skirtingas kvapas	Vienodi kvapai	CN teisingi atsakymai (%)	MCI-AD teisingi atsakymai (%)	MD-AD teisingi atsakymai (%)
2-feniletanolis	Izoamilacetatas [b]	29 (96.67)	20 (66.67) *	6 (20) *
(+)-Limonenas	(+)-Fenchonas [a, b]	29 (96.67)	13 (43.33) *	15 (50) *
Pyridinas	(-)-Limonenas [b]	28 (93.33)	21 (70)	13 (43.33) *

Skirtingas kvapas	Vienodi kvapai	CN teisingi atsakymai (%)	MCI-AD teisingi atsakymai (%)	MD-AD teisingi atsakymai (%)
Oktilacetatas	Cinamaldehidas [b]	28 (93.33)	19 (63.33) *	11 (36.67) *
2-feniletanolis	(+)-Mentolis [a, b]	28 (93.33)	16 (53.33) *	12 (40) *
1-butanolis	(+)-Fenchonas [b]	27 (90)	21 (70)	11 (36.67) *
Eukaliptolis	α-Iononas [b]	27 (90)	17 (56.67) *	10 (33.33) *
(-)-Limonenas	Citronelalas [b]	25 (83.33)	14 (46.67) *	9 (30) *
Anethole	Eugenolis [a, b]	23 (76.67)	10 (33.33) *	11 (36.67) *
Izoamilacetatas	Anetolis	21 (70)	22 (73.33)	17 (56.67)
Citronelalas	Linalolas	21 (70)	10 (33.33) *	16 (53.33)

Sutrumpinimai: CN grupė – pažintinių funkcijų sutrikimų neturinčių pagyvenusių asmenų grupė; MCI-AD grupė – pacientų, kuriems diagnozuotas lengvas kognityvinis sutrikimas dėl Alzheimerio ligos, grupė; MD-AD grupė – pacientų, kuriems diagnozuota lengva demencija dėl Alzheimerio ligos, grupė.

* Reikšmingai skyrėsi nuo CN grupės ($p < 0.016$).

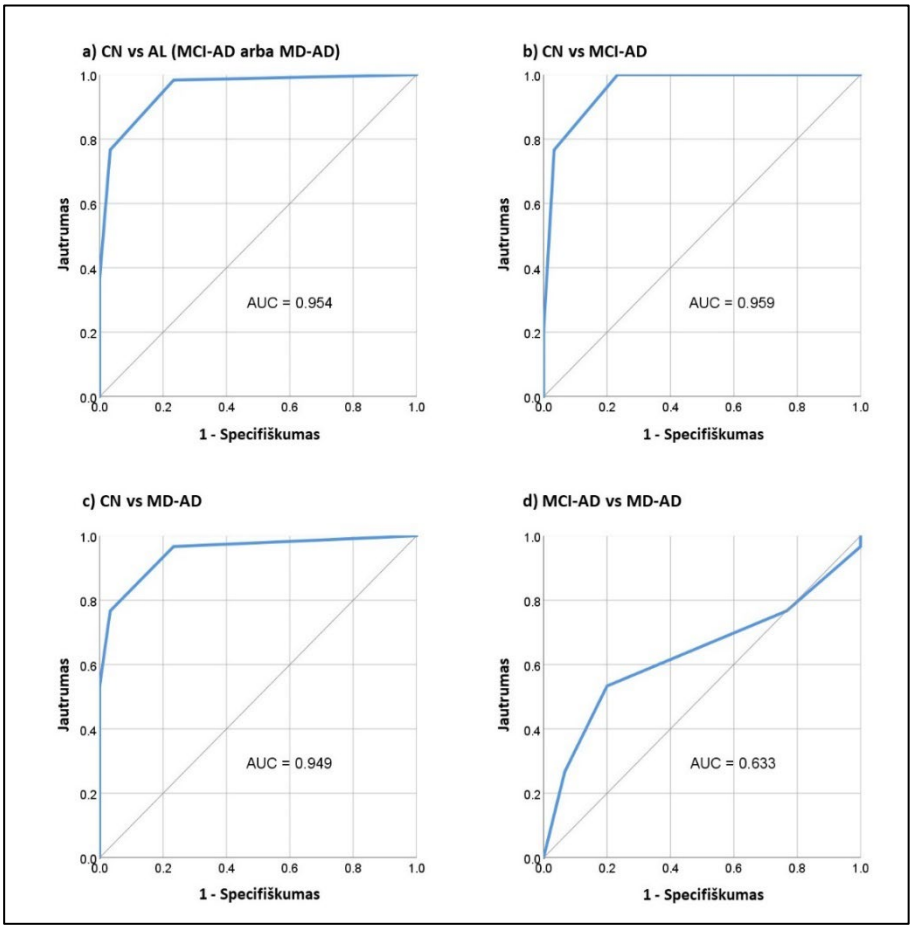
[a] Kvapai, kurie labiausiai skyrėsi tarp MCI-AD ir CN grupių ($p < 0.001$)

[b] Kvapai, kurie labiausiai skyrėsi tarp MD-AD ir CN grupių ($p < 0.001$)

3.4.4. Sutrumpinto kvapų diskriminacijos testo diagnostinės savybės

Keturių kvapų diskriminacijos testui buvo atrinkti keturi tripletai: trys tripletai, kurių diskriminacija labiausiai skyrėsi tarp MCI-AD ir CN grupių, bei tripletas, kurio diskriminacija reikšmingai skyrėsi tarp MCI-AD ir MD-AD grupių.

Atlikta ROC kreivių analizė, siekiant įvertinti keturių kvapų diskriminacijos testo galimybes atskirti CN grupės tiriamuosius nuo AL sergančių pacientų (MCI-AD ir MD-AD grupių tiriamieji), MCI-AD grupės tiriamųjų ir MD-AD grupės tiriamųjų, bei galimybes atskirti MCI-AD grupės tiriamuosius nuo MD-AD grupės tiriamųjų. ROC kreivės ir plotai po kreivėmis (AUC) vaizduojami **9 pav.**



9 pav. Keturių kvapų diskriminacijos testo diagnostinės savybės diferencijuojant tiriamųjų grupes

Sutrumpinimai: CN grupė – pažintinių funkcijų sutrikimų neturinčių pagyvenusių asmenų grupė; MCI-AD grupė – pacientų, kuriems diagnozuotas lengvas kognityvinis sutrikimas dėl Alzheimerio ligos, grupė; MD-AD grupė – pacientų, kuriems diagnozuota lengva demencija dėl Alzheimerio ligos, grupė; AUC – plotas po ROC (angl. *Receiver operating characteristic*) kreive.

Buvo pasirinkta ribinė vertė, kai ≤3 teisingų atsakymų rodė AL.

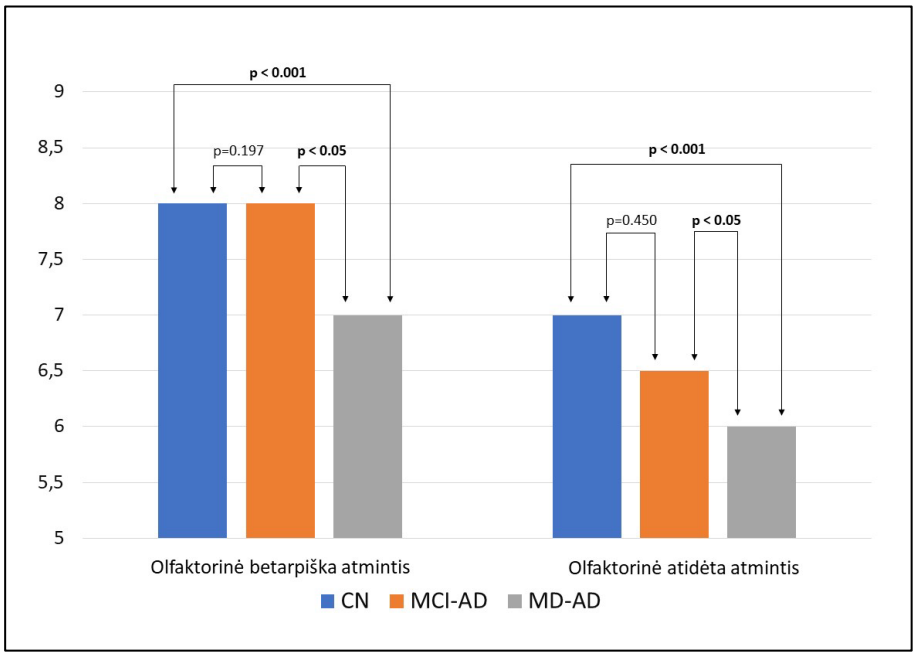
Naudojant šią ribinę vertę AL pacientams (MCI-AD arba MD-AD grupių tiriamiesiems) atskirti nuo CN tiriamųjų, keturių kvapų diskriminacijos testo jautrumas ir specifiškumas buvo atitinkamai 98,33 % (95 % PI: 91,06 %–99,96 %) ir 76,67 % (95 % PI: 57,72 %–90,07 %). Neigiama ir teigiama prognostinė vertė buvo atitinkamai 95,83 % (95 % PI: 76,53 %–99,39 %) ir 89,39 % (95 % PI: 81,49 %–94,16 %). Bendras diagnostinis tikslumas buvo 91,11 % (95 % PI: 83,23 %–96,08 %).

Diagnostinės savybės išliko geros ir diferencijuojant MCI-AD grupės tiriamuosius nuo CN grupės tiriamųjų. Naudojant tą pačią ribinę vertę (≤3), keturių kvapų diskriminacijos testo jautrumas ir specifiškumas buvo atitinkamai 100 % (95 % CI: 88,43 %–100 %) ir 76,67 % (95 % CI: 57,72 %–90,07 %). Neigiama ir teigiama prognostinė vertė buvo atitinkamai 100 % ir 81,08 % (95 % CI: 69,14 %–89,13 %). Bendras diagnostinis tikslumas buvo 88,33 % (95 % CI: 77,43 %–95,18 %).

3.5. Olfaktorinė atmintis

MD-AD grupės tiriamųjų olfaktorinė betarpiška atmintis ir olfaktorinė atidėta atmintis buvo reikšmingai blogesnė nei MCI-AD ir CN grupių tiriamųjų. MCI-AD ir CN grupių tiriamieji reikšmingai nesiskyrė (abiem atvejais Kruskalo ir Voliso testas, $p < 0,05$; *post hoc* analizė parodė reikšmingus skirtumus tarp MD-AD ir MCI-AD grupių bei MD-AD ir CN grupių [$p < 0,05$], o tarp MCI-AD ir CN grupių reikšmingų skirtumų nenustatyta [$p > 0,05$]). Olfaktorinės atminties užduočių rezultatai pateikiami **10 pav**. Išsamūs olfaktorinės atminties užduočių rezultatai pateikiami **IV straipsnyje**.

Pacientų, sergančių AL (MCI-AD ir MD-AD grupių tiriamieji), olfaktorinės betarpiškos atminties įvertinimo rezultatai reikšmingai koreliavo su AL simptomų trukme (Spirmeno rho = -0,366, $p < 0,05$), CDR įverčių suma (Spirmeno rho = -0,328, $p < 0,05$) ir VFT (Spirmeno rho = 0,355, $p < 0,05$). Olfaktorinės atidėtos atminties įvertinimo rezultatai reikšmingai koreliavo su AL simptomų trukme (Spirmeno rho = -0,360, $p < 0,05$), CDR įverčių suma (Spirmeno rho = -0,317, $p < 0,05$), VFT (Spirmeno rho = 0,303, $p < 0,05$) ir verbaliniu atidėtu atsiminimu po 5 minučių (Spirmeno rho = 0,258, $p < 0,05$). Nenustatyta nei olfaktorinės betarpiškos atminties, nei olfaktorinės atidėtos atminties vertinimo rezultatų reikšmingos koreliacijos su amžiumi (atitinkamai Spirmeno rho = -0,194, $p = 0,138$ ir Spirmeno rho = -0,226, $p = 0,082$).



10 pav. Olfaktorinės atminties vertinimo rezultatai (stulpeliai rodo medianas)

Sutrumpinimai: CN grupė – pažintinių funkcijų sutrikimų neturinčių pagyvenusių asmenų grupė; MCI-AD grupė – pacientų, kuriems diagnozuotas lengvas kognityvinis sutrikimas dėl Alzheimerio ligos, grupė; MD-AD grupė – pacientų, kuriems diagnozuota lengva demencija dėl Alzheimerio ligos, grupė.

Siekiant nustatyti, kurie kintamieji, koreliuojantys su pacientų, sergančių AL, olfaktorinės atminties rodikliais, gali juos reikšmingai prognozuoti, buvo sukurti daugialypės tiesinės regresijos modeliai. Be to, į modelius buvo įtraukti amžius, lytis ir GDS balai kaip žinomi veiksniai, turintys įtakos atminčiai ir uoslei bendroje populiacijoje.

Modelyje, kuriame amžius, lytis, GDS įvertis, AL simptomų trukmė, CDR įverčių suma ir VFT buvo nepriklausomi kintamieji, o olfaktorinės betarpiškos atminties vertinimo rezultatai – priklausomas kintamasis, bendra regresija buvo reikšminga (R^2 = 0,247, F = 2,904, p = 0,016). Stipriausias prognostinis veiksnys buvo AL simptomų trukmė (β = -0,261, p = 0,066). Taikant pakopinės regresijos modelį, AL simptomų trukmė (β = -0,290, p = 0,021) ir VFT (β = 0,306, p = 0,015) išliko vieninteliais veiksniais, reikšmingai prognozuojančiais olfaktorinės betarpiškos atminties vertinimo rezultatus.

Modelyje, kuriame amžius, lytis, GDS įvertis, AL simptomų trukmė, CDR įverčių suma, VFT ir verbalinis atidėtas atsiminimas po 5 minučių buvo nepriklausomi kintamieji, o olfaktorinės atidėtos atminties vertinimo

rezultatai – priklausomas kintamasis, bendra regresija taip pat buvo reikšminga (R^2 = 0,232, F = 2,249, p = 0,045). Stipriausias prognostinis veiksnys taip pat buvo AL simptomų trukmė (β = -0,302, p = 0,043). Taikant pakopinės regresijos modelį, AL simptomų trukmė (β = -0,291, p = 0,022) ir VFT (β = 0,268, p = 0,035) išliko vieninteliais veiksniais, reikšmingai prognozuojančiais olfaktorinės atidėtos atminties vertinimo rezultatus.

Buvo palyginti AL sergančių pacientų (MCI-AD ir MD-AD grupių tiriamųjų), kurie vartoja AChEI, ir AL sergančių pacientų, kuriems gydymo nebuvo skiriama, olfaktorinės atminties užduočių rezultatai. Pacientų, vartojančių AChEI, tiek olfaktorinės betarpiškos atminties užduočių (mediana ir IQR atitinkamai 6 (6–7) ir 7 (6–8); Mano ir Vitnio U testas, p = 0,024), tiek olfaktorinės atidėtos atminties užduočių (mediana ir IQR atitinkamai 6 (6–6) ir 6 (5–6); Mano ir Vitnio U testas, p = 0,025) rezultatai buvo reikšmingai blogesni nei pacientų, kuriems gydymo nebuvo skiriama.

Išanalizavus atskirų grupių rezultatus, tokių reikšmingų skirtumų nebebuvo. MCI-AD grupėje nebuvo reikšmingų skirtumų tarp pacientų, vartojančių ir nevartojančių AChEI, nei olfaktorinės betarpiškos atminties (mediana ir IQR atitinkamai 6 (6–6) ir 8 (6–8); Mano ir Vitnio U testas, p = 0,600), nei olfaktorinės atidėtos atminties užduočių (mediana ir IQR atitinkamai 6 (5–6) ir 7 (6–8); Mano ir Vitnio U testas, p = 0,387).

MD-AD grupėje taip pat nebuvo reikšmingų skirtumų tarp pacientų, vartojančių AChEI ir jų nevartojančių, nei olfaktorinės betarpiškos atminties (mediana ir IQR atitinkamai 6 (5,75–7) ir 7 (6–7); Mano ir Vitnio U testas, p = 0,208), nei olfaktorinės atidėtos atminties užduočių (mediana ir IQR atitinkamai 5,5 (5–6) ir 6 (5–7); Mano ir Vitnio U testas, p = 0,313).

Olfaktorinės atminties testų diagnostinės savybės nebuvo tiriamos, nes MCI-AD ir CN grupių tiriamieji tarpusavyje reikšmingai nesiskyrė.

4. IŠVADOS

1. Kvapų identifikacija yra sutrikusi ankstyvosiose AL stadijose, o kvapų identifikacijos testai pasižymi geromis diagnostinėmis savybėmis ankstyvosioms AL stadijoms nustatyti.
2. Kvapų diskriminacija yra sutrikusi ankstyvosiose AL stadijose, o kvapų diskriminacijos testai pasižymi geromis diagnostinėmis savybėmis ankstyvosioms AL stadijoms nustatyti.
3. Kvapų identifikacijos ir kvapų diskriminacijos testų rezultatai koreliuoja su kognityvinių funkcijų įvertinimo duomenimis ir suteikia papildomos diagnostinės vertės ankstyvosioms AL stadijoms nustatyti.
4. Sutrumpinti kvapų identifikacijos ir kvapų diskriminacijos testų variantai pasižymi geromis diagnostinėmis savybėmis ankstyvosioms AL stadijoms nustatyti.
5. Olfaktorinė atmintis yra sutrikusi ankstyvosiose AL stadijose, tačiau šie sutrikimai skiriasi nuo matomų verbalinės atminties sutrikimų.

REFERENCES

1. Global Status Report on the Public Health Response to Dementia (World Health Organization, Geneva, 2021). Licence: CC BY-NC-SA 3.0 IGO.
2. Gauthier, S., Rosa-Neto, P., Morais, J. A. & Webster, C. World
3. Alzheimer Report 2021: Journey through the Diagnosis of Dementia (Alzheimer's Disease Int., London, England, 2021).
   Global Action Plan on the Public Health Response to Dementia 2017–2025 (World Health Organization, Geneva, 2017). Licence: CC BY-NC-SA 3.0 IGO.
4. Albert MS, DeKosky ST, Dickson D, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):270-279. doi:10.1016/j.jalz.2011.03.008.
5. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):263-269. doi:10.1016/j.jalz.2011.03.005.
6. Park JE, Gunasekaran TI, Cho YH, et al. Diagnostic Blood Biomarkers in Alzheimer's Disease. Biomedicines. 2022;10(1):169. Published 2022 Jan 13. doi:10.3390/biomedicines10010169.
7. Cummings J, Lee G, Nahed P, et al. Alzheimer's disease drug development pipeline: 2022. Alzheimers Dement (N Y). 2022;8(1):e12295. Published 2022 May 4. doi:10.1002/trc2.12295.
8. Waldton S. Clinical observations of impaired cranial nerve function in senile dementia. Acta Psychiatr Scand. 1974;50(5):539-547. doi:10.1111/j.1600-0447.1974.tb09714.x.
9. Doty RL, Reyes PF, Gregor T. Presence of both odor identification and detection deficits in Alzheimer's disease. Brain Res Bull. 1987;18(5):597-600. doi:10.1016/0361-9230(87)90129-8.
10. Marine N, Boriana A. Olfactory markers of depression and Alzheimer's disease. Neurosci Biobehav Rev. 2014;45:262-270. doi:10.1016/j.neubiorev.2014.06.016.
11. Jung HJ, Shin IS, Lee JE. Olfactory function in mild cognitive impairment and Alzheimer's disease: A meta-analysis. Laryngoscope. 2019;129(2):362-369. doi:10.1002/lary.27399.

12. Roalf DR, Moberg MJ, Turetsky BI, et al. A quantitative meta-analysis of olfactory dysfunction in mild cognitive impairment. J Neurol Neurosurg Psychiatry. 2017;88(3):226-232. doi:10.1136/jnnp-2016-314638.
13. Djordjevic J, Jones-Gotman M, De Sousa K, Chertkow H. Olfaction in patients with mild cognitive impairment and Alzheimer's disease. Neurobiol Aging. 2008;29(5):693-706. doi:10.1016/j.neurobiolaging.2006.11.014.
14. Jobin B, Zahal R, Bussières EL, Frasnelli J, Boller B. Olfactory Identification in Subjective Cognitive Decline: A Meta-Analysis. J Alzheimers Dis. 2021;79(4):1497-1507. doi:10.3233/JAD-201022.
15. Wang Q, Chen B, Zhong X, et al. Olfactory Dysfunction Is Already Present with Subjective Cognitive Decline and Deepens with Disease Severity in the Alzheimer's Disease Spectrum. J Alzheimers Dis. 2021;79(2):585-595. doi:10.3233/JAD-201168.
16. Braak H, Braak E. Diagnostic criteria for neuropathologic assessment of Alzheimer's disease. Neurobiol Aging. 1997;18(4 Suppl):S85-S88. doi:10.1016/s0197-4580(97)00062-6.
17. Braak H, Del Tredici K. The preclinical phase of the pathological process underlying sporadic Alzheimer's disease. Brain. 2015;138(Pt 10):2814-2833. doi:10.1093/brain/awv236.
18. Chen B, Wang Q, Zhong X, et al. Structural and Functional Abnormalities of Olfactory-Related Regions in Subjective Cognitive Decline, Mild Cognitive Impairment, and Alzheimer's Disease. Int J Neuropsychopharmacol. 2022;25(5):361-374. doi:10.1093/ijnp/pyab091.
19. Wang J, Eslinger PJ, Doty RL, et al. Olfactory deficit detected by fMRI in early Alzheimer's disease. Brain Res. 2010;1357:184-194. doi:10.1016/j.brainres.2010.08.018.
20. Vasavada MM, Wang J, Eslinger PJ, et al. Olfactory cortex degeneration in Alzheimer's disease and mild cognitive impairment. J Alzheimers Dis. 2015;45(3):947-958. doi:10.3233/JAD-141947.
21. Förster S, Vaitl A, Teipel SJ, et al. Functional representation of olfactory impairment in early Alzheimer's disease. J Alzheimers Dis. 2010;22(2):581-591. doi:10.3233/JAD-2010-091549.
22. Doty RL. Psychophysical testing of smell and taste function. Handb Clin Neurol. 2019;164:229-246. doi:10.1016/B978-0-444-63855-7.00015-0.
23. Doty RL, Shaman P, Dann M. Development of the University of Pennsylvania Smell Identification Test: a standardized microencapsulated test of olfactory function. Physiol Behav. 1984;32(3):489-502. doi:10.1016/0031-9384(84)90269-5.

24. Kobal G, Hummel T, Sekinger B, Barz S, Roscher S, Wolf S. "Sniffin' sticks": screening of olfactory performance. Rhinology. 1996;34(4):222-226.
25. Wilson RS, Arnold SE, Tang Y, Bennett DA. Odor identification and decline in different cognitive domains in old age. Neuroepidemiology. 2006;26(2):61-67. doi:10.1159/000090250.
26. Schubert CR, Carmichael LL, Murphy C, Klein BE, Klein R, Cruickshanks KJ. Olfaction and the 5-year incidence of cognitive impairment in an epidemiological study of older adults. J Am Geriatr Soc. 2008;56(8):1517-1521. doi:10.1111/j.1532-5415.2008.01826.x.
27. Wilson RS, Schneider JA, Arnold SE, Tang Y, Boyle PA, Bennett DA. Olfactory identification and incidence of mild cognitive impairment in older age. Arch Gen Psychiatry. 2007;64(7):802-808. doi:10.1001/archpsyc.64.7.802.
28. Wheeler PL, Murphy C. Olfactory Measures as Predictors of Conversion to Mild Cognitive Impairment and Alzheimer's Disease. Brain Sci. 2021;11(11):1391. Published 2021 Oct 23. doi:10.3390/brainsci11111391.
29. Devanand DP, Michaels-Marston KS, Liu X, et al. Olfactory deficits in patients with mild cognitive impairment predict Alzheimer's disease at follow-up. Am J Psychiatry. 2000;157(9):1399-1405. doi:10.1176/appi.ajp.157.9.1399.
30. Tahmasebi R, Zehetmayer S, Pusswald G, Kovacs G, Stögmann E, Lehrner J. Identification of odors, faces, cities and naming of objects in patients with subjective cognitive decline, mild cognitive impairment and Alzheimer´s disease: a longitudinal study. Int Psychogeriatr. 2019;31(4):537-549. doi:10.1017/S1041610218001114.
31. Lojkowska W, Sawicka B, Gugala M, et al. Follow-up study of olfactory deficits, cognitive functions, and volume loss of medial temporal lobe structures in patients with mild cognitive impairment. Curr Alzheimer Res. 2011;8(6):689-698. doi:10.2174/156720511796717212.
32. Zhao A, Li Y, Yan Y, et al. Increased prediction value of biomarker combinations for the conversion of mild cognitive impairment to Alzheimer's dementia. Transl Neurodegener. 2020;9(1):30. Published 2020 Aug 3. doi:10.1186/s40035-020-00210-5.
33. Devanand DP, Lee S, Luchsinger JA, et al. Intact global cognitive and olfactory ability predicts lack of transition to dementia. Alzheimers Dement. 2020;16(2):326-334. doi:10.1016/j.jalz.2019.08.200.

