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RESEARCH ARTICLE

Diagnostic performance of light reflex pupillometry in Alzheimer's disease

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Abstract

Easily applied diagnostic tools such as digital biomarkers for Alzheimer's disease (AD) are urgently needed due to the recent approval of disease-modifying therapies. We aimed to determine the diagnostic performance of hand-held, quantitative light reflex pupillometry (qLRP) in patients with AD in a proof-of-concept, cross-sectional study. Participants underwent qLRP at a university memory clinic from August 2022 to October 2023. We fitted multivariable logistic regression models with qLRP, sex, and age as predictors evaluated with area under the receiver operating characteristics curve (AUROC). In total, 107 patients with AD, 44 patients with mixed AD and vascular cognitive dysfunction (VCD), 53 patients with dementia with Lewy bodies (DLB), and 50 healthy controls (HCs) were included. Our diagnostic models showed similar discriminatory ability (AUROC range 0.74-0.81) when distinguishing patients with AD from HCs and other dementias. The qLRP seems promising as a bedside digital biomarker to aid in diagnosing AD.

KEYWORDS
Alzheimer's disease, diagnosis, digital biomarker, light reflex, proof-of-concept, quantitative pupillometry

Highlights

- We demonstrated the diagnostic performance of qLRP in Alzheimer's disease.
- The diagnostic models were robust in sensitivity analyses.
- qLRP may assist in the bedside diagnostic evaluation of Alzheimer's disease.

1 | BACKGROUND

The prevalence of Alzheimer's disease (AD) is estimated to double every 20 years through 2050,[1] and recently, breakthrough disease-modifying therapies have been approved by the US Food and Drug Administration (FDA).[2,3] These events are likely to lead to an increased burden on diagnostic services. As a consequence, there is a need for low-cost, easily applied diagnostic assessment tools such as digital biomarkers for AD.[4,5]

Quantitative light reflex pupillometry (qLRP) can provide objective measures of the pupillary action in response to light stimuli. It can be carried out with a point-and-shoot, hand-held digital device, which

has shown excellent test-retest reliability in memory clinic cohorts, as recently demonstrated.[6] The light reflex is mainly subserved by the cholinergic preganglionic cell group of the Edinger-Westphal nucleus (EWn) which receives modulatory input from the locus coeruleus located inferior to the EWn in the mesencephalon.[7] The EWn is also connected with cortical areas, such as the frontal eye field, and the colliculus superior.[8] Cholinergic brain circuits are affected in AD as cholinergic neurons are particularly vulnerable to the pathological insults of AD, namely the build-up of $A\beta_{1\text{-}42}$-rich plaques and neurofibrillary tangles consisting of tau leading to neurodegeneration.[9,10] Beyond general cholinergic dysfunction, neuropathological studies have provided evidence of specific neuronal damage in the EWn in patients with AD.[11,12]

Several studies have shown a high discriminatory ability of qLRP in AD cohorts, but studies were mainly small with differing methods, and relevant diagnostic group comparators were not always included.[13–15]

Other approaches for exploring the pupillary response in AD have focused on the influence of cognitive load on pupil dilation to distinguish between healthy and AD giving credence to the hypothesis of utilizing the pupil as a biomarker in AD.[16]

We sought to develop a diagnostic application of hand-held qLRP in AD in a proof-of-concept setting in a large, representative cohort of memory clinic patients with high diagnostic certainty, including relevant comparators such as patients with mixed AD (+vascular pathology), vascular cognitive dysfunction (VCD), patients with dementia with Lewy bodies (DLB), and healthy controls (HCs). We hypothesized that qLRP could provide sensitive and specific diagnostic measures and could aid in distinguishing AD from both HCs and patients with other relevant pathologies, which could mimic AD in the clinic, namely patients with cerebrovascular disease and DLB.

RESEARCH IN CONTEXT

1. **Systematic review**: A systematic PubMed search was performed to identify studies investigating quantitative light reflex pupillometry (qLRP) in Alzheimer's disease (AD). Few studies included relevant differential diagnostic groups to evaluate the performance of qLRP in an envisioned use case, namely memory clinics.
2. **Interpretation**: We demonstrated the performance of hand-held quantitative light reflex pupillometry in the diagnosis of Alzheimer's disease in a real-world setting, and identified its usefulness in differentiating dementia disorders, thus proving the potential of the tool in the diagnostic assessment of AD.
3. **Future directions**: Future studies should aim at validating these findings in other settings, such as primary care, along with assessing its added value to known biomarkers for AD.

2 | METHODS

2.1 | Study design

Single-center, proof-of-concept, cross-sectional diagnostic study.

2.2 | Participants

We included patients seen for diagnostic work-up due to cognitive complaints in a tertiary memory clinic at a university hospital (Danish Dementia Research Centre at Rigshospitalet) in the period January 2022-October 2023 and HCs. Diagnostic assessment at the Danish Dementia Research Centre (DDRC) comprises examination by a doctor and nurse preferably with an informant/caregiver present. As a minimum patients undergo cognitive testing with two routine screening tests (Mini-Mental State Examination[17] and Addenbrooke's Cognitive Examination[18]), a structural scan (computed tomography or magnetic resonance imaging), and routine blood sampling. The center utilizes state-of-the-art neuroimaging techniques to ascertain various aspects of neurodegenerative pathology (e.g., 18-F-FDG-positron emission tomography [PET], dopamine agonist tracer scans, amyloid PET), cerebrospinal fluid biomarker analysis, genetic counseling, and neuropsychological testing if deemed relevant. The diagnosis is given following a multi-disciplinary (neurology, geriatrics, psychiatry, neuropsychology, nursing care) consensus conference.

