Odor identification impairment and cholinesterase inhibitor treatment in Alzheimer’s disease

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Abstract

Introduction: This study evaluated acute change in odor identification following atropine nasal spray challenge, and 8-week change in odor identification ability, as a predictor of long-term improvement in patients with mild to moderate Alzheimer’s disease (AD) who received open-label cholinesterase inhibitor treatment.

Methods: In patients with clinical AD, the University of Pennsylvania Smell identification Test (UPSIT) was administered before and after an anticholinergic atropine nasal spray challenge. Patients were then treated with donepezil for 52 weeks.

Results: In 21 study participants, acute atropine-induced decrease in UPSIT was not associated with change in the Alzheimer’s Disease Assessment Scale - Cognitive Subscale (ADAS-Cog) or Selective Reminding Test (SRT). Decline in odor identification performance from baseline to week 8 was indicative of a future decline in cognitive performance over 52 weeks.

Discussion: Change in odor identification with atropine challenge is not a useful predictor of treatment response to cholinesterase inhibitors. Short-term change in odor identification performance needs further investigation as a potential predictor of cognitive improvement with cholinesterase inhibitor treatment.

KEYWORDS
acetylcholine, Alzheimer’s disease, atropine, odor identification, olfaction

1 INTRODUCTION

Alzheimer’s disease (AD) brain pathology is characterized by neurofibrillary tangles and amyloid plaques. In the early stages of the disease, these pathognomonic signs of AD, particularly neurofibrillar tangles, are found in the olfactory bulb and tract, and cholinergic neurons start to degenerate. Cholinergic neurons are prominent in the olfactory bulb and entorhinal cortex; therefore, deficits in odor identification in early AD may indicate the loss of cholinergic inputs to the olfactory brain regions and higher order projection areas for olfactory processing, including the anterior olfactory nucleus, orbitofrontal cortex, piriform cortex, amygdala, entorhinal cortex, and hippocampus. These deficits manifest clinically as poor performance on tests of standardized odor identification.

The cholinergic system uses the neurotransmitter acetylcholine (ACh), which plays a significant role in learning and memory processes. As AD progresses, the activity of ACh becomes greatly reduced, contributing to memory deficits, which are the hallmark of the disease. The cholinergic hypothesis posits that AD onset and progression relate to the decrease in ACh. Acetylcholinesterase inhibitors (AChEs) increase...
the availability of ACh in the synapse and have modest efficacy above placebo for improving cognition in AD. A meta-analysis of 19 ACheI trials for patients with AD found a mild-moderate effect (d = -0.38) favoring the ACheI donepezil over placebo for improving global cognition. However, all trials except one had a duration of 6 months or less; therefore it is unclear how long gains are sustained. Brain imaging predictors of cognitive improvement on cholinesterase inhibitors have been inconsistent across studies, and identifying a simple peripheral marker of likely improvement may have clinical application.

Atropine is an anticholinergic drug that acts primarily on muscarinic receptors and may have potential for identifying individuals with preclinical AD. Administration of atropine as a nasal spray is an anticholinergic “challenge” that can be made to cross the “nose-brain barrier” by positioning the individual in the “Mecca” position. Using this approach, atropine has been shown to cause a temporary decrease in odor identification performance in patients with underlying AD pathology. In a sample of 56 elderly individuals (14 probable AD, 13 cognitive impairment no dementia, 29 cognitively intact), decline in odor identification scores from pre- to post-atropine nasal spray challenge was strongly correlated with lower memory performance and reduced magnetic resonance imaging (MRI) hippocampal volume. The atropine effect of reducing odor identification may be more pronounced in patients with already compromised cholinergic pathways, as in AD. Therefore, the degree of reduction in odor identification test scores following atropine nasal spray would serve as an indicator of reduced cholinergic neurotransmission, whereby individuals with the greatest reduction in odor identification stand to benefit the most from a medication that blocks the breakdown of ACh and increases ACh synaptic availability. Taken together, the atropine effect could serve as a prognostic indicator for who will respond to ACheI treatment.