34. Joseph T, Auger SD, Peress L, et al. Screening performance of abbreviated versions of the UPSIT smell test. J Neurol. 2019;266(8):1897-1906. doi:10.1007/s00415-019-09340-x.
35. Duff K, McCaffrey RJ, Solomon GS. The Pocket Smell Test: successfully discriminating probable Alzheimer's dementia from vascular dementia and major depression. J Neuropsychiatry Clin Neurosci. 2002;14(2):197-201. doi:10.1176/jnp.14.2.197.
36. Kjelvik G, Sando SB, Aasly J, Engedal KA, White LR. Use of the Brief Smell Identification Test for olfactory deficit in a Norwegian population with Alzheimer's disease. Int J Geriatr Psychiatry. 2007;22(10):1020-1024. doi:10.1002/gps.1783.
37. Woodward MR, Amrutkar CV, Shah HC, et al. Validation of olfactory deficit as a biomarker of Alzheimer disease. Neurol Clin Pract. 2017;7(1):5-14. doi:10.1212/CPJ.0000000000000293.
38. Tabert MH, Liu X, Doty RL, et al. A 10-item smell identification scale related to risk for Alzheimer's disease. Ann Neurol. 2005;58(1):155-160. doi:10.1002/ana.20533.
39. Woodward MR, Hafeez MU, Qi Q, et al. Odorant Item Specific Olfactory Identification Deficit May Differentiate Alzheimer Disease From Aging. Am J Geriatr Psychiatry. 2018;26(8):835-846. doi:10.1016/j.jagp.2018.02.008.
40. Mueller C, Renner B. A new procedure for the short screening of olfactory function using five items from the "Sniffin' Sticks" identification test kit. Am J Rhinol. 2006;20(1):113-116.
41. Hedner M, Larsson M, Arnold N, Zucco GM, Hummel T. Cognitive factors in odor detection, odor discrimination, and odor identification tasks. J Clin Exp Neuropsychol. 2010;32(10):1062-1067. doi:10.1080/13803391003683070.
42. Sohrabi HR, Bates KA, Weinborn MG, et al. Olfactory discrimination predicts cognitive decline among community-dwelling older adults. Transl Psychiatry. 2012;2(5):e118. Published 2012 May 22. doi:10.1038/tp.2012.43.
43. Kim HR, Kim SM, Seong W, et al. Cut-Off Scores of an Olfactory Function Test for Mild Cognitive Impairment and Dementia. Psychiatry Investig. 2020;17(10):1021-1030. doi:10.30773/pi.2020.0203.
44. Hsieh JW, Keller A, Wong M, Jiang RS, Vosshall LB. SMELL-S and SMELL-R: Olfactory tests not influenced by odor-specific insensitivity or prior olfactory experience. Proc Natl Acad Sci U S A. 2017;114(43):11275-11284. doi:10.1073/pnas.1711415114.

45. Larsson M, Nilsson LG, Olofsson JK, Nordin S. Demographic and cognitive predictors of cued odor identification: evidence from a population-based study. Chem Senses. 2004;29(6):547-554. doi:10.1093/chemse/bjh059.
46. Oberg C, Larsson M, Bäckman L. Differential sex effects in olfactory functioning: the role of verbal processing. J Int Neuropsychol Soc. 2002;8(5):691-698. doi:10.1017/s1355617702801424.
47. Cassano T, Romano A, Macheda T, et al. Olfactory memory is impaired in a triple transgenic model of Alzheimer disease. Behav Brain Res. 2011;224(2):408-412. doi:10.1016/j.bbr.2011.06.029.
48. Young JW, Sharkey J, Finlayson K. Progressive impairment in olfactory working memory in a mouse model of Mild Cognitive Impairment. Neurobiol Aging. 2009;30(9):1430-1443. doi:10.1016/j.neurobiolaging.2007.11.018.
49. Peng KY, Mathews PM, Levy E, Wilson DA. Apolipoprotein E4 causes early olfactory network abnormalities and short-term olfactory memory impairments. Neuroscience. 2017;343:364-371. doi:10.1016/j.neuroscience.2016.12.004.
50. Gilbert PE, Murphy C. Differences between recognition memory and remote memory for olfactory and visual stimuli in nondemented elderly individuals genetically at risk for Alzheimer's disease. Exp Gerontol. 2004;39(3):433-441. doi:10.1016/j.exger.2004.01.001.
51. Larsson M, Hedner M, Papenberg G, Seubert J, Bäckman L, Laukka EJ. Olfactory memory in the old and very old: relations to episodic and semantic memory and APOE genotype. Neurobiol Aging. 2016;38:118-126. doi:10.1016/j.neurobiolaging.2015.11.012.
52. Kapoulea EA, Murphy C. Older, non-demented apolipoprotein ε4 carrier males show hyperactivation and structural differences in odor memory regions: a blood-oxygen-level-dependent and structural magnetic resonance imaging study. Neurobiol Aging. 2020;93:25-34. doi:10.1016/j.neurobiolaging.2020.04.013.
53. Dhilla Albers A, Asafu-Adjei J, Delaney MK, et al. Episodic memory of odors stratifies Alzheimer biomarkers in normal elderly. Ann Neurol. 2016;80(6):846-857. doi:10.1002/ana.24792.
54. Gilbert PE, Barr PJ, Murphy C. Differences in olfactory and visual memory in patients with pathologically confirmed Alzheimer's disease and the Lewy body variant of Alzheimer's disease. J Int Neuropsychol Soc. 2004;10(6):835-842. doi:10.1017/s1355617704106024.
55. Bahar-Fuchs A, Moss S, Pike K, et al. P1-153: The relationship between olfactory identification and memory and Abeta burden in Alzheimer’s

disease, mild cognitive impairment and healthy aging: A PIB-PET study. Alzheimer's & Dementia. 2008;4:T251-T252. doi:https://doi.org/10.1016/j.jalz.2008.05.741.
56. Croy I, Zehner C, Larsson M, Zucco GM, Hummel T. Test-retest reliability and validity of the Sniffin' TOM odor memory test. Chem Senses. 2015;40(3):173-179. doi:10.1093/chemse/bju069.
57. Sorokowska A, Sabiniewicz A, Larsson M. TOM-32-An extended test for the assessment of olfactory memory. J Neurosci Methods. 2020;344:108873. doi:10.1016/j.jneumeth.2020.108873.
58. Nordin S, Murphy C. Odor memory in normal aging and Alzheimer's disease. Ann N Y Acad Sci. 1998;855:686-693. doi:10.1111/j.1749-6632.1998.tb10646.x.
59. Demencijų diagnostiniai kriterijai ir vertinimo skalės. Neurologijos seminarai 2000;2(10):18-38.
60. Hughes CP, Berg L, Danziger WL, Coben LA, Martin RL. A new clinical scale for the staging of dementia. Br J Psychiatry. 1982;140:566-572. doi:10.1192/bjp.140.6.566.
61. Rumeau C, Nguyen DT, Jankowski R. How to assess olfactory performance with the Sniffin' Sticks test(®). Eur Ann Otorhinolaryngol Head Neck Dis. 2016;133(3):203-206. doi:10.1016/j.anorl.2015.08.004.
62. Katotomichelakis M, Balatsouras D, Tripsianis G, Tsaroucha A, Homsioglou E, Danielides V. Normative values of olfactory function testing using the 'sniffin' sticks'. Laryngoscope. 2007;117(1):114-120. doi:10.1097/01.mlg.0000246518.79894.7e.
63. Hummel T, Kobal G, Gudziol H, Mackay-Sim A. Normative data for the "Sniffin' Sticks" including tests of odor identification, odor discrimination, and olfactory thresholds: an upgrade based on a group of more than 3,000 subjects. Eur Arch Otorhinolaryngol. 2007;264(3):237-243. doi:10.1007/s00405-006-0173-0.
64. Steinbach S, Hundt W, Vaitl A, et al. Taste in mild cognitive impairment and Alzheimer's disease. J Neurol. 2010;257(2):238-246. doi:10.1007/s00415-009-5300-6.
65. Quarmley M, Moberg PJ, Mechanic-Hamilton D, et al. Odor Identification Screening Improves Diagnostic Classification in Incipient Alzheimer's Disease. J Alzheimers Dis. 2017;55(4):1497-1507. doi:10.3233/JAD-160842.
66. Peters JM, Hummel T, Kratzsch T, Lötsch J, Skarke C, Frölich L. Olfactory function in mild cognitive impairment and Alzheimer's disease: an investigation using psychophysical and electrophysiological

techniques. Am J Psychiatry. 2003;160(11):1995-2002. doi:10.1176/appi.ajp.160.11.1995.
67. 2022 Alzheimer's disease facts and figures. Alzheimers Dement. 2022;18(4):700-789. doi:10.1002/alz.12638.
68. Velayudhan L, Gasper A, Pritchard M, Baillon S, Messer C, Proitsi P. Pattern of Smell Identification Impairment in Alzheimer's Disease. J Alzheimers Dis. 2015;46(2):381-387. doi:10.3233/JAD-142838.
69. Zendehbad AS, Noroozian M, Shakiba A, Kargar A, Davoudkhani M. Validation of Iranian Smell Identification Test for screening of mild cognitive impairment and Alzheimer's disease. Appl Neuropsychol Adult. 2022;29(1):77-82. doi:10.1080/23279095.2019.1710508.
70. Yoshitake M, Maeshima E, Maeshima S, et al. Olfactory identification ability in patients with mild cognitive impairment and Alzheimer's disease. J Phys Ther Sci. 2022;34(11):710-714. doi:10.1589/jpts.34.710.
71. Jimbo D, Inoue M, Taniguchi M, Urakami K. Specific feature of olfactory dysfunction with Alzheimer's disease inspected by the Odor Stick Identification Test. Psychogeriatrics. 2011;11(4):196-204. doi:10.1111/j.1479-8301.2011.00387.x.
72. Umeda-Kameyama Y, Ishii S, Kameyama M, et al. Heterogeneity of odorant identification impairment in patients with Alzheimer's Disease. Sci Rep. 2017;7(1):4798. Published 2017 Jul 6. doi:10.1038/s41598-017-05201-7.
73. Hori Y, Matsuda O, Ichikawa S. Olfactory function in elderly people and patients with Alzheimer's disease. Psychogeriatrics. 2015;15(3):179-185. doi:10.1111/psyg.12092.
74. Makowska I, Kloszewska I, Grabowska A, Szatkowska I, Rymarczyk K. Olfactory deficits in normal aging and Alzheimer's disease in the polish elderly population. Arch Clin Neuropsychol. 2011;26(3):270-279. doi:10.1093/arclin/acr011.
75. Solomon GS, Petrie WM, Hart JR, Brackin HB Jr. Olfactory dysfunction discriminates Alzheimer's dementia from major depression. J Neuropsychiatry Clin Neurosci. 1998;10(1):64-67. doi:10.1176/jnp.10.1.64.
76. McCaffrey RJ, Duff K, Solomon GS. Olfactory dysfunction discriminates probable Alzheimer's dementia from major depression: a cross-validation and extension. J Neuropsychiatry Clin Neurosci. 2000;12(1):29-33. doi:10.1176/jnp.12.1.29.
77. Zucco GM, Hummel T, Tomaiuolo F, Stevenson RJ. The influence of short-term memory on standard discrimination and cued identification

olfactory tasks. J Neurosci Methods. 2014;222:138-141. doi:10.1016/j.jneumeth.2013.11.006.
78. Tong MT, Peace ST, Cleland TA. Properties and mechanisms of olfactory learning and memory. Front Behav Neurosci. 2014;8:238. doi:10.3389/fnbeh.2014.00238.
79. Meunier D, Fonlupt P, Saive AL, Plailly J, Ravel N, Royet JP. Modular structure of functional networks in olfactory memory. Neuroimage. 2014;95:264-275. doi:10.1016/j.neuroimage.2014.03.041.
80. Cerf-Ducastel B, Murphy C. Neural substrates of cross-modal olfactory recognition memory: an fMRI study. Neuroimage. 2006;31(1):386-396. doi:10.1016/j.neuroimage.2005.11.009.

COPIES OF PUBLICATIONS

1st publication/ 1 publikacija

Properties of odor identification testing in screening for early-stage Alzheimer's disease

Audronyte E, Pakulaite-Kazliene G, Sutnikiene V, Kaubrys G

Sci Rep. 2023;13(1):6075

doi: 10.1038/s41598-023-32878-w

scientific reports

Check for updates

OPEN

Properties of odor identification testing in screening for early-stage Alzheimer's disease

Egle Audronyte✉, Gyte Pakulaite-Kazliene, Vaiva Sutnikiene & Gintaras Kaubrys

Odor identification (OI) is impaired in the early stages of Alzheimer's disease (AD). However, data regarding the diagnostic properties of OI tests are lacking, preventing their clinical use. We aimed to explore OI and determine the accuracy of OI testing in screening for patients with early AD. In total, 30 participants with mild cognitive impairment due to AD (MCI-AD), 30 with mild dementia due to AD (MD-AD), and 30 cognitively normal elderly participants (CN) were enrolled, and cognitive examination (CDR, MMSE, ADAS-Cog 13, and verbal fluency tests) and assessment of OI (Burghart Sniffin' Sticks odor identification test) were performed. MCI-AD patients scored significantly worse in OI than CN participants, and MD-AD patients had worse OI scores than MCI-AD patients. The ratio of OI to ADAS-Cog 13 score had good diagnostic accuracy in differentiating AD patients from CN participants and in differentiating MCI-AD patients from CN participants. Substitution of ADAS-Cog 13 score with the ratio of OI to ADAS-Cog 13 score in a multinomial regression model improved the classification accuracy, especially of MCI-AD cases. Our results confirmed that OI is impaired during the prodromal stage of AD. OI testing has a good diagnostic quality and can improve the accuracy of screening for early-stage AD.

Dementia is a leading cause of disability and dependency globally[1]. With its increasing prevalence, which is expected to reach 78 million cases by 2030, and consequently increasing socioeconomic impact, dementia is becoming a healthcare priority worldwide[1,2]. Despite this, most patients remain undiagnosed, with Alzheimer's Disease International (ADI) estimating 75% of undiagnosed dementia cases and the number potentially reaching 90% in some low- and middle-income countries[2]. Therefore, identifying affordable and widely accessible measures to achieve accurate diagnosis is increasingly important.

Alzheimer's disease (AD) is the most common cause of dementia and is estimated to cause 60–70% of all cases[3]. At present, biomarkers used for diagnosing AD require cerebrospinal fluid analysis or advanced neuroimaging techniques, as blood-based biomarkers are not yet accessible for use in clinical practice worldwide. These biomarkers measure brain amyloid-beta (Aβ) protein deposition and neuronal degeneration[4,5]. However, their wide use is limited because testing for them is invasive and expensive. To select patients who would benefit from these testing methods, identifying markers that would be easily accessible in community settings is necessary for the screening of wide populations.

These screening markers should not only reliably identify patients with AD but should also be able to do so in the early stages of the disease. In 2022, most medications used in clinical trials for AD were disease-modifying therapies (83.2%)[6]. These therapies are predominantly aimed at patients with early-stage AD, and as they enter clinical use, the need for accurate and affordable screening measures becomes more apparent.

Olfactory dysfunction in AD patients has been studied for nearly 50 years at this point[7]. Numerous studies have confirmed that it is a common symptom, present in up to 90% of patients with AD[8,9]. However, some uncertainties remain regarding its prevalence and magnitude during the early stages of the disease. Olfactory impairment has been found not only in patients with mild cognitive impairment (MCI)[10–12] but also in those with subjective cognitive decline (SCD)[13,14]. However, studies with MCI and SCD patients often use variable definitions of subtypes or do not subtype cognitive impairments at all[11,13]. This complicates the interpretation of the results with regard to AD, as it is likely that a heterogeneous mix of MCI and SCD patients with varying neurological conditions is being analyzed together[11].

Results from longitudinal studies support the early occurrence of olfactory dysfunction in patients with AD and suggest that olfactory testing could be used to predict future cognitive decline during follow-up. In longitudinal studies, olfactory impairment was found to be associated with an increased risk of MCI in healthy

Clinic of Neurology and Neurosurgery, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Vilnius, Lithuania. ✉email: egle.audronyte@santa.lt

individuals[15–18] as well as with an increased risk of conversion to dementia in patients with MCI[19–22]. In contrast, intact olfactory abilities were found to be reliable in identifying individuals who rarely transition to dementia in the future[23]. However, longitudinal studies often experience the same limitation of inconsistent subtyping of cognitive impairments. In many instances, MCI has not been typed, whereas in some studies, the cause of dementia is not specified[16,21]. Nevertheless, longitudinal studies confirm the rationale for further research on olfactory testing as a screening marker of AD.

The pathological evidence supports these clinical findings. Structures involved in the processing of olfactory information (especially entorhinal and transentorhinal areas) are affected by AD pathology early in the course of the disease[24,25]. Functional magnetic resonance imaging (fMRI) and [18F]-fluorodeoxyglucose PET (FDG-PET) also confirmed structural and functional abnormalities of olfaction-related regions in AD patients as early as SCD[26–29].

Thus, previous studies indicate that olfactory testing is a promising method for improving the accuracy of screening for early-stage AD and could be introduced into clinical practice if more data are obtained using generally accepted AD diagnostic criteria and standardized assessment methods.

In the current study, we aimed to analyze odor identification in patients with early-stage AD and explore its diagnostic qualities as a screening measure. We hypothesized that odor identification is impaired in the early stages of the disease and can be reliably used to differentiate patients with AD from cognitively normal participants, even in the prodromal stage of AD.

Results

Demographic and clinical characteristics. Cognitively normal elderly participants (CN), patients with mild cognitive impairment due to AD (MCI-AD), and patients with mild dementia due to AD (MD-AD) did not differ according to education, depressive symptoms (Geriatric Depression Scale results), or Hachinski Ischemic Score (Kruskal–Wallis $p > 0.05$). In addition, none of the three groups differed according to sex (chi-square test, $p > 0.05$) (Table 1).

The ages of participants in the MCI-AD and CN groups were similar (median age = 72 years, age range = 60–84 years for MCI-AD; median age = 74 years, age range = 63–89 years for CN). Participants in the MD-AD group were significantly older (median age = 78 years, age range = 65–85 years) (Kruskal–Wallis $p < 0.05$; post hoc analysis revealed significant differences between the CN and MD-AD groups; MCI-AD and MD-AD groups, and no significant difference between the CN and MCI-AD groups).

The demographic and clinical characteristics of the participants are presented in Table 1.

The results of the cognitive tests were significantly different among all three groups, as expected. Median MMSE score was 29 (range = 28–30) in CN, 26 (range = 24–27) in MCI-AD and 22 (range = 21–24) in MD-AD groups. Median ADAS-Cog score was 10.83 (range = 3.33–16.0) in CN, 20.84 (range = 12.67–33.0) in MCI-AD and 29.34 (range = 16.0–39.0) in MD-AD groups. Median CDR Sum of Boxes was 0 (range = 0–0) in CN, 2 (range = 1–3.5) in MCI-AD and 5 (range = 4–6) in MD-AD groups. Median fluency PAS score was 37 (range = 20–57) in CN, 28.5 (range = 14–56) in MCI-AD and 21 (range = 8–44) in MD-AD groups. Median fluency animals score was 20 (range = 11–36) in CN, 13 (range = 5–19) in MCI-AD and 10 (range = 3–19) in MD-AD groups. In all cases, Kruskal–Wallis $p < 0.05$, and post hoc analysis revealed significant differences among all three groups. The cognitive assessment results of the participants are presented in Table 2.

Odor identification. One-way ANOVA revealed significant differences in odor identification scores among all three groups (mean and standard deviation: CN, 12.77 ± 1.43; MCI-AD, 9.3 ± 2.23; MD-AD, 7.0 ± 2.13; $p < 0.001$, the post-hoc analysis revealed significant differences among all three groups). The odor identification scores are presented in Fig. 1.

In the sample of all participants, odor identification scores were strongly correlated with the results of the cognitive tests: mini-mental state examination (MMSE) (Spearman's rho: 0.735; $p < 0.001$), Alzheimer's Disease Assessment Scale-Cognitive Subscale, version 13 (ADAS-Cog 13) (Spearman's rho: − 0.771; $p < 0.001$), Clinical Dementia Rating (CDR) Sum of Boxes (Spearman's rho: − 0.775; $p < 0.001$), and verbal fluency tests combined results (Spearman's rho: 0.720; $p < 0.001$).

Upon analysis of the separate groups, no significant correlations were found between odor identification scores and cognitive test results in the MD-AD group. In the MCI-AD group, odor identification scores correlated with verbal fluency tests combined results (VFT) (Spearman's rho: 0.627; $p < 0.001$). In the CN group, there were

	CN (N = 30)	MCI-AD (N = 30)	MD-AD (N = 30)
Male (%) *	13 (43.3%)	13 (43.3%)	12 (40.0%)
Years of education *	15 (3)	16 (2)	16 (3)
Age **	74 (7)	72 (10)	78 (4)
GDS*	5.5 (2)	5.5 (2)	5 (2)
HIS*	1 (1)	1 (1)	1 (0)

Table 1. Demographic and clinical characteristics of the participants. GDS, Geriatric depression scale; HIS, Hachinski Ischemic Score. Data are represented as median and interquartile range unless specified otherwise. *The groups did not differ significantly. **MD-AD group differed significantly from CN and MCI groups. The CN and MCI groups did not show any significant differences.

	CN (N = 30)	MCI-AD (N = 30)	MD-AD (N = 30)
MMSE *	29 (1)	26 (1)	22 (2)
ADAS-Cog 13 *	10.83 (4.58)	20.84 (4.42)	29.34 (5.66)
CDR Sum of Boxes *	0 (0)	2 (1)	5 (1)
Fluency PAS *	37 (13)	28.5 (9)	21 (13)
Fluency animals *	20 (7)	13 (6)	10 (5)

Table 2. Cognitive assessment results of the participants. MMSE, Mini-mental state examination; ADAS-Cog 13, Alzheimer's Disease Assessment Scale-Cognitive Subscale; CDR, Clinical Dementia Rating. Data are represented as median and interquartile range. *All three groups differed significantly.

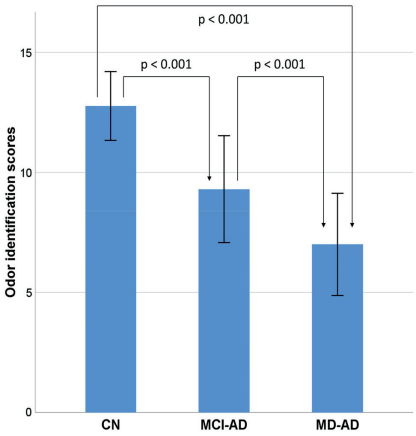


Figure 1. Odor identification scores in three groups of participants. Bars represent mean values, and error bars represent standard deviations.

significant, although weak, correlations between odor identification scores and ADAS-Cog 13 scores (Spearman's rho: −0.386; p = 0.035), as well as verbal fluency tests combined results (Spearman's rho: 0.479; p = 0.007).

The relationship between odor identification scores and the ADAS-Cog 13 results is shown in Fig. 2. The relationships between odor identification scores and MMSE, CDR Sum of Boxes and VFT are shown in Supplementary Fig. S1, S2 and S3, respectively (online).

There was a significant, although weak, correlation between odor identification scores and age when the results of all participants were analyzed (Spearman's rho: −0.334; p = 0.001). The correlation between odor identification and age remained significant in the CN group (Spearman's rho: −0.365; p = 0.047) but not in the MCI-AD and MD-AD groups when analyzed separately. The relationship between odor identification scores and age is shown in Supplementary Fig. S4 (online).

Multiple linear regression models with age, sex, education, and cognitive test scores (MMSE, ADAS-Cog-13, CDR Sum of Boxes, and composite verbal fluency test score [VFT = fluency PAS + fluency animals]) as independent variables were tested to determine whether they significantly predicted odor identification scores.

The overall regression was statistically significant in all four models: model with MMSE ($R^2 = 0.525$, $F = 23.452$, $p < 0.001$), model with ADAS-Cog 13 ($R^2 = 0.560$, $F = 27.079$, $p < 0.001$), model with CDR Sum of Boxes ($R^2 = 0.569$, $F = 28.068$, $p < 0.001$), and model with VFT ($R^2 = 0.464$, $F = 18.403$, $p < 0.001$).

However, only cognitive test scores significantly predicted odor identification scores in each case (MMSE: $\beta = 0.702$, $p < 0.001$; ADAS-Cog 13: $\beta = -0.735$, $p < 0.001$; CDR Sum of Boxes: $\beta = -0.735$, $p < 0.001$; VFT: $\beta = 0.719$; $p < 0.001$). None of the other predictors (age, sex, and education) significantly predicted odor identification scores in any of the models ($p > 0.05$).

