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RESEARCH ARTICLE

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Loss of corneal nerves and brain volume in mild cognitive impairment and dementia

Georgios Ponirakis[1] | Hanadi Al Hamad[2] | Adnan Khan[1] | Ioannis N. Petropoulos[1] |
Hoda Gad[1] | Mani Chandran[2] | Ahmed Elsotouhy[1,3] | Marwan Ramadan[2] |
Priya V. Gawhale[2] | Marwa Elorrabi[2] | Masharig Gadelseed[2] | Rhia Tosino[2] |
Anjum Arasn[2] | Pravija Manikoth[2] | Yasmin H.M. Abdelrahim[2] | Mahmoud A Refaee[2] |
Noushad Thodi[4] | Surjith Vattoth[5] | Hamad Almuhannadi[1] | Ziyad R. Mahfoud[1] |
Harun Bhat[1] | Ahmed Own[3] | Ashfaq Shuaib[6] | Rayaz A. Malik[1,7,8]

[1] Department of Medicine, Weill Cornell
Medicine-Qatar, Qatar Foundation, Doha,
Qatar

[2] Geriatric & Memory Clinic, Rumailah
Hospital, Hamad Medical Corporation, Doha,
Qatar

[3] Neuroradiology, Hamad General Hospital,
Hamad Medical Corporation, Doha, Qatar

[4] MRI Unit, Rumailah Hospital, Hamad Medical
Corporation, Doha, Qatar

[5] Radiology, University of Arkansas for
Medical Sciences, Arkansas, USA

[6] Department of Medicine, University of
Alberta, Alberta, Canada

[7] Faculty of Biology, Medicine and Health,
University of Manchester, Manchester, UK

[8] Faculty of Science and Engineering,
Manchester Metropolitan University,
Manchester, UK

Correspondence
Rayaz. A. Malik, Weill Cornell Medicine-Qatar,
Qatar Foundation, Education City, Doha, Qatar.
Email: ram2045@qatar-med.cornell.edu

Abstract

Introduction: This study compared the capability of corneal confocal microscopy (CCM) with magnetic resonance imaging (MRI) brain volumetry for the diagnosis of mild cognitive impairment (MCI) and dementia.

Methods: In this cross-sectional study, participants with no cognitive impairment (NCI), MCI, and dementia underwent assessment of Montreal Cognitive Assessment (MoCA), MRI brain volumetry, and CCM.

Results: Two hundred eight participants with NCI (n = 42), MCI (n = 98), and dementia (n = 68) of comparable age and gender were studied. For MCI, the area under the curve (AUC) of CCM (76% to 81%), was higher than brain volumetry (52% to 70%). For dementia, the AUC of CCM (77% to 85%), was comparable to brain volumetry (69% to 93%). Corneal nerve fiber density, length, branch density, whole brain, hippocampus, cortical gray matter, thalamus, amygdala, and ventricle volumes were associated with cognitive impairment after adjustment for confounders (All P's < .01).

Discussion: The diagnostic capability of CCM compared to brain volumetry is higher for identifying MCI and comparable for dementia, and abnormalities in both modalities are associated with cognitive impairment.

KEYWORDS
brain volumetry, corneal confocal microscopy, dementia, mild cognitive impairment, neurodegeneration

RESEARCH IN CONTEXT

1. **Systematic Review**: We searched PubMed and Google Scholar with the terms dementia, neurodegeneration, biomarker, and magnetic resonance imaging (MRI) brain for studies published in the English language from database inception to April 25, 2021. We identified a need for non-invasive and reliable biomarkers of neurodegeneration for mild cognitive impairment (MCI) and dementia. The diagnostic capability of corneal confocal microscopy (CCM) an ophthalmic marker of neurodegeneration for MCI and dementia has not been tested against MRI brain volumetry.
2. **Interpretation**: Compared to MRI brain volumetry, the diagnostic capability of CCM is higher for identifying people with MCI and comparable for dementia.
3. **Future directions**: Longitudinal studies are required to compare the capability of CCM and MRI brain volumetry for predicting progression of individuals with MCI to dementia.

1 | INTRODUCTION

Dementia is a progressive neurodegenerative disease affecting 40 to 50 million people worldwide.[1,2] Therapeutic and psychological interventions for people with early stage dementia can improve cognition, independence, and quality of life.[3] However, dementia is an insidious disease, and it is therefore important to establish biomarkers that provide direct or indirect evidence of the underlying pathology in the asymptomatic stages.[4] The National Institute on Aging–Alzheimer's Association (NIA-AA) has proposed that diagnostic biomarkers of Alzheimer's disease (AD) should include amyloid beta (Aß) and tau alongside biomarkers of neurodegeneration to stage the severity of the disease.[5] The "*diagnostic accelerator program*" call from the Alzheimer's Drug Discovery Foundation has targeted the need for accurate, reliable, and non-invasive biomarkers that identify mild cognitive impairment (MCI) and predict the development of dementia.

