Universitat Autònoma de Barcelona

DIABETES MELLITUS AS AN ACCELERATOR OF COGNITIVE IMPAIRMENT AND ALZHEIMER'S DISEASE

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DOCTORAL THESIS PRESENTED BY

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Abbreviations.

AD: Alzheimer´s disease.

AUC: Area under the curve.

BCEA63: Bivariate contour ellipse area 63($º^2$).

BCEA95: Bivariate contour ellipse area 95($º^2$).

BMI: Body mass index.

DR: Diabetic retinopathy.

DSDRS: Diabetes Specific Dementia Risk Score.

FDG: Fluorodeoxyglucose.

GF: Gaze fixation.

HR: Hazard ratio.

MAIA: 3rd generation microperimeter.

MCI: Mild cognitive impairment.

MMSE: Mini-Mental State Examination.

MOPEAD: Models of patient engagement for Alzheimer´s disease.

MRI: Magnetic resonance imaging.

NA: Not applicable.

NC: Normo-cognitive.

PET: Positron emission tomography.

RBANS: Repeatable Battery for the Assessment of Neuropsychological Status.

RS: Retinal sensitivity.

SD: Standard Deviation.

T2D: Type 2 diabetes.

VEP: Visual evoked potentials.

Index.

Resumen

La diabetes y la enfermedad de Alzheimer son dos patologías altamente prevalentes, con alto coste sanitario y relacionadas muy estrechamente entre sí. Por este motivo la *American Diabetes Association* (ADA) recomienda el cribaje de deterioro cognitivo en personas con diabetes mayores de 65 años. El evento de mayor interés es el diagnóstico de deterioro cognitivo leve (estado cognitivo situado entre la normo-cognición y la demencia), debido que sobre él se puede realizar medidas dirigidas a evitar el empeoramiento del estado cognitivo, por ejemplo, evitar hipoglucemias graves simplificando pautas terapéuticas. Las guías de práctica clínica actuales recomiendan para el cribado el uso del *Mini-mental State* (MMSE) y el *Montreal Cognitive Assessment* (MoCA), la primera con una baja sensibilidad para el diagnóstico de deterioro cognitivo leve (DCL) y la segunda requiere de certificación y personal entrenado para su uso. Para el establecer el diagnóstico se requiere de baterías neuropsicológicas complejas como la *Repeatable Battery for the Assessment of Neuropsychological* (RBANS), hoy imposible de implementar en la práctica clínica habitual. Bajo este escenario se plantea el objetivo principal de esta tesis doctoral que es evaluar la prevalencia de DCL en población con diabetes tipo 2 (DM2) > 65 años en un ámbito de atención terciaria y evaluar la posible aplicabilidad de otras pruebas de cribado como el *Diabetes Specific Dementia Risk Score* (DSDRS) y la M*icroperimetría Retiniana* (MAIA).

El DSDRS es un cuestionario desarrollado para predecir el riesgo de desarrollar deterioro cognitivo en personas con diabetes. Dado que muchos de los factores de riesgo para desarrollar demencia se encuentran en sus ítems. Uno de los objetivos secundarios de esta tesis fue valorar si DSDRS no solo es útil como predictor, sino también como pruebas de cribado de deterioro cognitivo.

La microperimetría de retina es una prueba no invasiva que mide la sensibilidad de la retina en términos de la mínima intensidad de luz que los pacientes pueden percibir cuando los puntos de luz estimulan áreas específicas de la retina y también evalúa la estabilidad de la fijación de la mirada. Su utilidad en la detección del deterioro cognitivo ha sido validada en estudios previos de nuestro equipo.

La primera parte de esta tesis doctoral se centra en establecer la prevalencia de DCL en la población con DM2 >65 años que acude a visitas de endocrinología en el ámbito hospitalario de tercer nivel. Para ello se diseñó un estudio clínico prospectivo observacional donde observamos una alta prevalencia de deterioro cognitivo

desconocido en pacientes DM2 que acudieron a un hospital de tercer nivel. Además, se estableció que el DSDRS podría ser una herramienta de detección útil para el deterioro cognitivo. Se requiere de nuevos estudios dirigidos a evaluar esta posible prueba de cribado.

La segunda parte de esta tesis doctoral se centra en primero validar nuestros resultados previos del uso del MAIA como una herramienta de detección confiable para DCL y se evaluó su utilidad como herramienta de monitorización anual del estado cognitivo. Para ello se diseñó un estudio prospectivo con seguimiento a 3 años, donde pudimos observar que los parámetros de la sensibilidad de la retina y sobre todo la fijación de la mirada se correlaciona con los dominios cognitivos del recuerdo diferido obtenidos por la RBANS, siendo una herramienta útil en la monitorización del estado cognitivo.

Finalmente, para robustecer nuestra teoría de que el MAIA explora diferentes circuitos neuronales, se diseñó un estudio transversal descriptivo con el objetivo de evaluar la relación de los parámetros obtenidos por MAIA con la vía visual valorada con el uso de potenciales visuales evocados (PVE). Se observó que correlación entre la sensibilidad de la retina (SR) obtenida por MAIA con los parámetros obtenidos con el uso de los PVE, estos resultados fortalecen a la teoría de que, la SR evalúa la vía óptica desde la retina hasta el cuerpo geniculado lateral del tálamo. Mientras que la fijación de la mirada parece depender de la compleja red de materia blanca, colículo superior, corteza parietal y frontal. Se requieren más estudio para poder profundizar esta relación.

Esperamos que este trabajo contribuya al conocimiento, exploración y aplicabilidad de nuevas pruebas de cribado sencillas y fácilmente implementables en la práctica clínica habitual para el diagnóstico de deterioro cognitivo leve en personas con DM2 > de 65 años.

Abstract

Diabetes and Alzheimer's disease are two highly prevalent pathologies, with high healthcare costs and very closely related to each other. For this reason, the *American Diabetes Association* (ADA) recommends screening for cognitive impairment in people with diabetes over 65 years of age. The most important event is the diagnosis of mild cognitive impairment (cognitive state situated between normo-cognition and dementia), because it is possible to carry out measures aimed at avoiding the worsening of the cognitive state, for example, avoiding severe hypoglycemia by simplifying treatment guidelines. Current clinical practice guidelines recommend the use of the *Mini-mental State* (MMSE) and the *Montreal Cognitive Assessment* (MoCA), the first one with a low sensitivity for the diagnosis of mild cognitive impairment (MCI) and the second one requires certification and trained personnel to use it. Complex neuropsychological batteries such as *Repeatable Battery for the Assessment of Neuropsychological* (RBANS) is required for the diagnosis, impossible to implement in standard clinical care. In this context, the main objective of this doctoral thesis is to evaluate the prevalence of MCI in a population with type 2 diabetes (DM2)> 65 years in a tertiary care setting and evaluate the possible applicability of other screening tests such as the *Diabetes Specific Dementia Risk Score* (DSDRS) and the retinal microperimetry (MAIA).

The DSDRS is a questionnaire developed to predict the risk of developing cognitive decline in people with diabetes. Because many of the risk factors for developing dementia are found in its items. One of the secondary objectives of this thesis was to evaluate the DSDRS, as a screening test for cognitive impairment and not as a predictor.

Retinal microperimetry is a non-invasive test that measures retinal sensitivity in terms of the minimum light intensity that patients can perceive when spots of light stimulate specific areas of the retina and evaluates gaze fixation stability. Its usefulness to detecting cognitive impairment has been validated in previous studies by our group.

The first part of this doctoral thesis focuses on determining the prevalence of MCI in the population with DM2> 65 years who attend endocrinology clinic in a tertiary hospital setting. For this purpose, a prospective observational clinical study was designed where we observed a high prevalence of unknown cognitive impairment in DM2 patients who attended a tertiary hospital. In addition, it was concluded that

DSDRS could be a useful screening tool for cognitive impairment. New studies are needed to evaluate this possible screening test.

The second part of this doctoral thesis focuses on, first to evaluate our previous results by MAIA as a reliable detection tool for MCI and its usefulness as an annual monitoring tool for cognitive status. For this purpose, a prospective study with a 3-year follow-up was designed, where we observed that the parameters of the retinal sensitivity (RS) and especially the gaze fixation have a correlation with the cognitive domains such as delayed memory obtained by the RBANS, being a useful tool in the monitoring of the cognitive state.

Finally, to strengthen our theory that the MAIA explores different neural circuits, a descriptive cross-sectional study was designed aiming at evaluating the relationship between the parameters obtained by MAIA and the visual pathway assessed by means of visual evoked potentials (VEP). A correlation between the RS obtained by MAIA with the parameters obtained by VEP was observed. These results strengthen the theory that RS evaluate the optic pathway from the retina to the lateral geniculate body of the thalamus, while gaze fixation seems to depend on the complex network of white matter, superior colliculus, parietal, and frontal cortex. More studies are required to deepen this relationship.

We hope that this work contributes to the knowledge, exploration, and applicability of new, simple and easily implemented screening tests in the daily clinical practice for the diagnosis of MCI in people with DM2> 65 years old.

1. Introduction

1.1. T2D AND DEMENTIA. EPIDEMIOLOGICAL DATA.

In 2019, a total of 463 million people is estimated to be living with diabetes, representing 9.3% of the global adult population (20–79 years). This number is expected to increase to 578 million (10.2%) in 2030 and 700 million (10.9%) in 2045 (1) as reflected by the **Figure 1**.

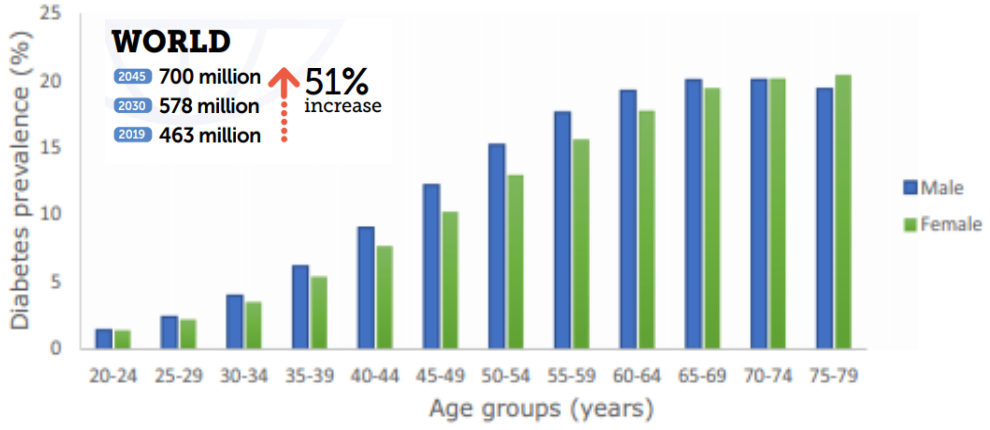


Figure 1.Diabetes prevalence by age and sex in 2019.

Current epidemiological data shows that type 2 diabetes (T2D) increases the risk of developing dementia, and in particular Alzheimer's disease (AD), by two to three times in comparison with the nondiabetic population matched by age and other risk factors (2).

AD is the most common cause of dementia (3) and is characterized by a progressive amnestic disorder with subsequent appearance of other cognitive, behavioural, and neuropsychiatric changes that impair social function and activities of daily living (4).

The prevalence of dementia is rapidly increasing in developed countries due to social and demographic changes. This trend is expected to worsen in the coming decades, with the number of cases possibly even tripling in the next 25 years. In fact, the World Health Organization has declared dementia control a global health priority (5).

T2D and AD are two prevalent diseases associated with a significant economic burden for the health-care systems of developed countries. The demographic and geographical relationship between T2D an AD is displayed in **Figure 2** (1)(6)(7). As can be appreciated, both diseases are age-related and,

therefore, it is not surprising that they run in parallel and are particularly prevalent in the developed world.

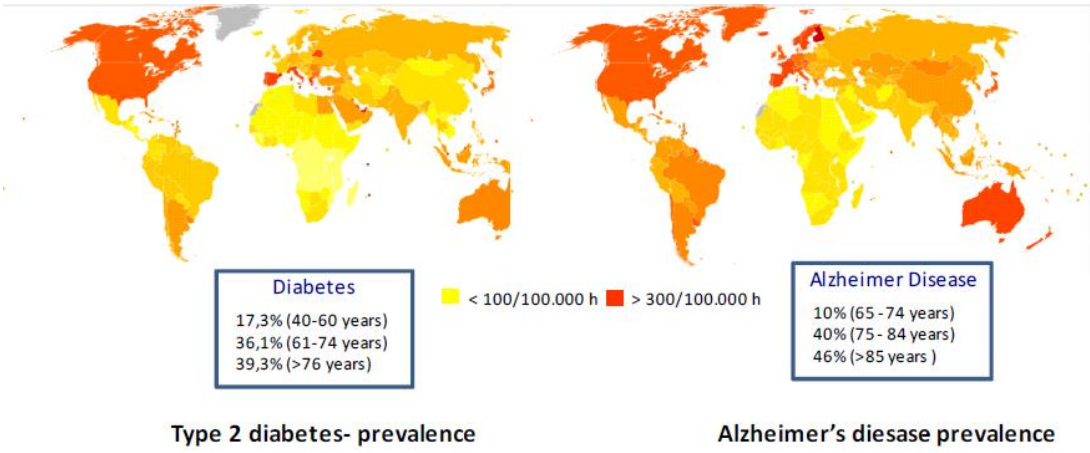


Figure 2. The prevalence of type 2 diabetes and Alzheimer's disease worldwide.