Diagnostic characteristics of odor identification. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the performance of the odor identification score in differentiating the CN group from AD (MCI-AD or MD-AD), MCI-AD, and MD-AD patients and MCI-AD patients from MD-AD patients. The ROC curves with areas under the curve (AUC) are shown in Supplementary Fig. S5 (online).

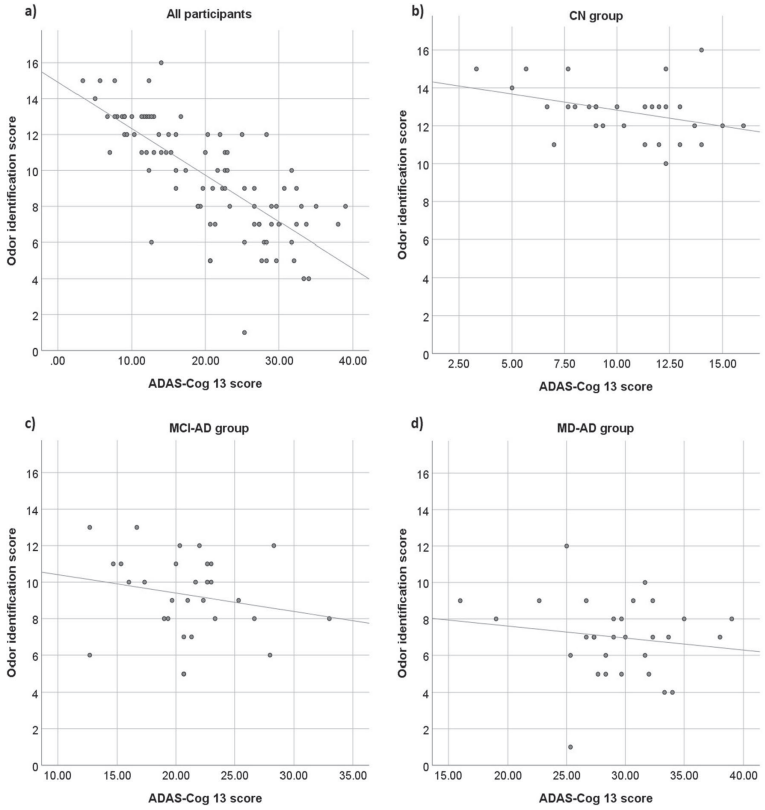


Figure 2. Relationship between odor identification scores and ADAS-Cog 13 results.

A cut-off score of ≤ 11 correct responses indicating AD was chosen.

Using this cut-off score for differentiating AD patients (MCI-AD or MD-AD) from CN participants, odor identification had sensitivity and specificity of 90% (95% CI: 79.49–96.24%) and 80% (95% CI: 61.43–92.29%), respectively. In addition, the negative and positive predictive values were 80% (95% CI: 64.71–89.72%) and 90% (95% CI: 81.41–94.87%), respectively. The overall diagnostic accuracy was 86.67% (95% CI: 77.87–92.92%).

The diagnostic characteristics remained good when differentiating MCI-AD patients from CN participants. Using the same cut-off score of ≤ 11, odor identification had a sensitivity and specificity of 83.33% (95% CI 65.28–94.36%) and 80% (95% CI: 61.43–92.29%), respectively. In addition, the negative and positive predictive values were 82.76% (95% CI: 67.89–91.59%) and 80.65% (95% CI: 66.68–89.66%), respectively. The overall diagnostic accuracy was 81.67% (95% CI: 69.56–90.48%).

The ratio of odor identification scores to ADAS-Cog 13 scores was calculated. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the performance of this ratio in differentiating the CN group from AD (MCI-AD or MD-AD), MCI-AD, and MD-AD patients and MCI-AD patients from MD-AD patients. The ROC curves with areas under the curve (AUC) are shown in Fig. 3.

A cut-off score of ≤ 0.75 indicating AD was chosen.

Using this cut-off score for differentiating AD patients (MCI-AD or MD-AD) from CN participants, ratio of odor identification score to ADAS-Cog 13 score had sensitivity and specificity of 96.67% (95% CI: 88.47 – 99.59%) and 96.67% (95% CI: 82.78– 99.92%), respectively. In addition, the negative and positive predictive values were 93.55% (95% CI: 78.75–98.27%) and 98.31% (95% CI: 89.41–99.75%), respectively. The overall diagnostic accuracy was 96.67% (95% CI: 90.57–99.31%).

The diagnostic characteristics remained good when differentiating MCI-AD patients from CN participants. Using the same cut-off score of ≤ 0.75, ratio of odor identification score to ADAS-Cog 13 score had a sensitivity

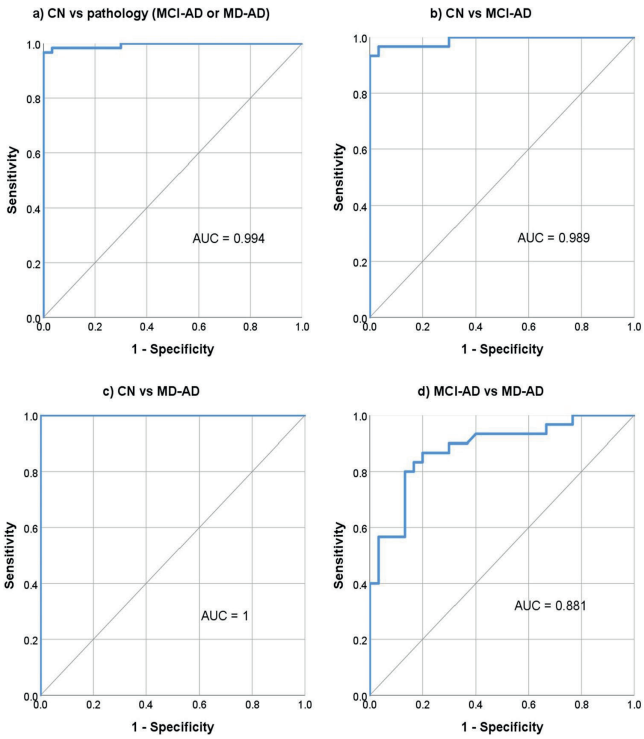


Figure 3. Performance of odor identification to ADAS-Cog 13 score ratio in differentiating between groups of participants.

and specificity of 93.33% (95% CI 77.93–99.18%) and 96.67% (95% CI: 82.78–99.92%), respectively. In addition, the negative and positive predictive values were 93.55% (95% CI: 79.14–98.23%) and 96.55% (95% CI: 80.26–99.48%), respectively. The overall diagnostic accuracy was 95% (95% CI: 86.08–98.96%).

Multinomial logistic regression was performed to analyze the relationship between predictor variables and membership in the three groups (CN, MCI-AD, and MD-AD).

First, a model using age, education, sex, and ADAS-Cog 13 scores as predictor variables was tested. The fit between the model containing only the intercept and the data improved with the addition of predictor variables ($X^2 = 139.656$, $p < 0.001$; Nagelkerke $R^2 = 0.887$). Pearson's X^2 and Deviance X^2 tests indicated that the model exhibited a good fit for the data ($p > 0.05$). The overall percentage of correctly classified cases using this model was 82.2% (93.3% CN, 70% MCI-AD, and 83.3% MD-AD cases correctly classified), with the ADAS-Cog 13 score as the strongest and most significant predictor ($X^2 = 122.652$, $p < 0.001$).

In the second model, ADAS-Cog 13 scores were replaced with the ratio of odor identification score to ADAS-Cog 13 score. The model with age, education, sex, and the ratio of odor identification score to ADAS-Cog 13 score as predictor variables also showed a significant improvement in fit over a null model ($X^2 = 139.767$, $p < 0.001$; Nagelkerke $R^2 = 0.887$). Pearson's X^2 and Deviance X^2 tests indicated that the model exhibited a good fit for the data ($p > 0.05$). The overall percentage of correctly classified cases using this model was 87.8% (96.7% CN, 83.3% MCI-AD, and 83.3% MD-AD cases correctly classified), with the ratio of odor identification score to ADAS-Cog 13 score as the strongest and most significant predictor ($X^2 = 122.763$; $p < 0.001$).

Discussion

In the current study, we found that odor identification was significantly impaired in the prodromal stage of AD (MCI-AD patients), and the impairment was even more severe in the later stages of the disease (mild dementia patients). This confirms the findings of previous studies, where olfactory impairment was also found to be present in the earliest stages of AD and further worsened during disease progression[10,14].

We compared our results with normative data and results from previous studies on AD patients, in which the Sniffin' Sticks odor identification test was used. The performance of healthy elderly participants varies from 12.06 ± 2.31 to 13.0 ± 0,92 according to normative data[30,31]. In addition, the performance of patients with MCI varied from 9.3 ± 4.0 to 10.2 ± 2.5 and that of patients with AD varied from 6.7 ± 2.3 to 7.8 ± 3.4 in various studies[14,32,33]. Therefore, our results were within the range of previous results and normative data. Collectively, the results from the current study and previous research suggest that impairment of odor identification is fairly consistent across various samples of patients with AD. Therefore, odor identification testing can be applied to various populations. Interestingly, odor identification is influenced by a patient's personal and cultural experiences and familiarity with different odors[34]. This makes it necessary to adapt odor identification tests to different populations[35–37]. However, when comparing results from studies conducted in Germany, China, the United States of America, and Lithuania (the current study), the results were rather similar, even though all of them used the standard Sniffin' sticks odor identification set[14,32,33].

Odor identification scores were strongly and significantly correlated with the results of the cognitive assessment. Linear regression analysis further demonstrated a significant relationship between these variables. However, age, sex, and education did not significantly predict odor identification scores in the linear regression models. These findings are in accordance with structural and functional changes in the olfactory system, which have been demonstrated in previous studies, and further prove that olfactory impairment is associated with processes of AD itself and cannot be explained by other factors that have been proven to influence olfaction in the general population, such as age and sex[24–29]. As olfactory and memory systems are known to significantly overlap anatomically and both are affected by cholinergic deficit, present in AD, the relationship between olfactory and cognitive impairment might be related pathogenetically. However, further studies are needed on this subject.

Odor identification demonstrated excellent characteristics for the differentiation of AD patients from healthy controls (AUC = 0.949) and for the differentiation of prodromal AD (MCI-AD) patients from healthy controls (AUC = 0.908). However, the results were not as good when differentiating between the different stages of AD (MCI-AD vs. MD-AD, AUC = 0.773). Similar results were found in previous studies, where odor identification also had better qualities in differentiating healthy participants from patients with AD than in differentiating between different stages of AD[33]. This suggests that changes in odor identification occur early in the course of the disease and are pronounced even in the prodromal stage of AD, thus making odor identification testing very suitable for screening for early AD and less reliable for monitoring disease progression.

However, diagnosing early AD is the most challenging task in clinical practice, especially in primary care settings. More than half (51%) of primary care physicians surveyed by the Alzheimer's Association said they were uncomfortable diagnosing MCI due to AD[38]. Currently available biomarkers are not able to solve this issue, as they are not easily accessible in community settings. Lack of specialists and facilities to perform diagnostic testing was the most commonly cited challenge by primary care physicians in the United States of America, when diagnosing mild cognitive impairment (MCI) due to AD[38]. Objective olfactory testing may be very useful for improving diagnostic certainty of screening for early-stage AD. In particular, 72% of primary care physicians stated that they find it challenging to differentiate MCI from normal aging[38]. As odor identification proved to have excellent characteristics in differentiating MCI-AD patients from healthy controls, it could be very helpful with this task.

The ratio of odor identification score to ADAS-Cog 13 score had even better characteristics for the differentiation of AD patients from healthy controls (AUC = 0.994) and for the differentiation of prodromal AD (MCI-AD) patients from healthy controls (AUC = 0.989). Using a combined measure of odor and cognitive testing would be very useful, as that could help to not only improve the detection of early-stage AD patients, but also differentiate them from patients with other disorders known to affect olfaction, such as Parkinson's disease[39]. However, further studies are needed to confirm that.

Substitution of ADAS-Cog 13 score with a ratio of odor identification score to ADAS-Cog 13 score in a multinomial logistic regression model containing demographic and cognitive data as predictive variables improved the overall classification accuracy from 82.2% to 87.8%. Correct classification of MCI-AD cases improved the most (from 70% to 83.3%), once again confirming the additional value of odor identification testing in screening for AD at the early stages.

The present study has several limitations. First, CSF and PET biomarkers were not used in this study. Studies involving these factors would help to explore the relationship between olfactory impairment and brain amyloid-beta (Aβ) deposition and neuronal degeneration markers. Also, CSF and PET biomarkers would help in excluding mild cognitive impairment due to dementia with Lewy bodies, which is difficult to differentiate from MCI due to AD with cognitive and olfactory testing alone, according to studies[40]. No participants in the current study had evidence of Parkinsonism, prominent visual hallucinations, or rapid eye movement sleep abnormalities, as required by criteria for probable AD and MCI due to AD by NIA-AA[4,5]. However, CSF and PET biomarkers would help in confirming the diagnosis. Second, the sample size was sufficient to prove significant changes; however, studies involving larger sample sizes would be useful in confirming these findings. Finally, the cross-sectional design of the study did not allow precise conclusions to be drawn regarding the progression of changes during the course of AD. Even though the results of the current study are encouraging, longitudinal studies are needed in order to confirm the value of odor identification testing in screening for AD.

Conclusion

In conclusion, our findings indicate that odor identification is impaired in the prodromal stage of AD and that these changes progress during the course of the disease. Odor identification testing demonstrates good diagnostic qualities and can improve the accuracy of screening for early-stage AD, serving as a reliable, noninvasive, and affordable marker.

Methods

Participants. Ninety participants were enrolled in the study: 30 CN participants, 30 patients with MCI-AD, and 30 patients with MD-AD. Cognitively normal participants had no cognitive complaints and a CDR score of 0. MCI-AD patients met the National Institute on Aging-Alzheimer's Association (NIA/AA) criteria for MCI-AD (Albert et al., 2011[4]) and had a CDR score of 0.5. Furthermore, patients with MD-AD met NIA/AA criteria for probable AD (McKhann et al., 2011[5]) and had a CDR score of 1.

Participants were excluded from the study if they had any central nervous system (CNS) disorder other than MCI-AD or MD-AD. Other exclusion criteria based on possible effects on cognitive functioning were cerebrovascular disorders (Hachinski Ischemic Score ≥ 4), severe head trauma, psychosis, depression (Geriatric Depression Scale > 9), psychoactive medications, and substance abuse. Participants were also excluded from the study if they had conditions potentially affecting olfactory function, such as nasal surgery, significant exposure to volatile substances, recent viral infections, or smoking.

The study was approved by the Vilnius Regional Bioethics Committee (approval number 2021/6–1355-830), and all the experiments were performed in accordance with the Declaration of Helsinki. In addition, written informed consent was obtained from all participants before the study.

Assessments of cognitive function. Cognitive and functional performance was evaluated using the CDR scale. MMSE was performed to evaluate global cognition. A more detailed evaluation of cognitive functioning was performed using the ADAS-Cog (scores ranging from 0–70) with additional delayed recall and number cancellation tasks (ADAS-Cog 13, scores ranging from 0–85). The delayed recall was scored from 0–10 (the number of words not recalled). The number cancellation task was scored from 0 to 5 (0 representing the best [≥ 30 correct responses] and 5 representing the worst [0–5 correct responses] performance). Verbal fluency was also tested (PAS and animal naming tasks).

Assessment of odor identification. The Sniffin' Sticks odor identification test was performed (Burghart®, Wedel, Germany). The Sniffin' Sticks odor identification test consists of 16 odors presented in felt-tip pens. The odors are orange, leather, cinnamon, peppermint, banana, lemon, liquorice, turpentine, garlic, coffee, apple, clove, pineapple, rose, anise and fish. Each odor was presented only once, for 3–4 s. The time interval between odors was 30 s. Participants were asked to select one of the four items from the answering card that best described the odor even if they were uncertain. The odor identification score is the number of correct responses out of sixteen. The examiner used odorless gloves, and the participants were instructed not to eat or drink anything at least 15 min prior to testing, as per the test instructions.

Data analysis. Statistical analysis was performed using IBM SPSS Statistics version 26.0. The normality of data distribution was tested using the Shapiro–Wilk test. A two-tailed chi-square test (for categorical variables) and Kruskal–Wallis or one-way analysis of variance (ANOVA) tests (for numerical variables) were used to analyze differences between groups. The correlations between variables were analyzed using Spearman rank correlation coefficients. Linear regression models were created to analyze the predictions of continuous variables. Categorical variables were predicted using multinomial logistic regression. ROC curve analysis was performed to evaluate the accuracy of the diagnostic tests. Statistical significance was set at a p-value of < 0.05.

Data availability

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Received: 23 January 2023; Accepted: 4 April 2023
Published online: 13 April 2023

References

1. Global Status Report on the Public Health Response to Dementia (World Health Organization, Geneva, 2021). Licence: CC BY-NC-SA 3.0 IGO.
2. Gauthier, S., Rosa-Neto, P., Morais, J. A. & Webster, C. World Alzheimer Report 2021: Journey through the Diagnosis of Dementia (Alzheimer's Disease Int., London, England, 2021).
3. *Global Action Plan on the Public Health Response to Dementia 2017–2025* (World Health Organization, Geneva, 2017). Licence: CC BY-NC-SA 3.0 IGO.
4. Albert, M. S. *et al.* The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* **7**, 270–279 (2011).
5. McKhann, G. M. *et al.* The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* **7**, 263–269 (2011).
6. Cummings, J. *et al.* Alzheimer's disease drug development pipeline: 2022. *Alzheimers Dement. (N Y)* **8**, e12295 (2022).
7. Waldton, S. Clinical observations of impaired cranial nerve function in senile dementia. *Acta Psychiatr. Scand.* **50**, 539–547 (1974).

8. Doty, R. L., Reyes, P. F. & Gregor, T. Presence of both odor identification and detection deficits in Alzheimer's disease. *Brain Res. Bull.* **18**, 597–600, ISSN 0361–9230 (1987).
9. Marine, N. & Boriana, A. Olfactory markers of depression and Alzheimer's disease. *Neurosci. Biobehav. Rev.* **45**, 262–270 (2014).
10. Jung, H. J., Shin, I. S. & Lee, J. E. Olfactory function in mild cognitive impairment and Alzheimer's disease: A meta-analysis. *Laryngoscope* **129**, 362–369 (2019).
11. Roalf, D. R. *et al.* A quantitative meta-analysis of olfactory dysfunction in mild cognitive impairment. *J. Neurol. Neurosurg. Psychiatry* **88**, 226–232 (2017).
12. Djordjevic, J., Jones-Gotman, M., De Sousa, K. & Chertkow, H. Olfaction in patients with mild cognitive impairment and Alzheimer's disease. *Neurobiol. Aging* **29**, 693–706 (2008).
13. Jobin, B., Zahal, R., Bussières, E. L., Frasnelli, J. & Boller, B. Olfactory identification in subjective cognitive decline: A meta-analysis. *J. Alzheimers Dis.* **79**, 1497–1507 (2021).
14. Wang, Q. *et al.* Olfactory dysfunction is already present with subjective cognitive decline and deepens with disease severity in the Alzheimer's disease spectrum. *J. Alzheimers Dis.* **79**, 585–595 (2021).
15. Wilson, R. S., Arnold, S. E., Tang, Y. & Bennett, D. A. Odor identification and decline in different cognitive domains in old age. *Neuroepidemiology* **26**, 61–67 (2006).
16. Schubert, C. R. *et al.* Olfaction and the 5-year incidence of cognitive impairment in an epidemiological study of older adults. *J. Am. Geriatr. Soc.* **56**, 1517–1521 (2008).
17. Wilson, R. S. *et al.* Olfactory identification and incidence of mild cognitive impairment in older age. *Arch. Gen. Psychiatry* **64**, 802–808 (2007).
18. Wheeler, P. L. & Murphy, C. Olfactory measures as predictors of conversion to mild cognitive impairment and Alzheimer's disease. *Brain Sci.* **11**, 1391 (2021).
19. Devanand, D. P. *et al.* Olfactory deficits in patients with mild cognitive impairment predict Alzheimer's disease at follow-up. *Am. J. Psychiatry* **157**, 1399–1405 (2000).
20. Tahmasebi, R. *et al.* Identification of odors, faces, cities and naming of objects in patients with subjective cognitive decline, mild cognitive impairment and Alzheimer´s disease: A longitudinal study. *Int. Psychogeriatr.* **31**, 537–549 (2019).
21. Lojkowska, W. *et al.* Follow-up study of olfactory deficits, cognitive functions, and volume loss of medial temporal lobe structures in patients with mild cognitive impairment. *Curr. Alzheimer Res.* **8**, 689–698 (2011).
22. Zhao, A. *et al.* Increased prediction value of biomarker combinations for the conversion of mild cognitive impairment to Alzheimer's dementia. *Transl. Neurodegener.* **9**, 30 (2020).
23. Devanand, D. P. *et al.* Intact global cognitive and olfactory ability predicts lack of transition to dementia. *Alzheimers Dement.* **16**, 326–334 (2020).
24. Braak, H. & Braak, E. Diagnostic criteria for neuropathologic assessment of Alzheimer's disease. *Neurobiol. Aging* **18**(4), S85–S88 (1997).
25. Braak, H. & Del Tredici, K. The preclinical phase of the pathological process underlying sporadic Alzheimer's disease. *Brain* **138**, 2814–2833 (2015).
26. Chen, B. *et al.* Structural and functional abnormalities of olfactory-related regions in subjective cognitive decline, mild cognitive impairment, and Alzheimer's disease. *Int. J. Neuropsychopharmacol.* **25**, 361–374 (2022).
27. Wang, J. *et al.* Olfactory deficit detected by fMRI in early Alzheimer's disease. *Brain Res.* **1357**, 184–194 (2010).
28. Vasavada, M. M. *et al.* Olfactory cortex degeneration in Alzheimer's disease and mild cognitive impairment. *J. Alzheimers Dis.* **45**, 947–958 (2015).
29. Förster, S. *et al.* Functional representation of olfactory impairment in early Alzheimer's disease. *J. Alzheimers Dis.* **22**, 581–591 (2010).
30. Katotomichelakis, M. *et al.* Normative values of olfactory function testing using the "sniffin" sticks'. *Laryngoscope* **117**, 114–120 (2007).
31. Hummel, T., Kobal, G., Gudziol, H. & Mackay-Sim, A. Normative data for the "Sniffin'Sticks" including tests of odor identification, odor discrimination, and olfactory thresholds: an upgrade based on a group of more than 3,000 subjects. *Eur. Arch. Otorhinolaryngol.* **264**, 237–243 (2007).
32. Steinbach, S. *et al.* Taste in mild cognitive impairment and Alzheimer's disease. *J. Neurol.* **257**, 238–246 (2010).
33. Quarmley, M. *et al.* Odor identification screening improves diagnostic classification in incipient Alzheimer's disease. *J. Alzheimers Dis.* **55**, 1497–1507 (2017).
34. Hsieh, J. W., Keller, A., Wong, M., Jiang, R. S. & Vosshall, L. B. SMELL-S and SMELL-R: Olfactory tests not influenced by odor-specific insensitivity or prior olfactory experience. *Proc. Natl Acad. Sci. U. S. A.* **114**, 11275–11284 (2017).
35. Ribeiro, J. C. *et al.* Cultural adaptation of the Portuguese version of the "Sniffin'Sticks" smell test: Reliability, validity, and normative data. *PLoS ONE* **11**, e0148937 (2016).
36. Sai-Guan, L., Husain, S., Zahedi, F. D., Ahmad, N. & Gendeh, B. S. Cultural adaptation of Sniffin' sticks smell identification test: The Malaysian version. *Iran. J. Otorhinolaryngol.* **32**, 213–222 (2020).
37. Balungwe, P. *et al.* Adaptation of the Sniffin' sticks test in south-kivu. *Eur. Ann. Otorhinolaryngol. Head Neck Dis.* **137**, 467–471 (2020).
38. Alzheimer's disease facts and figures. *Alzheimers Dement.* **18**, 700–789 (2022).
39. Ubeda-Bañon, I. *et al.* The human olfactory system in two proteinopathies: Alzheimer's and Parkinson's diseases. *Transl. Neurodegener.* **9**, 22 (2020).
40. Thomas, A. J. *et al.* Olfactory impairment in mild cognitive impairment with Lewy bodies and Alzheimer's disease. *Int. Psychogeriatr.* **34**, 585–592 (2022).

Acknowledgements
The authors would like to thank all the participants who participated in this study.

Author contributions
E.A. and G.K. were responsible for the conception and design of the study. E.A., V.S., and G.P.-K. collected the data. E.A., V.S., and G.P.-K. analyzed the data. E.A. and G.K. wrote the manuscript. V.S. and G.P.-K. revised the manuscript. All the authors approved the final manuscript.

Funding
The authors have no funding to report.

Competing interests
The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-023-32878-w.