Structural neuroimaging is an established method to identify neurodegeneration in AD.[4,6] Studies employing magnetic resonance imaging (MRI) brain volumetry have shown progressive brain atrophy in people with MCI and dementia compared to people with no cognitive impairment (NCI).[7,8] Visual rating of medial temporal lobe atrophy can differentiate probable[9] and established AD from NCI[10,11] and between amnesic and non-amnesic MCI.[12] The rate of hippocampal atrophy may also identify people with MCI who are at risk of developing dementia.[13,14]

Corneal confocal microscopy (CCM) is a rapid noninvasive ophthalmic imaging technique that allows objective quantification of corneal nerve fiber morphology.[15–18] It has been used to identify neurodegeneration in diabetic neuropathy,[19–21] HIV neuropathy,[22]

Friedreich ataxia,[23] multiple sclerosis,[24,25] and Parkinson disease.[26–28] More recently, we have shown that corneal nerve loss is associated with the severity of cognitive impairment and functional independence in people with MCI and dementia.[29,30]

The primary objective of this study was to compare the diagnostic accuracy of CCM to brain volumetry for distinguishing patients with MCI and dementia from people with NCI. The secondary objective was to assess the association of corneal nerve morphometry and MRI brain volumetry with cognitive function in MCI and dementia.

2 | METHODS

Subjects with MCI, dementia—including AD, vascular dementia (VaD), and mixed AD—and no cognitive impairment (NCI) 60 to 85 years of age were recruited from the geriatric and memory clinic in Rumailah Hospital, Doha, Qatar between September 18, 2016 and February 9, 2020. We excluded subjects with reversible cognitive impairment, complex and young-onset dementia, severe dementia, frontotemporal dementia, Lewy body dementia, Parkinson disease, severe anxiety, severe depression, mood disorders, psychosis, hypomania, peripheral neuropathy including severe vitamin B_{12} deficiency, hypothyroidism, HIV infection, and hepatitis C. We also excluded subjects with severe dry eye, corneal dystrophies, ocular trauma, or surgery in the preceding 12 months, and subjects who were unable to cooperate during the assessments. A history of severe dry eye was obtained by reviewing the medical records and direct interview with the participant. Dry eye assessment was not performed in the study. Diabetes is highly prevalent in patients 50 years of age or older in Qatar[31] and is a common comorbidity in VaD and mixed dementia and was not excluded.

This study was approved by the institutional review board of Weill Cornell Medicine in Qatar and Hamad Medical Corporation, and all participants gave informed consent to take part in the study. The research adhered to the tenets of the Declaration of Helsinki.

2.1 | Demographic and metabolic measures

Age, gender, blood pressure, body weight, body mass index (BMI), hemoglobin A1c (HbA1c), cholesterol, triglycerides, hemoglobin, mean corpuscular volume (MCV), vitamin B_{12}, thyroid-stimulating hormone (TSH), free thyroxine (FT4), and medical history were recorded from the electronic medical register (Cerner).

2.2 | Cognitive function assessment

Cognitive function was assessed using the Montreal Cognitive Assessment (MoCA) basic test, version 7.1, which includes seven cognitive domains including visuospatial/executive, naming, memory, attention, language, abstraction, and delayed recall, with a score of ≤26/30 indicating cognitive impairment.[32] An extra point was added for individuals who were illiterate or had only attended primary school, as it was

suggested in the original validation study that an extra point should be added to the total score if the individual had ≤12 years of education. The duration of cognitive dysfunction was recorded from the patient's medical history.

2.3 | Diagnosis

The diagnosis of MCI and dementia, including AD, VaD, and mixed AD, was based on the International Classification of Diseases, Tenth Revision (ICD-10) criteria.[33] A consensus diagnosis was reached by geriatricians, geriatric psychiatrists, and neurologists based on a comprehensive history of cognitive impairment, psychiatric history, medical history including episodes of delirium and other medical comorbidities, medication history, and functional history of basic daily living activities. MRI brain was undertaken to exclude potentially reversible causes of cognitive decline such as brain tumors, subdural hematoma, or normal-pressure hydrocephalus.

The diagnosis of AD was based on typical features of AD such as atrophy in hippocampi, entorhinal cortex, and amygdala on MRI and no significant decline in functioning. Brain atrophy was assessed by neuroradiologists using the criteria of Dubois et al[34] blinded to the diagnosis and clinical data.

The diagnosis of probable or possible VaD was based on the National Institute of Neurological Disorders and Stroke-Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN) criteria,[35] which includes multiple large vessel infarcts or a single strategically placed infarct in the angular gyrus, thalamus, basal forebrain, or posterior (PCA) or anterior cerebral artery (ACA) territories, and multiple basal ganglia and white matter lacunes, extensive periventricular white matter lesions, or combinations thereof.

The diagnosis of mixed dementia was based on the presence of AD and significant vascular changes.

2.4 | MRI brain acquisition

MRI was performed on a 3T MRI system (MAGNETOM Skyra, Siemens AG, Erlangen). A T1-weighted three-dimensional (3D) magnetization prepared rapid acquisition gradient echo sequence (MPRAGE) was obtained in the sagittal plane with a 1 mm slice thickness, repetition time of 1900 ms, echo time of 2.67 ms and 2.46 ms, inversion time of 1100 ms and 900 ms, flip angle of 9 degrees and 15 degrees, and field of view (FOV) of 240 × 100. Coronal and axial reformatted MPRAGE images were made from the sagittal 3D sequence.