The current study aimed to evaluate acute change in odor identification following atropine nasal spray challenge as a predictor of long-term improvement in patients with mild to moderate AD who receive ACheI (donepezil) treatment. Results for patients with mild cognitive impairment (MCI) from an independent sample enrolled in a separate study conducted in parallel have been reported previously. In the current study of patients with mild to moderate AD treated with donepezil, we hypothesized that: (1) the atropine effect (an acute decrease in odor identification test scores from pre- to post-atropine challenge at baseline) would predict improved cognitive and global functioning from baseline to weeks 26 and 52; and (2) increase in odor identification test scores after 8 weeks would predict improved cognitive and global functioning from baseline to weeks 26 and 52.

RESEARCH IN CONTEXT

1. Systematic review: The authors reviewed the published literature (eg PubMed, Scopus) on nasal atropine challenge and Alzheimer’s disease (AD). Atropine produces temporary odor identification deficits that are more pronounced in individuals with AD biomarkers; however, the utility of atropine as a prognostic indicator for AD patients undergoing cholinesterase inhibitor treatment is unclear, as previous null trials are limited and have focused on patients with mild cognitive impairment.

2. Interpretation: In the present study, immediate change in odor identification performance following nasal atropine challenge was not a useful predictor of treatment response to cholinesterase inhibitors. Decline in odor identification performance over the first 8 weeks of the trial was related to decline in cognitive performance over the 52-week trial.

3. Future directions: Although atropine challenge was not prognostically informative, expanded investigation of acute change in odor identification during cholinesterase treatment is needed.

METHODS

2.1 Participants

This study was approved by the New York State Psychiatric Institute (NYSPI)/Columbia University Institutional Review Board (IRB). The trial is registered on clinicaltrials.gov (identifier: NCT01951118). Participants were recruited from the Memory Disorders Clinic at NYSPI and the Behavioral Neurology practice at Columbia University Medical Center (CUMC), and by advertising in local media. Recruitment began in October 2013, with the final patient completing the trial in March 2019. Inclusion criteria were age 55-95 years, diagnosis of probable AD by National Institute on Neurological and Communicative Diseases and Stroke/Alzheimer’s Disease and Related Disorders Association criteria and core clinical diagnosis of “Probable AD dementia” by the new National Institute on Aging (NIA) criteria, Folstein Mini-Mental State Exam (MMSE) 18-27 of 30, availability of an informant, and ability to provide informed consent.

Exclusion criteria included current use of cholinesterase inhibitors, history of intolerance or contraindication to donepezil, and use of medications with anticholinergic properties including diphenhydramine, tricyclic antidepressants, and antipsychotics. Benzodiazepine use in lorazepam dose equivalents less than 2 mg daily was permitted. Other
exclusion criteria were severe unstable medical illness; specific neuro-
ological disorders including Parkinson disease, multiple sclerosis, and
stroke with residual neurological deficits; psychotic disorders including
schizophrenia, bipolar disorder, and schizoaffective disorder; alcohol/
substance dependence in the past 6 months; current major depression;
and suicidality. Exclusion criteria for olfaction testing were current
smoker > 1 pack daily, current upper respiratory infection, nasal
trauma or sinus surgery, and head trauma with loss of consciousness.

2.2 Measures

The screening visit comprised a medical, psychiatric, and neurological
evaluation; cognitive assessment to determine inclusion criteria; and
blood was drawn for hematocrit, electrolytes, liver, kidney, and thyroid
function tests, folate, vitamin B12 levels, and urinalysis to exclude pri-
mary medical causes of cognitive impairment.