Correspondence and requests for materials should be addressed to E.A.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

2nd publication/ 2 publikacija

Odor Discrimination as a Marker of Early Alzheimer's Disease

Audronyte E, Pakulaite-Kazliene G, Sutnikiene V, Kaubrys G

J Alzheimers Dis. 2023;94(3):1169-1178

doi: 10.3233/JAD-230077

Journal of Alzheimer's Disease 94 (2023) 1169–1178
DOI 10.3233/JAD-230077
IOS Press

Odor Discrimination as a Marker of Early Alzheimer's Disease

Egle Audronyte*, Gyte Pakulaite-Kazliene, Vaiva Sutnikiene and Gintaras Kaubrys
Clinic of Neurology and Neurosurgery, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Vilnius, Lithuania

Handling Associate Editor: Martin Vyhnalek

Accepted 25 May 2023
Pre-press 23 June 2023

Abstract.
Background: Olfactory dysfunction is an early symptom of Alzheimer's disease (AD). However, olfactory tests are rarely performed in clinical practice because their diagnostic efficacy in detecting early AD is unclear.
Objective: To investigate odor discrimination in patients with early AD and the efficacy of olfactory discrimination tests in differentiating these patients from subjects with normal cognition (CN).
Methods: Thirty patients each with mild dementia due to AD (MD-AD) and mild cognitive impairment due to AD (MCI-AD) and 30 older subjects with CN were enrolled. All participants underwent cognitive examinations (CDR, MMSE, ADAS-Cog 13, and verbal fluency) and odor discrimination tests (Sniffin' Sticks test, Burghart®, Germany).
Results: The MD-AD group achieved significantly worse scores on the olfactory discrimination test than the MCI-AD group, and the MCI-AD group achieved significantly worse results than the CN group ($p < 0.05$). A cut-off score of ≤ 10 had a diagnostic accuracy of 94.44% (95%CI, 87.51–98.17%) in differentiating patients with MCI-AD/MD-AD from subjects with CN and of 91.67% (95%CI, 81.61–97.24%) in differentiating those with MCI-AD from subjects with CN. Our multinomial logistic regression model with demographic data and ADAS-Cog 13 scores as predictor variables correctly classified 82.2% of the cases (CN, 93.3%; MC-AD, 70%; MD-AD, 83.3%); on adding the olfactory discrimination score to the model, the percentage increased to 92.2% (CN, 96.7%; MCI-AD, 86.7%; MD-AD, 93.3%).
Conclusion: Odor discrimination is impaired in cases of early AD and continues to deteriorate as the disease progresses. The olfactory discrimination test showed good diagnostic efficacy in detecting early AD.

Keywords: Alzheimer's disease, mild cognitive impairment, olfaction, olfactory impairment

INTRODUCTION

In 2019, more than 55 million persons were estimated to have dementia worldwide [1]. However, most patients remain undiagnosed. According to the estimates of Alzheimer's Disease International, up to 75% of the patients worldwide and up to 90% in low- and middle-income countries are not diagnosed [2]. The prevalence of dementia is continuously increasing and is estimated to reach 139 million cases by 2050 [1, 2]. Considering the present difficulties with accurate diagnostics and the increasing prevalence of dementia, identifying affordable and widely accessible diagnostic markers is of major importance.

Alzheimer's disease (AD) is the most common cause of dementia, accounting for up to 70% of all dementia cases [3]. Currently used biomarkers for diagnosing AD include biomarkers of brain amyloid-β (Aβ) protein deposition and of downstream neuronal degeneration or injury [4, 5]. However, these investigations require lumbar puncture (for cere-

*Correspondence to: Egle Audronyte, Clinic of Neurology and Neurosurgery, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Santariskiu str. 2, Vilnius, Lithuania. Tel.: +370 688 62753; E-mail: egle.audronyte@santa.lt.

brospinal fluid analysis) or advanced neuroimaging techniques (positron emission tomography [PET] or structural magnetic resonance imaging [MRI]). Thus, the routine use of these biomarkers is limited because of the cost and invasive nature of the tests.

It is also becoming increasingly important to develop methods for accurate and early detection of AD. In 2022, 143 agents were in clinical trials for AD, with most (83.2%) being disease-modifying therapies, predominantly aimed at patients with preclinical AD, prodromal AD, or mild dementia due to AD [6]. As these medications enter clinical practice, there is a growing need for reliable and sensitive markers that would be accessible in community settings, making it possible to screen wider populations of patients that would benefit from further testing.

Olfactory dysfunction was reported in patients with AD nearly 50 years ago [7]. It has since been proven to be a common symptom present in 85–90% of the patients with AD [8, 9]. It is not only a common symptom, but also a very early sign of AD. Olfactory impairment is consistently found in patients with mild cognitive impairment (MCI) [10–12]. It is also present in patients with subjective cognitive decline (SCD) and is thought to precede cognitive symptoms for several years [13, 14]. However, studies involving patients with MCI and SCD often vary in terms of subtyping patients or do not provide information on the subtypes [11, 13]. Thus, it is difficult to apply these results in clinical practice, as it is likely that patients with disorders other than AD may have been included in the study samples [11].

Nevertheless, the value of olfactory testing is also supported by longitudinal studies, as olfactory dysfunction in healthy individuals was found to be associated with an increased risk of developing MCI on follow-up [15–18]. Furthermore, olfactory impairment is associated with an increased risk of conversion to dementia [19–22]. On the other hand, intact olfactory abilities are associated with a low likelihood of future dementia [23]. Although longitudinal studies have the same limitations pertaining to variable selection and lack of subtyping data for MCI, they confirm the value of further research on olfactory impairment as a marker of early AD.

Previous clinical findings are corroborated by pathological evidence. It has been reported that structures of the olfactory system (olfactory bulb, anterior olfactory nucleus, entorhinal, and transentorhinal areas) are affected by AD pathology in the early stages of the disease [24, 25]. Functional MRI and [^{18}F]fluorodeoxyglucose PET studies have revealed structural and functional abnormalities in olfaction-related regions in the earliest stages of AD, and these changes were found to progress during the course of the disease [26–29].

Considering the evidence from previous studies, olfactory testing undoubtedly has the potential to be introduced into clinical practice and improve AD diagnostics if data are obtained using generally accepted AD diagnostic criteria and standardized assessment methods. In most studies, olfactory testing includes odor identification, and the odor discrimination ability of the patients has rarely been analyzed [13–23], even though both odor identification and odor discrimination tasks are considered to reflect higher processing of odors, that is impaired in the case of AD [30]. Moreover, performance on odor identification tests is heavily influenced by patients' personal and cultural experiences and familiarity with different odors, making it impossible to use the same odor identification tests across different populations without adaptations [31]. Odor identification is also known to be influenced by a subject's language abilities, making it difficult for researchers to interpret the results [32, 33]. Odor discrimination, although not completely independent of these factors, does not experience these limitations to the same extent as odor identification.

The aim of our study was to analyze odor discrimination in patients with early AD and explore its diagnostic qualities. We hypothesized that odor discrimination is impaired in the early stages of the disease and can reliably differentiate patients with AD, even those in prodromal stage, from subjects with CN.

MATERIALS AND METHODS

Participants

Thirty patients diagnosed with mild dementia due to Alzheimer's disease (MD-AD), 30 with mild cognitive impairment due to Alzheimer's disease (MCI-AD), and 30 elderly subjects with normal cognition (CN) were enrolled in the study.

The patients with MD-AD met the NIA/AA (National Institute on Aging-Alzheimer's Association) criteria for probable Alzheimer's disease [5] and had a Clinical Dementia Rating (CDR) of 1. All the patients were recruited from the memory clinic of Vilnius University hospital Santaros Klinikos. Probable AD was diagnosed by a specialist based on core clinical criteria with increased level of certainty, as all

the patients had documented progressive cognitive decline [5]. Biomarker probability of AD etiology was intermediate, as all the patients had evidence of neuronal injury based on structural MRI that was performed as a standard clinical practice when diagnosing AD based on regulations by the Ministry of Health of The Republic of Lithuania [5]. Biomarkers of brain Aβ protein deposition were not available.

Patients with MCI-AD met the NIA/AA criteria for MCI due to AD [4] and had a CDR of 0.5. All the patients were recruited from the memory clinic of Vilnius University hospital Santaros Klinikos. MCI due to AD was diagnosed by a specialist when clinical and cognitive criteria were established and etiology of MCI was consistent with AD pathophysiological process based on exclusion of vascular, traumatic, medical causes of cognitive decline, and documented longitudinal decline in cognition [4]. Biomarker probability of AD etiology was intermediate, as all the patients had evidence of neuronal injury based on structural MRI that was performed as a standard clinical practice when diagnosing AD based on regulations by the Ministry of Health of The Republic of Lithuania [4]. Biomarkers of brain Aβ protein deposition were not available.

Elderly subjects with CN had no cognitive complaints, a CDR of 0, and no neurological disorders.

The exclusion criteria were as follows: central nervous system disorders other than MCI-AD and MD-AD, head trauma, cerebrovascular disorders (Hachinski Ischemic Score ≥ 4), psychosis, depression (Geriatric Depression Scale score > 9), substance abuse, psychoactive medications, significant exposure to volatile substances affecting olfactory function, smoking, nasal surgery, and recent viral infections affecting olfactory function. The study was approved by the Vilnius Regional Bioethics Committee (Approval Number 2021/6-1355-830). Written informed consent was obtained from all the participants before participation in the study.

Assessments of cognitive function

Global cognition was evaluated using the Mini-Mental State Examination (MMSE). The Alzheimer's Disease Assessment Scale-Cognitive Subscale, version 13 (with additional delayed word recall and number cancellation tasks [ADAS-Cog 13], scores range: 0–85) was used for a detailed cognitive evaluation. Delayed recall was evaluated on a scale of 0 to 10 (number of words not recalled). The number cancellation task was evaluated using a scale from 0 to 5, with 0 indicating the best (≥ 30 correct responses) and 5 indicating the worst (0–5 correct responses) performance. Verbal fluency tests were also performed (PAS and animal naming), and the severity of cognitive impairment was evaluated using the Clinical Dementia Rating scale.

Assessment of odor discrimination

Olfactory discrimination was evaluated by Sniffin' Sticks tesst (Burghardt ®, Wedel, Germany); 16 triplets of odors were presented during the test. The participants were asked to identify which sample of the three had a different odor from the other two. The olfactory discrimination score was the number of correct responses out of 16.

The odors were presented in the order provided by the test instructions. Each odor was presented only once, for 3–4 s. The time interval between odors in the same triplet was 3 s. A time interval of 30 s was maintained between the sets of triplets. The subjects were instructed not to eat or drink anything for at least 15 min prior to testing. The examiner used odorless gloves, and the subjects wore a blindfold as per the test instructions.

Data analysis

Statistical analysis was performed using IBM SPSS Statistics version 26.0. The Shapiro–Wilk test was used to check whether data distribution was normal. Differences between groups were tested using the Kruskal–Wallis test for numerical variables and two-tailed chi-square test for categorical variables. Spearman rank correlation coefficient was used to determine the correlation between the variables. Linear regression was used to analyze prediction of continuous dependent variables, and multinomial logistic regression was used to analyze prediction of categorical variables. The performance of the diagnostic tests was evaluated by receiver operating characteristic (ROC) curve analysis. A p-value of < 0.05 was considered significant.

RESULTS

Demographic and clinical characteristics

The demographic and clinical characteristics of the patients are presented in Table 1. The participants in the three groups showed no difference in sex distribution (chi-square test, $p > 0.05$). There

Table 1
Demographic and clinical characteristics of the participants

	CN ($N = 30$)	MCI-AD ($N = 30$)	MD-AD ($N = 30$)	Statistics ($\chi^2(2)$ /H(2), p)
Male (%) *	13 (43.33%)	13 (43.33%)	12 (40%)	0.09, 0.96
Years of education *	15 (3)	16 (2)	16 (3)	0.84, 0.66
Age **	74 (7)	72 (10)	78 (4)	16.8,<0.001
GDS *	5.5 (2)	5.5 (2)	5 (2)	0.08, 0.96
HIS *	1 (1)	1 (1)	1 (0)	2.09, 0.35

Data are presented as median and interquartile range unless specified otherwise. *The groups did not differ significantly. **MD-AD group differed significantly from the CN and MCI-AD groups. The CN and MCI-AD groups did not show any significant differences. MD-AD, mild dementia due to Alzheimer's disease; MCI-AD, mild cognitive impairment due to Alzheimer's disease; CN, normal cognition; GDS, Geriatric depression scale; HIS, Hachinski ischemic score.

Table 2
Cognitive performance of the participants

	CN ($N = 30$)	MCI-AD ($N = 30$)	MD-AD ($N = 30$)	Statistics (H(2), p)
MMSE *	29 (1)	26 (1)	22 (2)	79.46,<0.001
ADAS-Cog 13 *	10.83 (4.58)	20.84 (4.42)	29.34 (5.66)	69.5,<0.001
CDR Sum of Boxes *	0 (0)	2 (1)	5 (1)	82.67,<0.001
Fluency PAS *	37 (13)	28.5 (9)	21 (13)	29.79,<0.001
Fluency Animals *	20 (7)	13 (6)	10 (5)	42.22,<0.001

Data are presented as median and interquartile range. *All three groups differed significantly. MD-AD, mild dementia due to Alzheimer's disease; MCI-AD, mild cognitive impairment due to Alzheimer's disease; CN, normal cognition.

were also no significant differences according to education, depressive symptoms (Geriatric Depression Scale results), and Hachinski ischemic score (for all, Kruskal–Wallis $p > 0.05$).

Participants in the MD-AD group were older than those in the MCI-AD and CN groups (Kruskal–Wallis $p < 0.05$; post-hoc analysis revealed significant differences between the CN and MD-AD, MCI-AD, and MD-AD groups, and no significant difference between the CN and MCI-AD groups).

As expected, the performance on the cognitive tests was significantly different between all three groups (for all, Kruskal–Wallis $p < 0.05$, post-hoc analysis revealed significant differences among the three groups). The results of cognitive tests are presented in Table 2.

Odor discrimination

The olfactory discrimination scores differed significantly among the three groups (medians and interquartile ranges: CN 12.5 (3), MCI-AD 9 (3), MD-AD 6 (2); Kruskal–Wallis $p < 0.05$, post hoc analysis revealed significant differences between all three groups). The results of the odor discrimination test are presented in Fig. 1.

In the sample of all participants, the olfactory discrimination scores strongly correlated with the results of the MMSE and ADAS-Cog 13 (Spearman's rho 0.78 and –0.77, respectively; $p < 0.001$). The olfactory discrimination scores also showed a strong correlation with the CDR sum of boxes (Spearman's rho=–0.82; $p < 0.001$). The correlation between the olfactory discrimination scores and verbal fluency tests was also significant but at a moderate level (Spearman's rho for PAS fluency = 0.64, for animal fluency = 0.64; $p < 0.001$ for both).

When analyzing the correlations in each group, no significant correlations were found between olfactory discrimination scores and cognitive test results in the MD-AD group. In the CN and MCI-AD groups, there were no significant correlations between odor discrimination and MMSE, ADAS-Cog 13, animal fluency, and CDR Sum of Boxes score. The correlation between olfactory discrimination scores and fluency PAS scores remained significant, although weak (Spearman's Rho CN 0.45, MCI-AD 0.44; $p < 0.05$ in both cases). The relationship between the olfactory discrimination scores and ADAS-Cog 13 results is shown in Fig. 2.

The olfactory discrimination scores correlated significantly, although very weakly, with age when

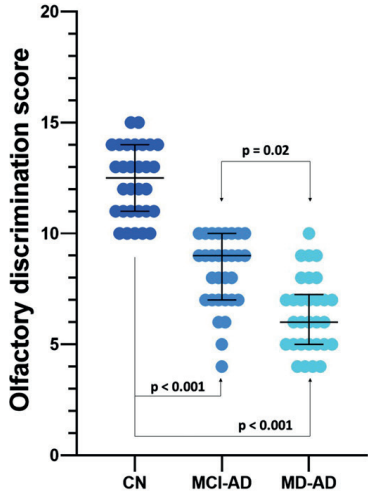


Fig. 1. Olfactory discrimination scores of the three groups. Lines represent medians, error bars represent Interquartile ranges, and dots represent individual data points. MD-AD, mild dementia due to Alzheimer's disease; MCI-AD, mild cognitive impairment due to Alzheimer's disease; CN, normal cognition.

analyzing the entire sample (Spearman's Rho –0.28; $p = 0.008$). There were no significant correlations between odor discrimination ability and age in the separate groups.

Multiple linear regression models, including age, sex, education, and cognitive test scores (MMSE, ADAS-Cog-13, CDR Sum of Boxes, and composite verbal fluency test score [VFT = PAS fluency+animal fluency]) as independent variables, were tested to determine whether they significantly predicted olfactory discrimination scores. The overall regression was statistically significant for all four models: model with MMSE $R^2 = 0.62$, $F = 35.05$, $p < 0.001$; model with ADAS-Cog 13 $R^2 = 0.58$, $F = 29.43$, $p < 0.001$; model with CDR Sum of Boxes $R^2 = 0.63$, $F = 36.25$, $p < 0.001$; model with VFT $R^2 = 0.46$, $F = 17.72$, $p < 0.001$. However, only cognitive test scores significantly predicted olfactory discrimination scores in each case (β for MMSE = 0.79, $p < 0.001$; for ADAS-Cog 13=–0.77, $p < 0.001$; for CDR Sum of Boxes=–0.8, $p < 0.001$; for VFT = 0.73; $p < 0.001$). None of the other predictors (age, sex, and education) significantly predicted the olfactory discrimination scores in any of the models ($p > 0.05$).

Diagnostic characteristics of odor discrimination

ROC analysis was performed to evaluate the performance of the olfactory discrimination score in differentiating the CN group from AD patients (MCI-AD or MD-AD), the CN group from MCI-AD patients, the CN group from MD-AD patients, and MCI-AD patients from MD-AD patients. The ROC curves with areas under the curve (AUC) are shown in Fig. 3.

A cut-off score of ≤ 10 correct responses was chosen for differentiating patients with AD (MCI-AD or MD-AD) from subjects with CN; the score had a sensitivity of 100% (95% confidence interval [CI], 94.04% to 100.00%) and specificity of 83.33% (95% CI, 65.28% to 94.36%). The negative predictive value was 100%, and the positive predictive value was 92.31% (95% CI, 84.35%–96.39%). The overall diagnostic accuracy was 94.44% (95% CI, 87.51% to 98.17%).

The diagnostic characteristics remained good when differentiating MCI-AD patients from subjects with CN. The same cut-off score of ≤ 10 had a sensitivity of 100% (95% CI, 88.43% to 100.00%) and specificity of 83.33% (95% CI, 65.28% to 94.36%). The negative predictive value was 100% and the positive predictive value was 85.71% (95% CI, 72.94% to 93.03%). The overall diagnostic accuracy was 91.67% (95% CI, 81.61% to 97.24%).

Multinomial logistic regression was performed to analyze the relationship between the predictor variables and membership in the three groups (CN, MCI-AD, and MD-AD). First, a model with age, education, sex, and ADAS-Cog 13 scores as predictor variables was tested. The fit between the model containing only the intercept and the data improved with the addition of predictor variables (chi-square =139.66, $p < 0.001$; Nagelkerke $R^2 = 0.89$). Pearson's chi-square and deviance chi-square tests indicated that the model exhibited a good fit to the data ($p > 0.05$). The overall percentage of correctly classified cases using this model was 82.2% (CN, 93.3%; MCI-AD, 70%; MD-AD, 83.3%), with the ADAS-Cog 13 score as the strongest and most significant predictor (chi-square =122.65, $p < 0.001$).

The olfactory discrimination scores were included in the model. The model with age, education, sex, ADAS-Cog 13 scores, and olfactory discrimination scores also showed a significant improvement in fit over a null model (chi-square=158.11, $p < 0.001$; Nagelkerke $R^2 = 0.93$). Pearson's chi-square and Deviance chi-square tests indicated that the model

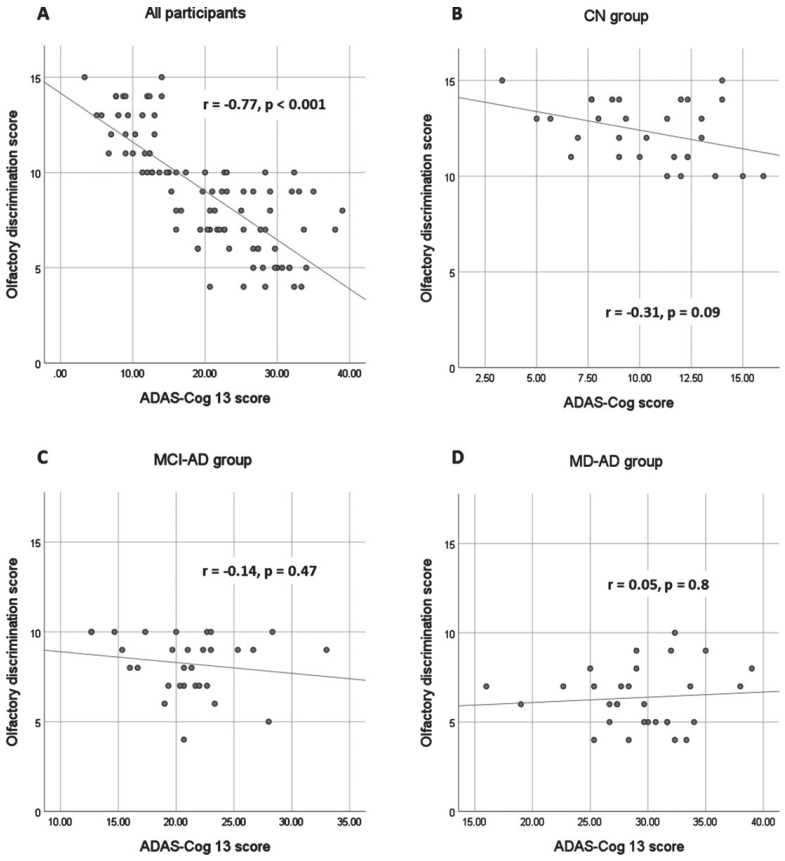


Fig. 2. Relationship between olfactory discrimination scores and ADAS-Cog 13 results. ADAS-Cog 13, Alzheimer's Disease Assessment Scale-Cognitive Subscale, version 13. MD-AD, mild dementia due to Alzheimer's disease; MCI-AD, mild cognitive impairment due to Alzheimer's disease; CN, normal cognition.

exhibited a good fit to the data ($p > 0.05$). The overall percentage of correctly classified cases using this model was 92.2% (CN, 96.7%; MCI-AD, 86.7%; MD-AD, 93.3%), with the ADAS-Cog 13 and olfactory discrimination scores both being strong and significant predictors (chi-square=28.01 and 18.45, respectively, $p < 0.001$ for both).

DISCUSSION

The levels of olfactory discrimination differed significantly among the three groups. We found that odor discrimination was impaired in the prodromal stage of AD (MCI-AD), and that the impairment became even more pronounced in patients with MD-AD. These findings are in accordance with the findings of previous studies, confirming the occurrence of olfactory impairment in the earliest stages of AD and the worsening of these changes as the disease progresses [10, 14].

The performance of the subjects in the current study was consistent with that reported in previous studies. According to normative data, the scores

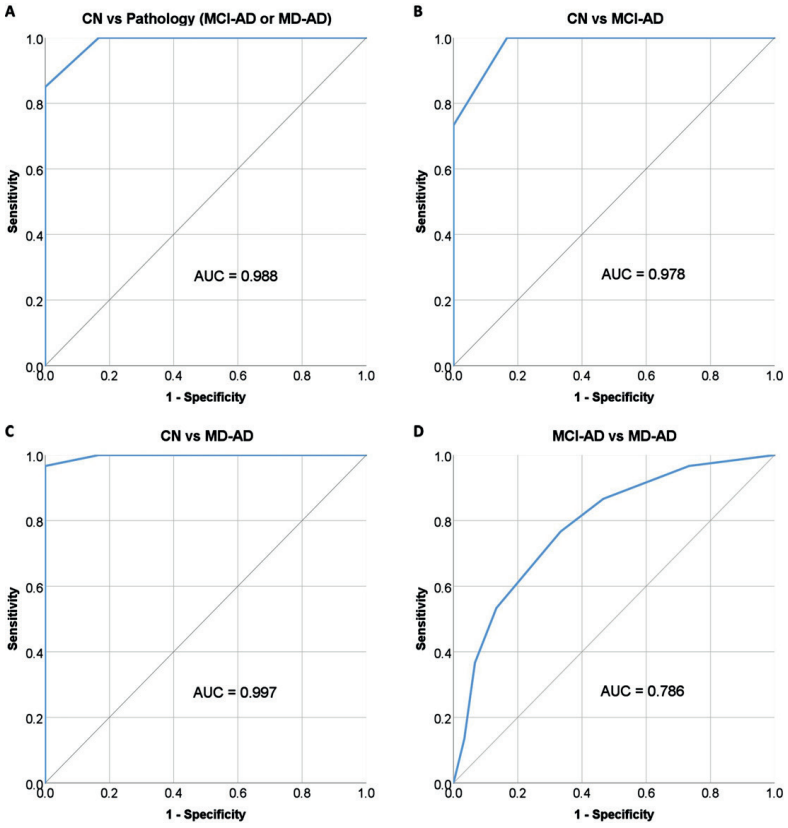


Fig. 3. Performance of olfactory discrimination score in differentiating between participants with AD and CN. MD-AD, mild dementia due to Alzheimer's disease; MCI-AD, mild cognitive impairment due to Alzheimer's disease; CN, normal cognition.

of healthy elderly subjects on odor discrimination tests range from 10.66 ± 2.5 to 13.80 ± 0.77 [34, 35]. The results of the CN group in the current study were within this range. The performance of patients with early AD was also similar to that reported in previous studies, where odor discrimination scores of the patients with MCI ranged from 7.9 ± 3.2 to 10.3 ± 2.6 and scores of the patients with AD ranged from 5.6 ± 3.8 to 9.6 ± 2.3 [12, 36, 37].