2.5 | Brain volume analysis

MRI brain volumetry was undertaken on a T1-weighted 3D MPRAGE sequence using NeuroQuant (NQ), a US Food and Drug Administration (FDA)–approved fully automated software.[36,37] The brain volume was adjusted for the percentage of intracranial volume (ICV), which includes all segmented structures to minimize the impact of the head size as a confounding factor. This study focused on 12 different brain structures, including the ICV percentage of the hippocampus, whole brain, ventricle, cortical gray matter, entorhinal cortex, thalamus, amygdala, cingulate gyrus, and frontal, temporal, parietal, and occipital lobes.

2.6 | Corneal confocal microscopy

CCM analysis was performed with the Heidelberg Retinal Tomograph III (HRT-3) Rostock Cornea Module (Heidelberg Engineering GmbH, Heidelberg, Germany). The cornea was locally anesthetized by instilling one drop of 0.4% benoxinate –hydrochloride (Chauvin Pharmaceuticals, Chefaro, UK). Viscotears (Carbomer 980, 0.2%, Novartis, UK) was used as the coupling agent between the cornea and the Tomo-Cap as well as between the TomoCap and the objective lens. Subjects were instructed to fixate on a target with the eye not being examined. Several scans of the sub-basal nerve plexus in the central cornea were captured per eye for ≈2 minutes. At a separate time, three high-clarity non-overlapping images per eye were selected based on depth, focus position, and contrast, as described previously[16–18] by one investigator who was blinded from the diagnosis, cognitive function, and MRI brain volumetry. To ensure that the selected images were representative, an image with low-, medium-, and high-fiber density was selected from a different location within the central corneal region. The mean corneal nerve fiber density (CNFD, fibers/mm^2), branch density (CNBD, branches/mm^2), and fiber length (CNFL, total fiber length mm/mm^2) were measured manually using CCMetrics.[15]

2.7 | Peripheral neuropathy assessment

Vibration perception threshold (VPT) was assessed using a Neurothesiometer (Horwell Scientific Laboratory Supplies) on the pulp of the large toe on both feet, and the average value of three measurements was recorded as a VPT in volts (V) ranging from 0 to 50 V.

2.8 | Sample size calculation

Based on our previous study,[30] the smallest effect size between the two groups (MCI and dementia) and the NCI group was 0.85 for CNFD, 0.75 for CNBD, and 0.90 for CNFL. For an 80% power and 2.5% significance level to account for at least two comparisons the sample size would be 34 per arm.

2.9 | Statistical analysis

Patient demographics and clinical characteristics were summarized using the mean and SD for numeric variables and frequency distribution for categorical variables. Variables were compared between the NCI, MCI, and dementia group using one-way analysis of variance

(ANOVA) with Bonferroni post hoc test for pairwise comparisons and chi-square test, respectively.

Univariate analysis by simple linear regression was performed with CCM measures and confounding factors as independent variables and cognitive function or brain volumetric MRI as the dependent variable. Multiple linear regression analysis included all variables with $P \leq .05$ at the bivariate level. The regression coefficient (beta) and the corresponding 95% confidence intervals (95% CIs) are presented.

Receiver-operating characteristic (ROC) curve analysis was used to determine the ability of CNFD, CNBD, CNFL, volume of hippocampus, whole brain, ventricle, cortical gray matter, thalamus, amygdala, entorhinal cortex, and frontal and temporal lobe to distinguish between subjects with MCI or dementia from subjects with NCI. The area under the ROC curve (AUC) and a cut-off point with the maximal sensitivity and specificity were calculated.

All analyses were performed using IBM-SPSS (version 26; SPSS Inc, Armonk, NY, USA). A two-tailed P value of $\leq.05$ was considered statistically significant.

3 | RESULTS

3.1 | Demographic and clinical characteristics

Of the 208 subjects studied, those with no cognitive impairment (NCI) (n = 42), mild cognitive impairment (MCI) (n = 98), and dementia (n = 68) had a comparable mean age (70.8 ± 6.2 vs 71.2 ± 5.9 vs 73.4 ± 5.8, $P = .06$), gender (females: 31.0% vs 39.8% vs 41.2%, $P = .53$), and prevalence of type 2 diabetes (T2D) (69.0% vs 58.2% vs 64.7%, $P = .43$), respectively. The dementia group was comprised of pure Alzheimer's disease (or AD) (32.2%), vascular dementia (VaD) (23.7%), and mixed AD with vascular lesions (44.1%). Systolic blood pressure (SBP), diastolic blood pressure (DBP), body weight, BMI, HbA1c, cholesterol, triglycerides, and MCV were comparable between the groups (Table 1).