The subsequent baseline (week 0) visit involved assessment with the
two main cognitive outcome measures: Alzheimer’s Disease Assess-
ment Scale - Cognitive Subscale (ADAS-Cog, 11-item version)18 and
12-item 6-trial Selective Reminding Test (SRT).19 For diagnostic pur-
poses, neuropsychological tests in the National Alzheimer’s Coordinat-
ing Center-Uniform Data Set (NACC–UDS) battery were administered:
Wechsler Memory Scale–III digit span forward and backward20; Wech-
sler Adult Intelligence Scale-Revised digit symbol21; Trail Making A and
B22; verbal fluency using the letters C, F, and L and animal, vegetable,
and fruit list generation22; and Boston Naming Test (60 items).23 If a
participant’s preferred language was Spanish, neuropsychological
tests were administered in Spanish using standardized versions that
have been validated in other studies.6 Otherwise, tests were adminis-
tered in English and preferred to be tested in English. The study physi-
cian completed the NACC clinical assessment, Clinician’s Interview
Based Impression of Change-plus (CIBIC-plus) and Clinician Interview
Based Impression of Severity (CIBIS) global assessment ratings,24 Clin-
ical Dementia Rating (CDR),25 and the Treatment Emergent Symptoms
Scale (TESS).26 which evaluates 26 common somatic side effects that
include gastrointestinal and central nervous system (CNS) side effects
known to occur with AChel. The study physician completing these mea-
sures remained blind to the cognitive outcome measures. An inform-
ant completed the Pfeffer Functional Activities Questionnaire (FAQ).27
Apolipoprotein E genotyping was conducted at Lgc Genomics, a ref-
erece laboratory. To determine AD diagnostic eligibility, two experi-
enced raters (Drs. Devanand and Stern) made a consensus diagnosis
while remaining blind to scores on predictor (UPSIT) and cognitive out-
come (ADAS-Cog total score and SRT total immediate recall) measures.

2.3 Olfactory assessment

The University of Pennsylvania Smell Identification test (UPSIT) was
administered. This standardized scratch and sniff test consists of 40
booklet pages with a single odor embedded in a microcapsule on each
page. Scratching with a pencil releases the odor and the participant
checks one of 4 choices, for example, chocolate, banana, onion, or fruit
punch. The total UPSIT score ranges from 0 (all answers incorrect) to
40 (all answers correct). At the baseline visit, immediately after UPSIT
administration, atropine solution 1 mg, with the dose divided approx-
imately equally between the two nostrils, was administered using the
“squirt system.”28 This was delivered via plastic tube attached to a
syringe while the patient reclined their head. The tube was placed in the
nasal cavity parallel to the nasal septum and directed toward the olfac-
tory cleft. Next, the patient assumed a crouching head-down posture
(the “Mecca” position) for 2 min to facilitate atropine crossing the crip-
riform plate into the olfactory bulb.34 The UPSIT was repeated 45 min
later to ensure sufficient time for the atropine to take effect.

2.4 Treatment

Research assessments were repeated at 8, 26, and 52 weeks, with
the exception of the diagnostic neuropsychological battery that was
repeated only at 26 and 52 weeks in order to reduce practice effects. At
baseline, donepezil was started at 5 mg daily followed by assessment at
4 weeks for tolerability before increasing the dose to 10 mg daily. This
dose was kept constant for the rest of the 52-week study. Patients who
could not tolerate donepezil 10 mg were maintained at 5 mg.

2.5 Statistical analyses

The baseline characteristics of the study sample were described by
mean and SD for continuous variables and percent and frequency
for categorical variables. Wilcoxon signed rank test was used to test
whether there is any change in UPSIT scores from pre- to post-atropine
challenge at week 0 (denoted as ΔUPSITa) and from pre-atropine
challenge at week 0 to 8 weeks of donepezil treatment (denoted as
ΔUPSITb). Spearman correlation coefficients examined bivariate asso-
ciations between baseline quantitative variables. The bivariate
association of ΔUPSITa and ΔUPSITb with baseline covariates were
evaluated using Spearman correlation and Kruskal-Wallis test for con-
tinuous and categorical baseline variables, respectively. The trajecto-
ries of UPSIT scores and cognitive measures were summarized using
mean ± SD. Fisher exact test and Wilcoxon rank sum test detected
differences between those who completed 52 weeks of follow-up and
those who dropped out for continuous and categorical baseline vari-
ables, respectively.