Of note, the study reporting the best odor discrimination results in the MCI group (10.3 ± 2.6) did not evaluate the subtype of MCI [37]. In order to apply the results in clinical practice, researchers must ensure that the study subjects meet the criteria for MCI due to AD in order to avoid including patients with disorders other than AD.

Olfactory discrimination scores showed a strong and significant correlation with the results of the cognitive tests in our study. Linear regression analysis demonstrated a significant relationship between these elements, with age, sex, and education having no significant influence on the odor discrimination score. These findings confirm that olfactory deficits in patients with AD are associated with the processes of the disease itself and cannot be explained by other factors known to affect olfaction in the general pop-

ulation, such as age and sex [34, 35]. These results are not unexpected, since structural and functional abnormalities have been detected in olfaction-related cortical regions in patients with AD [26–29].

The diagnostic qualities of olfactory tests have been examined in previous studies; however, information on the diagnostic qualities of olfactory discrimination tests specifically is still lacking as olfactory identification is usually the test of choice [12–23, 36, 37]. Our study yielded promising results, especially for improving the diagnosis of prodromal AD (MCI-AD).

In the current study, odor discrimination demonstrated excellent capabilities in differentiating patients with early AD (MCI-AD or MD-AD) from healthy controls (AUC = 0.988) and in differentiating patients with prodromal AD (MCI-AD) from healthy controls (AUC = 0.978). This is in accordance to findings from previous studies, where odor discrimination was found to be a significant and more reliable predictor of future cognitive decline, than odor identification [38]. However, other authors did not find odor discrimination to be superior to odor identification, even though both of them performed better then odor threshold in differentiating patients with AD and patients with MCI from cognitively normal participants [39].

Furthermore, the inclusion of olfactory discrimination scores into the multinomial logistic regression models improved the overall classification accuracy by 10% (the accuracy of classification into three groups [CN, MCI-AD, and MD-AD] improved from 82.2% to 92.2%). The correct classification of MCI-AD cases improved the most, by 16.7% (from 70% to 86.7%). This finding is important since prodromal AD (MCI-AD) might be difficult to diagnose, especially in primary care settings. According to a survey by the Alzheimer's Association, nearly two-thirds of primary care physicians (65%) said they were comfortable diagnosing MCI, while less than half (49%) reported being comfortable diagnosing MCI-AD [38]. Objective olfactory testing may be very useful for improving diagnostic certainty. Especially the certainty in differentiation of MCI from normal aging, which was the most frequently cited challenge when making the diagnosis (72%) [40].

Although odor discrimination had excellent diagnostic qualities in differentiating patients with early AD from healthy controls, its performance in differentiating between prodromal AD (MCI-AD) and patients with mild dementia (MD-AD) was not as good (AUC = 0.786) as expected in the current study. Similar results were found in recent studies on odor identification (SST12 test had an AUC of 0.741 for differentiation of MCI-AD from MD-AD) [41]. This is most likely because olfactory impairment occurs early in the course of the disease and is pronounced even in the prodromal stage of AD, making it not suitable for monitoring disease progression.

Early involvement of the olfactory system in the course of AD, which was once again confirmed by the findings of the current study, has recently led to research on olfactory training as a treatment method. Olfactory training was found to improve cognitive functioning in patients with dementia [42]. Moreover, olfactory training had a positive effect on frontal lobe activation in response to odors and increased the cortical thickness of the hippocampus [43, 44]. Even though the data are still limited at this time, these findings warrant further research on olfaction in AD.

The present study has several limitations. First, the cross-sectional design limits the accuracy of the conclusions regarding the progression of changes during the course of AD. Also, although the results regarding diagnostic properties of the odor discrimination test are encouraging, they need to be confirmed in further studies, especially estimations of negative and positive predictive values of the test. Second, CSF analysis and PET were not used, and including these modalities would have helped us analyze the relationship between olfactory changes and brain Aβ deposition and neuronal degeneration. Moreover, CSF and PET biomarkers would help in confirming the diagnosis. In the current study, all the participants met criteria for probable AD or MCI consistent with AD pathophysiological process [4, 5]. However, CSF and PET biomarkers would be helpful in increasing the level of certainty. Third, although the differences were significant in our small sample, studies involving larger sample sizes would help confirm these findings.

In conclusion, the current study showed that odor discrimination is already impaired in the prodromal stage of AD and that these changes progress during the course of the disease. Olfactory discrimination testing has good diagnostic qualities and can help clinicians accurately diagnose early AD, serving as a simple, noninvasive, affordable, and reliable marker.

ACKNOWLEDGMENTS

The authors would like to thank all the participants who participated in this study.

FUNDING

The authors have no funding to report.

CONFLICT OF INTEREST

All authors have no conflict of interest to report.

DATA AVAILABILITY

Data supporting the findings of this study are available upon request from the corresponding author.

REFERENCES

[1] World Health Organization (2021) *Global Status Report on the Public Health Response to Dementia*. World Health Organization, Geneva. License: CC, BY-NC-SA 3.0 IGO.

[2] Gauthier S, Rosa-Neto P, Morais JA, Webster C (2021) *World Alzheimer Report 2021: Journey through the Diagnosis of Dementia*, Alzheimer's Disease International, London, England.

[3] World Health Organization (2017) *Global Action Plan on the Public Health Response to Dementia 2017–2025*. World Health Organization, Geneva. License: CC BY-NC-SA 3.0 IGO.

[4] Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, Gamst A, Holtzman DM, Jagust WJ, Petersen RC, Snyder PJ, Carrillo MC, Thies B, Phelps CH (2011) The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* **7**, 270-279.

[5] McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH (2011) The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* **7**, 263-269.

[6] Cummings J, Lee G, Nahed P, Kambar MEZN, Zhong K, Fonseca J, Taghva K (2022) Alzheimer's disease drug development pipeline: 2022. *Alzheimers Dement* **8**, e12295.

[7] Waldton S (1974) Clinical observations of impaired cranial nerve function in senile dementia. A*cta Psychiatr Scand* **50**, 539-547.

[8] Doty RL, Reyes PF, Gregor T (1987) Presence of both odor identification and detection deficits in Alzheimer's disease. *Brain Res Bull* **18**, 597-600.

[9] Marine N, Boriana A (2014) Olfactory markers of depression and Alzheimer's disease. *Neurosci Biobehav Rev* **45**, 262-270.

[10] Jung HJ, Shin IS, Lee JE (2019) Olfactory function in mild cognitive impairment and Alzheimer's disease: A meta-analysis. *Laryngoscope* **129**, 362-369.

[11] Roalf DR, Moberg MJ, Turetsky BI, Brennan L, Kabadi S, Wolk DA, Moberg PJ (2017) A quantitative meta-analysis of olfactory dysfunction in mild cognitive impairment. *J Neurol Neurosurg Psychiatry* **88**, 226-232.

[12] Djordjevic J, Jones-Gotman M, De Sousa K, Chertkow H (2008) Olfaction in patients with mild cognitive impairment and Alzheimer's disease. *Neurobiol Aging* **29**, 693-706.

[13] Jobin B, Zahal R, Bussières EL, Frasnelli J, Boller B (2021) Olfactory identification in subjective cognitive decline: A meta-analysis. *J Alzheimers Dis* **79**, 1497-1507.

[14] Wang Q, Chen B, Zhong X, Zhou H, Zhang M, Mai N, Wu Z, Huang X, Haehner A, Chen X, Auber LA, Peng Q, Hummel T, Ning Y (2021) Olfactory dysfunction is already present with subjective cognitive decline and deepens with disease severity in the Alzheimer's disease spectrum. *J Alzheimers Dis* **79**, 585-595.

[15] Wilson RS, Arnold SE, Tang Y, Bennett DA (2006) Odor identification and decline in different cognitive domains in old age. *Neuroepidemiology* **26**, 61-67.

[16] Schubert CR, Carmichael LL, Murphy C, Klein BE, Klein R, Cruickshanks KJ (2008) Olfaction and the 5-year incidence of cognitive impairment in an epidemiological study of older adults. *J Am Geriatr Soc* **56**, 1517-1521.

[17] Wilson RS, Schneider JA, Arnold SE, Tang Y, Boyle PA, Bennett DA (2007) Olfactory identification and incidence of mild cognitive impairment in older age. *Arch Gen Psychiatry* **64**, 802-808.

[18] Wheeler PL, Murphy C (2021) Olfactory measures as predictors of conversion to mild cognitive impairment and Alzheimer's disease. *Brain Sci* **11**, 1391.

[19] Devanand DP, Michaels-Marston KS, Liu X, Pelton GH, Padilla M, Marder K, Bell K, Stern Y, Mayeux R (2000) Olfactory deficits in patients with mild cognitive impairment predict Alzheimer's disease at follow-up. *Am J Psychiatry* **157**, 1399-1405.

[20] Tahmasebi R, Zehetmayer S, Pusswald G, Kovacs G, Stögmann E, Lehrner J (2019) Identification of odors, faces, cities and naming of objects in patients with subjective cognitive decline, mild cognitive impairment and Alzheimer´s disease: A longitudinal study. *Int Psychogeriatr* **31**, 537-549.

[21] Lojkowska W, Sawicka B, Gugala M, Sienkiewicz-Jarosz H, Bochynska A, Scinska A, Korkosz A, Lojek E, Ryglewicz D (2011) Follow-up study of olfactory deficits, cognitive functions, and volume loss of medial temporal lobe structures in patients with mild cognitive impairment. *Curr Alzheimer Res* **8**, 689-698.

[22] Zhao A, Li Y, Yan Y, Qiu Y, Li B, Xu W, Wang Y, Liu J, Deng Y (2020) Increased prediction value of biomarker combinations for the conversion of mild cognitive impairment to Alzheimer's dementia. *Transl Neurodegener* **9**, 30.

[23] Devanand DP, Lee S, Luchsinger JA, Andrews H, Goldberg T, Huey ED, Schupf N, Manly J, Stern Y, Kreisl WC, Mayeux R (2020) Intact global cognitive and olfactory ability predicts lack of transition to dementia. *Alzheimers Dement* **16**, 326-334.

[24] Braak H, Braak E (1997) Diagnostic criteria for neuropathologic assessment of Alzheimer's disease. *Neurobiol Aging* **18**, S85-S88.

[25] Braak H, Del Tredici K (2015) The preclinical phase of the pathological process underlying sporadic Alzheimer's disease. *Brain* **138**, 2814-2833.

[26] Chen B, Wang Q, Zhong X, Mai N, Zhang M, Zhou H, Haehner A, Chen X, Wu Z, Auber LA, Rao D, Liu W, Zheng J, Lin L, Li N, Chen S, Chen B, Hummel T, Ning Y (2022) Structural and functional abnormalities of olfactory-related regions in subjective cognitive decline,

mild cognitive impairment, and Alzheimer's disease. *Int J Neuropsychopharmacol* **25**, 361-374.
[27] Wang J, Eslinger PJ, Doty RL, Zimmerman EK, Grunfeld R, Sun X, Meadowcroft MD, Connor JR, Price JL, Smith MB, Yang QX (2010) Olfactory deficit detected by fMRI in early Alzheimer's disease. *Brain Res* **1357**, 184-194.
[28] Vasavada MM, Wang J, Eslinger PJ, Gill DJ, Sun X, Karunanayaka P, Yang QX (2015) Olfactory cortex degeneration in Alzheimer's disease and mild cognitive impairment. *J Alzheimers Dis* **45**, 947-958.
[29] Förster S, Vaitl A, Teipel SJ, Yakushev I, Mustafa M, la Fougère C, Rominger A, Cumming P, Bartenstein P, Hampel H, Hummel T, Buerger K, Hundt W, Steinbach S (2010) Functional representation of olfactory impairment in early Alzheimer's disease. *J Alzheimers Dis* **22**, 581-591.
[30] Hedner M, Larsson M, Arnold N, Zucco GM, Hummel T (2010) Cognitive factors in odor detection, odor discrimination, and odor identification tasks. *J Clin Exp Neuropsychol* **32**, 1062-1067.
[31] Hsieh JW, Keller A, Wong M, Jiang RS, Vosshall LB (2017) SMELL-S and SMELL-R: Olfactory tests not influenced by odor-specific insensitivity or prior olfactory experience. *Proc Natl Acad Sci U S A* **114**, 11275-11284.
[32] Larsson M, Nilsson LG, Olofsson JK, Nordin S (2004) Demographic and cognitive predictors of cued odor identification: Evidence from a population-based study. *Chem Senses* **29**, 547-554.
[33] Oberg C, Larsson M, Bäckman L (2002) Differential sex effects in olfactory functioning: The role of verbal processing. *J Int Neuropsychol Soc* **8**, 691-698.
[34] Katotomichelakis M, Balatsouras D, Tripsianis G, Tsaroucha A, Homsioglou E, Danielides V (2007) Normative values of olfactory function testing using the 'Sniffin' sticks'. *Laryngoscope* **117**, 114-120.
[35] Hummel T, Kobal G, Gudziol H, Mackay-Sim A (2007) Normative data for the "Sniffin' Sticks" including tests of odor identification, odor discrimination, and olfactory thresholds: An upgrade based on a group of more than 3,000 subjects. *Eur Arch Otorhinolaryngol* **264**, 237-243.
[36] Peters JM, Hummel T, Kratzsch T, Lötsch J, Skarke C, Frölich L (2003) Olfactory function in mild cognitive impairment and Alzheimer's disease: An investigation using psychophysical and electrophysiological techniques. *Am J Psychiatry* **160**, 1995-2002.
[37] Steinbach S, Hundt W, Vaitl A, Heinrich P, Förster S, Bürger K, Zahnert T (2010) Taste in mild cognitive impairment and Alzheimer's disease. *J Neurol* **257**, 238-246.
[38] Sohrabi HR, Bates KA, Weinborn MG, Johnston AN, Bahramian A, Taddei K, Laws SM, Rodrigues M, Morici M, Howard M, Martins G, Mackay-Sim A, Gandy SE, Martins RN (2012) Olfactory discrimination predicts cognitive decline among community-dwelling older adults. *Transl Psychiatry* **2**, e118.
[39] Kim HR, Kim SM, Seong W, Min HJ, Kim KS, Ga H, Han DH (2020) Cut-off scores of an olfactory function test for mild cognitive impairment and dementia. *Psychiatry Investig* **17**, 1021-1030.
[40] (2022) 2022 Alzheimer's disease facts and figures. *Alzheimers Dement* **18**, 700-789.
[41] Li J, Bur AM, Villwock MR, Shankar S, Palmer G, Sykes KJ, Villwock JA (2021) Olfactory phenotypes differentiate cognitively unimpaired seniors from Alzheimer's disease and mild cognitive impairment: A combined machine learning and traditional statistical approach. *J Alzheimers Dis* **81**, 641-650.
[42] Cha H, Kim S, Kim H, Kim G, Kwon KY (2022) Effect of intensive olfactory training for cognitive function in patients with dementia. *Geriatr Gerontol Int* **22**, 5-11.
[43] Chen B, Espin M, Haussmann R, et al. (2022) The effect of olfactory training on olfaction, cognition, and brain function in patients with mild cognitive impairment. *J Alzheimers Dis* **85**, 745-754.
[44] Haehner A, Chen B, Espin M, Haussmann R, Matthes C, Desser D, Loessner L, Brandt MD, Donix M, Hummel T (2022) Training with odors impacts hippocampal thickness in patients with mild cognitive impairment. *J Alzheimers Dis* **88**, 743-755.

3rd publication/ 3 publikacija

Brief Test of Olfactory Dysfunction Based on Diagnostic Features of Specific Odors in Early-Stage Alzheimer Disease

Audronyte E, Sutnikiene V, Pakulaite-Kazliene G, Kaubrys G

Med Sci Monit. 2023;29:e940363

doi: 10.12659/MSM.940363

MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2023; 29: e940363
DOI: 10.12659/MSM.940363

Received: 2023.03.15
Accepted: 2023.04.24
Available online: 2023.05.15
Published: 2023.05.27

Brief Test of Olfactory Dysfunction Based on Diagnostic Features of Specific Odors in Early-Stage Alzheimer Disease

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF Egle Audronyte
BCEF Vaiva Sutnikiene
CDEF Gyte Pakulaite-Kazliene
ABCDEF Gintaras Kaubrys

Clinic of Neurology and Neurosurgery, Faculty of Medicine, Vilnius University, Vilnius, Lithuania

Corresponding Author: Egle Audronyte, e-mail: egle.audronyte@gmail.com
Financial support: None declared
Conflict of interest: None declared

Background: Olfactory impairment is an early symptom of Alzheimer disease (AD). However, it is rarely assessed in clinical practice. This study aimed to assess the identification and discrimination of specific odors in patients with early-stage AD using the Sniffin' Sticks test and determine the items that would be most valuable in the diagnosis of early-stage AD in order to create a brief test of olfactory dysfunction.

Material/Methods: Three groups of participants were enrolled, including 30 patients with mild cognitive impairment due to AD (MCI-AD group), 30 with mild dementia due to AD (MD-AD group), and 30 older participants with normal cognition (NC group). All participants underwent cognitive (Clinical Dementia Rating, Mini-Mental State Examination, Alzheimer's Disease Assessment Scale-Cognitive Subscale, and verbal fluency tests) and olfactory (Burghart Sniffin' Sticks odor identification and odor discrimination tests) assessments.

Results: The MD-AD group scored significantly lower than the MCI-AD group and the MCI-AD group scored significantly lower than the NC group in both the odor identification ($P<0.001$) and discrimination ($P<0.05$) tasks. The shortened versions of the odor identification and discrimination tasks showed good diagnostic properties in differentiating patients with AD from the NC participants (receiver operating characteristic [ROC] area under the curve [AUC]=0.912 and 0.954, respectively) and differentiating patients with MCI-AD from the NC participants (ROC AUC=0.871 and 0.959, respectively).

Conclusions: The brief versions of olfactory tests, containing selected items that were found to differ the most between cognitively normal participants and early-stage AD patients, have good diagnostic qualities and can aid clinicians in screening for early-stage AD.

Keywords: Alzheimer Disease • Cognitive Dysfunction • Olfaction Disorders

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/940363

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

Despite recent advances in neuroimaging, cerebrospinal fluid, and blood biomarkers, diagnosing early-stage Alzheimer disease (AD) remains challenging [1-3]. This is especially true in primary care, as these biomarkers are often expensive and not available in community settings. Currently available biomarkers for diagnosing AD measure brain amyloid-beta (Aβ) protein deposition and neuronal degeneration and require cerebrospinal fluid analysis or advanced neuroimaging techniques [1,2].

According to a survey by the Alzheimer's Association, the lack of specialists and facilities to perform diagnostic testing was the most commonly cited challenge faced by primary care physicians in the United States when diagnosing mild cognitive impairment (MCI) due to AD [4]. The situation is even worse in low- and middle-income countries, where up to 90% of dementia cases are not diagnosed, according to estimations by Alzheimer's Disease International [5]. Additionally, the survey by the Alzheimer's Association showed that cognitive testing was often not sufficient to accurately identify patients with early changes, with 72% of primary care physicians stating that they found it challenging to differentiate MCI from normal aging deficits [4]. Therefore, additional objective measures that would be sensitive, affordable, and widely accessible for predicting early-stage AD are needed.

Olfactory impairment is an early and common symptom of AD. According to various studies, olfactory impairment manifests prior to cognitive decline and is present in up to 90% of patients with AD [6-9]. Odor identification tests have been the most studied olfactory assessment methods, while other olfactory functions have rarely been analyzed in patients with AD. Odor identification testing was recognized as an inexpensive and short method that can serve as an excellent screening tool by helping to accurately identify early changes and prevent a delay in diagnosis [10]. However, olfactory dysfunction assessment is rarely used in clinical practice for diagnosing AD.

One possible limitation of olfactory testing in clinical practice is the relatively long duration of assessment. The University of Pennsylvania Smell Identification Test (UPSIT) is the most commonly used odor identification test and consists of 40 odors [11,12]. The Sniffin' Sticks test, which is particularly popular in Europe, consists of 16 odors [11,13]. However, intervals of at least 30 s are recommended between the presentation of odors to prevent olfactory desensitization [13].

Various shortened versions of the UPSIT, such as the Brief Smell Identification Test, Quick Smell Identification Test, and Pocket Smell Test, were created to provide a more convenient method for screening patients [11,14]. Some of these shortened versions have even been tested in patients with AD and have shown encouraging results [15,16]. Attempts have also been made to select UPSIT items that would be the most specific for detecting AD [10,17,18]. A shortened version of the Sniffin' Sticks odor identification test was also created [19]. However, it was not tested in patients with AD.

Since odor discrimination has rarely been tested in previous studies, information regarding the discrimination of specific odorants and shortened versions of the tests are lacking.

The objective of this study was to explore the processing of specific odors in patients with early-stage AD using Sniffin' Sticks odor identification and odor discrimination tasks. We aimed to assess the identification and discrimination of specific odors in patients with early-stage AD using the Sniffin' Sticks test and determine the items that would be most valuable in the diagnosis of early-stage AD in order to create a brief test of olfactory dysfunction and explore its diagnostic qualities.

Material and Methods

Participants

The study was approved by the Vilnius Regional Bioethics Committee (approval number: 2021/6-1355-830). The study was conducted according to the principles of the Declaration of Helsinki. All participants were informed of the study procedures. All participants agreed to participate in the study and provided written informed consent by signing relevant written informed consent forms.

Three groups of participants were enrolled in the study. The first group included patients diagnosed with mild dementia (MD) due to AD: MD-AD group, based on the National Institute on Aging-Alzheimer's Association (NIA/AA) criteria for probable AD and Clinical Dementia Rating (CDR) total score of 1 [2]. The second group consisted of patients diagnosed with MCI due to AD: MCI-AD group, according to the NIA/AA criteria for MCI due to AD and CDR total score of 0.5 [1]. The third group comprised older participants with normal cognition: NC group. Each group included 30 participants.

Participants were enrolled only if they had no other central nervous system disorders except MCI due to AD or AD and no significant cerebrovascular pathology (Hachinski Ischemic Score <4).

Other exclusion criteria were history of severe brain trauma, significant psychiatric conditions (psychosis, depression [Geriatric Depression Scale score > 9], substance abuse, or psychoactive medications), history of nasal trauma or surgery, smoking, and recent viral infections potentially affecting olfaction.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Assessment of Cognitive Functions

Demographic information (age, sex, duration of AD symptoms, and medical history) was obtained from each participant.

For the assessment of global cognitive functioning, the Mini-Mental State Examination (MMSE) and CDR were performed [20,21]. Further evaluation was performed using the Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog) [20]. Phonemic (PAS) and categorical (animals) verbal fluency were also evaluated.

Assessment of Olfactory Function

The Sniffin' Sticks odor identification test and odor discrimination test were performed (Burghart®, Wedel, Germany).

The Sniffin' Sticks odor identification test consists of 16 odors: orange, leather, cinnamon, peppermint, banana, lemon, licorice, turpentine, garlic, coffee, apple, clove, pineapple, rose, anise, and fish. Each odor was presented using a felt tip pen. The cap was removed, and the odor was presented only once for 3 to 4 s. The participants were asked to choose which of the 4 items in the answering card best described the odor. They were prompted to choose an item, even if they were uncertain. A time interval of 30 s was maintained between the odor presentations. The odor identification score is the number of correct responses out of 16 [22].

The Sniffin' Sticks odor discrimination test consists of 16 triplets of odors, which are presented using felt tip pens. The participant was asked to identify which item had a different odor from the other two in each triplet. The odors were presented in the order provided by the test instructions. Each odor was presented only once, for 3 to 4 s. A time interval of 3 s was maintained between odors in the same triplet. A time interval of 30 s was maintained between the sets of triplets. The odor discrimination score is the number of correct responses out of 16 [22].

The examiner used odorless gloves during the olfactory testing. The participants were asked not to drink or eat anything for at least 15 min prior to testing [22].

Data Analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp, Armonk, NY, USA).

The Shapiro-Wilk test was used to determine the normality of data distribution.