A meta-analysis published in 2006 by Biessels et al (8) showed that diabetic patients had an almost two-fold higher risk of developing AD than age-matched non-diabetic subjects. These findings have been recently confirmed in a large Nationwide Population-based Study performed in Taiwan with a follow-up of 10 years, which clearly shows that the risk of developing AD in the diabetic population is significantly higher than in the non-diabetic population with a hazard ratio (HR) of 1.7(9). In addition, there is evidence that not only the presence of diabetes, but the duration of the disease is an important risk factor. In the Harmony Study, which was performed in a large Swedish cohort of twins, it was shown that diabetic patients, diagnosed at age ≤65 years old, presented a higher risk of AD than those patients in whom diabetes was diagnosed ≥65 years (9) (10).

Also, poor glycaemic control is also an independent risk factor for accelerated cognitive decline and AD in the T2D population (11) (12) and it has been suggested that higher glucose levels may be a risk factor for dementia, even among persons without diabetes (13).

1.2. MILD COGNITIVE IMPAIRMENT AND T2D.

The effectiveness of treatment strategies is limited when dementia is already established. For this reason, efforts should focus on early diagnosis strategy that could lead to significant economic and social impact (2).

The term “mild cognitive impairment” was first suggested in 1980 by Reisberg et al (14) but only in 1999 Petersen et al.(15) further developed and defined the concept by proposing criteria based on an observational study on ageing.

At a Key Symposium held in Stockholm in the year 2003, MCI was defined as a heterogeneous entity divided into three categories: amnestic MCI with greater risk of AD; MCI of multiple cognitive domains; and MCI with impairment of a single cognitive function different from memory (16). Subsequently, it is classified into two categories, amnestic (single or multiple domain) and non-amnestic (single or multiple domain) (17).

Main diagnostic points of MCI were redefined: the individual having neither dementia nor normal cognition. There is evidence of cognitive decline measured objectively or based on subjective perception combined with objective cognitive impairment and preservation of basic living and complex instrumental activities or minimally compromised(16).

Mild cognitive impairment (MCI) represents a greater impairment in cognition than normal age-related memory loss, but not severe enough to cause significant impairment on daily function.(18)(19). MCI was included in the International Classification of Diseases (ICD).

Generally, amnestic MCI is characterized by a decreased ability to learn new information or retrieve stored information, without affecting the basic activities of the daily living. Despite the fact that patients with MCI can conduct a normal life, the important problem associated with MCI is that about 10–30% will progress annually to dementia (20). The **Figure 3,** shows the charting the course from healthy ageing to AD.

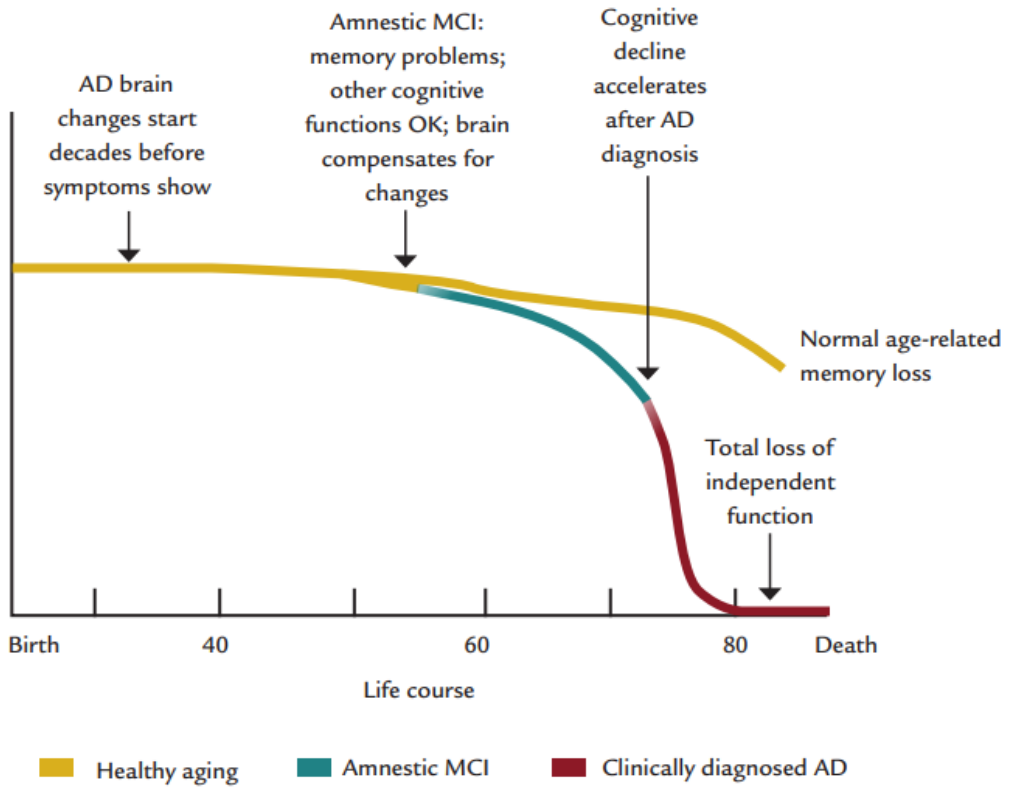


Figure 3. Charting the course from healthy ageing to AD

Despite some discrepancies among studies, the researchers agree that individuals diagnosed with MCI develop dementia at a faster rate than the rest of the population (21).

In addition, the disappointing results of clinical trials carried out in individuals with established dementia have generated the hypothesis that interventions have occurred too late in the process of the disease. Probably the earlier we act, the better the chances of success (5).

However, it is clear that in most health systems, dementia is underdiagnosed, and when diagnosis occurs, it is typically at a relatively late and irreversible stage in the process of the disease.

Evidence from a primary care-based screening and diagnosis program in the United States revealed that only 19% of patients with a confirmed dementia diagnosis had been screened for dementia during routine medical care (22).

Additionally, a population-based study showed that approximately 20% of family informants failed to recognize memory problems in elderly subjects who were found to have dementia on a standardized examination (23).

Early diagnosis of MCI is important in T2D population for several reasons. First, the early detection may allow specific interventions aimed at slowing the conversion to dementia. For example, using drugs without or with minimal hypoglycemia capacity is strongly recommended (24). Hypoglycemia and dementia are clinically underestimated and are related to poor outcomes; thus, they may compromise the life expectancy of patients with T2D (25). Severe hypoglycemic episodes induce chronic subclinical brain damage, cognitive decline, and subsequent dementia (25). However, the effects of recurrent moderate hypoglycemia on cognitive decline and dementia remain largely uninvestigated (25) (26).

The possible pathophysiological hypotheses include post-hypoglycemic neuronal damage, inflammatory processes, coagulation defects, endothelial abnormalities, and synaptic dysfunction of hippocampal neurons during hypoglycemia episodes (25) Secondly, the presence of MCI can lead to mismanagement of the specific treatment for T2D. This can cause episodes of severe hypoglycemia and increasing cardiovascular mortality, and hypoglycemia itself worsens the patient's cognitive state, all of which translates into an increase in healthcare costs directly or indirectly (27).

Furthermore, an accurate diagnosis is the prerequisite to found reversible causes of cognitive impairment, eg, depression (28).

The diagnose of cognitive impairment in the early stages in patients with T2D, will allow the implementation of a patient-centered treatment based on its simplicity and the prioritizing of antidiabetic treatments with a low capacity to provoke hypoglycemia.

In fact, the American Diabetes Association ADA clinical guidelines recommend screening for early detection of cognitive impairment in adults older than 65 at the initial visit and annual follow-ups as appropriate (29).

For this purpose, screening tools such as the mini-mental state examination (MMSE) test (30) and the Montreal cognitive assessment (MoCA) are recommended. Nevertheless, once the screening is performed, diagnosis of MCI is based on a complex neuropsychological test battery (18), strategy which makes unfeasible their incorporation into current standards of care for the whole population with T2D. At present, there are no reported reliable examinations or phenotypic indicators to identify patients with T2D with MCI.

Screening is a key step in the diagnosis of dementia, which is why the recommended methods should have a high sensitivity for the detection of MCI (31).

In addition, early diagnosis can allow the patient to participate in research studies and support groups in the community if desired (32). For these reasons, early diagnosis is important in this specific population.

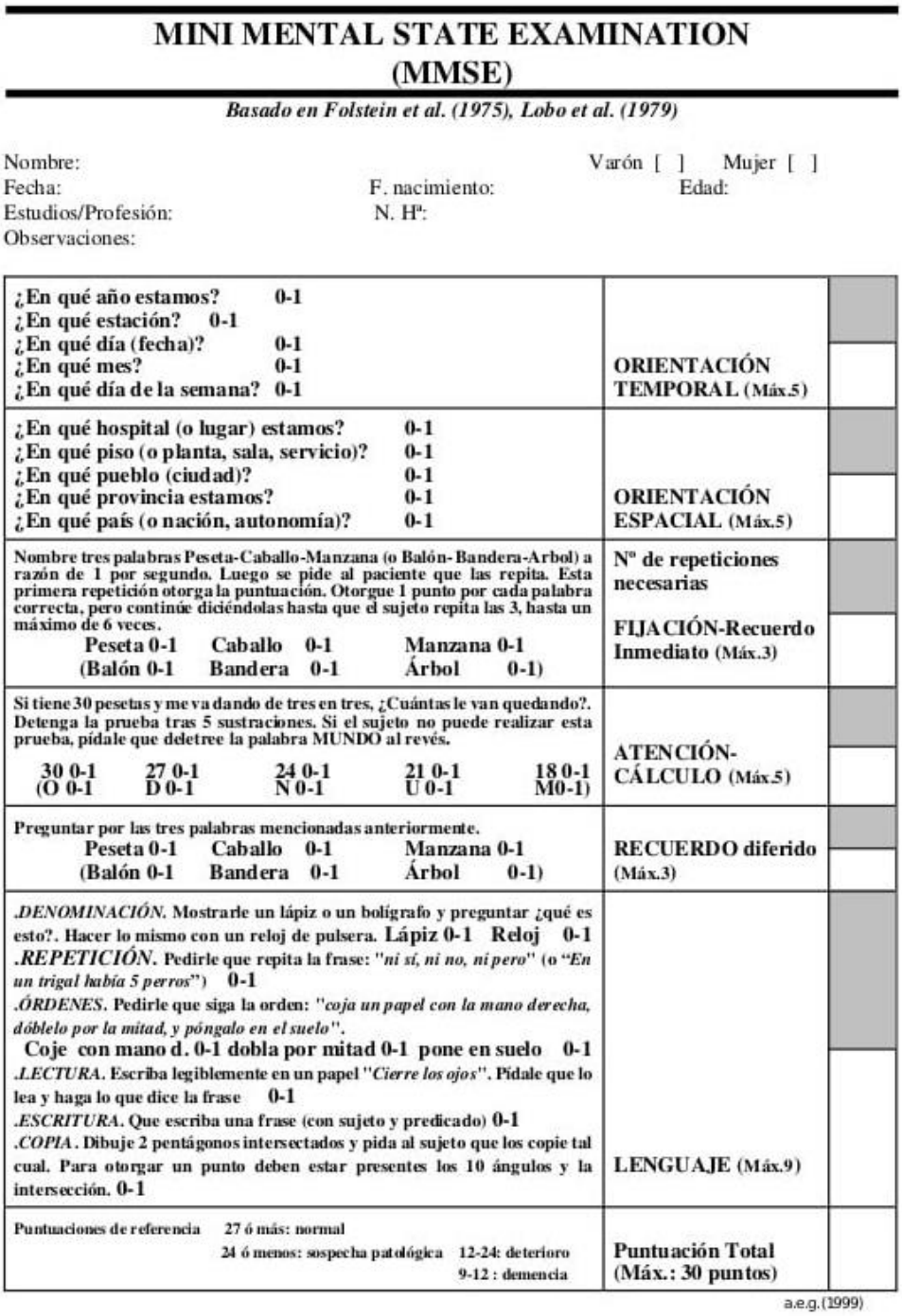
1.3. SCREENING TESTS AIMED AT IDENTIFYING COGNITIVE IMPAIRMENT.

1.3.1. MINI-MENTAL STATE EXAMINATION (MMSE).

Screening tests play a crucial role in dementia diagnostics, thus they should be very sensitive for mild cognitive impairment (MCI) assessment. Nowadays, the Mini-Mental State Examination (MMSE) is the most commonly used scale in cognitive function evaluation, albeit it is claimed to be imprecise for MCI detection (33).

The Mini-Mental State Examination (MMSE) was published 47 years ago, in 1975 as a practical method for cognitive function assessment (30). Currently it is the most commonly used screening method in the assessment of the severity of dementia in both: clinical and research field. According to Milne et al (34), 79% of the healthcare professionals use at least one test, 51% of them – the MMSE and its variants (34). Research of Davey et al. shows that 91% of interviewees use MMSE during their medical practice (35).