Linear models with repeated measures were applied to assess the
effect of acute change in odor identification following atropine nasal
spray challenge on the cognitive outcomes measured at baseline, 26,
and 52 weeks. Those outcomes include ADAS-cog, SRT, CIBIC-plus
SRT-delayed, and CIBIS. The ADAS-Cog was skewed and therefore
transformed to reduce the impact of extreme values. The general-
ized estimating equation (GEE) approach was employed to estimate
model parameters. For each outcome variable, patients with data missing at baseline were excluded from analysis. We choose GEE over mixed-effects models, as it is more robust to misspecification of correlation structure in repeated measures.

For each outcome, two models were considered. The first model started with the time indicator (week 0, 26, or 52), ΔUPSITa, their interaction, baseline pre-nasal challenge UPSIT score (UPSIT0), and any baseline variables that were significantly correlated with ΔUPSITa in the bivariate analysis. The non-zero coefficients of the time-by-ΔUPSITa interaction indicated whether a time trend in the outcome was modified by ΔUPSITa, or whether there was time-varying association between ΔUPSITa and outcome. The final model excluded non-significant interaction terms and non-significant baseline covariates other than UPSIT0. The second model was constructed similarly as the first model with ΔUPSITa replaced by ΔUPSITb wk. All statistical analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC).

3 RESULTS

Descriptive statistics for baseline demographic and clinical characteristics, neuropsychological assessments, and olfactory test scores are presented in Table 1 for patients with AD enrolled in the trial. The patients between 56 and 70 years of age had a mean age of 70.3 (SD 9.6) years, with 42.9% being female. Twenty patients remained in the trial at week 8 and 17 remained at week 52. Patients withdrew from the study for the following reasons: death due to natural causes, withdrew consent/no longer interested in participation, moved, and medical illness/moved. There were no significant differences in baseline characteristics between dropouts (n = 4) and those who completed 52 weeks of follow-up.

Bivariate analysis showed that smoking status was not associated with education (P = .80) or UPSIT0 (P = .81), whereas it tended to differ by baseline age (P = .085) and by sex (P = .076) in that 64% (7/11) non-smokers were female, and 60% (3/5) smokers and all 5 with unknown smoking status were male. UPSIT0 was not significantly associated with age, sex, education, MMSE, smoking status, donepezil dose, or apolipoprotein E (APOE) genotype (P > .10). Similarly, we did not observe significant associations between these variables and pre-post nasal challenge UPSIT score difference ΔUPSITa, as well as pre-to 8-week treatment UPSIT score difference ΔUPSITb wk. There was no significant change between pre- (M = 20.86, SD = 5.04) and post- (M = 19.76, SD = 5.73) nasal challenge UPSIT (t(20) = 1.51, P = 0.146). The atropine effect was variable, with n = 11 patients exhibiting a decrease in UPSIT, n = 5 remaining the same, and n = 5 exhibiting an increase in UPSIT.

Summary statistics of cognitive measures and UPSIT scores are given in Table 2. Cognitive measures varied over 52 weeks, with an increase in ADAS-Cog scores (denoting worse performance) and an increase in SRT total recall scores (denoting improved performance). ADAS-Cog score changed from mean 21.7 (SD 6.1) at baseline to 19.3 (SD 5.8) at 8 weeks, 19.6 (SD 7.2) at 26 weeks, and 22.7 (SD 9.0) at