Differences in categorical variables between groups were analyzed using a 2-tailed chi-square test (for determining differences between groups in the sex of the participants) and Fisher's exact test (for determining differences between groups in the frequency of correct identification and discrimination of specific odors).

Differences between 2 groups of non-normally distributed numerical variables were analyzed using the Mann-Whitney U test. The Mann-Whitney test was used for determining the difference between MCI-AD and MD-AD groups in the duration of AD symptoms.

Comparisons between 3 groups of numerical variables were performed using one-way analysis of variance (ANOVA) for normally distributed variables (overall odor identification score) and the Kruskal-Wallis test for non-normally distributed variables (age of participants, years of education, Hachinski Ischemic Score, results of the Geriatric Depression Scale, results of the MMSE, CDR sum of boxes, results of the ADAS-Cog, results of fluency tests, and overall odor discrimination score).

Items that were found to differ the most between cognitively normal participants and early-stage AD patients were selected for the 4-odor identification score and the 4-odor discrimination score. The multiple linear regression models with age, sex, education, duration of symptoms, ADAS-Cog score, and composite verbal fluency test score (fluency PAS + fluency animals) as independent variables, and the 4-odor identification score or the 4-odor discrimination score as dependent variables were tested to determine whether these independent variables significantly predicted the 4-odor identification score and the 4-odor discrimination score.

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the accuracy of the 4-odor identification score and the 4-odor discrimination score.

Statistical significance was set at $P<0.05$. In the case of multiple comparisons using Fisher's exact test, Bonferroni correction was applied, and a P value <0.016 was considered significant.

Results

Demographic and Clinical Characteristics

Patients in the MD-AD group were significantly older than those in the MCI-AD and NC groups. However, the MCI-AD and NC groups did not show any significant differences in age. The median age [age range] was 78 [65-85], 72 [60-84], and 74 [63-89] years in the MD-AD, MCI-AD, and NC groups, respectively (Kruskal-Wallis test, $P<0.05$; post hoc analysis revealed significant differences between the NC and MD-AD groups as well as the MCI-AD and MD-AD groups but no significant difference between the NC and MCI-AD groups).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 1. Demographic and clinical characteristics of the participants.

	NC (N=30)	MCI-AD (N=30)	MD-AD (N=30)
Male (%)*	13 (43.3%)	13 (43.3%)	12 (40.0%)
Years of education*	15 [13.5-16]	16 [14-16]	16 [13-16]
Age**	74 [68.75-76]	72 [67.75-77.25]	78 [75-79.25]
GDS*	5.5 [4-6.25]	5.5 [4-6]	5 [4-6.25]
HIS*	1 [0-1]	1 [0-1]	1 [1-1.25]
Duration of AD symptoms (in years)***	N/A	3 [2-3]	4 [3-5]

GDS – Geriatric Depression Scale; HIS – Hachinski Ischemic Score; NC – normal cognition; AD – Alzheimer disease; MCI-AD – mild cognitive impairment due to Alzheimer disease; MD-AD – mild dementia due to Alzheimer disease; N/A – not applicable. Data are represented as median and interquartile range unless otherwise specified. * The groups did not differ significantly. ** The MD-AD group differed significantly from the MCI-AD and NC groups. No significant differences were observed between the NC and MCI-AD groups. *** The MD-AD group differed significantly from the MCI-AD group.

Table 2. Results of the cognitive assessments.

	NC (N=30)	MCI-AD (N=30)	MD-AD (N=30)
MMSE*	29 [29-30]	26 [25-26]	22 [21-23]
CDR sum of boxes*	0 [0-0]	2 [1.5-2.5]	5 [4.5-5.5]
ADAS-Cog*	5.33 [4.59-7]	11.33 [9.17-13.75]	17.67 [15.17-20.33]
Categorical fluency (animals)*	20 [14.75-22]	13 [11-17]	10 [7-12]
Phonemic fluency (PAS)*	37 [28-41]	28.5 [25-34.25]	21 [14.75-27.25]

NC – normal cognition; MCI-AD – mild cognitive impairment due to Alzheimer disease; MD-AD – mild dementia due to Alzheimer disease; CDR – Clinical Dementia Rating; MMSE – Mini-Mental State Examination; ADAS-Cog – Alzheimer Disease Assessment Scale-Cognitive Subscale. Data are presented as median and interquartile range. * All 3 groups differ significantly.

The 3 groups did not differ significantly according to years of education, Hachinski Ischemic Score, or Geriatric Depression Scale results (Kruskal-Wallis test, $P>0.05$). They also did not differ significantly according to sex (2-tailed chi-square test, $P>0.05$; **Table 1**).

The median duration [range of duration] of AD symptoms was 4 [2-6] years in the MD-AD group and 3 [1-5] years in the MCI-AD group. The difference between the groups was significant (Mann-Whitney U test, $P<0.001$).

The results of the cognitive tests differed significantly among the 3 groups. In all cases, the Kruskal-Wallis test and post hoc analysis revealed significant differences among the 3 groups ($P<0.05$). The median and interquartile range of the MMSE, CDR sum of boxes, ADAS-Cog scores, and results of fluency tests are provided in **Table 2**.

Odor Identification

Overall odor identification scores differed significantly among the 3 groups. Mean (standard deviation [SD]) scores were 12.77 (1.43) in the NC group, 9.3 (2.23) in the MCI-AD group, and 7.0 (2.13) in the MD-AD group (one-way ANOVA, $P<0.001$; post hoc analysis revealed significant differences among all 3 groups).

Five odors (leather, lemon, licorice, apple, and pineapple) were excluded from further analysis because of poor identification (<70% correct responses) in the NC group.

The identification scores for the remaining odors are presented in **Table 3**.

Nine of the remaining 11 odors (all except anis and turpentine) had significantly worse identification scores in the MD-AD group than in the NC group (Fisher's exact test, $P<0.016$). Three odors (clove, garlic, and banana) had significantly worse identification scores in the MCI-AD group than in the NC group (Fisher's exact test, $P<0.016$). The identification scores of all 11 odors did not differ significantly between the MCI-AD and MD-AD groups (Fisher's exact test, $P>0.016$).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 3. Results of the odor identification test.

Odor	NC correct responses (%)	MCI-AD correct responses (%)	MD-AD correct responses (%)
Clove[a,b]	30 (100.00)	19 (63.33)*	18 (60.00)*
Fish	29 (96.67)	28 (93.33)	20 (66.67)*
Orange	29 (96.67)	23 (76.67)	19 (63.33)*
Garlic[b]	29 (96.67)	22 (73.33)	17 (56.67)*
Coffee	28 (93.33)	24 (80.00)	19 (63.33)*
Cinnamon[b]	28 (93.33)	19 (63.33)*	10 (33.33)*
Peppermint	27 (90.00)	24 (80.00)	16 (53.33)*
Rose	27 (90.00)	22 (73.33)	15 (50.00)*
Banana[b]	26 (86.67)	16 (53.33)*	7 (23.33)*
Anis	25 (83.33)	19 (63.33)	17 (56.67)
Turpentine	22 (73.33)	12 (40.00)	16 (53.33)

NC – normal cognition; AD – Alzheimer disease; MCI-AD – mild cognitive impairment due to Alzheimer disease; MD-AD – mild dementia due to Alzheimer disease. * Significantly different from the NC group ($P<0.016$). [a] The odor that differed the most ($P<0.001$) between the MD-AD and NC groups. [b] The odors that differed the most ($P<0.001$) between the MCI-AD and NC groups.

Four odors that had the greatest differences in identification scores between the MD-AD and NC groups were selected (Fisher's exact test, $P<0.001$). The identification score for these 4 odors (clove, garlic, cinnamon, and banana) was calculated. ROC curve analysis was performed to evaluate the performance of the 4-odor identification score in differentiating the NC participants from patients with AD (MCI-AD or MD-AD), MCI-AD, and MD-AD and the patients with MCI-AD from those with MD-AD. The ROC curves with the area under the curve (AUC) are shown in **Figure 1**.

A cut-off score of ≤3 for detecting AD was chosen. Using this cut-off score for differentiating patients with AD (MCI-AD or MD-AD) from NC participants, the 4-odor identification score had a sensitivity and specificity of 91.67% (95% confidence interval [CI]: 81.61-97.24%) and 76.67% (95% CI: 57.72-90.07%), respectively. The negative and positive predictive values were 82.14% (95% CI: 66.01-91.59%) and 88.71% (95% CI: 80.35-93.79%), respectively. The overall diagnostic accuracy was 86.67% (95% CI: 77.87-92.92%).

The diagnostic characteristics remained good when differentiating patients with MCI-AD from NC participants. Using the same cut-off score of ≤3, the 4-odor identification score had a sensitivity and specificity of 86.67% (95% CI: 69.28-96.24%) and 76.67% (95% CI: 57.72-90.07%), respectively. The negative and positive predictive values were 85.19% (95% CI: 69.33-93.60%) and 78.79% (95% CI: 65.67-87.82%), respectively. The overall diagnostic accuracy was 81.67% (95% CI: 69.56-90.48%).

A multiple linear regression model with age, sex, education, duration of symptoms, ADAS-Cog score, and composite verbal fluency test score (fluency PAS + fluency animals) as independent variables was tested to determine whether these variables significantly predicted the 4-odor identification score.

The overall regression was statistically significant (R2=0.471, F=14.205, $P<0.001$), with symptom duration (β=-0.446, $P<0.001$) being the only significant predictor of the 4-odor identification score.

Odor Discrimination

Overall odor discrimination scores differed significantly among the 3 groups. Median [interquartile range] scores were 12.5 [11-14] in the NC group, 9 [7-10] in the MCI-AD group, and 6 [5-7.25] in the MD-AD group (Kruskal-Wallis test, $P<0.05$; post hoc analysis revealed significant differences between all 3 groups).

Five triplets were excluded from further analysis because of poor identification (<70% of correct responses) in the NC group. The discrimination scores of the remaining odors are presented in **Table 4**.

Odor discrimination scores in 9 of the remaining 11 triplets were significantly worse in the MD-AD group than in the NC group (Fisher's exact test, $P<0.016$), while odor discrimination scores in 8 triplets were significantly worse in the MCI-AD group than in the NC group (Fisher's exact test, $P<0.016$). Odor discrimination scores in the triplet containing 2-phenylethanol as

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

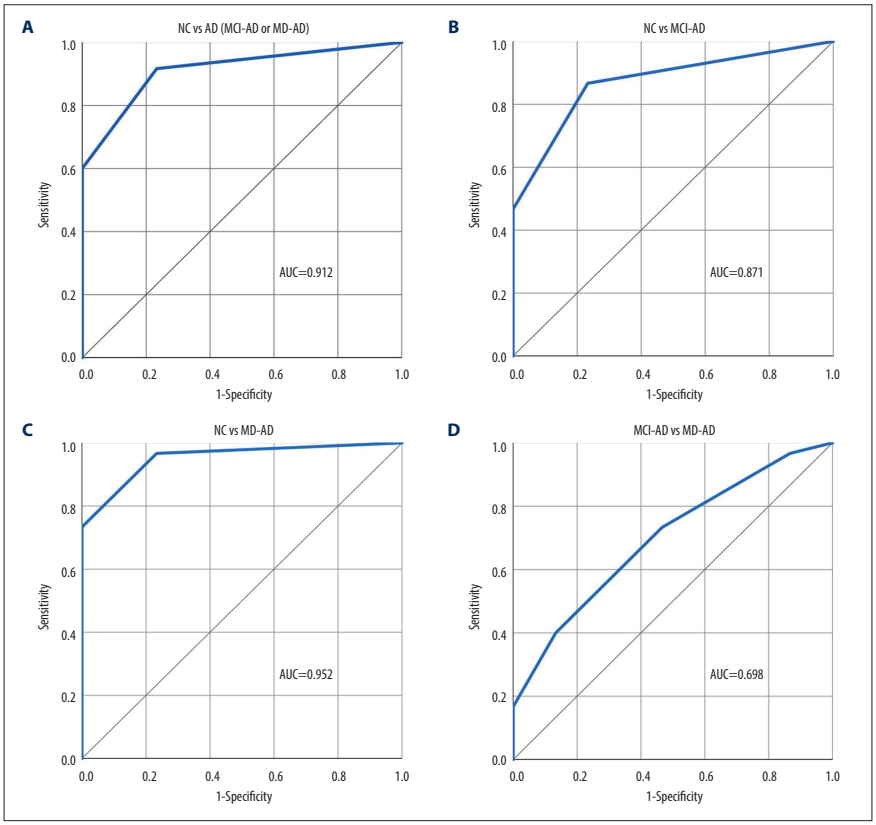


Figure 1. (**A-D**) Performance of the 4-odor identification score in differentiating participants of the various groups. NC – normal cognition; AD – Alzheimer disease; MCI-AD – mild cognitive impairment due to Alzheimer disease; MD-AD – mild dementia due to Alzheimer disease; AUC – receiver operating characteristic (ROC) area under the curve. *Created using IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.*

the target odor and isoamyl acetate as the non-target odor differed significantly between the MCI-AD and MD-AD groups (Fisher's exact test, $P<0.001$). Odor discrimination scores in all the remaining 10 triplets did not differ significantly between the MCI-AD and MD-AD groups (Fisher's exact test, $P>0.016$).

The 3 triplets that differed the most between the MCI-AD and NC groups and the triplet with 2-phenylethanol as the target odor and isoamyl acetate as the non-target odor that differed significantly between the MCI-AD and MD-AD groups were selected for the 4-odor discrimination score. ROC curve analysis was performed to evaluate the performance of the 4-odor discrimination score in differentiating the NC participants from patients with AD (MCI-AD or MD-AD), MCI-AD, and MD-AD and the patients with MCI-AD from those with MD-AD. The ROC curves with AUC are shown in **Figure 2**.

A cut-off score of ≤3 for detecting AD was chosen. Using this cut-off score for differentiating patients with AD (MCI-AD or MD-AD) from NC participants, the 4-odor discrimination score had a sensitivity and specificity of 98.33% (95% CI: 91.06-99.96%) and 76.67% (95% CI: 57.72-90.07%), respectively. The negative and

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 4. Results of the odor discrimination test.

Target odor	Non-target odor	NC correct responses (%)	MCI-AD correct responses (%)	MD-AD correct responses (%)
2-Phenylethanol[a]	Isoamyl acetate[a]	29 (96.67)	20 (66.67)*	6 (20.00)*
(+)-Limonene[a,b]	(+)-Fenchone[b]	29 (96.67)	13 (43.33)*	15 (50.00)*
Pyridine[a]	(-)-Limonene[a]	28 (93.33)	21 (70.00)	13 (43.33)*
Octyl acetate[a]	Cinnamaldehyde[a]	28 (93.33)	19 (63.33)*	11 (36.67)*
2-Phenylethanol[a,b]	(+)-Menthol[b]	28 (93.33)	16 (53.33)*	12 (40.00)*
1-Butanol[a]	(+)-Fenchone[a]	27 (90.00)	21 (70.00)	11 (36.67)*
Eucalyptol[a]	α-Ionone[a]	27 (90.00)	17 (56.67)*	10 (33.33)*
(-)-Limonene[a]	Citronellal[a]	25 (83.33)	14 (46.67)*	9 (30.00)*
Anethole[a,b]	Eugenol[b]	23 (76.67)	10 (33.33)*	11 (36.67)*
Isoamyl acetate	Anethole	21 (70.00)	22 (73.33)	17 (56.67)
Citronellal	Linalool	21 (70.00)	10 (33.33)*	16 (53.33)

NC – normal cognition; AD – Alzheimer disease; MCI-AD – mild cognitive impairment due to Alzheimer disease; MD-AD – mild dementia due to Alzheimer disease. * Significantly different from the NC group (P<0.016). [a] Triplets that differed the most (P<0.001) between the MD-AD and NC groups. [b] Triplets that differed the most (P<0.001) between the MCI-AD and NC groups.

positive predictive values were 95.83% (95% CI: 76.53-99.39%) and 89.39% (95% CI: 81.49-94.16%), respectively. The overall diagnostic accuracy was 91.11% (95% CI: 83.23-96.08%).

The diagnostic characteristics remained good when differentiating patients with MCI-AD from NC participants. Using the same cut-off score of ≤3, the 4-odor discrimination score had a sensitivity and specificity of 100% (95% CI: 88.43-100%) and 76.67% (95% CI: 57.72-90.07%), respectively. The negative and positive predictive values were 100% and 81.08% (95% CI: 69.14-89.13%), respectively. The overall diagnostic accuracy was 88.33% (95% CI: 77.43-95.18%).

A multiple linear regression model with age, sex, education, duration of symptoms, ADAS-Cog score, and composite verbal fluency test score (fluency PAS + fluency animals) as independent variables was used to determine whether these variables significantly predicted the 4-odor discrimination score.

The overall regression was statistically significant (R2=0.552, F=17.056, P<0.001), with symptom duration (β=-0.485, P<0.001) being the only significant predictor of the 4-odor discrimination score.

Discussion

In this study, odor identification and odor discrimination were impaired in patients with prodromal AD (MCI due to AD). This impairment was even more pronounced in patients with MD due to AD. These findings are in accordance with previous research in which olfactory dysfunction was demonstrated in patients with early-stage AD [6-9,23]. In previous studies, the changes were also found to be already present in MCI due to AD and further worsen in the dementia stage [24].

The duration of AD symptoms was the only significant predictor of odor identification and odor discrimination scores. This result confirms that olfactory dysfunction in AD is associated with the disease processes and cannot be explained by cognitive deficits that are observed in patients with AD or by other factors that influence olfaction in the general population, such as age and sex [6,7].

The identification scores of clove, garlic, cinnamon, and banana odors differed the most between the patients with AD and healthy participants in the present study. Although the results of previous studies are not completely uniform, they also indicate that clove [10,17], banana [25,26], and garlic [26-28] are among the most sensitive odors for testing patients with AD. However, cinnamon was not found to be sensitive for detecting olfactory dysfunction in patients with AD in previous studies. Furthermore, it was not included in the sets of 10 odors that were determined to be the most suitable for testing patients with AD [10,17].

In previous studies, identification of the rose odor was most consistently found to be impaired in patients with AD

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

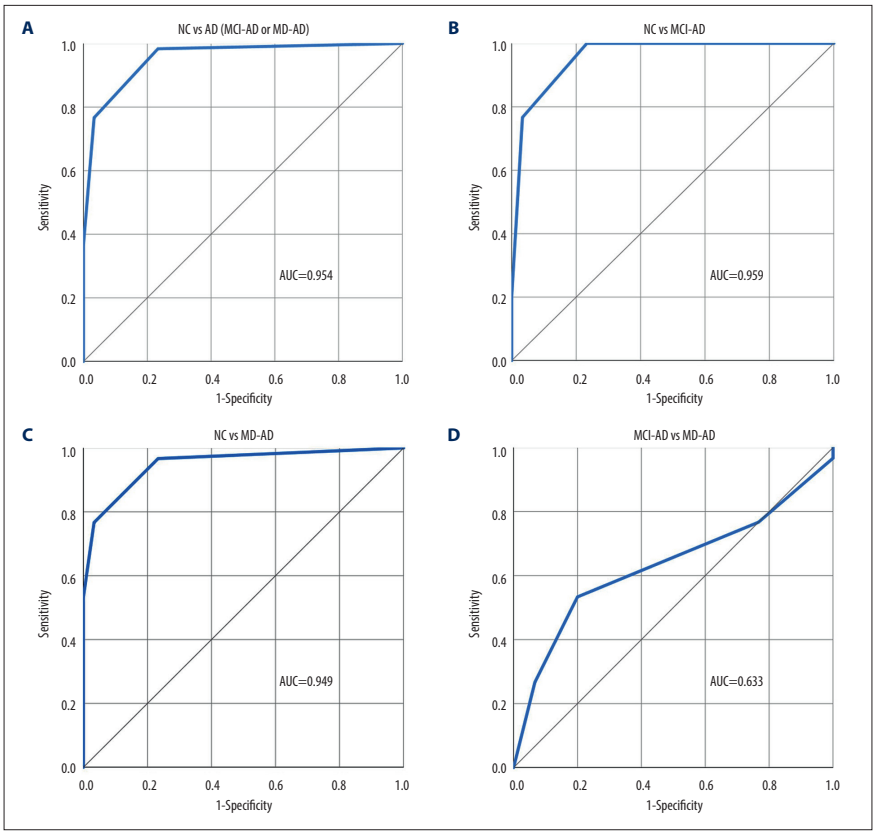


Figure 2. (**A-D**) Performance of the 4-odor discrimination score in differentiating participants of the various groups. NC – normal cognition; AD – Alzheimer disease; MCI-AD – mild cognitive impairment due to Alzheimer disease; MD-AD – mild dementia due to Alzheimer disease; AUC – receiver operating characteristic (ROC) area under the curve. *Created using IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.*

[10,18,27-30]. In our study, the identification of the rose odor was also significantly impaired in the MD-AD group; however, it was not among the odors that differed the most between MD-AD and NC groups. Thus, it was not included in the shortened version of the odor identification score.

The shortened odor identification score, consisting of 4 odors (clove, garlic, cinnamon, and banana), had good diagnostic qualities for detecting AD and even MCI due to AD in the present study. Previous studies had determined that the 3-item Pocket Smell Test had acceptable, albeit slightly worse, diagnostic accuracy [15,31-33]. However, in previous studies, standard tests containing lemon/lilac/smoke or apple/gas/rose odorants were used, and these combinations were not chosen specifically for patients with AD. Considering these results of previous studies, our findings indicate that short versions (3-4 items) of the odor identification test have good diagnostic properties and could be useful in diagnosing early-stage AD, particularly if specific items that are the most sensitive for predicting AD are chosen.

The short versions of olfactory tests could improve their applicability in clinical practice because they are less time

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

consuming. In the present study, the Sniffin' Sticks test was used and proved to have good diagnostic qualities. Gathering data on different olfactory tests is crucial because the cost of the most commonly used tests could be a factor limiting their wider application. The UPSIT and all other tests derived from it are single-use "scratch-and-sniff" tests that must be purchased separately for each patient [11,12]. The Sniffin' Sticks test was designed to be used repetitively over a period of several months [13].

Odor discrimination demonstrated a different pattern of impairment than odor identification. In the case of odor identification, the identification scores for most odors were significantly worse in patients with MD-AD than in the healthy participants. However, the identification scores of only 3 odors were significantly worse in patients with MCI-AD than in the healthy participants. In contrast, the scores of the odor discrimination task for most odors were significantly worse in patients with MCI-AD than in the healthy participants, indicating pronounced impairment in odor discrimination during the early stage of the disease. Accordingly, the shortened version of the odor discrimination task had better diagnostic qualities for prodromal AD than the shortened version of odor identification task. Early changes in the performance of the odor discrimination task might be related to the different nature of the tests. Both odor identification and discrimination reflect higher processing of odors; however, olfactory short-term memory is involved to a greater extent in odor discrimination tasks [34].

Additionally, both olfactory tasks had poor abilities to differentiate between the different stages of AD (MCI-MD vs MD-AD; ROC AUC=0.698 for the 4-odor identification score and ROC AUC=0.633 for the 4-odor discrimination score). Therefore, since changes in olfactory abilities appear early in the disease course, olfactory testing may be extremely helpful for diagnosing early-stage AD and less reliable for monitoring disease progression. Our findings were similar to those of previous studies, in which odor identification was determined to have better qualities for differentiating healthy participants from patients with AD than differentiating between patients with different stages of AD [35].

The present study had several limitations. First, although the study results were significant, the sample size was rather small. Thus, the results should be confirmed in studies with larger sample sizes. Second, the cross-sectional design of the study limits the accuracy of the conclusions with respect to the longitudinal changes during the disease course. Finally, cerebrospinal fluid and positron emission tomography biomarkers were not tested in this study. The use of these factors could help exclude the possibility of other neurodegenerative conditions and analyze the relationship between olfactory changes, amyloid beta deposition in the brain, and neuronal degeneration.

Conclusions

In conclusion, the present study confirmed that odor identification and discrimination are impaired in the prodromal stage of AD and that these changes progress during the course of AD. Furthermore, we demonstrated that the brief versions of olfactory tests, containing selected items that were found to differ the most between cognitively normal participants and early-stage AD patients, have good diagnostic qualities and can aid clinicians in screening for early-stage AD, facilitating the accurate and timely identification of patients requiring further assessment and treatment.

Acknowledgements

The authors would like to thank all the participants who participated in this study.

Declaration of Figures' Authenticity

All figures submitted have been created by the authors, who confirm that the images are original with no duplication and have not been previously published in whole or in part.