The MoCA score was lower in the MCI (22.0 ± 5.8, $P < .0001$) and dementia (13.0 ± 5.9, $P < .0001$) group compared to the NCI group (27.6 ± 3.8) (Table 1). The mean duration of cognitive impairment was significantly longer in the dementia (3.1 ± 2.7 years) compared to the MCI (1.6 ± 2.1 years) group, $P < .0001$.

All subjects completed the assessment without expressing any concerns about the eye drop or contact of the cornea with the CCM Tomo-Cap. There was a reduction in corneal nerve fibers in subjects with MCI and dementia compared to subjects with NCI (Figure 1). Compared to those with NCI corneal nerve fiber density (CNFD, fibers/mm^2) (31.9 ± 7.4 vs 24.0 ± 9.3 and 20.1 ± 8.3, $P \leq .0001$), branch density (CNBD, branches/mm^2) (86.4 ± 44.9 vs 52.9 ± 35.8 and 46.1 ± 27.0, $P \leq .0001$) and length (CNFL, mm/mm^2) (22.5 ± 6.0 vs 16.5 ± 6.5 and 14.7 ± 5.8, $P \leq .0001$) were significantly lower in subjects with MCI and dementia (Table 1). After excluding those with diabetes, compared to those with NCI (n = 13), CNFD (35.3 ± 5.6 vs 26.0 ± 9.1 and 21.4 ± 7.7 fibers/mm^2, $P = .001-.0001$), CNBD (95.2 ± 49.2 vs

57.3 ± 38.2 and 52.8 ± 26.5 branches/mm^2, $P = .002-.001$), and CNFL (24.7 ± 4.5 vs 17.7 ± 6.8 and 15.9 ± 4.8 mm/mm^2, $P \leq .0001$) remained significantly lower in subjects with MCI (n = 41) and dementia (n = 24). CNFD ($P = .66$), CNBD ($P = .40$), and CNFL ($P = .43$) were comparable between those with pure AD, VaD, and mixed AD with vascular lesions.

Vibration perception threshold (VPT) on the feet was significantly higher in subjects with dementia ($P \leq .0001$) but not in those with MCI compared to subjects with NCI (17.3 ± 9.4 vs 11.4 ± 8.4, $P = .07$) and comparable between subjects with MCI and dementia (17.3 ± 9.4 vs 21.2 ± 10.6, $P = .16$).

The volume of the whole brain (73.1 ± 2.8 vs 70.8 ± 3.5 vs 67.6 ± 2.9, $P \leq .0001$) and hippocampus (0.46 ± 0.05 vs 0.42 ± 0.08 vs 0.34 ± 0.08, $P \leq .0001$) were lower in MCI and dementia groups compared to NCI (Table 1). The volume of cortical gray matter ($P \leq .0001$) and amygdala ($P < .01$) was lower and ventricle volume was larger in subjects with dementia compared to NCI, but there was no difference between MCI and NCI. The volume of the entorhinal cortex ($P < .05$), thalamus ($P = .01$), and frontal ($P < .05$) and temporal lobes ($P = .001$) was significantly lower in subjects with dementia compared to MCI but was comparable between MCI and NCI. There was no significant difference in the volume of the parietal lobe, occipital lobe, or cingulate gyrus between subjects with MCI and dementia.

3.2 | Diagnostic accuracy for distinguishing MCI from NCI

Table 2 and Figure 2 show the diagnostic accuracy of CCM measures and brain volumetry for identifying subjects with MCI and dementia. CNFL distinguished subjects with MCI from NCI with 81% AUC (95% CI 71-91%). Using an CNFL cutoff of ≤21 mm/mm^2 the sensitivity and specificity were 80% and 76%, respectively. CNFD, CNBD, MoCA score, volume of whole brain, ventricle, and hippocampi showed an AUC ranging from 63% to 79%. The volume of the cortical gray matter, frontal, temporal, parietal, occipital lobe, entorhinal cortex, thalamus, amygdala, and cingulate gyrus could not distinguish MCI from NCI.

3.3 | Diagnostic accuracy for distinguishing dementia from NCI

Measures to distinguish dementia from NCI with ≥80% AUC were: CNFD (AUC: 85%, 95% CI 75-95%), CNFL (AUC: 84%, 95% CI 73-95%), whole brain (AUC: 93%, 95% CI 85-100%), hippocampus (AUC: 89%, 95% CI 81-98%), ventricle (AUC: 82%, 95% CI 70-93%) volumes, and MoCA (AUC: 97%, 95% CI 92-100%). The sensitivity and specificity using a cutoff point with a ≥80% specificity were 81% and 80% for a CNFD ≤27 fibers/mm^2, 81% and 76% for a CNFL ≤21 mm/mm^2, 85% and 92% for whole brain ≤70 ICV%, 85% and 92% with hippocampi ≤0.40 ICV%, 70% and 84% with ventricle ≥3.58 ICV%, and 100% and 88% with MoCA score ≤26. The volume of cortical gray matter,