### TABLE 1 Baseline clinical and demographic characteristics of 21 AD participants

| Variable          | % (n) or Mean± SD |
|-------------------|-------------------|
| Sex               |                   |
| Male              | 57.14 (12)        |
| Female            | 42.86 (9)         |
| Race              |                   |
| White             | 52.38 (11)        |
| African American  | 9.52 (2)          |
| Hispanic          | 23.81 (5)         |
| Asian             | 9.52 (2)          |
| Other             | 4.76 (1)          |
| Smoking status    |                   |
| Never             | 52.38 (11)        |
| Past              | 19.05 (4)         |
| Current           | 4.76 (1)          |
| Unknown           | 23.81 (5)         |
| Age in years      | 70.33 ± 9.63      |
| Years of schooling| 16.71 ± 3.20      |
| MMSE              | 23.048 ± 2.40     |
| UPSIT score at baseline | 20.86 ± 5.04 |
| Pre-post nasal challenge test |         |
| UPSIT score change| -1.10 ± 3.16      |
| UPSIT reduction >25%| 14.29% (3)       |
| Over first 8 weeks (n = 20) |                 |
| UPSIT score change| -2.40 ± 4.64*     |
| UPSIT reduction >25%| 20.0% (4)       |
| ADAS-Cog          | 21.67 ± 6.12      |
| SRT-Total         | 21.19 ± 8.23      |
| SRT-delayed       | 0.81 ± 1.44       |
| WAIS-R digit symbol (n = 19) | 25.47 ± 9.27 |
| FAQ (n = 16)      | 11.25 ± 6.19      |
| ECOG (n = 20)     | 90.80 ± 28.11     |
| CIBIS             | 3.62 ± 0.50       |
| TESS (n = 18)     | 1.89 ± 2.05       |

Abbreviations: ADAS-Cog, Alzheimer’s Disease Assessment Scale-cognitive subscale; ApoE, apolipoprotein E; CIBIC, Clinician’s Interview Based Impression of Change; CIBIS, Clinician Interview Based Impression of Severity; ECOG, Everyday Cognition Scale; FAQ, Functional Activities Questionnaire; MMSE, Mini-Mental Status Exam; SD, standard deviation; SRT, Selective Reminding Test; TESS, Treatment Emergent Symptoms Scale; UPSIT, University of Pennsylvania Smell Identification Test; WAIS-R, Wechsler Adult Intelligence Scale-Revised.

*Paired t-test to detect UPSIT score change: from baseline to week 8, P = .0320; pre-post nasal challenge test P = .1457.
TABLE 2  Summary statistics of cognitive measures, UPSIT score, and clinical variables by time

|                          | Baseline Mean ± SD (n) | Week 8 Mean ± SD (n) | Week 26 Mean ± SD (n) | Week 52 Mean ± SD (n) |
|--------------------------|------------------------|----------------------|-----------------------|-----------------------|
| UPSIT                    | 20.86 ± 5.04 (21)      | 18.26 ± 5.18 (20)    | 17.50 ± 5.19 (20)     | 17.40 ± 5.07 (15)     |
| Primary outcomes         |                        |                      |                       |                       |
| ADAS-Cog                 | 21.67 ± 6.12 (21)      | 19.30 ± 5.80 (20)    | 19.63 ± 7.15 (20)     | 22.69 ± 8.99 (15)     |
| SRT-Total                | 21.19 ± 8.23 (21)      | 24.10 ± 9.82 (20)    | 23.00 ± 8.07 (20)     | 24.38 ± 9.69 (16)     |
| CIBIC-plus               | —                      | 3.55 ± 0.69 (20)     | 3.75 ± 0.64 (20)      | 4.38 ± 0.96 (16)      |
| Secondary outcomes       |                        |                      |                       |                       |
| SRT-delayed              | 0.81 ± 1.44 (21)       | 1.25 ± 2.31 (20)     | 0.70 ± 1.34 (20)      | 0.75 ± 1.81 (16)      |
| CIBIS                    | 3.62 ± 0.50 (21)       | 3.70 ± 0.47 (20)     | 3.60 ± 0.50 (20)      | 3.88 ± 0.96 (16)      |

Abbreviations: ADAS-Cog, Alzheimer’s Disease Assessment Schedule-cognitive subscale; CIBIC, Clinician’s Interview Based Impression of Change; CIBIS, Clinician Interview Based Impression of Severity; SD, standard deviation; SRT, Selective Reminding Test; UPSIT, University of Pennsylvania Smell Identification Test.