Patients were included after diagnosis (with few exceptions, see below) if they fulfilled the following inclusion criteria (1) a diagnosis of AD (both mild cognitive impairment [MCI] and dementia stages),[19,20] AD with mixed pathology (cerebrovascular),[20] cognitive dysfunction due to cerebrovascular disease,[21] or MCI or DLB,[22,23] (2) An MMSE total score > 15, (3) able to provide informed consent, (4) able to cooperate to investigations with pupillometry, and none of the following exclusion criteria: (1) diagnosis of concurrent neurological (other than AD) or psychiatric disorder (mild depression allowed), (2) excessive alcohol intake > 5 units per day or substance use, (3) concurrent participation in interventional studies, (4) other known brain disorder which could cause cognitive dysfunction, (5) severe ophthalmological disorders (severe cataract, severe glaucoma, age-related macular degeneration, vitreous body bleed, vitreous body collapse, retinal bleed), which may interfere with pupillometry, as evaluated by the investigator. Mild eye disease was allowed, defined as glaucoma receiving monotherapy glaucoma medication, cataract treatment with uncomplicated cataract surgery, or similar uncomplicated disorders as evaluated by the primary investigator (M.G.) due to the high prevalence of these disorders, especially age-related cataract (~50% in +75-year olds[24]). No restriction was put on the medication that patients could receive (including acetylcholinesterase inhibitors, antidepressants, and hypnotics). The reference test for this diagnostic study was the diagnostic criteria applied as listed above. The HCs were included if they fulfilled the following inclusion criteria: (1) able to cooperate with

the investigations, (2) normal cognitive function as evaluated by the investigator, (3) age between 50 and 90 years, and none of the listed exclusion criteria applied to patients.

The HCs were recruited via a list of healthy participants recruited for previous finished trials and clinical studies and through advertising. No compensation was given to participants. Eleven participants were included before diagnosis during diagnostic work-up (eight patients later diagnosed with AD, two with DLB, and one patient with VCD) as part of a previous study.[6] The pupillometry measurements were not used to diagnose these patients and the diagnosis was given within 1 month after inclusion. The study referenced[6] showed no changes within 1 month for pupillary light response (PLR) parameters. We registered the study at clinicaltrials.gov (NCT05175664) and obtained permission from the regional ethics committee before the commencement of the study (file number: H-21045068). The tenets of the 1975 Helsinki Declaration were followed, and written informed consent was obtained from all participants. For participants with cognitive impairment, a caregiver had to be present during obtainment of written consent. The memory clinic physician was always consulted to ensure capacity to provide consent. The study is reported according to the TRIPOD reporting guidelines (see Supplementary Material for TRIPOD checklist).[25]

2.3 | Procedure

2.3.1 | Quantitative light reflex pupillometry

The hand-held PLR-3000 (NeurOptics®) Pupillometer system was used to measure the light reflex (for description of procedure see Supplementary Material).

2.3.2 | Primary outcome measures

The PLR-3000 provides the following parameters: baseline pre-stimulus pupil diameter (mm), peak constriction pupil diameter (mm), delta change between baseline and peak pupil diameter (%), latency (msec), average constriction velocity (mm/sec), maximum constriction velocity (mm/sec), average dilation velocity (mm/sec), and time to reach 75% of baseline value after peak constriction (T75) (sec).

2.3.3 | Pre-processing of data

The method for pre-processing follows the same procedure as described previously (see Supplementary Material).[6]

2.3.4 | Cognitive testing and demographic data

The MMSE[17] assesses global cognitive function and was administered by a trained nurse at an initial evaluation visit to the memory clinic for patients, whereas the investigator administered the MMSE for the HCs. In addition to the MMSE, patients underwent Addenbrooke's Cognitive Examination and a tailored diagnostic work-up, in some cases including neuropsychological testing, whereas this was done for HCs. A history of ophthalmological conditions, subjective visual acuity (to screen for major ocular disorders), medications, and eye surgery were taken from the patient and caregiver as well as extracted from medical files. HCs filled out a questionnaire, where these items were addressed as well. Demographic data, information on medication use, and eye surgery were extracted from medical files and entered into an electronic database (REDCap, Vancouver).

2.3.5 | Statistical analysis

R (ver. 4.2.2) was used for all analyses.[26] The source code used to produce the output can be found at https://github.com/Matgram/dqLRP/tree/main.

The sample size was estimated from a power calculation with the following parameters: alpha 0.05 and power 80%. As a surrogate measure of diagnostic performance, the study was powered to detect a difference of 0.19 mm/sec in the qLRP variable constriction velocity assuming a standard deviation of 0.33 mm/sec as reported in healthy individuals using the ClinCalc power calculator (https://clincalc.com/stats/samplesize.aspx).[27]

We calculated Spearman's correlation coefficient, *rho*, for correlations between all pupillometric and demographic variables.