References:

1. Albert MS, DeKosky ST, Dickson D, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):270-79
2. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):263-69
3. Park JE, Gunasekaran TI, Cho YH, et al. Diagnostic blood biomarkers in Alzheimer's disease. Biomedicines. 2022;10(1):169
4. 2022 Alzheimer's disease facts and figures. Alzheimers Dement. 2022;18(4):700-89
5. Gauthier S, Rosa-Neto P, Morais JA, Webster C. World Alzheimer Report 2021: Journey through the diagnosis of dementia. London, England: Alzheimer's Disease International. 2021
6. Jobin B, Zahal R, Bussières EL, et al. Olfactory identification in subjective cognitive decline: A meta-analysis. J Alzheimers Dis. 2021;79(4):1497-507
7. Wang Q, Chen B, Zhong X, et al. Olfactory dysfunction is already present with subjective cognitive decline and deepens with disease severity in the Alzheimer's disease spectrum. J Alzheimers Dis. 2021;79(2):585-95
8. Jung HJ, Shin IS, Lee JE. Olfactory function in mild cognitive impairment and Alzheimer's disease: A meta-analysis. Laryngoscope. 2019;129(2):362-69
9. Marine N, Boriana A. Olfactory markers of depression and Alzheimer's disease. Neurosci Biobehav Rev. 2014;45:262-70
10. Woodward MR, Amrutkar CV, Shah HC, et al. Validation of olfactory deficit as a biomarker of Alzheimer disease. Neurol Clin Pract. 2017;7(1):5-14

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

11. Doty RL. Psychophysical testing of smell and taste function. Handb Clin Neurol. 2019;164:229-46
12. Doty RL, Shaman P, Dann M. Development of the University of Pennsylvania Smell Identification Test: A standardized microencapsulated test of olfactory function. Physiol Behav. 1984;32(3):489-502
13. Kobal G, Hummel T, Sekinger B, et al. "Sniffin' sticks": Screening of olfactory performance. Rhinology. 1996;34(4):222-26
14. Joseph T, Auger SD, Peress L, et al. Screening performance of abbreviated versions of the UPSIT smell test. J Neurol. 2019;266(8):1897-906
15. Duff K, McCaffrey RJ, Solomon GS. The Pocket Smell Test: Successfully discriminating probable Alzheimer's dementia from vascular dementia and major depression. J Neuropsychiatry Clin Neurosci. 2002;14(2):197-201
16. Kjelvik G, Sando SB, Aasly J, et al. Use of the Brief Smell Identification Test for olfactory deficit in a Norwegian population with Alzheimer's disease. Int J Geriatr Psychiatry. 2007;22(10):1020-24
17. Tabert MH, Liu X, Doty RL, et al. A 10-item smell identification scale related to risk for Alzheimer's disease. Ann Neurol. 2005;58(1):155-60
18. Woodward MR, Hafeez MU, Qi Q, et al. Odorant item specific olfactory identification deficit may differentiate Alzheimer disease from aging. Am J Geriatr Psychiatry. 2018;26(8):835-46
19. Mueller C, Renner B. A new procedure for the short screening of olfactory function using five items from the "Sniffin' Sticks" identification test kit. Am J Rhinol. 2006;20(1):113-16
20. Demencijų diagnostiniai kriterijai ir vertinimo skalės. Neurologijos seminarai. 2000;2(10):18-38 [in Lithuanian]
21. Hughes CP, Berg L, Danziger WL, et al. A new clinical scale for the staging of dementia. Br J Psychiatry. 1982;140:566-72
22. Rumeau C, Nguyen DT, Jankowski R. How to assess olfactory performance with the Sniffin' Sticks test(®). Eur Ann Otorhinolaryngol Head Neck Dis. 2016;133(3):203-6
23. Kim SM, Kim HR, Min HJ, et al. A novel olfactory threshold test for screening cognitive decline among elderly people. PLoS One. 2021;16(7):e0254357
24. Yu Q, Guo P, Li D, et al. Olfactory dysfunction and its relationship with clinical symptoms of Alzheimer disease. Aging Dis. 2018;9(6):1084-95
25. Velayudhan L, Gasper A, Pritchard M, et al. Pattern of smell identification impairment in Alzheimer's disease. J Alzheimers Dis. 2015;46(2):381-87
26. Zendehbad AS, Noroozian M, Shakiba A, et al. Validation of Iranian Smell Identification Test for screening of mild cognitive impairment and Alzheimer's disease. Appl Neuropsychol Adult. 2022;29(1):77-82
27. Yoshitake M, Maeshima E, Maeshima S, et al. Olfactory identification ability in patients with mild cognitive impairment and Alzheimer's disease. J Phys Ther Sci. 2022;34(11):710-14
28. Jimbo D, Inoue M, Taniguchi M, Urakami K. Specific feature of olfactory dysfunction with Alzheimer's disease inspected by the Odor Stick Identification Test. Psychogeriatrics. 2011;11(4):196-204
29. Umeda-Kameyama Y, Ishii S, Kameyama M, et al. Heterogeneity of odorant identification impairment in patients with Alzheimer's Disease. Sci Rep. 2017;7(1):4798
30. Hori Y, Matsuda O, Ichikawa S. Olfactory function in elderly people and patients with Alzheimer's disease. Psychogeriatrics. 2015;15(3):179-85
31. Makowska I, Kloszewska I, Grabowska A, et al. Olfactory deficits in normal aging and Alzheimer's disease in the polish elderly population. Arch Clin Neuropsychol. 2011;26(3):270-79
32. Solomon GS, Petrie WM, Hart JR, Brackin HB Jr. Olfactory dysfunction discriminates Alzheimer's dementia from major depression. J Neuropsychiatry Clin Neurosci. 1998;10(1):64-67
33. McCaffrey RJ, Duff K, Solomon GS. Olfactory dysfunction discriminates probable Alzheimer's dementia from major depression: A cross-validation and extension. J Neuropsychiatry Clin Neurosci. 2000;12(1):29-33
34. Zucco GM, Hummel T, Tomaiuolo F, Stevenson RJ. The influence of short-term memory on standard discrimination and cued identification olfactory tasks. J Neurosci Methods. 2014;222:138-41
35. Quarmley M, Moberg PJ, Mechanic-Hamilton D, et al. Odor identification screening improves diagnostic classification in incipient Alzheimer's disease. J Alzheimers Dis. 2017;55(4):1497-507

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

4th publication/ 4 publikacija

Olfactory memory in mild cognitive impairment and Alzheimer's disease

Audronyte E, Sutnikiene V, Pakulaite-Kazliene G, Kaubrys G

Front. Neurol. 2023;14:1165594

doi: 10.3389/fneur.2023.1165594

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 02 June 2023
DOI 10.3389/fneur.2023.1165594

OPEN ACCESS

EDITED BY
Giovanni Rizzo,
IRCCS Institute of Neurological Sciences of Bologna (ISNB), Italy

REVIEWED BY
Charles Hall,
Albert Einstein College of Medicine, United States
Alexandra Badea,
Duke University, United States

*CORRESPONDENCE
Egle Audronyte
egle.audronyte@santa.lt

RECEIVED 14 February 2023
ACCEPTED 15 May 2023
PUBLISHED 02 June 2023

CITATION
Audronyte E, Sutnikiene V, Pakulaite-Kazliene G and Kaubrys G (2023) Olfactory memory in mild cognitive impairment and Alzheimer's disease.
Front. Neurol. 14:1165594.
doi: 10.3389/fneur.2023.1165594

Olfactory memory in mild cognitive impairment and Alzheimer's disease

Egle Audronyte*, Vaiva Sutnikiene, Gyte Pakulaite-Kazliene and Gintaras Kaubrys

Clinic of Neurology and Neurosurgery, Faculty of Medicine, Institute of Clinical Medicine, Vilnius University, Vilnius, Lithuania

Introduction: Olfaction is impaired in Alzheimer's disease (AD). However, olfactory memory has rarely been examined. As the pathogenesis of AD remains largely unknown, collecting more data regarding the occurrence and progression of its symptoms would help gain more insight into the disease.

Objective: To investigate olfactory memory and its relationship with verbal memory and other clinical features in patients with early-stage AD.

Methods: Three groups of participants were enrolled in this study: patients with mild dementia due to AD (MD-AD, N=30), patients with mild cognitive impairment due to AD (MCI-AD, N=30), and cognitively normal older participants (CN, N=30). All participants underwent cognitive evaluation (Clinical Dementia Rating scale, Mini Mental State Examination, Alzheimer's Disease Assessment Scale–Cognitive Subscale, delayed verbal recall, and verbal fluency tests) and assessment of olfactory immediate and delayed recognition memory.

Results: Olfactory immediate and delayed recognition memory scores were significantly lower in the MD-AD group than in the MCI-AD and CN groups. The MCI-AD and CN groups did not differ significantly [in both cases, Kruskal–Wallis test, p<0.05; *post hoc* analysis revealed significant differences between the MD-AD and MCI-AD groups and between the MD-AD and CN groups (p<0.05), and no significant difference between the MCI-AD and CN groups (p>0.05)]. Verbal immediate recall, delayed recall after 5min, and delayed recall after 30min scores were significantly worse in the MD-AD and MCI-AD groups than in the CN group. MD-AD and MCI-AD groups did not differ significantly [in all cases Kruskal–Wallis test, p<0.05; *post hoc* analysis revealed significant differences between MD-AD and CN groups, and MCI-AD and CN groups (p<0.05) and no significant difference between MD-AD and MCI-AD groups (p>0.05)]. Duration of AD symptoms was a strong predictor of both immediate and delayed olfactory recognition memory scores.

Conclusion: Olfactory memory impairment was observed in patients with AD. The changes progress during the course of the disease. However, unlike verbal memory, olfactory memory is not significantly impaired in the prodromal stage of AD.

KEYWORDS

Alzheimer's disease, mild cognitive impairment – MCI, olfactory impairment, olfaction, olfactory memory

1. Introduction

The prevalence of Alzheimer's disease (AD) and other dementias has increased by 160.84% in the 30 years from 1990 to 2019 and continues to increase (1). Consequently, the social and economic impacts of the disease are becoming major issues, making it a global healthcare priority (2, 3).

To achieve accurate diagnosis and identify effective treatment methods, a deeper understanding of the pathogenesis and progression of the disease is required. The amyloid β cascade hypothesis was proposed more than 30 years ago and has been continuously investigated ever since (4, 5). Another hallmark of AD is the hyperphosphorylation of tau proteins and formation of neurofibrillary tangles (6). However, AD is a complex condition that cannot be explained exclusively by these mechanisms. Many other processes are proposed as contributing to AD, especially neuroinflammation and mitochondrial dysfunction (6, 7). Nevertheless, despite extensive research on the subject, the pathogenesis of AD remains largely unknown. An imbalance of various neurotransmitters is observed in patients with AD, with cholinergic deficit being the most recognized feature (7). As cholinergic pathways are involved in various processes, including memory and olfactory information processing, a deeper understanding of AD symptoms and their progression would help gain more insight into the processes of the disease.

Olfactory impairment has recently gained attention as a common and early sign of AD that precedes cognitive decline by several years (8–10). However, most studies have focused on odor identification testing, and other olfactory functions have rarely been analyzed. Especially sparse are data regarding olfactory memory.

Animal studies have yielded promising results. Olfactory memory deficits have been observed in mouse models of AD (11, 12). Olfactory memory impairment and altered functioning of olfactory network was also found in apolipoprotein E ε4 (ApoE4) knock-in mice (13). Studies on cognitively unimpaired human subjects at higher risk of AD (ApoE4 carriers) have confirmed these findings. ApoE4 carriers have impaired olfactory memory abilities (14, 15) and altered activation on functional magnetic resonance imaging (fMRI) during olfactory memory tasks (16). In healthy elderly subjects reduced olfactory memory abilities were found to be associated with deficits in executive functioning (17).

However, few studies have been conducted in patients with AD. Furthermore, the results are inconsistent, with some authors finding olfactory memory to be affected in patients with AD (18, 19), while others found only odor identification to be impaired, with no deficits in olfactory memory (20). The lack of research on olfactory memory probably stems from the difficulties in assessing this function. Typically, odor familiarity ratings or various odor recognition tasks are employed for this purpose (18, 21, 22). However, there are no universally accepted methods for assessing olfactory memory, with some authors even measuring the verbal recall of previously presented odors (23).

The objective of our study was to address this knowledge gap and investigate olfactory memory function in patients with early stage AD. We further aimed to investigate the relationship between olfactory and verbal memory, as well as other clinical features. Since the anatomical structures involved in olfactory and verbal memory processes differ, collecting more data regarding specific patterns of olfactory and verbal memory impairment in patients with AD would help gain more insight into the progression of AD.

2. Materials and methods

2.1. Participants

Ninety participants were enrolled in the study: 30 cognitively normal older participants (CN), 30 with mild cognitive impairment due to Alzheimer's disease (MCI-AD), and 30 with mild dementia due to AD (MD-AD).

AD was diagnosed according to the National Institute on Aging-Alzheimer's Association (NIA/AA) criteria for probable AD (24). MCI-AD was diagnosed according to the NIA/AA criteria for MCI due to AD (25). The cognitively healthy older participants had no cognitive complaints or neurological disorders.

MD-AD patients had a Clinical Dementia Rating (CDR) total score of 1, MCI-AD patients had a CDR total score of 0.5, and CN participants had a CDR total score of 0.

The participants with MCI-AD and with MD-AD were recruited form the Memory Clinic in Vilnius University Hospital Santaros Klinikos. Cognitively normal older participants were recruited form the primary care clinic in the same hospital.

Patients were only enrolled in the study if they were treatment-naïve or were taking a stable dose of an acetylcholinesterase inhibitor (AChEI) for at least 3 months.

Participants were excluded from the study if they had: other central nervous system disorders; a Hachinski Ischemic Score ≥ 4, indicating possible significant cerebrovascular disease; or previous significant head trauma. Participants with psychiatric conditions such as psychosis, substance abuse, significant depression (Geriatric Depression Scale score > 9), and those taking psychoactive medications were also excluded. Participants with conditions potentially affecting olfaction were also excluded from the study (smoking, nasal trauma or surgery, significant exposure to volatile substances, and recent viral infections).

This study was approved by the Vilnius Regional Bioethics Committee (Approval Number 2021/6–1,355-830). All participants agreed to participate in the study, were informed of the study procedures, and provided written informed consent by signing relevant written informed consent forms.

2.2. Assessments of cognitive function

The Mini Mental State Examination (MMSE) was performed to evaluate global cognition.

For more detailed evaluation, the Alzheimer's Disease Assessment Scale–Cognitive Subscale (ADAS-Cog) was administered. Additionally, delayed recall was evaluated after 5 min and after 30 min.

The verbal fluency score (VFS), comprising phonemic verbal fluency (PAS) and categorical verbal fluency (animals), was also tested.

Severity of symptoms was quantified using the CDR scale.

2.3. Assessment of olfactory memory

The olfactory memory assessment task was designed using odors from the standard validated Sniffin' Sticks odor identification test (Burghart, Wedel, Germany).

The Sniffin' Sticks odor identification test consists of 16 odors presented in felt-tip pens.

In the encoding phase of the olfactory memory task, five odors were randomly assigned to each participant (target odors). Each of these five odors was presented for 3 s with the tip of the pen placed approximately 2 cm in front of both nostrils. The participants were instructed to memorize the odors without verbal clues.

Immediate olfactory recognition memory was assessed immediately after the encoding phase. Five new odors were randomly assigned to each participant (distractors). Distractors were presented with the target odors in a randomized manner. Each of the 10 odors was presented for 3 s with the tip of the pen placed approximately 2 cm in front of both nostrils. Participants were instructed to choose whether the odor was new or presented previously (target odor). The immediate odor recognition score was the number of correct answers (0–10).

Delayed olfactory recognition memory was tested 30 min after the encoding phase. Five new odors were randomly assigned to each participant (second group of distractors). Distractors were presented with the target odors in a randomized manner. Each of the 10 odors was presented for 3 s with the tip of the pen placed approximately 2 cm in front of both nostrils. Participants were instructed to choose whether the odor was new or presented previously (target odor). The delayed odor recognition score was the number of correct answers (0–10).

A time interval of 30 s was kept between odors.

All participants were instructed not to drink or eat anything for at least 15 min prior to testing. The examiner wore odorless gloves and the participants wore a blindfold.

2.4. Data analysis

Statistical analysis was performed with IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, United States).

The normality of the data distribution was tested using the Shapiro–Wilk test. Differences between groups were analyzed using a two-tailed chi-square test (categorical variables), Mann–Whitney U test (numerical variables, comparison between two groups), and Kruskal–Wallis test (numerical variables, comparison between three groups).

The Spearman rank correlation coefficient was used to determine correlation between variables.

Linear regression models were created to analyze the predictions of continuous variables.

A value of p of <0.05 was considered statistically significant.

3. Results

3.1. Demographic and clinical characteristics

Cognitively normal older participants (CN), patients with mild cognitive impairment due to Alzheimer's disease (MCI-AD), and patients with mild dementia due to Alzheimer's disease (MD-AD) did not differ according to sex (two-tailed chi-square test, $p > 0.05$). There were also no differences according to education, Geriatric Depression Scale (GDS) results, or Hachinski Ischemic Score (HIS) (Kruskal–Wallis test, $p > 0.05$).

The CN and MCI-AD groups did not differ in age. The median age of the MD-AD group was significantly higher (Kruskal–Wallis $p < 0.05$; *post hoc* analysis revealed no significant difference between the CN and MCI-AD groups, and significant differences between the CN and MD-AD, and MCI-AD and MD-AD groups; Cohen's $d = 0.906$).

As expected, patients with MD-AD had a significantly longer duration of AD symptoms than those with MCI-AD (Mann–Whitney U test, $p < 0.001$; Cohen's $d = 1.132$). There were significantly more patients taking AChEIs in the MD-AD group than in the MCI-AD group (two-tailed chi-square test, $p < 0.05$).

The demographic and clinical characteristics of the participants are presented in Table 1.

3.2. Cognitive characteristics

The three groups differed significantly in cognitive assessment tasks (CDR sum of boxes, MMSE, ADAS-Cog, and combined VFS) (in all cases, Kruskal–Wallis test $p < 0.05$, and *post hoc* analysis revealed significant differences between all three groups).

Results of cognitive tests are presented in Table 2.

Verbal memory was also analyzed. Immediate recall (third trial on the ADAS-Cog word recall task), delayed recall after 5 min, and delayed recall after 30 min all differed significantly between the CN and MCI-AD groups, and the CN and MD-AD groups, but did not differ significantly between the MCI-AD and MD-AD groups [in all three cases, Kruskal–Wallis test $p < 0.05$; *post hoc* analysis revealed significant differences between the CN and MCI-AD groups, and CN and MD-AD groups ($p < 0.05$), and no significant difference between the MCI-AD and MD-AD groups ($p > 0.05$); Cohen's $d = 2.518$, 2.992 and 3.108, respectively].

The results [median and interquartile range (IQR)] of immediate recall in the CN, MCI-AD, and MD-AD groups were 8 (8–9), 6 (5–7), and 5 (4–6), respectively. Results of delayed recall after 5 min were 7

TABLE 1 Demographic and clinical characteristics of the participants.

	CN (N=30)	MCI-AD (N=30)	MD-AD (N=30)
Male, n (%)*	13 (43.33%)	13 (43.33%)	12 (40%)
Age (years)**	74 (68.75–76)	72 (67.75–77.25)	78 (75–79.25)
Years of education*	15 (13.5–16)	16 (14–16)	16 (13–16)
HIS*	1 (0–1)	1 (0–1)	1 (1–1.25)
GDS*	5.5 (4–6.25)	5.5 (4–6)	5 (4–6.25)
Duration of AD symptoms (in years)***	N/A	3 (2–3)	4 (3–5)
Use of AChEI, n (%)***	N/A	3 (10%)	14 (46.7%)

CN, cognitively normal; MCI-AD, mild cognitive impairment due to Alzheimer's disease; MD-AD, mild dementia due to Alzheimer's disease; HIS, Hachinski Ischemic Score; GDS, geriatric depression scale; AChEI, acetylcholinesterase inhibitor. Data are presented as median and interquartile range unless otherwise specified.
*Groups do not differ significantly.
**MD-AD group differs significantly from CN and MCI groups. CN and MCI groups do not differ significantly from each other.
***MD-AD group differs significantly from MCI-AD group.

TABLE 2 Cognitive assessment of the participants.

	CN (*N*=30)	MCI-AD (*N*=30)	MD-AD (*N*=30)
CDR sum of boxes*	0 (0–0)	2 (1.5–2.5)	5 (4.5–5.5)
MMSE*	29 (29–30)	26 (25–26)	22 (21–23)
ADAS-Cog*	5.33 (4.59–7)	11.33 (9.17–13.75)	17.67 (15.17–20.33)
VFS*	57.5 (43–63)	41 (35–50.75)	29.5 (21–39)

CN, cognitively normal; MCI-AD, mild cognitive impairment due to Alzheimer's disease; MD-AD, mild dementia due to Alzheimer's disease; CDR, clinical dementia rating; MMSE, mini mental state examination; ADAS-Cog, Alzheimer's disease assessment scale–cognitive subscale; VFS, combined verbal fluency score.
Data are presented as median and interquartile range.
*All three groups differ significantly.

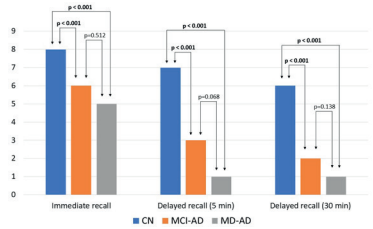


FIGURE 1
Results of verbal recall memory tasks in three groups of participants. Bars represent medians. Kruskal–Wallis test found the three groups to differ significantly ($p<0.05$). *Post hoc* analysis revealed significant differences between the CN and MCI-AD groups, and CN and MD-AD groups ($p<0.05$), and no significant difference between the MCI-AD and MD-AD groups ($p>0.05$). CN, cognitively normal; MCI-AD, mild cognitive impairment due to Alzheimer's disease; MD-AD, mild dementia due to Alzheimer's disease.

(6–7), 3 (1–4.25), and 1 (0–2), respectively. Results of delayed recall after 30 min were 6 (6–7), 2 (1–3.25), and 1 (0–1.25), respectively. The results of the verbal recall memory task are shown in Figure 1.

The results of the verbal memory tasks were compared between patients with AD (MCI-AD and MD-AD participants) taking AChEIs and treatment-naïve patients.

Patients on AChEIs did not differ from treatment-naïve patients in immediate recall [median and IQR 5 (4.5–6.5) and 6 (4–6), respectively; Mann–Whitney *U* test, $p=0.762$], delayed recall after 5 min [median and IQR 2 (1–2.5) and 2 (1–4), respectively; Mann–Whitney *U* test, $p=0.617$], or delayed recall after 30 min [median and IQR 1 (0–2) and 1 (0–3), respectively; Mann–Whitney *U* test, $p=0.852$] tasks. However, the duration of AD symptoms was significantly longer in patients taking AChEIs than in those who were not [median and IQR 4 (3.5–5) and 3 (2–3), respectively; Mann–Whitney *U* test, $p<0.001$; Cohen's $d=1.323$].

Upon analyzing the separate groups, the results remained the same. In the MCI-AD group there were no significant differences between patients on AChEIs and patients not taking them in immediate recall [median and IQR 6 (5–6) and 7 (5–7), respectively; Mann–Whitney *U* test, $p=0.283$], delayed recall after 5 min [median and IQR range 4 (1–4) and 3 (1–4), respectively; Mann–Whitney *U* test, $p=0.554$], and delayed recall after 30 min [median and IQR 3 (0–3) and 2 (1–3), respectively; Mann–Whitney *U* test, $p=0.600$].

In the MD-AD group there were also no significant differences between patients on AChEIs and patients not taking them in immediate recall [median and IQR 5 (4–6) and 4.5 (3.25–6), respectively; Mann–Whitney U test, $p=0.400$], delayed recall after 5 min [median and IQR 2 (0.75–2) and 1 (0–2.75), respectively; Mann–Whitney *U* test, $p=0.423$], and delayed recall after 30 min [median and IQR 1 (0–2) and 0 (0–1), respectively; Mann–Whitney *U* test, $p=0.179$].

The difference in the duration of AD symptoms between patients on treatment and untreated participants remained significant both in the MCI-AD [median and IQR 4 (3–4) and 2 (2–3), respectively; Mann–Whitney *U* test, $p=0.026$; Cohen's $d=0.85$], and in the MD-AD groups [median and IQR 6 (5–6) and 3 (3–4), respectively; Mann–Whitney *U* test, $p=0.01$; Cohen's $d=1.045$].

3.3. Olfactory memory characteristics

Olfactory immediate recognition memory and olfactory delayed recognition memory were significantly worse in patients with MD-AD than in those with MCI-AD or CN. The MCI-AD and CN groups did not differ significantly from each other [in both cases, Kruskal–Wallis test, $p<0.05$; *post hoc* analysis revealed significant differences between the MD-AD and MCI-AD groups and the MD-AD and CN groups ($p<0.05$), and no significant difference between the MCI-AD and CN groups ($p>0.05$); Cohen's $d=0.966$ and 0.852, respectively].

The median and IQR of immediate recognition memory in the MD-AD, MCI-AD, and CN groups were 7 (6–7), 8 (6–8.25), and 8 (7–9), respectively. Results of delayed recognition memory were 6 (5–6.25), 6.5 (5.75–8), and 7 (6–8), respectively. The results of the olfactory recognition memory tasks are shown in Figure 2.