TABLE 1 Demographic and clinical characteristics of the study population

	NCI (n = 42)	MCI (n = 98)	Dementia (n = 68)	P value[a]	P value[b]	P value[c]
Cognitive function						
MoCA score	27.6 ± 3.8	22.0 ± 5.8	13.0 ± 5.9	≤.0001	≤.0001	≤.0001
Cognitive impairment duration, years	N/A	1.6 ± 2.1	3.1 ± 2.7			≤.0001
Corneal nerve fiber measures						
CNFD, fibers/mm^2	31.9 ± 7.4	24.0 ± 9.3	20.1 ± 8.3	≤.0001	≤.0001	.01
CNBD, branches/mm^2	86.4 ± 44.9	52.9 ± 35.8	46.1 ± 27.0	≤.0001	≤.0001	NS
CNFL, mm/mm^2	22.5 ± 6.0	16.5 ± 6.5	14.7 ± 5.8	≤.0001	≤.0001	NS
Brain volumetric MRI						
Whole brain, ICV %	73.1 ± 2.8	70.8 ± 3.5	67.6 ± 2.9	<.01	≤.0001	≤.0001
Cortical gray matter, ICV %	28.9 ± 3.2	28.6 ± 3.6	24.8 ± 3.8	NS	≤.0001	≤.0001
Ventricle, ICV %	2.7 ± 1.3	3.3 ± 1.4	5.1 ± 2.5	NS	≤.0001	≤.0001
Hippocampus, ICV %	0.46 ± 0.05	0.42 ± 0.08	0.34 ± 0.08	<.05	≤.0001	≤.0001
Entorhinal cortex, ICV %	0.31 ± 0.12	0.33 ± 0.10	0.27 ± 0.09	NS	NS	<.05
Thalamus, ICV %	0.91 ± 0.08	0.91 ± 0.13	0.84 ± 0.10	NS	NS	.01
Amygdala, ICV %	0.19 ± 0.02	0.19 ± 0.03	0.16 ± 0.04	NS	<.01	<.01
Cingulate gyrus, ICV %	0.89 ± 0.28	0.95 ± 0.18	0.88 ± 0.12	NS	NS	NS
Frontal lobe, ICV %	9.5 ± 3.0	10.1 ± 1.8	8.8 ± 1.4	NS	NS	<.05
Temporal lobe, ICV %	7.0 ± 2.2	7.4 ± 1.4	6.2 ± 1.2	NS	NS	.001
Parietal lobe, ICV %	6.1 ± 2.0	6.2 ± 1.2	5.8 ± 0.9	NS	NS	NS
Occipital lobe, ICV %	3.2 ± 1.1	3.5 ± 0.8	3.2 ± 0.6	NS	NS	NS

Characteristics of 208 participants presented as mean ± SD for numeric variables and frequency distribution for NCI, MCI, and dementia. Continuous and categorical variables were compared using one-way ANOVA with Bonferroni post hoc test and chi-square test, respectively. Abbreviations: Montreal cognitive assessment (MoCA); no cognitive impairment (NCI), mild cognitive impairment (MCI), corneal nerve fiber density (CNFD); corneal nerve branch density (CNBD); corneal nerve fiber length (CNFL); and mean corpuscular volume (MCV).

[a]NCI versus MCI.

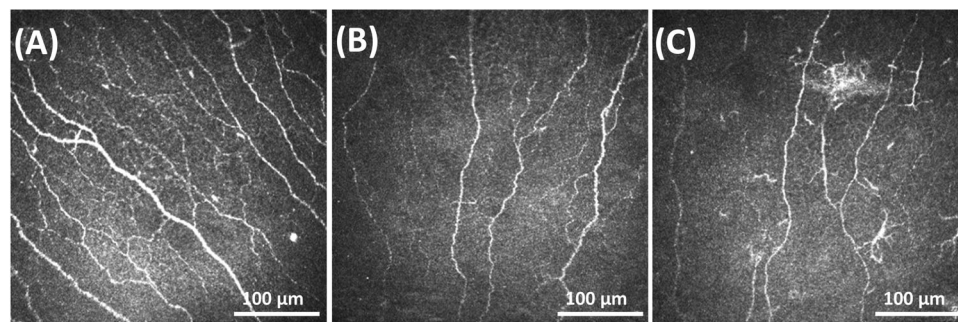[b]NCI versus Dementia.

[c]MCI versus Dementia.

FIGURE 1 Corneal nerve fiber morphology in a subject with no cognitive impairment, mild cognitive impairment (MCI), and dementia. Corneal confocal microscopy (CCM) images of the sub-basal nerve plexus from a subject with (A) no cognitive impairment, (B) MCI, and (C) dementia showing decreased corneal nerve fiber density, length, and branch density in subjects with MCI and dementia compared to subjects with no cognitive impairment

TABLE 2 Receiver-operating characteristic (ROC) curve analysis for the diagnostic accuracy of corneal confocal microscopy and MRI brain volumetry for MCI and dementia