MMSE is an easy and quick test to perform, the Spanish version of the test is shown at **Figure 4**. Despite the fact that American Academy of Neurology in its guidance suggested MMSE as an important tool in detecting early cognitive disorders, many researchers doubts the accuracy of this scale (36).



MINI MENTAL STATE EXAMINATION (MMSE)

Basado en Folstein et al. (1975), Lobo et al. (1979)

Nombre: Varón [ ] Mujer [ ]
Fecha: F. nacimiento: Edad:
Estudios/Profesión: N. Hª:
Observaciones:

¿En qué año estamos? 0-1 ¿En qué estación? 0-1 ¿En qué día (fecha)? 0-1 ¿En qué mes? 0-1 ¿En qué día de la semana? 0-1	**ORIENTACIÓN TEMPORAL** (Máx.5)
¿En qué hospital (o lugar) estamos? 0-1 ¿En qué piso (o planta, sala, servicio)? 0-1 ¿En qué pueblo (ciudad)? 0-1 ¿En qué provincia estamos? 0-1 ¿En qué país (o nación, autonomía)? 0-1	**ORIENTACIÓN ESPACIAL** (Máx.5)
Nombre tres palabras Peseta-Caballo-Manzana (o Balón-Bandera-Arbol) a razón de 1 por segundo. Luego se pide al paciente que las repita. Esta primera repetición otorga la puntuación. Otorgue 1 punto por cada palabra correcta, pero continúe diciéndolas hasta que el sujeto repita las 3, hasta un máximo de 6 veces. Peseta 0-1 Caballo 0-1 Manzana 0-1 (Balón 0-1 Bandera 0-1 Árbol 0-1)	**Nº de repeticiones necesarias** **FIJACIÓN-Recuerdo Inmediato** (Máx.3)
Si tiene 30 pesetas y me va dando de tres en tres, ¿Cuántas le van quedando?. Detenga la prueba tras 5 sustraciones. Si el sujeto no puede realizar esta prueba, pídale que deletree la palabra MUNDO al revés. 30 0-1 27 0-1 24 0-1 21 0-1 18 0-1 (O 0-1 D 0-1 N 0-1 U 0-1 M0-1)	**ATENCIÓN-CÁLCULO** (Máx.5)
Preguntar por las tres palabras mencionadas anteriormente. Peseta 0-1 Caballo 0-1 Manzana 0-1 (Balón 0-1 Bandera 0-1 Árbol 0-1)	**RECUERDO diferido** (Máx.3)
.DENOMINACIÓN. Mostrarle un lápiz o un bolígrafo y preguntar ¿qué es esto?. Hacer lo mismo con un reloj de pulsera. Lápiz 0-1 Reloj 0-1 *.REPETICIÓN.* Pedirle que repita la frase: "*ni sí, ni no, ni pero*" (o "*En un trigal había 5 perros*") 0-1 *.ÓRDENES.* Pedirle que siga la orden: "*coja un papel con la mano derecha, dóblelo por la mitad, y póngalo en el suelo*". Coje con mano d. 0-1 dobla por mitad 0-1 pone en suelo 0-1 *.LECTURA.* Escriba legiblemente en un papel "*Cierre los ojos*". Pídale que lo lea y haga lo que dice la frase 0-1 *.ESCRITURA.* Que escriba una frase (con sujeto y predicado) 0-1 *.COPIA.* Dibuje 2 pentágonos intersectados y pida al sujeto que los copie tal cual. Para otorgar un punto deben estar presentes los 10 ángulos y la intersección. 0-1	**LENGUAJE** (Máx.9)
Puntuaciones de referencia 27 ó más: normal 24 ó menos: sospecha patológica 12-24: deterioro 9-12 : demencia	**Puntuación Total (Máx.: 30 puntos)**

a.e.g.(1999)

Figure 4. Mini-mental state examination (MMSE) Spanish version.

The problem of this test is the insufficient sensitivity of the different tests which assess individual domains only and the lack of correlation between the final result and age, education, gender or ethnic differences (37) (38) (39).

Most individuals meeting clinical criteria for MCI score above 26 on the MMSE, which is also the range for normal elderly individuals (40).

A meta-analysis in 2016 by Ciesielska et al (33) showed that 13 out of 20 studies provide information regarding the sensitivity and specificity. Only 2 studies reported results for 5 or more cut-off points. The remaining 11 studies took into account the most promising parameters based on the cut-off points. Most often sensitivity and specificity for scoring: 27/28 (n = 6 studies); 25/26 (n = 5); and recommended 26/27 (n = 4) was presented. On the basis on ROC analysis, score 27/28 proved to be most promising (sensitivity of 66.34% and a specificity of 72.94%) in differentiating MCI vs. NC. The author concluded that MMSE is characterized by low detection of early cognitive deficits (33).

1.3.2. MONTREAL COGNITIVE ASSESSMENT (MoCA).

The Montreal Cognitive Assessment (MoCA) was developed in 2005 as a tool to screen patients who present with mild cognitive complaints and usually perform in the normal range on the MMSE (40).

The last version contains eight domains to explore as a MMSE. The Spanish version as reflected by the **Figure 5.**

A meta-analysis in 2016 by Ciesielska et al (33) evaluated the reliability of 20 published studies analyzing MoCA and MMSE in distinguishing MCI among the healthy population aged over 60. Whole sample of MCI group consisted of 3,024, and a group of healthy controls consisted of 4,862 people (33).To analyze the sensitivity and specificity of MoCA for all the cut-offs, it was observed that only 5 studies had data for eight or more cut-off points.

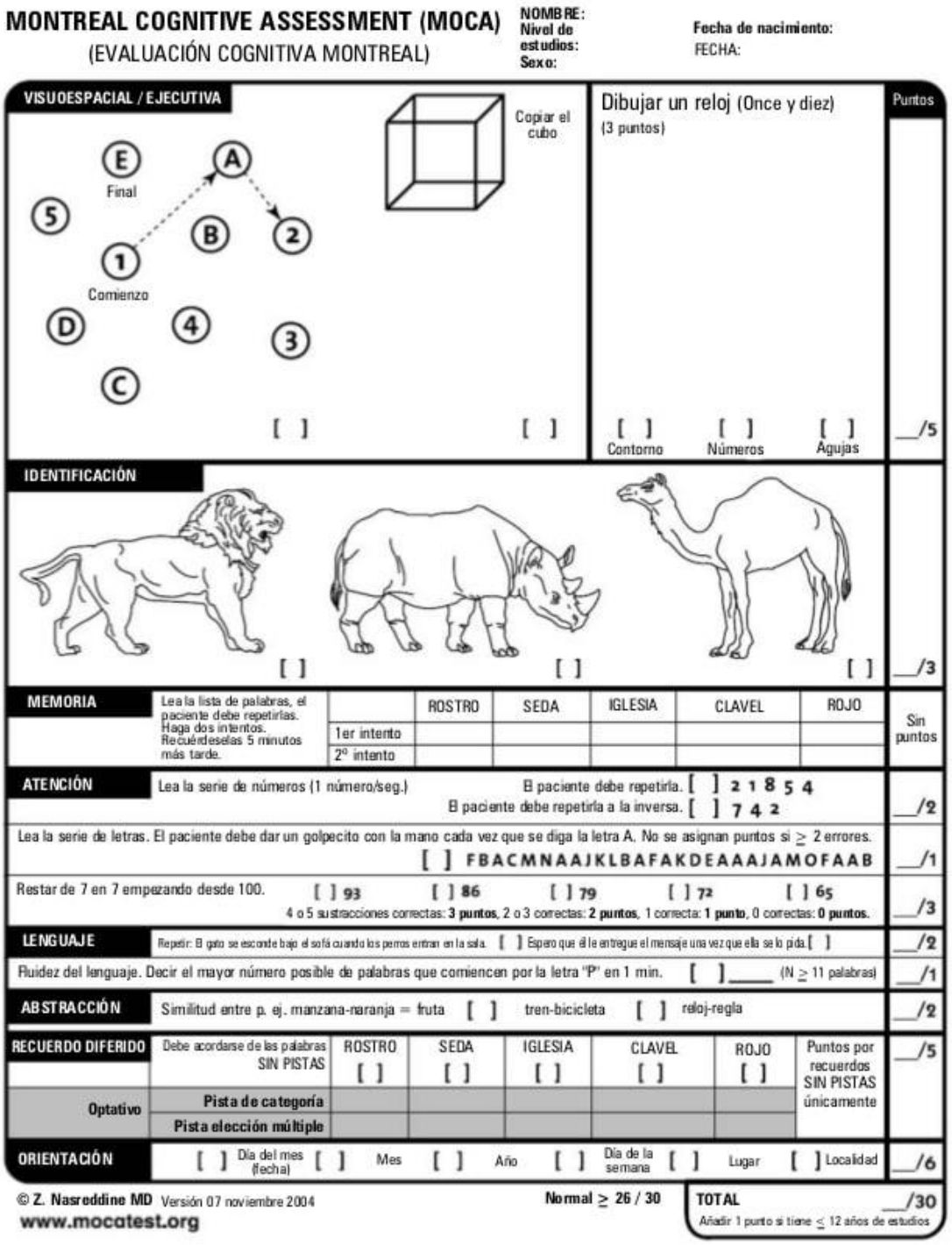


Figure 5. Montreal Cognitive Assessment (MoCA) Spanish version.

The remaining 16 studies included only data for the most reliable – according to the authors – cut-off points. In most cases sensitivity and specificity were documented for the recommended parameter 25/26 (n = 13 studies), for 23/24 (n = 10) and 24/25 (n = 9) (33).

Finally, the ROC analysis showed that the most diagnostically reliable cut-off point in differentiating MCI vs. HC is 24/25 (sensitivity of 80.48% and specificity of 81.19%) (33).

Analyzing the diagnostic reliability of the test based on multiple cut-offs – where AUC was 0.736 for MMSE (95% CI: 0.718–0.767) and for MoCA 0.846 (95% CI: 0.823–0.868) – one can make an assumption that MoCA is better in detecting MCI than MMSE. A similar conclusion was made by Mitchell et al. (36) in a meta-analysis evaluating the accuracy of MMSE in detecting MCI (33).

The major limitations for the use of MoCA at large scale in daily clinical practice is the need of previous training and certification and that the patient need to be fully literated.

Figure 6 shows the comparison between the cognitive domains tested in MMSE and MoCA conducted by Magierska et al (41).

Cognitive function		MMSE (No. of points/trials)	MoCA (No. of points/trials)
Orientation		10 tasks (10 points)	6 tasks (6 points)
Memory	Learning	Learning of 3 words (3points/1 trial allowed)	learning of 5 words (no points/2 trials allowed)
	Delayed recall	3 words (3 points)	5 words (5points)
	Cued recall (optional)	not present	5 words (no points)
	Recognition (optional	not present	5 words (no points)
Naming		2 items (2 points)	3 pictures (3 points)
Visuospatial functions		copy of pentagons (1 point)	copy of cube (1 point) clock drawing (3 points)
Comprehension		3-stage command (3)	not present
Vigilance		not present	tapping with hand at letter A (1 point)
Language		repetition of sentence (1 point)	Repetition of 2 sentences (2 points)
Reading		Sentence (1 point)	not present
Abstract thinking		not present	similarities (2 points)
Writing		patient's sentence (1 point)	not present
Alternating Trial Making		not present	1 trial (1 points)

Figure 6. Comparison of MMSE and MoCA in terms of the studied areas of cognition and scoring.

1.4. POSSIBLE TOOLS TO SCREENING FOR THE DIAGNOSIS OF COGNITIVE IMPAIRMENT.

1.4.1. DIABETES SPECIFIC DEMENTIA RISK SCORE (DSDRS).

In the recent years, attention has turned to early intervention strategies at a stage when there still is time to modify disease progression (42) (43).

Nevertheless, prevention trials have not yet shown the desired effect. To increase the chances of success of future trials, enrichment of study cohorts through risk stratification has been recommended.

A similar approach has been followed successfully in the cardiovascular field, where risk scores are used to select people for targeted treatment (44) (45).

Recently, several risk scores to predict the risk of dementia have been published (see research in context panel) (46) (47) (48) (49).

Since people with T2DM are particularly vulnerable to dementia, it is even more critical to identify those at high risk in early stages.

The Diabetes Specific Dementia risk Score (DSDRS) predicts an individual's absolute risk of developing dementia within the subsequent decade based on diabetes-related comorbidities and complications, age, and education. The DSDRS stratifies individuals into 14 categories from −1 to ≥12, showing a 15-fold difference in dementia risk between the lowest and the highest sum score and performs well in all age categories (50). **Figure 7**

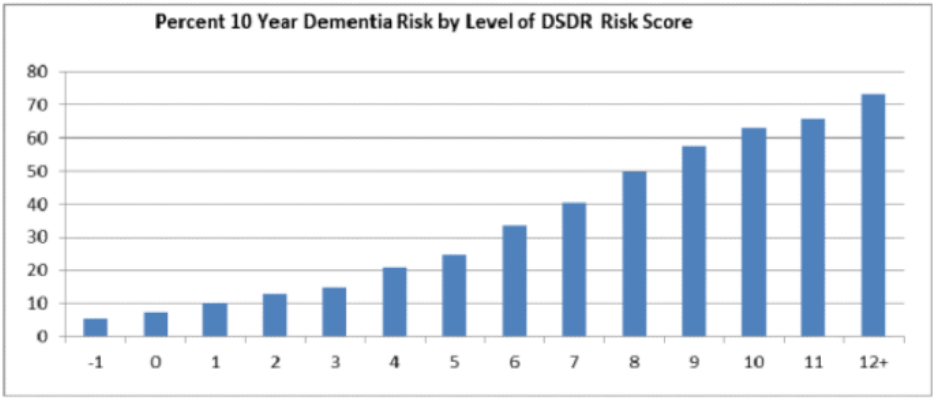


Figure 7. Ten years dementia risk by levels of DSDR categories in the development cohort (50).