52 weeks (Table 2). SRT total immediate recall score changed from 21.2 (SD 8.2) at baseline to 24.1 (SD 9.8) at 8 weeks, 23.0 (SD 8.1) at 26 weeks, and 24.4 (SD 9.7) at 52 weeks.

3.1 | Effect of acute decrease in UPSIT scores with atropine challenge

Effect of ΔUPSIT8wk on cognitive outcomes was presented in model A in Table 3. The ADAS-Cog model for the effect of ΔUPSIT8wk (change in UPSIT score due to nasal challenge) controlling for baseline UPSIT score was fit to 56 observations from 21 patients. The estimated coefficient of time variable indicated that ADAS-Cog significantly decreased (corresponding to improved performance) over 26 weeks from baseline (B = −0.13, 95% CI = −0.25 to −0.02), whereas not over 52 weeks, that is, the mean within-patient change in level of ADAS-Cog over 52 weeks was not significantly different from zero. ADAS-Cog score was unrelated to ΔUPSIT8wk (Table 3).

The SRT-Total model for the effect of ΔUPSIT8wk was fit to 57 observations from 21 patients. SRT-Total score increased significantly over time (improved) from baseline to 52 weeks (B = 2.36, 95% CI = 0.19 to 4.53). Baseline UPSIT score was unrelated to SRT-Total score. The change in UPSIT score due to atropine nasal challenge, ΔUPSIT8wk, was unrelated to SRT-Total score.

SRT Delayed Recall was a secondary outcome measure. The SRT-Delay model for the effect of ΔUPSIT8wk was fit to 57 observations from 21 patients. SRT-Delay scores were unchanged over time. Baseline UPSIT score was positively associated with SRT-Delay score, suggesting that lower UPSIT score at baseline was related to lower SRT-delayed score over time. SRT-Delay score over time was unrelated to ΔUPSIT8wk.

The CIBIC-plus model for the effect of ΔUPSIT8wk was fitted to 36 observations from 20 patients. There was positive change (increase) over time with larger change over 52 weeks than over 26 weeks. The change was unrelated to ΔUPSIT8wk. Baseline UPSIT scores were unrelated to change in CIBIC-plus scores over time.

3.2 | Effect of change in UPSIT scores from 0 to 8 weeks

Effect of ΔUPSIT0-8wk on cognitive outcomes was presented in model B in Table 3. The ADAS-Cog outcome model for the effect of ΔUPSIT0-8wk was fit to 55 observations from 20 patients because one patient had missing UPSIT at week 8. ADAS-Cog significantly decreased (corresponding to improved performance) over 26 weeks from baseline, whereas not over 52 weeks, as seen in the model for effect of ΔUPSIT8wk. Higher ADAS-Cog score was significantly associated with lower baseline UPSIT score (B = −0.03, 95% CI = −0.05 to −0.02). Negative ΔUPSIT0-8wk (decline in UPSIT over the first 8 weeks) was significantly associated with increased ADAS-Cog score over time (B = −0.02, 95% CI = −0.05 to −0.03).

The SRT-Total outcome model for the effect of ΔUPSIT0-8wk was fit to 56 observations from 20 patients because one patient had missing UPSIT at week 8. SRT-total score significantly increased over time (improved) from baseline to 52 weeks for patients with no change in UPSIT score over the first 8 weeks (B = 4.12, 95% CI = 2.36 to 5.89). The significant interaction of ΔUPSIT0-8wk by 52 weeks indicated that a unit decline in UPSIT over 8 weeks was associated with a decline in SRT-Total score (B = 0.63, 95% CI = 0.30 to 0.98) over 52 weeks.

The SRT-Delay outcome model for the effect of ΔUPSIT0-8wk was fit to 56 observations from 20 patients because one patient had missing UPSIT at week 8. SRT-Delay scores were unchanged over time. Baseline UPSIT score was positively associated with SRT-delayed score. SRT-Delay score over time was unrelated to ΔUPSIT0-8wk.