MCI	AUC % (95% CI)	P value	Cutoff point	Sensitivity (%)	Specificity (%)
AUC ≥ 80%					
CNFL, mm/mm²	81 (71-91)	≤.0001	≤21	80	76
AUC 60-80%					
MoCA score	79 (68-90)	≤.0001	≤27	73	84
CNFD, fibers/mm²	76 (66-87)	≤.0001	≤27	59	80
CNBD, branches/mm²	76 (66-87)	≤.0001	≤58	59	80
Whole brain, ICV %	70 (59-81)	.001	≤71	53	80
Ventricle, ICV %	67 (54-80)	.01	≥2.86	58	68
Hippocampus, ICV %	67 (55-79)	<.01	≤0.41	50	80
Unable to distinguish					
Amygdala, ICV %	53 (40-67)	.63	N/A	N/A	N/A
Cortical gray matter, ICV %	52 (38-66)	.77	N/A	N/A	N/A
Thalamus, ICV %	52 (39-64)	.76	N/A	N/A	N/A
Parietal lobe, ICV %	51 (38-65)	.84	N/A	N/A	N/A
Entorhinal cortex, ICV %	51 (37-65)	.86	N/A	N/A	N/A
Cingulate gyrus, ICV %	49 (36-62)	.89	N/A	N/A	N/A
Temporal lobe, ICV %	48 (34-63)	.82	N/A	N/A	N/A
Frontal lobe, ICV %	48 (34-61)	.75	N/A	N/A	N/A
Occipital lobe, ICV %	46 (32-60)	.54	N/A	N/A	N/A

Dementia	AUC % (95% CI)	P value	Cutoff point	Sensitivity (%)	Specificity (%)
AUC ≥ 80%					
MoCA score	97 (92-100)	≤.0001	≤26	100	88
Whole brain, ICV %	93 (85-100)	≤.0001	≤70	92	84
Hippocampi, ICV %	89 (81-98)	≤.0001	≤0.40	85	80
CNFD, fibers/mm²	85 (75-95)	≤.0001	≤27	81	76
CNFL, mm/mm²	84 (73-95)	≤.0001	≤21	81	84
Ventricle, ICV %	82 (70-93)	≤.0001	≥3.58	70	84
AUC 60-80%					
Cortical gray matter, ICV %	79 (67-91)	≤.0001	≤25.4	63	80
CNBD, branches/mm²	77 (64-90)	≤.0001	≤58	80	88
Thalamus, ICV %	76 (63-89)	≤.0001	≤0.83	56	84
Frontal lobe, ICV %	75 (61-89)	≤.0001	≤8.8	59	80
Temporal lobe, ICV %	75 (62-89)	≤.0001	≤6.5	63	80
Amygdala, ICV %	72 (58-86)	<.01	≤0.18	63	84
Entorhinal cortex, ICV %	69 (53-84)	<.05	≤0.26	56	76
Parietal lobe, ICV %	69 (54-84)	<.05	≤5.8	59	
Cingulate gyrus, ICV %	66 (51-81)	<.05	≤0.87	52	
Unable to distinguish					
Occipital lobe, ICV %	63 (48-79)	.10	N/A	N/A	N/A

Abbreviations: no cognitive impairment (NCI), mild cognitive impairment (MCI), corneal nerve fiber density (CNFD), corneal nerve branch density (CNBD), corneal nerve fiber length (CNFL).
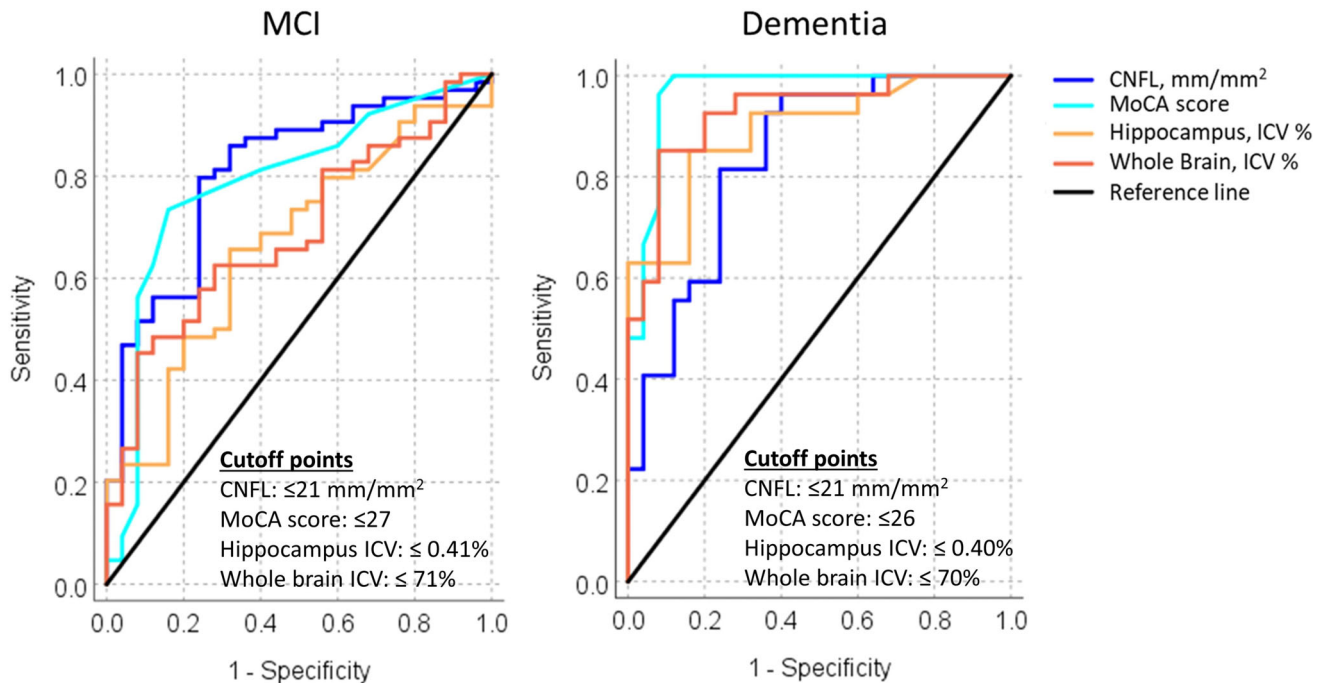
FIGURE 2 The diagnostic accuracy of corneal nerve fiber length, Montreal Cognitive Assessment (MoCA), hippocampus, and whole brain intracranial volume percentage for MCI and dementia. Receiver-operating characteristic (ROC) curve analysis showing the area under the curve for corneal nerve fiber length, MoCA, hippocampus, and whole brain intracranial volume percentage