The DSDRS is a simple and practical tool. All included predictors are easy to assess and available in the medical record of the patient, even in primary care setting. More importantly, no additional labor-intensive or expensive tests, such as cognitive testing or brain imaging, are required for calculation (50).

DSDRS is a prognostic model by definition created to predict future risk. Because of the stochastic nature, the estimated probabilities are of primary interest. Both discrimination and calibration are essential components of model evaluation (51). Apart from accuracy, the practical value of a prediction model depends on other items, such as the potentials for extrapolation, relevance of the outcome, and usability. The discriminative ability of the DSDRS (c-statistic 0.733-0.744) is higher or equal to that of other commonly used risk scores that rely on similar, readily available clinical predictors, such as the CHADS2 (0.707) or Framingham cardiovascular risk score (0.686).

The DSDRS is at present, the only risk score specifically targeting older people with T2D, a population particularly vulnerable to dementia (50). The clinical variables of DSDRS are shown in **Figure 8.**

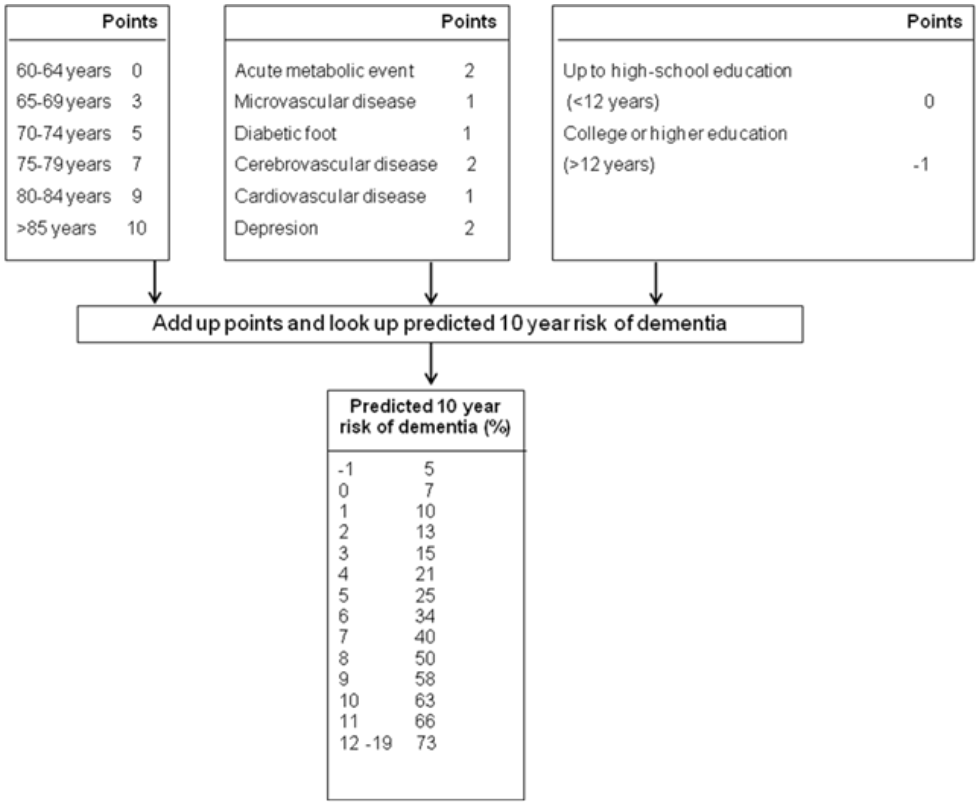


Figure 8. Summary of the diabetes-specific dementia risk score According to the figure.

As reflected by **Figure 8**, some of the predictors included in the DSRDS are end-organ complications of T2D.

The macrovascular disease such as a stroke was related with vascular dementia (5), but also, other macrovascular diseases, such as coronary heart disease is related whit dementia (52).

Major modifiable risk factors for cognitive impairment and dementia relate or impact the vascular system including hypertension, smoking, obesity, diabetes, hypercholesterolemia and lack of physical exercise. Notably, these factors are also risk factors for coronary heart disease (53) (54).

In a recent systematic review of the literature on modifiable risk factors, several studies on heart disease were identified, of which the majority reported a higher risk for cognitive impairment or dementia (55).

A me meta-analysis developed by Deckers et al in 2017 suggests that coronary heart disease is prospectively associated with increased odds of developing cognitive impairment or dementia (52).

In addition, the presence of hyperglycemic crisis episode, including diabetic ketoacidosis, hyperosmolar hyperglycemic state, and mixed syndromes are increased the risk of dementia in diabetic patients aged 45-64 years (56) and dementia was predicted by ≥3 hyperglycemic crisis episode (56).

Consequently, macrovascular complications and acute decompensations related to the development of cognitive impairment, it seems reasonable to us that the DSDRS could be use not only as a predictive test, but also as a screening test for cognitive impairment. It is a hypothesis that has not been explored until yet. This is one of the objectives of this work.

1.4.2 THE RETINA AS A WINDOW INTO THE BRAIN.

During embryonic development, the retina and optic nerve extend from the diencephalon, and are thus considered part of the CNS. The retina is composed

of layers of specialized neurons that are interconnected through synapses **Figure 9.**

Light that enters the eye is captured by photoreceptor cells in the outermost layer of the retina, which initiates a cascade of neuronal signals that eventually reach the retinal ganglion cells,the axons of which form the optic nerve. These axons extend to the lateral geniculate nucleus in the thalamus and the superior colliculus in the midbrain, from which information is further relayed to the higher visual processing centres that enable us to perceive an image of our world (57).

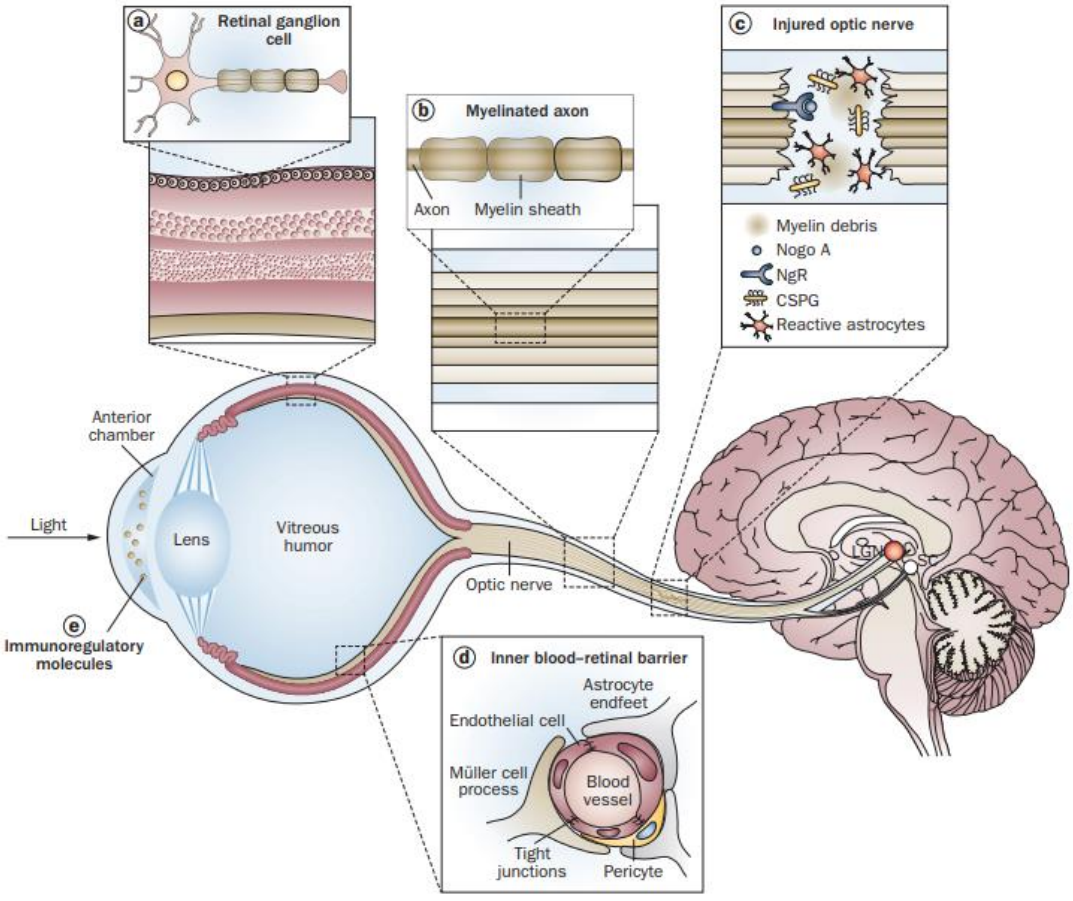


Figure 9. The eye as an extension of the brain. **A)** The retinal layers are composed of several neuronal types including retinal ganglion cells, which share structural morphology with other CNS neurons**. B)** The axons of these cells are myelinated by oligodendrocytes posterior to the globe, and form the optic nerve, which extends to the LGN and SC of the brain. **C)** Injury to the optic nerve produces, in a manner similar to other CNS neurons, an environment that is both hostile to survival of neurons that were spared in the primary insult and inhibitory to regeneration of severed axons. **D and E)** Similar to the CNS, the eye has a unique relationship with the immune system that involves specialized barriers such as the inner blood–retinal barrier, the retinal counterpart of the CNS blood–brain barrier **D)** and the constitutive presence of immunoregulatory molecules **E)** Abbreviations: CSPG, chondroitin sulphate proteoglycan; LGN, lateral geniculate nucleus; NgR, Nogo receptor; SC, superior colliculus (57).

Diabetic retinopathy (most common complication of diabetes and cause of preventable blindness among working-age individuals in most developed countries (58), has a great importance for the study of problems related to cognition (59).

The ACCORD-MIND and ACCORD Eye sub-studies showed that DR is associated with lower gray matter volume and predicted future cognitive decline in the T2D population (60). In addition, it was recently reported that T2D patients with advanced DR presented a 42% higher risk of incident dementia (61).

The concept of diabetic retinopathy (DR) as a microvascular disease has evolved, in that it is now considered a more complex diabetic complication in which neurodegeneration plays a significant role (62) (63) (64).

In fact, the ADA has recently defined diabetic retinopathy as a highly tissue-specific neurovascular complication involving progressive disruption of the interdependence between multiple cell-types in the retina (65).

Both DR and AD are characterized by a number of pathological mechanisms that coalesce around the neurovascular unit, including neuroinflammation and degeneration, vascular degeneration, and glial activation (66). Recent advancements have identified a number of common mechanisms in T2D and AD, which are centered around the neurovascular unit (NVU) (66).

Retinal neurodegeneration is an early event in the pathogenesis of diabetic retinopathy. Low-grade inflammation, immune cell activation, extracellular glutamate accumulation and an imbalance of local production of neurotrophic factors are crucial for retinal neurodegeneration development (59). Glial activation and neuron apoptosis are the two prominent hallmarks of this condition. The main consequences are:

- NVU impairment and BRB disruption.
- Impairment of vision-related quality of life:
    - Decreased hue discrimination.
    - Decreased contrast sensitivity.
    - Delayed dark adaptation.

- Reduced visual field sensitivity.
- Loss of visual acuity.

The assessment of retinal neurodegeneration could help to identify individuals with type 2 diabetes who are at risk of developing Alzheimer's disease (59).

1.4.2.1. RETINAL MICROPERIMETRY (MAIA).

Figure 10. Retinal microperimetry third generation (MAIA).

As we have said previously, the retina is ontogenically a brain-derived tissue, and it has been suggested that it may provide an easily accessible and non-invasive way of examining the pathology of the brain (63) (67).

Therefore, it seems reasonable to propose that the evaluation of retinal parameters related to neurodegeneration, such as retinal function, could be useful for identifying those patients with type 2 diabetes who have a high risk of developing AD.

Several methods can be used to measure retinal function. Among them, fundus-driven microperimetry has emerged as a simple and rapid test that can be used in clinical practice. (68) (69).