We fitted multivariable logistic regression class prediction models with AD set as the case versus HCs, patients with cerebrovascular disease (mixed AD + VCD, and VCD, separately), and DLB in separate models. Three models were investigated for each class distinction, namely a model incorporating all qLRP parameters as mentioned in the previous section (Model 1), a sparse model with age and sex (excluding qLRP parameters with multicollinearity) (Model 2), and third an exploratory model, where qLRP parameters included in the sparse model were fitted with cubic splines using the *splines* package in *R* (Model 3). We considered a complete case analysis in all analyses. Assumption of no multicollinearity was assessed by examining variance inflation factors and was violated for Model 1. Linearity assumption was checked by visual inspection of residuals of sparse models and the influence of outliers was checked by inspecting differences in fits and DFBETAs. The performance of models was evaluated by comparing their area under the receiver operating characteristics curve (AUROC) and a corresponding 95% confidence interval ($CI_{95\%}$) using the DeLong test. The AUROC takes on values between 0.5 (chance level) and 1 (perfect discrimination). We investigated thresholds that maximized sensitivity and specificity respectively in a two-cutpoint procedure, where the first and the latter measure were set as a minimum criterion of 85% while maximizing the other measure resulting in a "rule-out" lower, sensitive threshold of the model as well as a "rule-in" higher, specific threshold and an interposed "grey zone." This was done to provide clinicians with cutpoints with the highest diagnostic applicability. A $CI_{95\%}$ for each corresponding measure was calculated using

TABLE 1 Cohort characteristics.

Parameter	Alzheimer's disease (*N* = 107)	Mixed AD (+vascular pathology) (*N* = 20)	Vascular cognitive dysfunction (*N* = 24)	Dementia with Lewy bodies (*N* = 53)	Healthy controls (*N* = 50)	*p*-Value
Age (years)						<0.001[a]
Mean (SD)	75.6 (7)	80.4 (5.2)	80.5 (5.1)	76.2 (6.5)	71.4 (7.9)	
Sex						<0.001[b]
Female	55 (51.4%)	12 (60%)	8 (33%)	8 (15.1%)	31 (62%)	
Male	52 (48.6%)	8 (40%)	16 (67%)	45 (84.9%)	19 (38%)	
Educational level (years)						<0.001[a]
Mean (SD)	11.8 (3.6)	11.4 (4.2)	9.8 (2.8)	11.8 (4)	14.8 (2.7)	
Range	7-20	7-17	7-17	7-19	6-18	
MMSE total score						<0.001[a]
Mean (SD)	25.8 (2.6)	22.4 (3.2)	25.5 (4)	25.3 (3.7)	29.1 (1.2)	
Range	19-30	18-28	12[c]-30	16-30	25-30	
Disease severity						
MCI	28 (25.9%)	0 (0%)	10 (42%)	5 (9.4%)	–	
Mild	75 (69.4%)	11 (55%)	9 (38%)	41 (77.4%)	–	
Moderate	5 (4.6%)	9 (45%)	5 (21%)	7 (13.2%)	–	
Medication with pupillary effect						<0.001[b]
No	95 (89.6%)	14 (70%)	16 (67%)	34 (66.7%)	45 (90%)	
Yes	11 (10.4%)	6 (30%)	8 (33%)	17 (33.3%)	5 (10%)	
Mild chronic eye disease						0.016[b]
No	69 (64.5%)	10 (50%)	12 (50%)	20 (37.7%)	32 (64%)	
Yes	38 (35.5%)	10 (50%)	12 (50%)	33 (62.3%)	18 (36%)	
Cataract surgery history						0.023[b]
No	81 (75.7%)	11 (55%)	16 (67%)	35 (66%)	44 (88%)	
Yes	26 (24.3%)	9 (45%)	8 (33%)	18 (34%)	6 (12%)	

[a]One-way ANOVA.
[b]Pearson's χ-squared test.
[c]One patient with cerebrovascular disease was included with an MMSE total score of 12, evaluated as partly due to a slight language barrier. Percentages may add up to more than 100% due to rounding. N missing < 5 not shown.
Abbreviations: AD = Alzheimer's disease; ANOVA, analysis of variance; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination.

the *boot_ci* function (*N* = 2000 runs and out-of-bag estimates) from the *cutpointr* package.

Sensitivity analyses were performed to assess the influence of variables that could confound the discriminatory ability of qLRP, namely the presence of mild eye disease, the use of medication that could exert pupillary influence, history of cataract surgery, and lastly the performance in early (MCI stage) and dementia stage in dichotomized groups. Further, internal validation with 10-fold 1000 times cross-validation was performed for Model 2 using the *caret* package in *R*.

To use the prediction model, qLRP parameters along with age and sex can be entered as coefficients in the modeling scenarios using the provided web applications (see Supplementary Material).

The alpha was set at 0.05 and differences considered significant below this level, and only two-tailed tests were used.