The CIBIC-plus model for the effect of ΔUPSIT0-8wk was fit to 36 observations from 20 patients. There was positive change (increase) in CIBIC-plus over time with larger change over 52 weeks than over 26 weeks. The change was unrelated to ΔUPSIT0-8wk. The negative coef-
### TABLE 3  Estimated coefficients with 95% confidence interval (CI) in the models for cognitive outcomes

|                       | Model A                                      | Model B                                      |
|-----------------------|----------------------------------------------|----------------------------------------------|
| log(ADAS-Cog)         | B (95% CI)                                   | B (95% CI)                                   |
| Week 26 vs 0          | -0.132 (-0.248, -0.016)*                     | -0.133 (-0.249, -0.016)*                     |
| Week 52 vs 0          | 0.045 (-0.087, 0.177)                       | 0.046 (-0.087, 0.178)                       |
| Baseline UPSIT        | -0.017 (-0.039, 0.0046)                      | -0.026 (-0.050, -0.002)*                     |
| ΔUPSIT_a              | -0.004 (-0.054, 0.047)                       | ---                                          |
| ΔUPSIT_{bk}           | ---                                          | -0.024 (-0.0448, -0.003)*                    |
| SRT-total             |                                              |                                              |
| Week 26 vs 0          | 1.510 (-0.6549, 3.6745)                      | 1.6649 (-0.553, 3.883)                       |
| Week 52 vs 0          | 2.360 (0.192, 4.527)*                        | 4.122 (2.357, 5.886)***                      |
| Baseline UPSIT        | 0.336 (-0.152, 0.823)                        | 0.585 (0.145, 1.026)*                        |
| ΔUPSIT_a              | -0.187 (-1.381, 1.008)                       | ---                                          |
| ΔUPSIT_{bk}           | ---                                          | 0.242 (-0.404, 0.887)                        |
| ΔUPSIT_{bk} by wk 26  | ---                                          | 0.090 (-0.186, 0.364)                        |
| ΔUPSIT_{bk} by wk 52  | ---                                          | 0.633 (0.300, 0.975)***                      |
| SRT-delay             |                                              |                                              |
| Week 26 vs 0          | -0.139 (-0.618, 0.341)                       | -0.150 (-0.636, 0.336)                       |
| Week 52 vs 0          | -0.273 (-0.803, 0.258)                       | -0.278 (-0.821, 0.266)                       |
| Baseline UPSIT        | 0.075 (0.023, 0.128)**                       | 0.084 (0.001, 0.167)*                       |
| ΔUPSIT_a              | 0.184 (-0.020, 0.387)                        | ---                                          |
| ΔUPSIT_{bk}           | ---                                          | 0.033 (-0.085, 0.151)                        |
| CIBIC-plus            |                                              |                                              |
| Week 52 vs 26         | 0.710 (0.315, 1.105)**                       | 0.718 (0.333, 0.102) **                      |
| Baseline UPSIT        | -0.044 (-0.099, 0.011)                       | -0.036 (-0.071, -0.002)*                     |
| ΔUPSIT_a              | 0.007 (-0.044, 0.059)                        | ---                                          |
| ΔUPSIT_{bk}           | ---                                          | 0.018 (-0.093, 0.129)                        |
| CIBIS                 |                                              |                                              |
| Week 26 vs 0          | -0.022 (-0.195, 0.152)                       | -0.050(-0.218, 0.118)                       |
| Week 52 vs 0          | 0.245 (-0.159,0.648)                        | 0.210 (-0.194, 0.614)                       |
| Baseline UPSIT        | 0.018 (-0.0280,0.665)                        | 0.024 (-0.035, 0.082)                       |
| ΔUPSIT_a              | -0.046 (-0.0990,0.007)                       | ---                                          |
| ΔUPSIT_{bk}           | ---                                          | -0.002 (-0.051,0.047)                       |