Retinal microperimetry is a non-invasive test that measures retinal sensitivity in terms of the minimum light intensity that patients can perceive when spots of light stimulate specific areas of the retina and also evaluates gaze fixation stability (70). All the parameters evaluated by the MAIA can be observed in the clinical report in **Figure 11.**

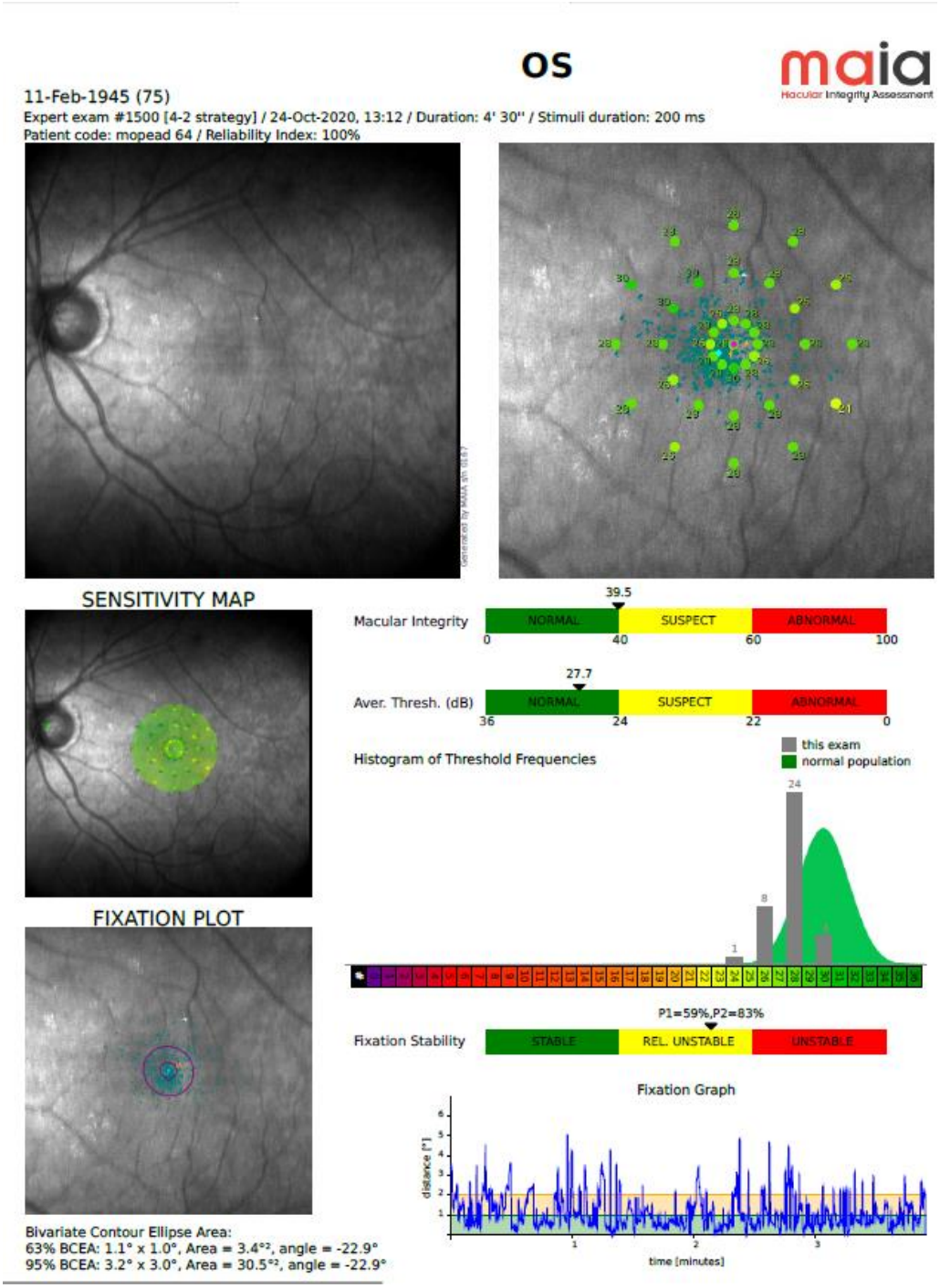


Figure 11. Example of a clinical report obtained by the MAIA.

Some recent studies have suggested that microperimetry is even more sensitive than multifocal electroretinography in detecting early functional changes of the retina (70).

Retinal sensitivity significantly correlated with brain imaging, such as magnetic resonance imaging (MRI) and positron emission tomography (PET) and

identified patients with MCI and dementia, as confirmed by complete neuropsychological battery.

Furthermore, by adding the gaze-fixation parameters to the retinal sensitivity, the probability of identifying cognitive impairment significantly increased in an independent manner, suggesting that retinal sensitivity and gaze fixation explore different neuronal circuits and are complementary (71).

As we mentioned previously, DR and AD are characterized by a pathological mechanisms common that coalesce around the neurovascular unit, including neuroinflammation and degeneration, vascular degeneration, and glial activation (66) (59).

The gaze fixation (parameter explored by MAIA) seems to depend on the complex white matter network. In addition, the superior colliculus and the parietal and frontal cortex play an essential role in gaze fixation (72) (73).

For this reason, it seems reasonable to us the MAIA could be explore different pathways related to cognitive impairment in patients with T2D>65 years.

1.5. TESTS FOR THE DIAGNOSIS OF COGNITIVE IMPAIRMENT.

1.5.1. REPEATABLE BATTERY FOR THE ASSESSMENT OF NEUROPSYCHOLOGICAL (RBANS).

The Repeatable Battery for the Assessment of Neuropsychological Status is a commonly used brief battery of cognitive function. The RBANS was primarily developed as a cognitive instrument for diagnostic in geriatric patients evaluated for suspected dementia but has been expanded to evaluate a wider age range and multiple neurological and psychiatric conditions (74).

The development of the RBANS was theoretically driven with the goal of providing a brief and repeatable assessment of cognitive abilities across multiple domains. It consists of 12 subtests that yield a Total Scale Score and five Index Scores for the following: Immediate Memory (List Learning and Story Memory), Visuospatial/Constructional (Figure Copy and Line Orientation), Language (Picture Naming and Semantic Fluency), Attention (Digit Span and Coding), and Delayed Memory (List Recall, List Recognition, Story Recall, and Figure Recall).

The original normative sample age range was 12 to 89 years, though additional age, education, gender, and race norms were published for the elderly (75) (76).

The RBANS fills a niche as an assessment instrument falling between briefer dementia screeners and full neuropsychological assessment, further diversifying the assessment abilities of clinical psychologists and expanding the repertoire of repeatable batteries available to neuropsychologists.

In addition to neuropsychological screening for neurodegenerative processes in the elderly, the RBANS can serve as a more general screening instrument of neurocognition (74).

Stimuli are contained in a wire-bound, easel-type booklet, making the test easily portable and allowing for bedside administration. Total administration time is 30-60 mins (74), you can see it at the **Figure 12.**

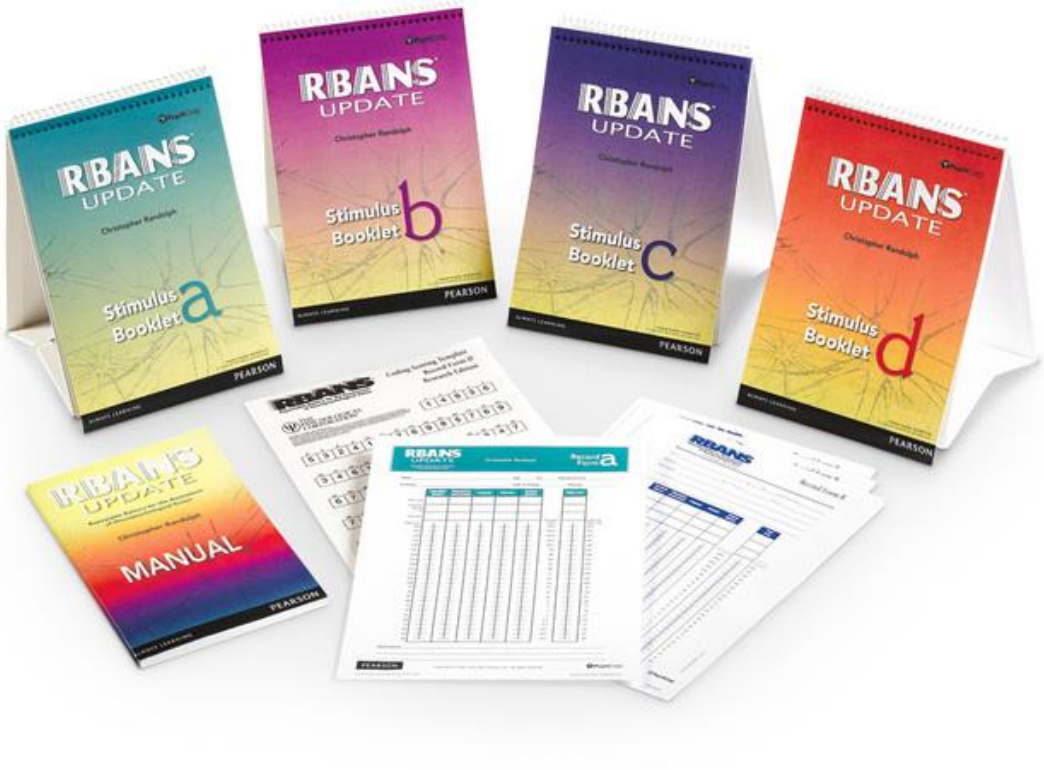


Figure 12. RBANS Stimulus Booklet.

The RBANS is a reliable test for the diagnosis of cognitive impairment and Alzheimer's disease and can discriminate rates of cognitive change between samples with cognitive impairment (77)..

As we can see, it is a neuropsychological test with added complexity that requires adequate interpretation by personnel specialized in neuropsychology, making it impossible in routine clinical practice to implement it as a screening test for the diagnosis of mild cognitive impairment in diabetic patients.

2. Hypothesis

Main:

There is a high prevalence of MCI among patients with T2D>65 years attended in a tertiary care setting.

Secondary:

DSDRS and retinal microperimetry can be useful and simple tools for screening and monitoring cognitive function in patients with T2D >65 years.

3. Objectives

Main:

To evaluate the prevalence of MCI in patients withT2D >65 years attending a tertiary care setting.

Secondary:

1. To evaluate the applicability of DSDRS as a screening tool for patients with T2D with cognitive impairment in a tertiary care setting.
2. To validate our previous results showing that retinal microperimetry is a reliable screening tool for MCI in patients with T2D>65 years and explore its usefulness for monitoring the cognitive function.
3. To explore the underlying mechanisms involved in sensitivity and eye fixation assessed by retinal microperimetry for the screening and monitoring the cognitive function.

4. Material and methods

4.1. PREVALENCE OF MCI IN THE T2D POPULATION >65 YEARS IN A TERTIARY CARE SETTING AND THE APPLICABILITY OF DSDRS AS A SCREENING TOOL FOR PATIENTS WITH T2D >65 YEARS WITH COGNITIVE IMPAIRMENT.

For the main objective, a clinical study was conducted as part of the project: “Models of patient engagement for Alzheimer´s disease" MOPEAD (78).

The MOPEAD project is a European Innovative Medicines Initiative (IMI2) Project-grant number 115985 5.

MOPEAD is a multinational project carried out by a multidisciplinary consortium of 14 partners, including academic institutions, pharmaceutical companies, technology companies, and relevant stakeholders such as patient associations, working across five work packages. The consortium partners are shown in **Figure 13.**

MOPEAD consortium members

Partner	Country	Type of institution	Leader	Activities
Fundació ACE	Spain	Academic and clinical center	Mercè Boada	Management, clinical core, analysis, and dissemination
Eli Lilly and Company Ltd	UK	Pharmaceutical company	Laura Campo	Management, clinical core, analysis, and dissemination
ASDM Consulting	Belgium	SME	Annette Dumas	Dissemination
Astra Zeneca	USA	Pharmaceutical company	Craig Shering	Clinical core
European Institute of Women’s Health	Ireland	NGO	Peggy Maguire	Dissemination
GMV Soluciones Globales Internet S.A.U.	Spain	IT company	Adrián Rodrigo	Clinical core and analysis
Karolinska Institutet	Sweden	Academic and clinical center	Bengt Winblad	Clinical core and analysis
Modus Research and Innovation Ltd	UK	Not-for-profit SME research organization	Neil Stewart	Management
Spomincica	Slovenia	Patient association	David Krivec	Dissemination
University of Cologne Medical Center	Germany	Academic and clinical center	Frank Jessen	Clinical core
University Medical Centre Ljubljana	Slovenia	Academic and clinical center	Milica Kramberger	Clinical core
Vall d’Hebron Research Institute	Spain	Academic and clinical center	Rafael Simó	Clinical core (Run 4)
VU Medical Center	The Netherlands	Academic and clinical center	Pieter Jelle Visser	Clinical core
Alzheimer Europe	Luxembourg	Patient organization	Jean Georges	Management and dissemination

Abbreviation: MOPEAD, Models of Patient Engagement for Alzheimer’s Disease; SME, small and medium enterprise.

Figure 13. MOPEAD consortium members

The complete project evaluated four models of patient engagement strategy (Runs) aimed to help identify individuals at risk of AD in a multicenter setting. Patients from five countries and four different settings/country were included), as follows:

(a) identify individuals in the community with hidden cognitive impairment or individuals without cognitive impairment at risk of AD; (b) promote cognitive wellbeing and healthy cognitive aging in the European Union using AD Citizen Science, Open House and developing campaigns in primary and tertiary care

setting; (c) test innovative patient engagement models and generate clinical and demographic data to evaluate the most efficient approaches; (d) develop common pre-screening methodologies for practitioners.