3 | RESULTS

The cohort characteristics are shown in Table 1. Patients with AD were generally older than HCs and younger than patients with mixed AD and cerebrovascular disease. There was a skewed distribution of men and women, as patients with AD had both sexes equally represented whereas patients with DLB were mainly male. Patients with AD were classified as MCI or mild dementia in 26% and 69% of cases, respectively, reflecting a relatively early disease stage. Patients with AD and HCs had equal proportions receiving medication that could influence the pupil, whereas a higher proportion was found for patients with cerebrovascular disease and DLB. All patients had a higher proportion than HCs of individuals who had a history of cataract surgery. MMSE total scores were different between groups, and patients with mixed pathology had the lowest MMSE score.

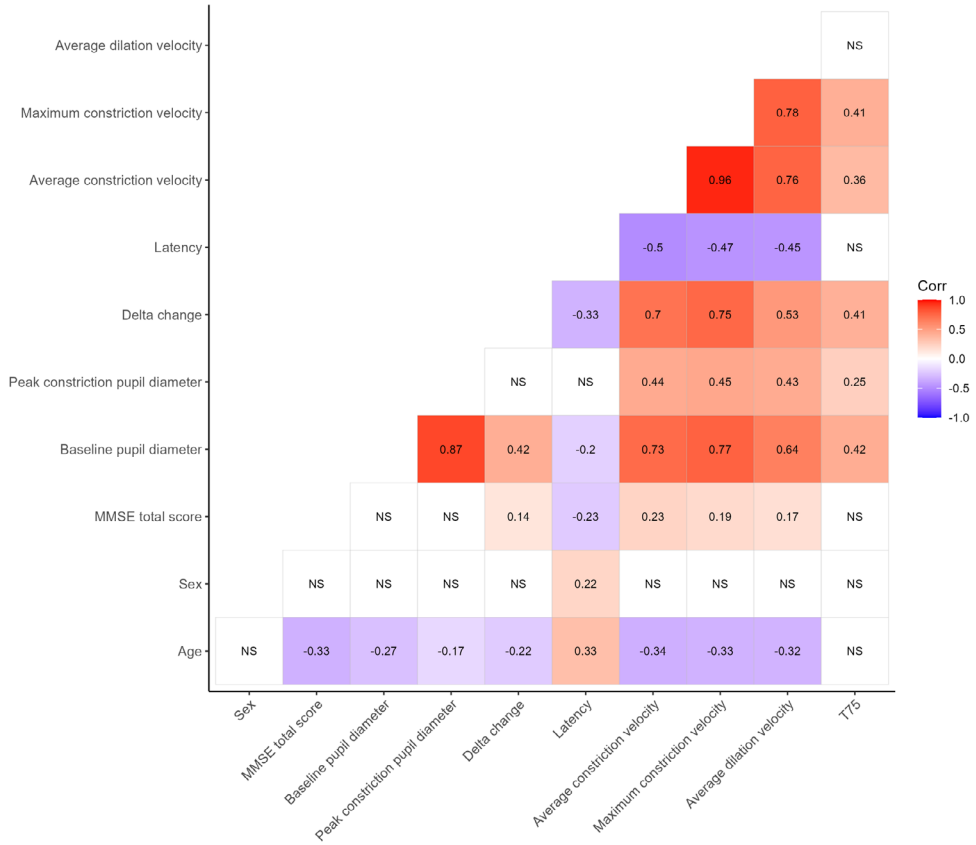

FIGURE 1 A correlogram showing Spearman's rank correlation coefficients for significant correlations. Correlation is shown for the direction of male sex (female sex is in the opposite direction). NS, not significant.

In correlation analysis (shown in Figure 1), we found moderate-strong positive correlations between the qLRP parameters baseline pupil diameter, peak constriction pupil diameter, average and maximum constriction velocity, and delta. MMSE total score correlated weakly positively with delta, constriction/dilation velocity, and negatively with latency. Age was weakly correlated negatively with all qLRP parameters, except latency (positive correlation) and T75 (no correlation). Male sex was weakly, positively correlated with latency.

All qLRP parameters differed between diagnostic groups, except T75 ($p = 0.34$) (Table 2 and Figures 2–3).

3.1 | Diagnostic models

3.1.1 | AD versus HC

The model which also incorporated age and sex showed diagnostic ability slightly higher than the model without age and sex (AUROC 0.74, $CI_{95\%}$: 0.66-0.83) and the cross-validated estimate was only slightly lower, indicating external validity of the model (Table 3). When we applied a two-cutpoint procedure to Model 2, it showed a specificity of 40% with a sensitivity at 85%. In the Supplementary Material (Figures S1-S8), the associated negative and positive predictive values are shown for varying disease prevalence.

3.1.2 | AD versus DLB

The diagnostic model with only qLRP parameters had the ability to discriminate between AD and DLB above chance level (AUROC 0.67, $CI_{95\%}$: 0.58-0.76), and the addition of age and sex improved the model slightly. Model 3, which explored a non-linear association, showed the highest diagnostic ability (AUROC 0.86) but could suffer from overfitting.

3.1.3 | AD versus mixed AD and VCD

When age and sex were added to the model, it showed a similar diagnostic ability to discriminate both mixed AD and VCD from AD, both above chance level (Table 3). The cross-validated AUROCs were lower for both group discriminations (0.73 and 0.7, respectively), which indicated that the models were slightly optimistic. The diagnostic models had similar specificity and sensitivity when applying a two-cutpoint procedure.

3.1.4 | Sensitivity analyses

The results of the sensitivity analyses are reported in the Tables S1-S4.