frontal, temporal and parietal lobe, amygdala, entorhinal cortex, thalamus, cingulate gyrus, and CNBD showed an AUC ranging from 66% to 79%. Occipital lobe volume could not distinguish dementia from NCI (Table 2).

3.4 | Association of CCM measures and brain volumetry with cognitive function

The association of cognitive function with CCM and brain volumetry was assessed after adjustment for duration of cognitive impairment, body weight, and MCV (Table 3). Cognitive function was positively associated with CNFD ($P = .001$), CNBD ($P < .01$), CNFL ($P < .01$), volume of whole brain ($P < .0001$), hippocampi ($P < .0001$), cortical gray matter ($P < .0001$), thalamus ($P < .01$), and amygdala ($P < .01$), and negatively associated with ventricle volume ($P = .001$). Cognitive function had no association with frontal lobe ($P = .34$), temporal lobe ($P = .07$), entorhinal cortex ($P = .44$), and cingulate gyrus ($P = .87$) volumes.

3.5 | Association of CCM measures with brain volumetric MRI

The association of whole brain volume with CCM measures was assessed after adjusting for age, cholesterol, and MCV (Table 3). Whole brain volume was positively associated with CNFD (β coefficient: 0.08 fibers/mm^2, 95% CI 0.003, -0.16; $P < .05$) but not CNBD ($P = .23$) or CNFL ($P = .12$).

4 | DISCUSSION

This study shows that the diagnostic capability of corneal confocal microscopy (or CCM) is superior to MRI brain volumetry for distinguishing MCI from NCI and comparable for distinguishing dementia from NCI. Furthermore, after adjustment for confounding factors, corneal nerve measures and MRI brain volumetry were associated with cognitive function in MCI and dementia.

Structural neuroimaging has been validated as a diagnostic biomarker of neurodegeneration in AD.[4,6] A significant reduction in the volume of the hippocampus, amygdala, and entorhinal cortex and an increase in the volume of the lateral ventricles are established features of dementia but not MCI.[13,38] This study shows that the volume of whole brain, hippocampi, and lateral ventricles identifies patients with dementia with high accuracy (AUC ≥80%), whereas the volume of cortical gray matter, thalamus, amygdala, entorhinal cortex, cingulate gyrus, frontal, temporal, and parietal lobes has only moderate accuracy (AUC = 66-79%). Furthermore, we and others[30,39] have shown that MRI brain volumetry performs poorly in identifying people with MCI, indicating that significant brain atrophy only occurs in established dementia, although the annual change in hippocampal volume is a good predictor of MCI progression to dementia.[13,14]

CCM, an ophthalmic imaging technique, shows corneal nerve degeneration in people with MCI and dementia, which was related to the severity of cognitive dysfunction and impaired activity of daily living.[29,30] Corneal nerve fiber measures are significantly lower in patients with AD, VaD, and mixed dementia compared to MCI and

TABLE 3 The association between corneal nerve fiber measures, MRI brain volumetry, and cognitive function

	Adjusted beta coefficient	95% Confidence Interval	P value
MoCA score as a dependent variable			
CNFD, fibers/mm^2	0.22	0.09 to 0.35	.001
CNBD, branches/mm^2	0.04	0.01 to 0.08	<.01
CNFL, mm/mm^2	0.27	0.09 to 0.45	<.01
Whole Brain, ICV %	0.98	0.63 to 1.33	<.0001
Hippocampi, ICV %	37.14	21.38 to 52.90	<.0001
Ventricle, ICV %	−1.42	−2.22 to −0.61	.001
Cortical gray matter, ICV %	0.72	0.38 to 1.06	<.0001
Frontal lobe, ICV %	0.30	−0.32 to 0.91	NS
Temporal lobe, ICV %	0.76	−0.06 to 1.57	NS
Entorhinal cortex, ICV%	5.04	−7.88 to 17.95	NS
Thalamus, ICV %	17.91	5.59 to 30.22	<.01
Amygdala, ICV %	58.56	18.55 to 98.58	<.01
Cingulate gyrus, ICV %	−0.54	−7.31 to 6.23	NS
Whole brain volume, ICV % as a dependent variable			
CNFD, fibers/mm^2	0.08	0.003 to 0.16	<.05
CNBD, branches/mm^2	0.01	−0.01 to 0.03	NS
CNFL, mm/mm^2	0.09	−0.02 to 0.20	NS