In each country, at least 100 patients were to be screened/model in order to refer at least 33 patients to the memory clinic/model. The four different models were called “Runs”, as follows: (a) Run 1: AD Citizen Science, on-line screening campaign; (b) Run 2: Open House campaign; (c) Run 3: Primary care based patient engagement; (d) Run 4: Tertiary care based patient engagement. The extended protocol was previously published and explained (78).

Our study focuses on a prospective observational study performed only in one center (Vall d’Hebron University Hospital (HVH), Barcelona, Spain) as the leader of Run-4 (Tertiary care–based patient engagement: Diabetologist setting) of the MOPEAD study.

The study was approved by the local ethics committee and conducted following the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.

The sample size was determined by protocol (100 patients per country) the inclusion criteria were recruited from the pool of patients that attended the outpatient clinic. As per MOPEAD protocol.

The inclusion criteria were:

(a) Age between 65–85 years.
(b) T2D with duration > 5 years.
(c) Functionally literate, without severe auditory and visual abnormalities.
(d) Written informed consent.
(e) Clinical data available for the DSDRS calculation.

Exclusion criteria were:

(a) Other types of diabetes.
(b) Previous diagnosis of AD, MCI or other types of dementia following the diagnostic criteria of the NIA-AA (79).
(c) History of stroke.

(d) History of unstable neurological or psychiatric conditions that may affect cognition, including the presence of depression.
(e) Severe metabolic or systemic disease such as unstable acute cardiovascular disease, renal failure with GFR < 30 mL/min/m2, decompensated liver cirrhosis or liver failure, hypothyroidism untreated or vitamin B12 deficiency.
(f) Drugs affecting cognitive status, for example, antipsychotics, opioids, long half-life benzodiazepines like diazepam.
(g) Uncorrected severe sensory deficits (blindness, deafness).

All participants underwent a complete medical history, physical examination, and biochemical analysis.

All the patients underwent the Minimental State Evaluation questionnaire (MMSE) (30), which was administered by the same member of the health-care team for all patients. During the visit, all the patients were asked up to three initial questions about their cognitive state: (1) “Do you feel that your memory is getting worse?” (2) “How long have you been noticing it?” and (3) “Are you worried about this alteration?”

Following the protocol of the MOPEAD study, all patients underwent the DSDRS was calculated based on the clinical data and the medical history, as described by Exalto et al (50).

The score is the sum of several points that are assigned as follows:

(a) Age (60–64 years: 0, 65–69 years: 3, 70–74 years: 5; 75–79 years: 7; 80–84 years: 9; >85 years: 10).
(b) Associated conditions (acute metabolic event: 2; macrovascular disease: 1; diabetic foot: 1; cerebrovascular disease: 2; cardiovascular disease: 1; depression: 2).
(c) Education level (<12 years: 0; >12 years: -1).

The acute metabolic event was defined as severe hypoglycemia requiring assistance from another person, hyperosmolar hyperglycemia or ketoacidosis.

As per MOPEAD protocol, (78) those subjects having a MMSE score ≤ 27 or DSDRS ≥ 7 and positive answer for ≥ 2 of the initial questions or DSDRS ≥ 10 were referred to the specialized memory clinic (Fundació ACE, Barcelona, Spain) to complete the neuropsychological evaluation to confirm or exclude mild cognitive impairment.

Statistical analysis:

The categorical variables were expressed as a percentage. For the quantitative variables, means and standard deviation are displayed if they follow a normal distribution, and those that do not are displayed in median and range.

To evaluate differences between groups Chi square test was used for qualitative variables and Analysis of Variance (ANOVA); following this, DMS post-hoc tests for quantitative variables were used. For variables that do not follow a normal distribution, a nonparametric test was used to compare between groups (Kruskal-Wallis).

To evaluate the correlation between MMSE and DSDRS, the Pearson correlation test was performed. Significance was accepted at $p < 0.05$. Regression was used to predict the Receiver Operating Characteristic (ROC) curves and the chi-square test for comparison of ROC area.

The statistical analyses were performed with the statistical package SPSS version 21. Data of all the patients included in the study was used for descriptive statistics. Data from the patients that underwent a complete neuropsychological evaluation at the memory clinic was used for correlations and regression analysis.

4.2. THE RETINAL MICROPERIMETRY AS A USEFUL TOOL FOR SCREENING AND THE MONITORING OF THE COGNITIVE FUNCTION.

For this objective, a clinical trial was designed: prospective, observational pilot study which consecutive patients > 65 years with T2D and without known cognitive impairment attending the outpatient clinic of our center between March–October 2018.

The study was conducted according to the tenets of the Helsinki Declaration. The Ethic Committee of the Vall d'Hebron University Hospital approved all procedures (PR(AG)28/2017), and it was conducted following the Strengthening of the Reporting of Observational Studies in Epidemiology guidelines.

The inclusion criteria were:

(a) Age between 65–85 years old.
(b) T2D with a duration > 5 years.
(c) Functional literacy, without severe auditory or visual abnormalities.
(d) Without or with mild to moderate diabetic retinopathy.
(e) The absence of other eye degenerative diseases apart from diabetes (e.g., glaucoma).
(f) Written informed consent.

The exclusion criteria were:

(a) Other types of diabetes.
(b) A previous diagnosis of AD or other types of dementia following the diagnostic criteria of the National Institute on Aging and Alzheimer's Association (NIA-AA) (79).
(c) A history of stroke.
(d) A history of unstable neurological or psychiatric conditions that may affect cognition, including the presence of depression.
(e) Severe metabolic or systemic disease, such as unstable acute cardiovascular disease, renal failure with glomerular filtration rate (GFR) < 30 mL/min/m2, decompensated liver cirrhosis or liver failure, or untreated hypothyroidism or vitamin B12 deficiency.

(f) Drugs affecting cognitive status, for example, antipsychotics, opioids, or long half-life benzodiazepines.
(g) Uncorrected severe sensory deficits (blindness, deafness).
(h) Severe diabetic retinopathy (photocoagulation, etc.).
(i) Other neurodegenerative diseases (e.g., Parkinson's).

All the evaluated patients underwent a complete medical history, physical examination, and biochemical analysis (including HbA1c, lipid profile, the albumin excretion rate).

To confirm or exclude MCI a complete neuropsychological evaluation was performed by a trained neuropsychologist using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) (80) at the baseline and 3-years follow-up.

The battery indexes scores were detailed below:

I.- Immediate Memory:

This index consists of the following two subtests:

(a) List Learning consists of immediate recall of a 10-item list of words over four learning trials.

The words are semantically unrelated, early age of-acquisition, relatively high-imagery, and as phonetically unique as possible.

(b) Story Memory consists of a 12-item story, read aloud for immediate recall over two trials. Recall is scored using a verbatim criterion, to avoid complicated scoring rules.

II.- Visuospatial/Constructional:

This index consists of the following two subtests:

(a) Figure Copy consists of copying a geometric figure comprised of 10 parts. Each part receives a 2-point score (accuracy and placement), for a total of 20 possible points.

(b) Line Orientation consists of a 10-item line orientation test. Each item involves a radiating array of 13 lines spanning 180 degrees; below the

array are two target lines that are identical in orientation to two of the lines from the array. The subject's task on each item is to identify the matching lines. One point is given for each correctly matched line, for a total score of 20.

III.- Language:

This index consists of the following two subtests:

(a) Picture Naming consists of 10-line drawings, which the subject must name. Semantic cues are given if the object is obviously misperceived (e.g., ''umbrella'' for mushroom).

(b) Semantic Fluency consists of the total number of exemplars generated for a given semantic category (e.g., fruits and vegetables) within 60 seconds. The semantic categories used were chosen in order to attempt to minimize retrieval demands and thereby more specifically tap semantic stores rather than retrieval strategies (cf. Randolph, Braun, et al., 1993).

IV.- Attention:

This index consists of the following two subtests:

(a) Digit Span is analogous to digits forward on the WAIS (Wechsler, 1955). There are two strings of digits in each item, at lengths increasing from 2 to 9 digits. The second string at a given length is only read if the first string is failed.

(b) Coding is similar in nature to the Symbol Digit Modalities Test (Smith, 1973) or the Digit Symbol subtest of the WAIS-R. Numbers rather than symbols were chosen for the response in order to avoid the possible detrimental effect of a constructional apraxia on performance. The score is the total number of items completed in 90 seconds.

V.- Delayed Memory:

This index includes four subtests:

(a) List Recall involves free recall of the words from the List Learning task.

(b) List Recognition involves yes/no recognition testing for memory of the words from the List Learning task.

(c) Story Recall involves free recall of the story from the story memory test.

(d) Figure Recall involves free recall of the figure from the figure copy subtest.

SCALING: Each index score will be separately scaled by age group to a scaled score *mean* of 100 with associated *standard deviation* of 15. This will allow for a profile of performance across cognitive domains, and a total scaled score will be derived from the sum of these, also with a normal *mean* of 100 and *standard deviation* of 15.

In this work, the neuropsychological battery (RBANS) was used as the gold standard for the diagnosis of normo-cognition and mild cognitive impairment.

Subsequently, at 3-years of follow-up, the patients were classified into 3 groups according to the results obtained by the RBANS:

NC-NC: Patients who were normo-cognitive at the beginning of the study and at the 3-year follow-up, they remained normo-cognitive due to their score on the RBANS.

NC-MCI: Patients who were normo-cognitive at the beginning of the study, but after 3-years of follow-up they were reclassified as mild cognitive impairment.

MCI-MCI: Patients who had mild cognitive impairment at the beginning of the study and who remained mild cognitive impairment after 3-years of follow-up due to their RBANS score.

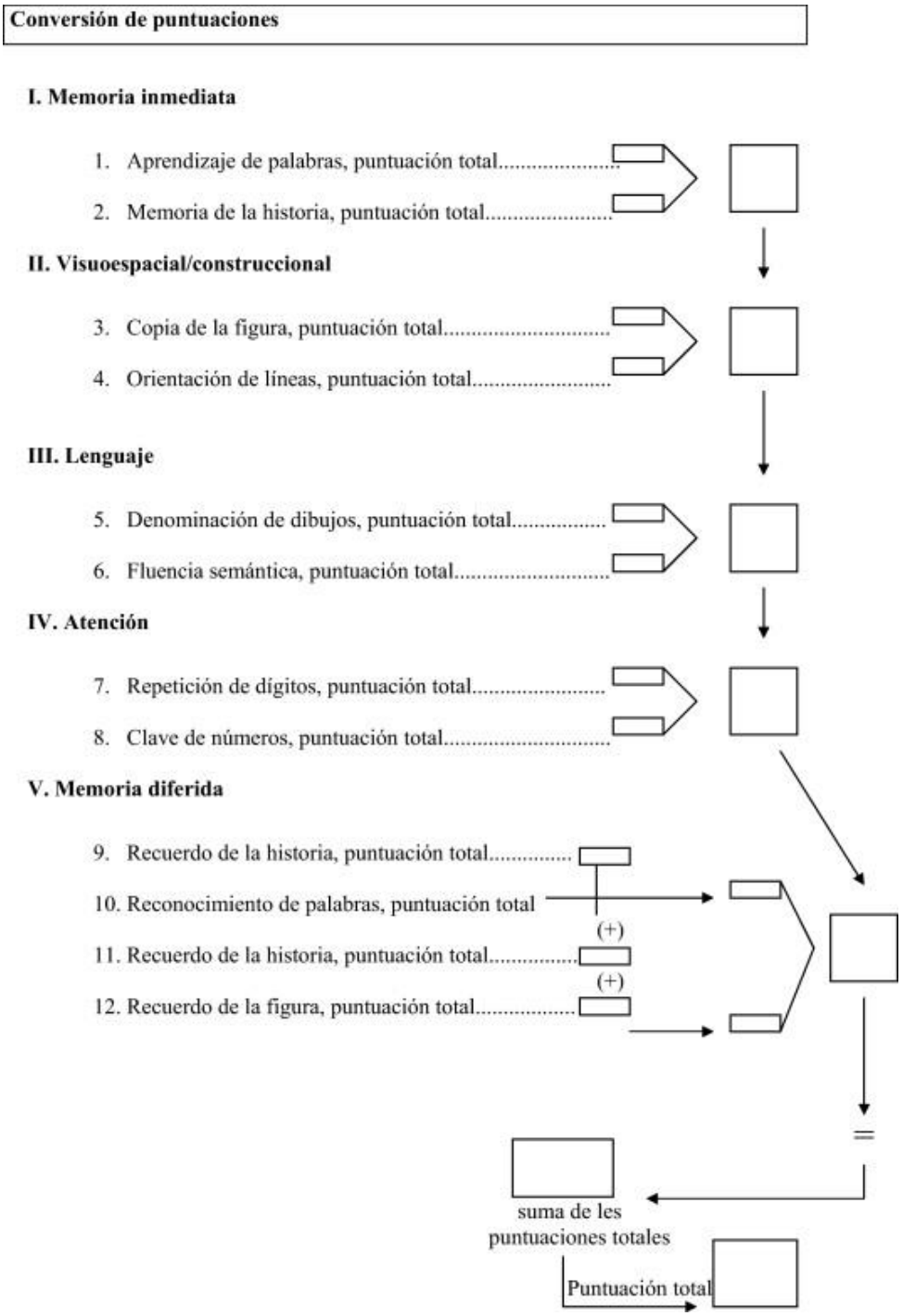


Figure 14. RBANS Score convention, Spanish version.