TABLE 2 Quantitative light reflex pupillometry parameters according to diagnostic group

Parameter	Alzheimer's disease	Mixed AD (+vascular pathology)	Vascular cognitive dysfunction	Dementia with Lewy bodies	Healthy controls	*p*-Value
Baseline pupil diameter (mm)						0.009[a]
Mean (SD)	3.03 (0.51)	2.95 (0.5)	3.06 (0.69)	2.87 (0.6)	3.27 (0.56)	
Range	1.9-4.33	2.13-4.03	1.87-4.53	1.8-4.6	2.13-5.13	
Peak constriction pupil diameter (mm)						0.023[a]
Mean (SD)	2.05 (0.33)	2.14 (0.35)	2.16 (0.48)	1.97 (0.33)	2.18 (0.32)	
Range	1.23-3	1.43-2.9	1.33-3.27	1.3-3.07	1.43-3.13	
Delta change (percent)						<0.001[a]
Mean (SD)	31.8 (6)	27.1 (7)	29 (5.4)	30.4 (6.1)	32.8 (4.5)	
Range	15-43	12.3-38.7	17.3-38.3	16.7-46	21.1-40.7	
Latency (sec)						<0.001[a]
Mean(SD)	0.240(0.027)	0.263 (0.031)	0.255 (0.027)	0.251 (0.025)	0.227 (0.021)	
Range	0.177-0.31	0.21-0.333	0.2-0.313	0.2-0.31	0.2-0.29	
Average constriction velocity (mm/sec)						<0.001[a]
Mean (SD)	1.88 (0.52)	1.6 (0.54)	1.7 (0.58)	1.65 (0.59)	2.18 (0.46)	
Range	0.53-3.62	0.71-2.64	0.59-2.58	0.67-3.92	1.14-3.24	
Maximum constriction velocity (mm/sec)						<0.001[a]
Mean (SD)	2.85 (0.78)	2.4 (0.78)	2.65 (0.87)	2.56 (0.89)	3.25 (0.72)	
Range	0.85-5.23	1.03-3.64	0.99-4.11	0.92-5.78	1.8-4.96	
Average dilation velocity (mm/sec)						0.009[a]
Mean (SD)	0.94 (0.24)	0.84 (0.34)	0.87 (0.24)	0.88 (0.27)	1.022 (0.19)	
Range	0.33-1.54	0.34-1.49	0.47-1.31	0.42-1.92	0.68-1.45	
Time to reach 75% of baseline (sec)						0.339[a]
Mean (SD)	1.62 (0.65)	1.45 (0.46)	1.472 (0.52)	1.63 (0.99)	1.78 (0.69)	
Range	0.71-4.19	0.9-2.34	0.97-3.55	0.73-7.29	0.85-3.87	

[a]One-way ANOVA.
Abbreviations: AD, Alzheimer's disease; ANOVA, analysis of variance;

4 | DISCUSSION

This study investigated the diagnostic performance of qLRP in diagnosing AD in a memory clinic cohort. We found that our models performed with an ability above chance level when distinguishing AD from HCs, DLB, and patients with cerebrovascular disease. The diagnostic models were robust to sensitivity analyses and suspected confounding ocular factors did not seem to drive the discriminatory ability of qLRP.

Our results indicate that qLRP might prove useful as a diagnostic aide, both when distinguishing healthy from patients with AD as well as in the differential diagnostic process of distinguishing different dementia etiologies, where the highest discriminatory ability was found for cerebrovascular disease. We acknowledge that this tool should not be used as a standalone diagnostic biomarker for AD, and we did not evaluate the added value of qLRP to current diagnostic tests, which remains to be elucidated in future studies. We have not identified studies, which have previously investigated the ability of qLRP to distinguish between dementia etiologies and our study provides the first evidence that qLRP parameters may be affected in both patients with DLB and patients with cerebrovascular disease. We mainly found more exaggerated slowing of the pupillary reflex as compared with AD, which was surprising. We found the highest AUROCs when we applied qLRP in the AD vs. cerebrovascular disease scenario. It seems that cerebrovascular disease may be distinguished with some certainty from AD using only qLRP. While we could not provide data that explain the driving factor behind the altered qLRP parameters in cerebrovascular disease, one hypothesized explanation could be extant white matter lesions, which are prevalent in cerebrovascular disposed individuals, and their anatomical localization.[28] A recent study investigated whether strategic infarctions might influence pupillary measurements but did not identify differences between groups, hinting at other possible explanations such as white matter hyperintensities.[29] The proposed pathophysiology of white matter hyperintensities, which includes axonal loss and demyelination,[30] could explain why certain qLRP parameters were shown to be affected, but further research is needed to establish this association.