All the variables considered in the fitted model had $P < .05$. MoCA score was adjusted for duration of cognitive impairment, body weight, and mean corpuscular volume. Whole brain volume was adjusted for age, cholesterol, and mean corpuscular volume. Abbreviations: corneal nerve fiber density (CNFD), length (CNFL), branch density (CNBD), and intra cranial volume (ICV).

NCI.[29,30] We have also shown that CCM is superior to the presence of medial temporal lobe atrophy (MTA) for distinguishing MCI from NCI.[30] The pathogenic processes common to dementia and corneal nerve fiber damage are not known. Corneal nerves are derived from the ophthalmic division of the trigeminal nerve and are anatomically components of the peripheral nervous system.[40,41] Tauopathy is a key feature of dementia,[42] and we have recently shown stromal corneal nerve loss in transgenic mice overexpressing human tau.[43] Corneal nerve loss has also been associated with many of the risk factors for MCI and dementia including hyperglycemia, hypertension and hyperlipidemia,[21] and the presence of white matter hyperintensities,[44] and cerebral ischemia.[45] Although diabetes is associated with corneal nerve loss,[17,46–49] this study shows that the loss of corneal nerve fibers in patients with MCI and dementia remained significant after excluding those with diabetes. Indeed, in our recent study in which diabetes was excluded, there was evidence of significant corneal nerve fiber loss in patients with MCI and dementia.[29]

MTA is associated with memory loss in MCI and AD.[50,51] Stelmokas et al[37] reported reduced hippocampal volume and enlarged lateral ventricles with delayed memory performance in MCI. This study shows that the volume of whole brain, hippocampi, ventricles, cortical gray matter, thalamus, and amygdala were significantly associated with cognitive function in MCI and dementia. In line with our previous findings,[29] this study also showed that corneal nerve fiber loss was

associated with cognitive decline. The association between neurodegeneration and cognitive function in dementia is complex, as some post-mortem studies have shown that there are cases of dementia with limited neurodegeneration and equally there are patients with neurodegeneration without cognitive impairment.[52–54] The association between neurodegeneration and cognitive impairment may also be influenced by cerebrovascular ischemia,[45,55] which reduces the brain reserve to tolerate neurodegeneration.[54,56] The association of corneal nerve loss with cognitive impairment may be influenced by diabetes, as it increases the odds of cerebral ischemia, infarct, and lacunes,[57] which increase the risk of cognitive impairment[58] and dementia.[59]

Further studies are needed to compare the diagnostic performance of CCM in MCI and dementia against established biomarkers of AD including [^{18}F] fluorodeoxyglucose (FDG) uptake on PET,[60,61] cerebrospinal fluid (CSF) concentrations of amyloid beta (Aβ) 42, Aβ40 or tau/phosphorylated tau,[62,63] or positron emission tomography (PET) for Aβ deposition.[64,65]

An ideal biomarker for dementia should be able to identify subclinical pathology in MCI and predict those who develop dementia.[5] In the present study, although all three corneal nerve fiber measures were reduced in MCI and dementia, a proportion of people with MCI had corneal nerve loss that was comparable to that of patients with dementia. A longitudinal study is currently underway to assess whether people who progress from MCI to dementia show greater evidence of

corneal nerve loss. We acknowledge as a study limitation that we have not assessed for severe dry eye, which is associated with corneal nerve abnormalities.[66] Our study shows that there was an increase in the vibration perception threshold in subjects with dementia. However, vibration perception threshold is a subjective psychophysical test, which may have been influenced by a lack of motivation, alertness, and concentration, especially in patients with dementia.

In conclusion, this study shows that CCM has high diagnostic accuracy for MCI and dementia, whereas MRI brain volumetry has high diagnostic accuracy for dementia only. Loss of corneal nerves and MRI brain volume was associated with cognitive impairment in MCI and dementia. These data support the contention that CCM could act as a surrogate marker of neurodegeneration in MCI and dementia, especially to identify people with MCI who progress to dementia.

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CONFLICTS OF INTEREST

The authors confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship and are not listed. They also confirm that the order of authors listed in the manuscript has been approved by all authors. Dr. Surjith Vattoth has Elsevier book author royalty, received consulting fee as an Elsevier master author consultant in head and neck imaging, and received payment for ESNR - ECHNR course faculty. None of the other authors have received or anticipate receiving income, goods, or benefit from a company that will influence the design, conduct, or reporting of the study.

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