Those patients that gave their informed consent and were recruited for follow-up underwent retinal microperimetry and the Mini-Mental State Evaluation (MMSE), validated in Spanish, (81) at baseline, 12-month follow-up and 3-years follow-up. The cut-off for a normal cognitive function score of MMSE is ≥ 26 (range 0–30).

Retinal sensitivity and gaze-fixation parameters were evaluated by fundus-driven microperimetry (MAIA 3rd generation) after a previous pupillary dilation of a minimum of 4 mm.

The standard MAIA test covers a 10-diameter area with 37 measurement points, and a red 1 radius circle was used as the fixation target. A four-level fixed strategy was used: Goldmann III size stimulus, background luminance of 4 as band maximum luminance of 1000 asb, with a 25-dB dynamic range. The microperimeter automatically compensates for eye movements during examination via a software module that tracks them.

For the evaluation of fixation, the MAIA uses high-speed eye trackers (25 Hz) to record the fixation pattern and to control fixation losses. To calculate the fixation indexes P1 and P2, the MAIA uses all fixation positions during the test, representing the percentage of fixation points within a circle of 2 and 4 degrees in diameter, respectively. Using the bivariate contour ellipse area (BCEA), the MAIA provides a more accurate fixation pattern station. The BCEA is calculated as the bivariate ellipse (measured in squared degrees "º2") that covers the fixing positions and takes into account 1 standard deviation (BCEA63%) or 2 standard deviations (BCEA95%). Through the major and minor axes, the area of this ellipse (2 orthogonal diameters) is calculated, describing the extension of the fixation points (horizontal and vertical diameters). For the analysis, we used data from the first explored eye (usually the right eye). The main variables included in the study were the MMSE score and the specific domains disclosed of the MMSE scores: temporal orientation, spatial orientation, immediate recall, attention, delayed recall, and language.

All patients underwent the DSDRS was calculated based on the clinical data and the medical history, as described by Exalto et al (50) at baseline.

The score is the sum of several points that are assigned as follows:

(d) Age (60–64 years: 0, 65–69 years: 3, 70–74 years: 5; 75–79 years: 7; 80–84 years: 9; >85 years: 10).

(e) Associated conditions (acute metabolic event: 2; macrovascular disease: 1; diabetic foot: 1; cerebrovascular disease: 2; cardiovascular disease: 1; depression: 2).

(f) Education level (<12 years: 0; >12 years: -1).

The acute metabolic event was defined as severe hypoglycemia requiring assistance from another person, hyperosmolar hyperglycemia or ketoacidosis.

This was a pilot study. At present, there are no similar studies; therefore, the sample size was not calculated.

The study visit schedule is shown in **Table 1.**

Table 1. The schedule for the study.

	Baseline.	12-month follow-up.	3-years follow-up.
Medical history.	X		X
RBANS.	X		X
MMSE.	X	X	X
Retinal Microperimetry.	X	X	X
DSDRS.	X		

RBANS: Repeatable Battery for the Assessment of Neuropsychological Status., MMSE: Mini-Mental State Examination, DSDRS: Diabetes Specific Dementia Risk Score.

Statistical analysis:

The categorical variables were expressed as a percentage. For quantitative variables that follow a normal distribution, they were expressed as means and standard deviation; for those that did not, they were expressed as median and range.

The chi-square test was used to evaluate the differences between groups for qualitative variables that follow a normal distribution. On the other hand, for the quantitative variables that follow a normal distribution, the ANOVA test was used. The non-parametric Kruskal–Wallis test was used to evaluate the differences between groups of variables that do not follow a normal distribution. For the 12-month follow-up on the quantitative variables, we used the Student's t-test to compare means for paired data.

To evaluate the correlation between MMSSE, RBANS and MAIA values, the Pearson correlations were performed. Significance was accepted at $p < 0.05$. The Bonferroni correction was used for multiple comparisons. Statistical analyses were performed with the STATA 15 statistical package.

4.3. UNDERLYING MECHANISMS BY WHICH RETINAL MICROPERIMETRY IS USEFUL FOR SCREENING AND MONITORING THE COGNITIVE FUNCTION.

A cross-sectional descriptive study was performed, comprising consecutive patients with T2D>65 years old, attended at the outpatient clinic of our hospital between June-December 2019. The study was conducted according to the Declaration of Helsinki and was approved by the local Ethics Committee (PR(AG)28/2017). Informed consent was obtained from all subjects involved in the study.

Inclusion criteria: 1) age >65 years; 2) T2D with a duration >5 years; 3) no apparent or only mild non-proliferative DR, according to the ETDRS (82); 4) patients with normal visual acuity (0.8-1).

Exclusion criteria: 1) patients with neurodegenerative diseases of (e.g., glaucoma, multiple sclerosis); 2) patients with HbA1c >10% (high blood glucose levels could affect retinal function (83)) or hyperglycemic or hypoglycemic decompensations in the last 6 months. 3) unable or unwilling to perform VEP and retinal microperimetry.

RS (dB) and GF were evaluated by fundus-driven microperimetry (third-generation Macular Integrity Assessment-MAIA). The characteristics of GF (location and stability) were quantified and categorized according to bivariate contour ellipse area, as the preferred retinal loci to calculate fixation stability (BCEA): BCEA63% (1 standard deviation) and BCEA95% (2 standard deviations) areas. The BCEA is measured as a bivariate contour ellipse, expressed in "º2". For the analysis we used BCEA95% as the most integrative parameter. The complete procedure was detailed elsewhere (71) (84).

It should be noted that our study was aimed to evaluate correlations between two functional independent tests (MAIA and VEP) in the same patient and not to test for ophthalmologic disease. We are exploring the potential of MAIA as a reliable tool for cognitive impairment screening and monitoring.

For this purpose, in the previous studies we only analyzed the data from the first eye that was evaluated, in order to avoid “learning-effect” in the second

eye. For the same reasons, in the present study we applied the same procedure, including in the analysis data from the right eye in all cased.

Visual evoked Potentials (VEP) represents an electrophysiological test used to quantify the functional integrity of the visual pathway and occipital cortex activity, even more sensitive and economical compared to MRI in detecting lesions of the visual pathway (85), considered at present the gold-standard.

VEP were evaluated with electrodiagnostic System (Nicolet EDX EMG). Stimuli were applied alternating geometric pattern of medium square (16x16) and small square (32X32). The variables categorized where: Latency (ms) at (N75, P100, N145) and Amplitude (75-100Uv) at (12x16) and (24x32), as per protocol. P100 wave is the most robust peak with comparatively minimal interindividual variability, nominal within-subject inter-eye difference, and negligible variation with high repeatability (85). P100 values from the right eye were used for correlations.

This was a pilot study. Since there is no previous data aimed to explore similar hypothesis, we could not perform sample size calculation.

Statistical Analysis:

To assess differences between the groups, a X^2 test was used for qualitative variables and ANOVA followed by a Least Significant Difference (LSD) post hoc test were used for quantitative variables. To evaluate the correlation between PEV and retinal sensitivity and gaze fixation variables, a Spearman correlation test and regression analyses adjusted by age were performed. All p values were based on a two-tail statistical significance test. Significance was accepted at $p=<0.05$. Statistical analysis was performed with the STATA 15 statistical package.

5. Results

5.1. PREVALENCE OF MCI IN THE T2D POPULATION >65 YEARS IN A TERTIARY CARE SETTING AND THE APPLICABILITY OF DSDRS AS A SCREENING TOOL FOR PATIENTS WITH T2D >65 YEARS WITH COGNITIVE IMPAIRMENT.

A total of 112 consecutive T2D patients were recruited. The baseline characteristics are presented in **Table 2**. Of all the enrolled patients, 82 (73.12%) fulfilled criteria for referral to the Fundació ACE's memory clinic for suspected cognitive impairment as per MOPEAD protocol.

Table 2. Baseline characteristics of the patients included in the study.

	Referred to the memory clinic	Not referred	p
N (%)	82 (73.2%)	30 (26.7%)	**0.001**
Age (years) mean±SD	74.8± 4.5	69.5±3.4	**0.02**
Sex (women) n%	36 (43.9%)	13 (43.3%)	0.72
BMI (kg/m2) mean±SD	28.9±5.1	32.0±5.8	0.09
Smoker n(%)	72 (87.8%)	29 (96.6%)	0.06
Hypertension n(%)	77 (93.9)%	27 (90)%	0.63
Dyslipidemia n(%)	76 (92.6%)	29 (96.6%)	0.21
Obstructive sleep apnea n(%)	17 (20.7%)	8 (26.6%)	0.35
Ischemic heart disease n(%)	25 (30.4%)	5 (16.1%)	0.06
Peripheral arteriopathy n(%)	20 (24.3%)	4 (13.3%)	0.08
T2D duration (mean±SD)	19.7±9.8	17.5±7.8	0.09
Insulin use n(%)	60 (73.1%)	18 (60%)	**0.04**
HbA1C (%) mean±SD	7.7±1.0	7.4±0.8	0.43
Severe hypoglycemia (n)	6	0	NA
Diabetic retinopathy n(%)	53 (64.3%)	15 (50%)	0.28
Diabetic nephropathy n(%)	34 (41.4%)	13 (43.3%)	0.64
Diabetic polineuropathy n(%)	27 (32.9%)	9 (30%)	0.53
MMSE mean±SD	26.84±2.01	28.8±0.66	**0.04**
DSDRS mean±SD	7.48±2.2	4.63±1.2	**0.02**

BMI: body mass index, T2D: type 2 diabetes, HbA1C: glycated hemoglobin A1C, MMSE: Mini-Mental State Evaluation, DSDRS: Diabetes Specific Dementia Risk Score.

Patients who met criteria for referring to memory clinic were older (mean of 74.8± 4.5 years versus 69.5±3.4 years, p = 0.02) and had a higher percentage of insulin treatment (73.1% versus 60%, p = 0.04) than patients who did not.

In addition, a trend to present an increased burden of cardiovascular disease and longer diabetes duration was also observed.

Of the 82 T2D patients meeting criteria of referral to memory clinic, 43 (52.4%) declined participation in the second phase of the study due to associated comorbidities (21, 48.8%), lack of interest (16, 37.2%), and absence of social support (6, 13.95%).

A total of 39 T2D were evaluated at the memory clinic and underwent a complete neuropsychological assessment, as previously described (78), and 34 individuals (87.2%) received a diagnosis of MCI, 3 (7.7%) of AD dementia and 2 (5.1%) of cognitively healthy. **Table 3** reflects the characteristics of these patients.

Table 3. Characteristics of the patients that were performed a complete neuropsychological evaluation at the memory clinic.

	Patients That Were Evaluated at the Memory Clinic
N	39
Race	Caucasian
Age (years) median (min–max)	75 (68–84)
Education level < 9 years (%)	83.87
Sex (women) %	44.1
BMI (kg/m^2) mean ± SD	28.51 ± 4.01
Smoker (%)	54.83
Hypertension (%)	87.09
Dyslipidemia (%)	96
Ischemic heart disease (%)	25.80
Peripheral arteriopathy (%)	19.2
T2D duration (mean ± SD) years	19.1 ± 6.2
Insulin use%	67.74
HbA1C (%) mean ± SD	7.69 ± 0.76
Hypoglycemia (*n*)	64
Severe hypoglycemia (*n*)	6
Diabetic retinopathy (%)	54.83
Diabetic nephropathy (%)	38.70
Diabetic polyneuropathy (%)	22.58
MMSE (mean ± SD)	26.84 ± 2.01
Prevalence of MCI%	87.2

BMI: body mass index, MCI: mild cognitive impairment, MMSE: Mini-Mental State Examination, DSDRS: diabetes specific dementia risk score. SD: Standard deviation.

Furthermore, as expected, this group of patients presented lower MMSE (26.84 ± 2.01 versus 28.8 ± 0.66, p = 0.04) and higher DSDRS scores (7.48 ± 2.2 versus 4.63 ± 1.2, p = 0.02), respectively. The detailed number of patients that fulfilled the criteria for referral to the memory clinic (MMSE or DSDRS or both) are presented in **Annex 10.2.** All the patients had at least two positive answers to the initial questions.

Pearson correlation analyses showed inversely proportional relationship between MMSE and DSDRS scores (r = -0.3640; CI (-0.414 – -0.135) p < 0.05), as reflected in **Figure 15.**

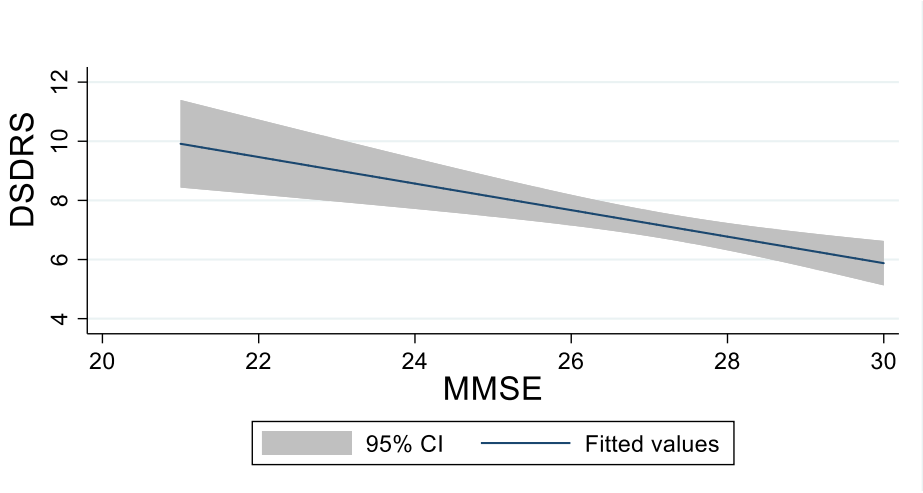


Figure 15. Correlation between DSDRS and MMSE scores**.** DSDRS: diabetes specific dementia risk score, MMSE: Mini-Mental State Examination

The regression logistic analysis revealed that MMSE and DSDRS scores were independent predictors of the global cognitive impairment (MCI plus dementia), as reflected in **Table 4.**

Table 4. Independent predictors of cognitive function (MCI and dementia).