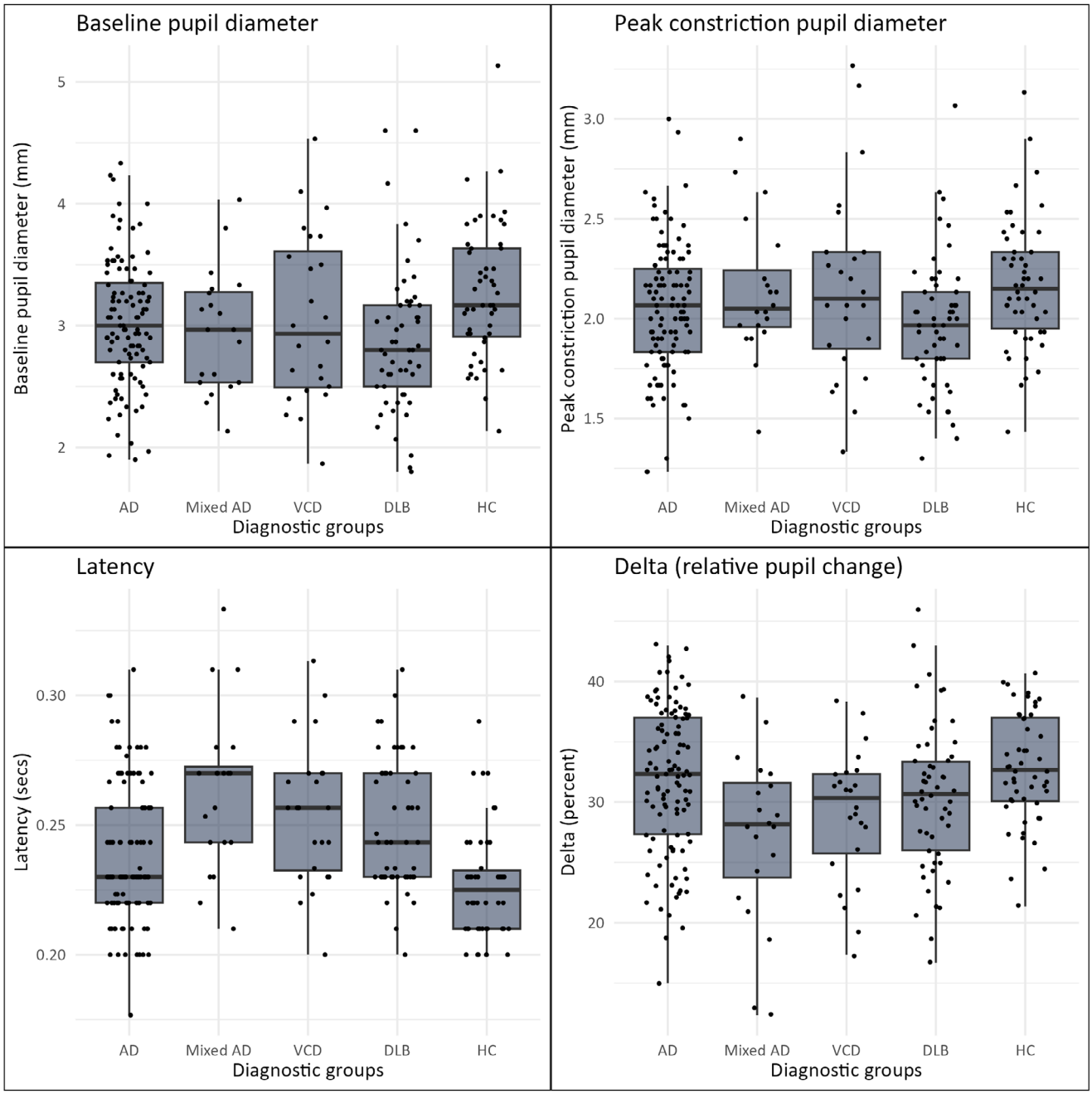

FIGURE 2 Boxplots with overlayed dot-plots of qLRP parameters. The horizontal stroke in the box indicates the median, and the boxes indicate the 25% and 75% interquartile range (IQR). Whiskers are 1.5 × IQR. AD, Alzheimer's disease; DLB, dementia with Lewy bodies; HC, healthy controls; mixed AD, Alzheimer's disease with mixed pathology (AD+vascular pathology); qLRP, quantitative light reflex pupillometry; VCD, vascular cognitive dysfunction.

qLRP was able to distinguish with some certainty AD from DLB, which is an important differential diagnostic to AD, as DLB shows a high degree of co-pathology and shares symptoms with AD.[31,32] Previous studies showed that Parkinson's disease, which shares neuropathological features with DLB, could be distinguished from HCs using qLRP,[33] but no studies have to our knowledge investigated the potential of qLRP in distinguishing AD and DLB. The mechanism that drives the diagnostic ability of qLRP for this discrimination is not known in detail, but autonomic dysfunction could be hypothesized to play a role as the light reflex is under the influence of the autonomic nervous system.[34] As such it could be assumed that autonomic dysfunction, which is pronounced in DLB and part of the diagnostic criteria,[22] could drive the effect observed on qLRP parameters, and qLRP could be a sensitive marker in gauging this aspect of the disease, although more research is needed.