In patients with AD (MCI-AD and MD-AD groups), olfactory immediate recognition memory scores were significantly correlated with the duration of AD symptoms (Spearman rho = −0.366, $p<0.05$), CDR sum of boxes (Spearman rho = −0.328, $p<0.05$), and VFS (Spearman rho = 0.355, $p<0.05$). Olfactory delayed recognition memory scores correlated significantly with the duration of AD symptoms (Spearman rho = −0.360, $p<0.05$), CDR sum of boxes (Spearman rho = −0.317, $p<0.05$), VFS (Spearman rho = 0.303, $p<0.05$), and delayed verbal recall after 5 min (Spearman rho = 0.258, $p<0.05$). Neither olfactory immediate recognition memory scores, nor olfactory delayed recognition memory scores correlated significantly with age (Spearman rho = −0.194, $p=0.138$ and Spearman rho = −0.226, $p=0.082$, respectively).

Multiple linear regression models were created to determine which variables that correlated with olfactory memory scores in AD patients could significantly predict them. Additionally, age, sex, and GDS scores were included in the models as factors known to influence memory and olfaction in the general population.

In the model with age, sex, GDS score, duration of AD symptoms, CDR sum of boxes, and VFS as independent variables and olfactory immediate recognition as the dependent variable, the overall regression was significant ($R^2=0.247$, $F=2.904$, $p=0.016$). The strongest predictor was duration of AD symptoms [$\beta=-0.261$, $B=-0.288$, 95% Confidence interval (95% CI) (−0.597, 0.02); $p=0.066$]. In a stepwise regression model, duration of AD symptoms

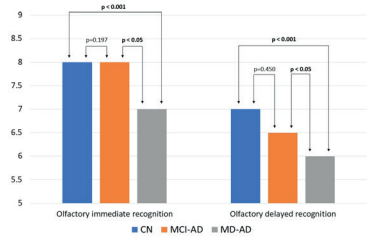


FIGURE 2
Results of olfactory recognition memory tasks in three groups of participants. Bars represent medians. Kruskal–Wallis test found the three groups to differ significantly ($p<0.05$). *Post hoc* analysis revealed significant differences between the MD-AD and MCI-AD groups and the MD-AD and CN groups ($p<0.05$), and no significant difference between the MCI-AD and CN groups ($p>0.05$). CN, cognitively normal; MCI-AD, mild cognitive impairment due to Alzheimer's disease; MD-AD, mild dementia due to Alzheimer's disease.

[$\beta=-0.290$, $B=-0.321$, 95% CI (−0.59, −0.051); $p=0.021$] and VFS [$\beta=0.306$, $B=0.033$, 95% CI (0.007, 0.06); $p=0.015$] remained the only significant predictors of olfactory immediate recognition memory score.

In the model with age, sex, GDS score, duration of AD symptoms, CDR sum of boxes, VFS, and delayed verbal recall after 5 min as independent variables and olfactory delayed recognition as dependent variable, the overall regression was also significant ($R^2=0.232$, $F=2.249$, $p=0.045$). The strongest predictor was duration of AD symptoms as well [$\beta=-0.302$, $B=-0.309$, 95% CI (−0.609, −0.010); $p=0.043$]. In a stepwise regression model duration of AD symptoms [$\beta=-0.291$, $B=-0.299$, 95% CI (−0.553, −0.044); $p=0.022$] and VFS [$\beta=0.268$, $B=0.027$, 95% CI (0.002, 0.052); $p=0.035$] remained the only significant predictors of olfactory delayed recognition memory score.

The results of the olfactory memory tasks were compared between patients with AD (MCI-AD and MD-AD participants) taking AChEIs and treatment-naïve patients. Patients on AChEIs had significantly worse results than treatment-naïve patients in both olfactory immediate recognition [median and IQR 6 (6–7) and 7 (6–8), respectively; Mann–Whitney *U* test, $p=0.024$; Cohen's $d=0.592$] and olfactory delayed recognition [median and IQR 6 (6–6) and 6 (5–6), respectively; Mann–Whitney *U* test, $p=0.025$; Cohen's $d=0.585$] tasks.

Analysis of the separate groups revealed that these differences were no longer significant at the group level. In the MCI-AD group, there were no significant differences between patients on AChEIs and patients not taking them, neither in olfactory immediate recognition [median and IQR 6 (6–6) and 8 (6–8) respectively; Mann–Whitney *U* test, $p=0.600$], nor in olfactory delayed recognition [median and IQR 6 (5–6) and 7 (6–8), respectively; Mann–Whitney *U* test, $p=0.387$].

In the MD-AD group, there were also no significant differences between patients on AChEIs and those not taking them, neither in olfactory immediate recognition [median and IQR 6 (5.75–7) and 7 (6–7), respectively; Mann–Whitney *U* test, $p=0.208$], nor in olfactory delayed recognition [median and IQR 5.5 (5–6) and 6 (5–7), respectively; Mann–Whitney *U* test, $p=0.313$].

4. Discussion

We found olfactory recognition memory to be impaired in AD patients in the current study. This confirms the findings of other authors who also found olfactory memory to be worsened in AD (18, 19). Although not all studies in patients with AD confirm this finding (20), animal studies suggest that impairment of olfactory memory is indeed a feature of AD (11, 12).

Data on patients with early-stage AD are limited. In a study performed in 2008, authors found no olfactory memory deficits in prodromal AD (MCI patients) (20). However, in a more recent work, researchers revealed olfactory memory to be impaired in MCI, as well as in patients with subjective cognitive decline (SCD) (18). In the current study, we found olfactory recognition memory (immediate, as well as delayed) to be impaired in patients with mild dementia, however, performance of MCI-AD participants did not differ significantly from cognitively normal participants.

Olfactory recognition memory (immediate, as well as delayed) scores correlated with the duration of the symptoms in AD patients. Furthermore, multiple linear regression analysis showed that duration of AD symptoms was a strong predictor of olfactory recognition memory (immediate, as well as delayed) scores. Thus, we can conclude that olfactory memory is a symptom of AD and that deficits in olfactory memory progress during the course of the disease. However, these changes did not reach the level of statistical significance during the prodromal stage of the disease (MCI due to AD).

However, verbal memory testing yielded different results. Verbal recall memory (immediate, as well as delayed) was already significantly impaired in MCI-AD patients. However, the patients with MD-AD did not differ significantly from those with MCI-AD. This is not surprising as episodic verbal memory impairment is an early and prominent symptom of AD, with subtle changes occurring even at the preclinical stage (26). In this study, verbal memory deficits were highly pronounced in patients with MCI-AD. As a result, even though patients with mild dementia tended to have worse results than patients with MCI, especially in the delayed recall task, these differences did not reach statistical significance. In addition, the ADAS-Cog recall task may lack sensitivity for differentiating between patients with MCI and mild dementia, and more complex tasks are needed for this purpose. This is consistent with the results from previous studies, where the addition of a delayed recall task to the ADAS-Cog also increased the accuracy of testing MCI participants but did not improve the accuracy of testing AD patients with dementia (27).

Even today, the specific brain regions responsible for human olfactory memory are poorly understood; however, multiple structures are known to be involved in this process, and this network differs significantly from verbal memory (28–30). The results of our study also highlighted the differences between these two types of memory, as their impairment occurs at different stages of the disease.

In the current study, treatment-naïve patients showed better olfactory memory results than patients taking AChEIs. However, the duration of AD symptoms was significantly shorter in treatment-naive patients. Longer disease duration accounts for the more pronounced olfactory memory impairment in patients taking AChEIs. It is interesting to note that verbal memory did not differ significantly between patients taking AChEIs and those who were not, despite the longer duration of the disease. Thus, we can conclude that AChEI treatment had a significant effect on verbal memory and was able to compensate for the longer progression of the disease;

however, the same effect was not observed for olfactory memory. Thus, not only do olfactory and verbal memory deficits manifest in different patterns in patients with AD, but the response to cholinergic stimulation is also distinct.

In animal studies, cholinergic activation has been found to improve olfactory dysfunction (31–33). However, results regarding the impact of AChEIs on olfactory function in patients with AD are inconsistent. Some studies have found that olfactory function is improved by treatment with AChEIs (34, 35) and have even suggested that atropine challenge is indicative of a cognitive response to AChEI treatment; however, further studies did not confirm these findings (36, 37). It is important to note that odor identification was tested in these studies, and olfactory memory was not specifically analyzed. Thus, the different effects of AChEIs on olfactory identification and memory could not be excluded. In the current study, there was a significant difference in olfactory memory scores depending on AChEI status in the sample of all patients with AD. However, similar differences were not found in the MCI-AD and MD-AD groups separately, even though the difference in disease duration remained significant. Therefore, a positive effect of cholinergic stimulation on olfactory memory cannot be excluded, although it is not as substantial as its effect on verbal memory.

This study has a few limitations. First, the cross-sectional design limited the accuracy of the conclusions regarding longitudinal changes during the course of AD. Second, biomarkers of amyloid deposition and neuronal degeneration were not tested, thus preventing the analysis of their relationship with the findings. Finally, in order to confirm these results, further research with larger samples of participants is needed.

In conclusion, our findings indicated that olfactory memory is impaired in patients with AD. These deficits progress over the course of the disease. However, unlike verbal memory, olfactory memory is not significantly impaired in the prodromal stage of AD.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Vilnius Regional Bioethics Committee. The patients/participants provided their written informed consent to participate in this study.

Author contributions

EA: study design and concept, data collection and analysis, and manuscript editing. VS and GP-K: data collection, data analysis, and manuscript editing. GK: study design and concept, data analysis, and manuscript editing. All authors contributed to this article and approved the final manuscript.

Acknowledgments

The authors would like to thank all the participants who participated in this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Li X, Feng X, Sun X, Hou N, Han F, Liu Y. Global, regional, and national burden of Alzheimer's disease and other dementias, 1990–2019. *Front Aging Neurosci*. (2022) 14:937486. doi: 10.3389/fnagi.2022.937486

2. World Health Organization. *Global status report on the public health response to dementia*. Geneva: World Health Organization (2021).

3. Gauthier S, Rosa-Neto P, Morais JA. *Webster C world Alzheimer report 2021: journey through the diagnosis of dementia*. London, England: Alzheimer's Disease International (2021).

4. Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. *Science*. (1992) 256:184–5. doi: 10.1126/science.1566067

5. Selkoe DJ. The molecular pathology of Alzheimer's disease. *Neuron*. (1991) 6:487–98. doi: 10.1016/0896-6273(91)90052-2

6. Hane FT, Lee BY, Leonenko Z. Recent progress in Alzheimer's disease research, part 1: pathology. *J Alzheimers Dis*. (2017) 57:1–28. doi: 10.3233/JAD-160882

7. Khan S, Barve KH, Kumar MS. Recent advancements in pathogenesis, diagnostics and treatment of Alzheimer's disease. *Curr Neuropharmacol*. (2020) 18:1106–25. doi: 10.2174/1570159X18666200528142429

8. Marine N, Boriana A. Olfactory markers of depression and Alzheimer's disease. *Neurosci Biobehav Rev*. (2014) 45:262–70. doi: 10.1016/j.neubiorev.2014.06.016

9. Jobin B, Zahal R, Bussières EL, Frasnelli J, Boller B. Olfactory identification in subjective cognitive decline: a meta-analysis. *J Alzheimers Dis*. (2021) 79:1497–507. doi: 10.3233/JAD-201022

10. Wang Q, Chen B, Zhong X, Zhou H, Zhang M, Mai N, et al. Olfactory dysfunction is already present with subjective cognitive decline and deepens with disease severity in the Alzheimer's disease spectrum. *J Alzheimers Dis*. (2021) 79:585–95. doi: 10.3233/JAD-201168

11. Cassano T, Romano A, Macheda T, Colangeli R, Cimmino CS, Petrella A, et al. Olfactory memory is impaired in a triple transgenic model of Alzheimer disease. *Behav Brain Res*. (2011) 224:408–12. doi: 10.1016/j.bbr.2011.06.029

12. Young JW, Sharkey J, Finlayson K. Progressive impairment in olfactory working memory in a mouse model of mild cognitive impairment. *Neurobiol Aging*. (2009) 30:1430–43. doi: 10.1016/j.neurobiolaging.2007.11.018

13. Peng KY, Mathews PM, Levy E, Wilson DA. Apolipoprotein E4 causes early olfactory network abnormalities and short-term olfactory memory impairments. *Neuroscience*. (2017) 343:364–71. doi: 10.1016/j.neuroscience.2016.12.004

14. Gilbert PE, Murphy C. Differences between recognition memory and remote memory for olfactory and visual stimuli in nondemented elderly individuals genetically at risk for Alzheimer's disease. *Exp Gerontol*. (2004) 39:433–41. doi: 10.1016/j.exger.2004.01.001

15. Larsson M, Hedner M, Papenberg G, Seubert J, Bäckman L, Laukka EJ. Olfactory memory in the old and very old: relations to episodic and semantic memory and APOE genotype. *Neurobiol Aging*. (2016) 38:118–26. doi: 10.1016/j.neurobiolaging.2015.11.012

16. Kapoulea EA, Murphy C. Older, non-demented apolipoprotein ε4 carrier males show hyperactivation and structural differences in odor memory regions: a blood-oxygen-level-dependent and structural magnetic resonance imaging study. *Neurobiol Aging*. (2020) 93:25–34. doi: 10.1016/j.neurobiolaging.2020.04.013

17. Schlintl C, Schienle A. Reduced olfactory memory performance is associated with executive function deficits in older adults. *Exp Aging Res*. (2022):1–13. doi: 10.1080/0361073X.2022.2122651

18. Dhilla Albers A, Asafu-Adjei J, Delaney MK, Kelly KE, Gomez-Isla T, Blacker D, et al. Episodic memory of odors stratifies Alzheimer biomarkers in normal elderly. *Ann Neurol*. (2016) 80:846–57. doi: 10.1002/ana.24792

19. Gilbert PE, Barr PJ, Murphy C. Differences in olfactory and visual memory in patients with pathologically confirmed Alzheimer's disease and the Lewy body variant of Alzheimer's disease. *J Int Neuropsychol Soc*. (2004) 10:835–42. doi: 10.1017/s1355617704106024

20. Bahar-Fuchs A, Moss S, Pike K, Villemagne V, Masters C, Rowe C, et al. P1-153: the relationship between olfactory identification and memory and Abeta burden in Alzheimer's disease, mild cognitive impairment and healthy aging: a PIB-PET study. *Alzheimers Dement*. 2008:4. doi: 10.1016/j.jalz.2008.05.741

21. Croy I, Zehner C, Larsson M, Zucco GM, Hummel T. Test-retest reliability and validity of the Sniffin' TOM odor memory test. *Chem Senses*. (2015) 40:173–9. doi: 10.1093/chemse/bju069

22. Sorokowska A, Sabiniewicz A, Larsson M. TOM-32-an extended test for the assessment of olfactory memory. *J Neurosci Methods*. (2020) 344:108873. doi: 10.1016/j.jneumeth.2020.108873

23. Nordin S, Murphy C. Odor memory in normal aging and Alzheimer's disease. *Ann N Y Acad Sci*. (1998) 855:686–93. doi: 10.1111/j.1749-6632.1998.tb10646.x

24. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. (2011) 7:263–9. doi: 10.1016/j.jalz.2011.03.005

25. Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. (2011) 7:270–9. doi: 10.1016/j.jalz.2011.03.008

26. Lange KL, Bondi MW, Salmon DP, Galasko D, Delis DC, Thomas RG, et al. Decline in verbal memory during preclinical Alzheimer's disease: examination of the effect of APOE genotype. *J Int Neuropsychol Soc*. (2002) 8:943–55. doi: 10.1017/s1355617702870096

27. Sano M, Raman R, Emond J, Thomas RG, Petersen R, Schneider LS, et al. Adding delayed recall to the Alzheimer disease assessment scale is useful in studies of mild cognitive impairment but not Alzheimer disease. *Alzheimer Dis Assoc Disord*. (2011) 25:122–7. doi: 10.1097/WAD.0b013e3181f883b7

28. Tong MT, Peace ST, Cleland TA. Properties and mechanisms of olfactory learning and memory. *Front Behav Neurosci*. (2014) 8:238. doi: 10.3389/fnbeh.2014.00238

29. Meunier D, Fonlupt P, Saive AL, Plailly J, Ravel N, Royet JP. Modular structure of functional networks in olfactory memory. *NeuroImage*. (2014) 95:264–75. doi: 10.1016/j.neuroimage.2014.03.041

30. Cerf-Ducastel B, Murphy C. Neural substrates of cross-modal olfactory recognition memory: an fMRI study. *Neuroimage*. (2006) 31:386–96. doi: 10.1016/j.neuroimage.2005.11.009

31. Takahashi K, Tsuji M, Nakagawasai O, Katsuyama S, Miyagawa K, Kurokawa K, et al. Activation of cholinergic system partially rescues olfactory dysfunction-induced learning and memory deficit in mice. *Behav Brain Res*. (2021) 408:113283. doi: 10.1016/j.bbr.2021.113283

32. Mandairon N, Ferretti CJ, Stack CM, Rubin DB, Cleland TA, Linster C. Cholinergic modulation in the olfactory bulb influences spontaneous olfactory discrimination in adult rats. *Eur J Neurosci*. (2006) 24:3234–44. doi: 10.1111/j.1460-9568.2006.05212.x

33. Takahashi K, Tsuji M, Nakagawasai O, Katsuyama S, Hong L, Miyagawa K, et al. Donepezil prevents olfactory dysfunction and α-synuclein aggregation in the olfactory bulb by enhancing autophagy in zinc sulfate-treated mice. *Behav Brain Res*. (2023) 438:114175. doi: 10.1016/j.bbr.2022.114175

34. Velayudhan L, Lovestone S. Smell identification test as a treatment response marker in patients with Alzheimer disease receiving donepezil. *J Clin Psychopharmacol*. (2009) 29:387–90. doi: 10.1097/JCP.0b013e3181aba5a5

35. Devanand DP, Lentz C, Chunga RE, Ciarleglio A, Scodes JM, Andrews H, et al. Change in odor identification impairment is associated with improvement with cholinesterase inhibitor treatment in mild cognitive impairment. *J Alzheimers Dis*. (2017) 60:1525–31. doi: 10.3233/JAD-170497

36. Devanand DP, Liu X, Chunga RE, Cohen H, Andrews H, Schofield PW, et al. Odor identification impairment and change with cholinesterase inhibitor treatment in mild cognitive impairment. *J Alzheimers Dis*. (2020) 75:845–54. doi: 10.3233/JAD-200021

37. Motter JN, Liu X, Qian M, Cohen HR, Devanand DP. Odor identification impairment and cholinesterase inhibitor treatment in Alzheimer's disease. *Alzheimers Dement*. (2021) 13:e12158. doi: 10.1002/dad2.12158

LIST OF PUBLICATIONS NOT INCLUDED IN THE THESIS

1. Pakulaitė G, Regelskytė V, **Audronytė E**, Kuzmickienė J, Kaubrys G. *Alzheimerio ligos gydymo metodų paieška: klinikinių tyrimų kryptys = In search of Alzheimer's disease treatment methods: trends of clinical trials*. Neurologijos seminarai. 2018; 22(75): 21–30. doi: 10.29014/ns.2018.03.
2. **Audronytė E**, Sutnikienė V, Kaubrys G. *Demencija sergančių pacientų priežiūros rekomendacijos COVID-19 pandemijos metu = Dementia care recommendations during the COVID-19 pandemic*. Neurologijos seminarai. 2020; 24(84): 136–137. doi: 10.29014/ns.2020.19.
3. Urbonaitė GV, **Audronytė E**, Valaikienė J. *Užpakalinės grįžtamosios encefalopatijos sindromas: klinikinio atvejo pristatymas ir literatūros apžvalga = Posterior reversible encephalopathy syndrome: a case report and review of the literature*. Neurologijos seminarai. 2020; 24(85): 263–270. doi: 10.29014/ns.2020.34.
4. Grigaitė J, Šiaurytė K, **Audronytė E**, et al. *Novel In-Frame Deletion in HTRA1 Gene, Responsible for Stroke at a Young Age and Dementia-A Case Study*. Genes (Basel). 2021;12(12):1955. Published 2021 Dec 7. doi:10.3390/genes12121955

LIST OF PRESENTATIONS

1. „*Olfactory Dysfunction as a Biomarker for the Early Diagnosis of Alzheimer's Disease*“. Oral presentation. „Evoliucinė medicina: sveikata ir ligos besikeičiančioje aplinkoje (Evolutionary Medicine: Health and Diseases in Changing Environment)“. Vilnius, Lietuva, April 5-10, 2018.
2. „*Olfactory perception and memory as a biomarker for the early diagnosis of Alzheimers's disease*“. Oral presentation. „Baltic Congress of Neurology 2018“. Kaunas, Lietuva, September 6-8, 2018.
3. „*Olfactory dysfunction in Alzheimer's disease: a Possible Biomarker for the Early Diagnosis*“. Oral presentation. „10th Conference of the Lithuanian Neuroscience Association / LNA and 2nd International Symposium on Visual Physiology, Environment and Perception / VISPEP“. Vilnius, Lithuania, November 30 – December 1, 2018.
4. „*Odour Identification in Early Alzheimer's Disease*“. Poster presentation. „8th Congress of the European Academy of Neurology - Europe 2022“. Vienna, Austria, June 25-28, 2022.
5. „*Odor Identification and Discrimination as Markers of Early Alzheimer's Disease*“. Poster presentation. „Alzheimer's Association International Conference 2022“. San Diego, USA, July 31 – August 4, 2022.
6. „*Olfactory Measures as a Marker of Early Alzheimer's Disease*“. Oral presentation. „5th International Conference of Evolutionary Medicine“. Vilnius, Lithuania, August 24-27, 2022.

ANNEX

VILNIAUS REGIONINIS BIOMEDICININIŲ TYRIMŲ ETIKOS KOMITETAS
sui generis darinys prie VILNIAUS UNIVERSITETO

LEIDIMAS
ATLIKTI BIOMEDICININĮ TYRIMĄ

2021 06 29 Nr.2021/6-1355-830

Tyrimo pavadinimas:

Uoslės jutimo ir olfaktorinės atminties sutrikimų reikšmės ankstyvai Alzheimerio ligos diagnostikai tyrimas

Protokolo Nr.:	OLFAD-1
Versija:	1.2
Data:	2021 06 18
Informuoto asmens sutikimo forma:	1.3 (tiriamajam) 2021 06 18 1.3 (kontrolinės grupės tiriamajam) 2021 06 18 1.3 (partneriui) 2021 06 18
Pagrindinis tyrėjas:	**Eglė Audronytė**
Įstaigos pavadinimas: Adresas:	VšĮ Vilniaus universiteto ligoninė Santaros klinikos Santariškių g. 2, Vilnius
Leidimas galioja iki:	**2026 03**

Leidimas išduotas Vilniaus regioninio biomedicininių tyrimų etikos komiteto posėdžio, vykusio 2021 m. birželio 29 d. sprendimu (protokolas Nr. 2021/6)

Pirmininkas doc. dr. Alfredas Laurinavičius

Viešoji įstaiga
Universiteto g. 3
01513 Vilnius

Duomenys kaupiami ir saugomi
Juridinių asmenų registre
Kodas 211950810

Komiteto duomenys:
M. K. Čiurlionio g. 21, LT- 03101 Vilnius
Tel. (8 5) 268 6998, el. p. rbtek@mf.vu.lt

NB!

Leidimas galioja iki biomedicininio tyrimo dokumentuose nurodytos biomedicininio **tyrimo pabaigos datos.**

Biomedicininių tyrimų užsakovas, jo įgaliotas atstovas ir (ar) pagrindinis tyrėjas per **30 kalendorinių dienų** privalo raštu pranešti leidimą atlikti biomedicininį tyrimą išdavusiai institucijai apie tyrimo **pabaigą** ir per **90 kalendorinių dienų** pateikti tyrimo vykdymo **ataskaitos santrauką**.

Biomedicininių tyrimų užsakovas, užsakovo įgaliotas atstovas ir (ar) pagrindinis tyrėjas, norėdami **atlikti** su biomedicininiu tyrimu susijusių **dokumentų pakeitimus**, turi gauti leidimą išdavusios institucijos **pritarimą**.

Lietuvos Respublikos sveikatos apsaugos ministro įsakymas 2010 m. gegužės 6 d. Nr. V-406 „Dėl Lietuvos Respublikos sveikatos apsaugos ministro 2008 m. sausio 4 d. įsakymo Nr. V-2 „Dėl leidimų atlikti biomedicininį tyrimą išdavimo tvarkos aprašo patvirtinimo" papildymo (*Valstybės žinios*, 2010-05-13, Nr. *55-2706*).

NOTES

Vilnius University Press
9 Saulėtekio Ave., Building III, LT-10222 Vilnius
Email: info@leidykla.vu.lt, www.leidykla.vu.lt
bookshop.vu.lt, journals.vu.lt
Print run copies 30