**Abbreviations:** ADAS-Cog, Alzheimer’s Disease Assessment Schedule-cognitive subscale; CI, confidence interval; CIBIC, Clinician’s Interview Based Impression of Change; CIBIS, Clinician Interview Based Impression of Severity; SRT, Selective Reminding Test; UPSIT, University of Pennsylvania Smell Identification Test. Outcome of ADAS-Cog was transformed by logarithmic function. Model A examines the effect of a predictor for the UPSIT score change in response to nasal challenge (ΔUPSIT_a) defined as pre-post nasal challenge UPSIT score difference at baseline. Model B examines the effect of a predictor for the UPSIT score change over the first 8 weeks from baseline pre-nasal challenge (ΔUPSIT_{8wk}).

*P < .05.
**P < .01.
***P < .001.
****P < .0001.

Acute decrease in odor identification performance following atropine challenge was not predictive of change in cognitive performance or functional measures of AD patients during the 52-week open treatment trial with donepezil. Our group previously reported atropine-induced decrease in UPSIT was associated with increased...
verbal memory (SRT total recall) and global improvement (CIBIC-plus), but not global cognition (ADAS-Cog) over 52 weeks in a sample of 37 patients with MCI treated with donepezil.29 These earlier findings were not replicated in a more recent trial with a larger sample of 100 MCI patients, where atropine-induced decrease in UPSIT was not associated with longitudinal change in any cognitive or functional outcome measures.7 Collectively, results of these studies do not support the use of atropine challenge to reliably improve selection of patients to receive clinical treatment with cholinesterase inhibitors, regardless of phase of clinically defined AD. One potential reason for this null finding is that atropine may not have reached the olfactory bulb. We could not localize or quantify the extent to which atropine crossed the cribriform plate, and given the nonsignificant change in UPSIT immediately following atropine challenge, it is possible that for some patients atropine did not reach the area required for its anticholinergic effects to fully manifest.

Although atropine-induced change in odor identification was not related to clinical outcomes, baseline pre-atropine odor identification was associated with baseline global cognition (ADAS-Cog) and verbal memory (SRT Total Score), consistent with the existing literature.30–32 Furthermore, decline in odor identification from baseline to week 8 was indicative of future decline in ADAS-Cog and SRT performance over 52 weeks. This finding raises the possibility that progressive decline in olfactory identification performance could be an indicator of disease progression and ACheI nonresponse in the long term. Olfactory deficits consistently precede cognitive decline in early phases of AD. Indeed, in longitudinal studies, impaired odor identification manifests before impairments in other cognitive domains and confers increased risk of conversion to dementia in community dwelling and MCI populations.33

A key limitation of this preliminary study is small sample size (n = 21) and absence of a placebo control condition. Another limitation is that the study was conducted with clinical diagnoses, and without biomarkers. This design consideration is balanced by the potential benefits of developing a cost-effective approach. The lack of an atropine placebo condition precludes us from estimating expected change in UPSIT performance immediately following a nasal challenge procedure. In the absence of a waitlist or placebo control for the treatment portion of the study, changes in cognitive test performance may be due in part to practice effects. Although practice effects likely influenced cognitive test performance as seen by the increase in SRT indices in the first 8 weeks, practice effects are generally absent for odor identification tests over short-term and long-term follow-up. There was no objective measure of olfactory functioning as an anosmic-based exclusionary criterion. It is possible that some patients already had significantly reduced olfactory capabilities to the point where atropine would not reduce them further. Another limitation is that there was no statistical correction performed for multiple samples analyzed in parallel across multiple studies from the same working group.

In conclusion, these results do not support the use of atropine challenge as a prognostic indicator for patients with AD treated with cholinesterase inhibitors. These results align with previous findings that odor identification performance is related to global cognition and verbal memory, and that short-term decline in odor identification indicates risk of long-term cognitive decline. Further work, including larger longitudinal studies, is needed to explore the value of repeated olfactory assessments in predicting cognitive and functional changes with ACheI treatment in AD.

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CONFLICT OF INTEREST
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