We provided sensitivity and specificity thresholds of our diagnostic models to increase clinical applicability. This two-cutpoint method and the different diagnostic models could be applied in specific

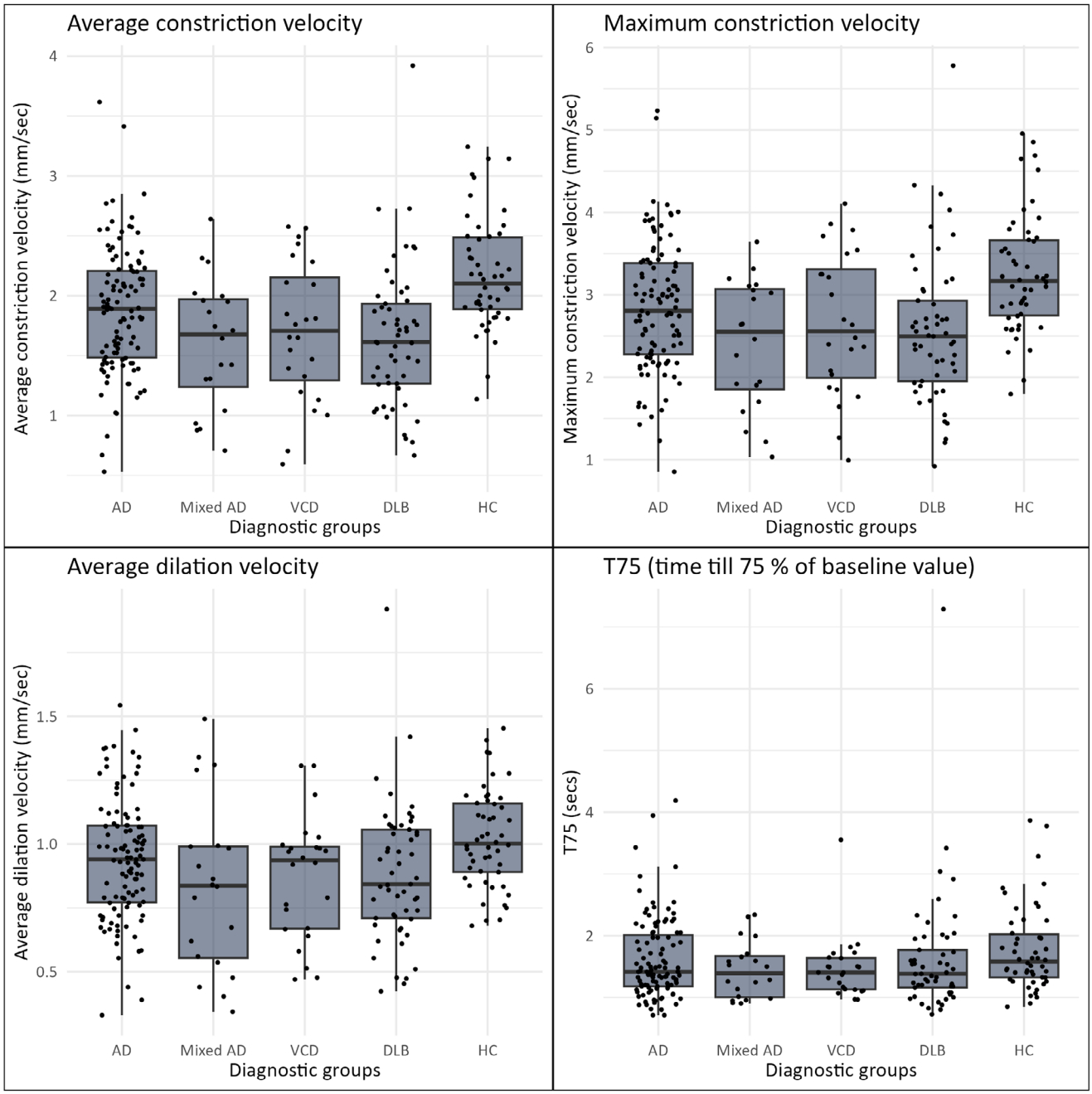

FIGURE 3 Boxplots with overlayed dot-plots of qLRP parameters. The horizontal stroke in the box indicates the median, and the boxes indicate the 25% and 75% interquartile range (IQR). Whiskers are 1.5 × IQR. AD, Alzheimer's disease; DLB, dementia with Lewy bodies; HC, healthy controls; mixed AD, Alzheimer's disease with mixed pathology (AD+vascular pathology); qLRP, quantitative light reflex pupillometry; VCD, vascular cognitive dysfunction.

situations, for example, at an initial triage entry-point to memory clinics such as primary care providers and in the setting of diagnostic centers (i.e., memory clinics). Applying both thresholds in a primary care setting, the sensitive threshold would indicate a "rule-out" level, whereby patients falling below the threshold have little chance of having AD vs. a healthy condition (non-neurodegenerative), and the upper threshold could indicate that the presence of AD may be likely and a referral to a specialist center should be made. The resulting interposed "gray zone" may benefit from further testing before a decision is made, and ultimately represents a downside to our cutpoint optimization. The other diagnostic models (vs. cerebrovascular disease and DLB) may have relevance in more specialized settings where an ability to differentiate dementia disorders is needed. These testing scenarios would need to be evaluated in future studies to evaluate the added value of qLRP in these particular settings.

Previous studies have shown a remarkable discriminatory ability of qLRP for distinguishing AD vs. healthy, with near-perfect AUC.[13–15] We could not replicate these results, and the reasons for this could

TABLE 3 Performance of diagnostic models.

Parameter	AUROC [95% CI]	Specificity at minimum 85% sensitivity [95% CI] (cutpoint)	Sensitivity at minimum 85% specificity [95% CI] (cutpoint)	Cross-validated AUROC
AD vs. HC Model 1	0.72 [0.64-0.8]			
AD vs. HC Model 2	0.74 [0.66-0.83]	40% [19-68%] (0.52)	38% [16-72%] (0.81)	0.68
AD vs. HC Model 3	0.81 [0.74-0.88]			
AD vs. DLB Model 1	0.67 [0.58-0.76]			
AD vs. DLB Model 2	0.75 [0.67-0.83]	47% [21-67%] (0.52)	46% [18-71%] (0.81)	0.69
AD vs. DLB Model 3	0.86 [0.79-0.92]			
AD vs. Mixed AD + VCD Model 1	0.75 [0.66-0.83]			
AD vs. Mixed AD + VCD Model 2	0.8 [0.72-0.88]	57% [31-79%] (0.59)	52% [36-78%] (0.82)	0.73
AD vs. Mixed AD + VCD Model 3	0.899 [0.85-0.95]			
AD vs. VCD Model 1	0.73 [0.61-0.85]			
AD vs. VCD Model 2	0.81 [0.72-0.9]	63% [25-89%] (0.72)	58% [33-85%] (0.86)	0.7
AD vs. VCD Model 3	–			

Notes: Model 1 = all qLRP parameters as explaining variables, Model 2 = Sparse model (excluding peak constriction and maximum velocity qLRP variables) with age and sex as covariates, Model 3 = as Model 2 with cubic spline terms fitted for all qLRP parameters. Cutpoint refers to the probability threshold of the logistic regression model prediction. Model algorithm did not converge.
Abbreviations: AD, Alzheimer's disease; AUROC, area under the receiver operating characteristics curve; qLRP, quantitative light reflex pupillometry; VCD, vascular cognitive dysfuntion.

be manifold: first, our cohort differed, as we included a larger cohort of patients with frequent ocular disorders, and allowed medication that could have affected the pupillary response, with a wide MMSE range, indicating a representative sample of a memory clinic, which is heterogenous.[35] Also, the pupillometry system applied differed between some previous studies and ours. We used a hand-held, research-grade pupillometer, while other groups applied in-house developed, stationary pupillometers with a faster sampling rate resulting in smaller measurement errors (see Table S5 for review of previous qLRP studies) and a lower percentage of discarded samples, which may explain the differences observed. In that regard, the low-cost and quick application of hand-held qLRP seems advantageous in the clinic, while the experimental approach seems more useful in a proof-of-mechanism setting. Further, recent studies have shown promise in targeting specific melanopsin retinal ganglion cells of the retina. This approach seems interesting given the fact that patients with AD show selective disruption of function in these cells.[36]

While a substantial proportion of patients across diagnostic groups had ocular pathology, which is common in older age and populations of patients with dementia,[37] these factors did not affect the diagnostic ability of qLRP in sensitivity analyses. Earlier studies on qLRP have, with few exceptions,[38] excluded patients with even mild ocular disorders.[13–15,39–47] This could have induced selection bias in previous studies, which seemingly can be avoided without influencing the discriminatory ability of qLRP to a large degree.

Our study had several strengths. First, we examined a large cohort of patients and relevant comparators in a real-world setting, reflecting the setting where one use case of qLRP seems relevant. Second, we applied broad inclusion criteria which included participants with mild ocular disorders, thus enhancing the generalizability of our results; and third, we applied a commercially available pupillometer, which increases the reproducibility of our results.

We also acknowledge several limitations of our study. First, we included a healthy control group without cognitive complaints. For a true diagnostic ability, an ideal comparison group for the detection of AD might be a subjective cognitive decline group with adequate follow-up and diagnostic work-up to rule out incipient dementia. Second, our VCD group was small which led to insufficient data in certain modeling situations. Third, we did not perform ophthalmological examination beyond ophthalmoscopy without a specific focus on fundus pathology,

which may have led to some patients erroneously not having relevant eye pathology, such as optic nerve pathology, described. This could have been mitigated by the inclusion of fundus exam and/or optical coherence tomography and represents a shortcoming of this study. Fourth, we did not validate our results in an independent dataset; however, we did include an out-of-sample estimate of the AUROC by using cross-validation. Fifth, we could not account for senile miosis, and while we ran sensitivity analyses excluding patients with mild eye disorders, this could also represent a considerable source of residual confounding; however, this may have been mitigated by the inclusion of age-matched controls. Sixth, as we mainly used data from the right eye, a between-eyes correlation was not considered which may have reduced the statistical power.

In this proof-of-concept study, we have shown that qLRP may serve as a supportive digital, bedside biomarker for AD in a specialized memory clinic setting, where qLRP could be used to assist in distinguishing between patients with AD, healthy individuals, patients with cerebrovascular disease, and DLB. Our findings may be used to validate qLRP as a sensitive digital, diagnostic biomarker for AD, and future studies should apply qLRP in relevant settings in an unbiased manner along with other diagnostic assessment modalities to validate our findings.

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CONFLICT OF INTEREST STATEMENT

S.G.H. has taught courses for Novo Nordisk with honoraria paid directly to the Danish Dementia Research Center. The remaining authors have no conflicts of interest. Author disclosures are available in the supporting information.

CONSENT STATEMENT

All human subjects provided informed consent.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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