Coefficients [a]						
		Unstardardized Coefficients		Standardized Coefficients		
Model		B	Error Estándar	Beta	t	Sig.
1	(Constant)	6.147	1.205		5.102	**0.000**
	Age	−0.012	0.013	−0.121	−0.895	0.373
	Insulin use	0.098	0.093	0.088	1.054	0.295
	MMSE score	−0.149	0.025	−0.528	−6.021	**0.000**
	DSDRS score	0.063	0.027	0.306	2.310	**0.023**

[a] Dependent variable: Cognitive impairment. MMSE: Mini-Mental State Examination, DSDRS: Diabetes Specific Dementia Risk Score.

The predictive value of DSDRS ≥ 7 for the diagnosis of cognitive impairment was significant AUC: 0.739, CI 95% (0.557–0.921), p < 0.024 (**Figure 16**).

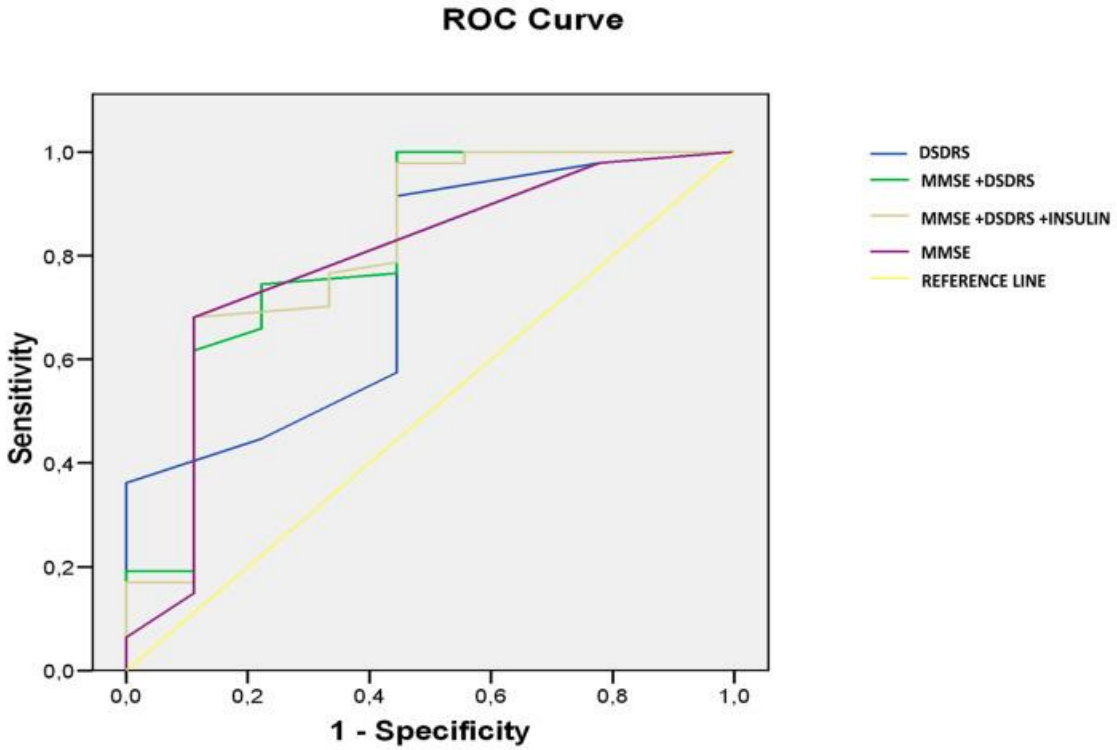


Figure 16. The ROC Curve for the MMSE and DSDRS as screening tools of cognitive impairment. MMSE: Mini-Mental State Examination, DSDRS: Diabetes Specific Dementia Rrisk Score.

The prediction capacity of combined MMSE and DSDRS for identifying subject with cognitive impairment (MCI + dementia) was AUROC 0.902 (p 0.003, CI 95% (0.840–0.992)), significantly higher (p 0.01) than MMSE (AUROC 0.785, p 0.007, CI 95% (0.814–0.948)) or DSDRS separately (AUROC 0.739, p 0.024, CI 95% (0.609–0.825)) (**Figure 16**). No significant differences were seen between MMSE and DSDRS separately. When insulin use was added to the model, the predictive value of the scores combined was not changed (AUROC 0.904, p 0.001, CI95% (0.841–0.966)).

5.2. THE RETINAL MICROPERIMETRY AS A USEFUL TOOL FOR SCREENING AND THE MONITORING OF THE COGNITIVE FUNCTION.

At baseline, 50 patients with T2D that were evaluated using RBANS, 36 were catalogued as NC, 14 as MCI. Of these patients, 4 patients with MCI denied their enrolled in a 3-years follow-up observational study.

The baseline characteristics are shown in the **Table 5**.

Table 5. Baseline characteristics of the patients.

	T2D NC	**T2D MCI**	***p* Value**
***n* (%)**	36 (78.2%)	10 (21.7%)	NA
Age (years)	75.9 ± 3.9	75.4 ± 3.5	0.284
Gender (men) %	14 (46.7%)	5 (50.0%)	0.136
Body mass index (BMI) (kg/m^2)	30.1 ± 4.1	30.2 ± 4.9	0.495
Smoker (%)	20 (66.7%)	6 (60.0%)	0.702
Hypertension (HTA) (%)	26 (86.7%)	10 (100%)	0.064
Dyslipidemia (DLP) (%)	30 (100%)	10 (100%)	N.A.
Obstructive sleep apnea (OSA) (%)	8 (26.7%)	2 (20.0%)	0.842
Ischemic heart disease (%)	10 (33.3%)	3 (30.0%)	0.406
Peripheral arteriopathy (%)	4 (11.1%)	1 (10.0%)	0.321
T2D duration (years)	19.3 ± 4.3	19.5 ± 2.9	0.884
Insulin use	16 (53.3%)	6 (60.0%)	0.083
Insulin dosed (UI/kg/day)	0.56 ± 0.3	0.57 ± 0.1	0.435
Glycosylated hemoglobin (HbA1C DCCT) (%)	7.4 ± 1.0	7.2 ± 0.6	0.680
Severe hypoglycemia at last year (*n*)	0	2	NA
Type 2 diabetes related complications			
Retinopathy (%)	16 (53.3%)	6 (60.0%)	0.465
Nephropathy (%)	6 (20.0%)	4 (42.0%)	0.206
Polyneuropathy (%)	8 (26.7%)	2 (20.0%)	0.673
Mini-Mental State Examination (MMSE)	28.4 ± 0.8	26.6 ± 1.1	**<0.001**
DSDRS mean±SD	5.8±2.4	8.4±1.4	**0.031**

T2D, type 2 diabetes; NC, normo-cognitive; MCI, mild cognitive impairment; BMI, body mass index. NA: not applicable.

Both groups were similar in terms of the BMI, cardiovascular risk factors, diabetes duration, insulin use, the degree of metabolic control, and the presence of micro and macroangiopathic complications.

Significant differences have been observed in total score of MMSE and DSDRS at baseline.

Regarding microperimetry, significant differences were seen between the two groups at baseline in all the parameters that were evaluated, as reflected by **Table 6**.

Table 6. Retinal microperimetry parameters at baseline.

	T2D NC	**T2D MCI**	***p* Value**
n	36	10	NA
Retinal sensitivity (dB)	22.9 ± 2.4	20.3 ± 1.8	**0.016**
Duration (min)	2.3 ± 0.9	2.8 ± 0.7	0.075
Fixation stability P1 (%)	85.9 ± 18.6	66.6 ± 19.0	**0.018**
Fixation stability P2 (%)	93.2 ± 10.5	79.6 ± 19.0	**0.006**
BCEA63 (^{o2}) (median (range))	1.35 (0.4–2)	4.4 (3.4–18.1)	**0.001**
BCEA63 (^{o2}) (mean ± 1 standard deviation)	1.35 ± 0.5	4.7 ± 0.6	**0.041**
BCEA95 (^{o2})(median (range))	9.5 (3.4–18.1)	24 (11.5–36.4)	**0.013**
BCEA95 (^{o2})(mean ± 1 standard deviation)	10.6 ± 8.7	45.7 ± 22.2	**0.002**

BCEA63, bivariate contour ellipse area 63; BCEA95, bivariate contour ellipse area 95. NC normo-cognitive, MCI mild cognitive impairment, NA not applicable.

At baseline, both retinal sensitivity and gaze fixation were independently correlated with the diagnosis of MCI (AUROC 0.7703, $p < 0.01$, and AUROC 0.765, $p < 0.01$, respectively). When the two variables were combined, the predictive value for MCI detection increased (AUROC 0.826, $p = 0.031$), confirming in this cohort our previous results (71).

The analysis of the paired data of the MMSE and microperimetry parameters showed that MMSE and retinal sensitivity did not change after 12-months in the patients included in the study. By contrast, significant worsening was found in all the parameters related to gaze fixation (**Table 7**).

Table 7. MMSE and microperimetry parameters at basal and 12-months follow-up.

	Basal	12-Months Follow-Up	*p* Value
MMSE			
Normo-cognitive	28.9 ± 0.2	28.4 ± 0.2	0.077
Mild cognitive impairment	25.7 ± 0.9	24.7 ± 1.4	0.110
Retinal sensitivity (dB)			
Normo-cognitive	23.7 ± 0.3	21.8 ± 1.1	0.075
Mild cognitive impairment	18.4 ± 0.8	17.5 ± 0.8	0.275
Fixation stability P1 (%)			
Normo-cognitive	84.4 ± 4.5	64.6 ± 7.8	**0.020**
Mild cognitive impairment	68.3 ± 3.4	58.1 ± 3.9	**0.004**
Fixation stability P2 (%)			
Normo-cognitive	91.9 ± 2.6	83.3 ± 4.7	**0.034**
Mild cognitive impairment	84.2 ± 3.5	77.1 ± 3.1	**0.004**
BCEA63 (^{o2}) (mean ± 1 standard deviation)			
Normo-cognitive	2.1 ± 0.5	3.9 ± 1.5	**0.005**
Mild cognitive impairment	3.7 ± 0.6	4.9 ± 0.7	**0.028**
BCEA63 (^{o2}) (median (range))			
Normo-cognitive	0.8 (0.4–2)	1.2 (0.4–2.7)	**0.012**
Mild cognitive impairment	2.1 (3.4–18.1)	2.3 (3.8–15.2)	**0.043**
BCEA95 (^{o2}) (mean ± 1 standard deviation)			
Normo-cognitive	18.7 ± 5.5	35.4 ± 10.3	**0.007**
Mild cognitive impairment	30.9 ± 4.8	41.4 ± 6.2	**0.040**
BCEA95 (^{o2}) (median (range))			
Normo-cognitive	7.5 (3.4–18.1)	9.6 (0.9–24.9)	**0.023**
Mild cognitive impairment	18.1 (11.5–36.4)	20.6 (16.7–67.2)	**0.038**

BCEA63, bivariate contour ellipse area 63; BCEA95, bivariate contour ellipse area 95; MMSE, Mini-Mental State Evaluation.

At 3-years follow-up, in our sample, there was a conversion rate to MCI in 18 patients (50%) diagnosed by RBANS (NC-MCI). The MMSE was able to diagnose 16 patients (44.4%). The 10 patients with MCI at the beginning of the study remained MCI at the end of the study.

Table 8 shows the comparative data between the baseline clinical characteristics of the NC patients that remained NC and those that converted to MCI after 3-years.

Table 8. Baseline characteristics between NC-NC and NC-MCI.

	NC-NC	NC-MCI	*p* Value
***n* (%)**	18 (50%)	18 (50%)	NA
Age (years)	74.5 ± 4.6	77.5 ± 4.1	**0.042**
Gender (men) %	10 (55.6%)	12 (66.7%)	0.494
Body mass index (BMI) (kg/m^2)	30.9 ± 4.0	30.3 ± 4.4	0.6452
Smoker (%)	12 (66.7%)	14 (77.8%)	0.457
Hypertension (HTA) (%)	14 (77.8%)	18 (100%)	**0.034**
Dyslipidemia (DLP) (%)	18 (100%)	18 (100%)	N.A.
Obstructive sleep apnea (OSA) (%)	4 (22.2%)	6 (33.3%)	0.457
Ischemic heart disease (%)	4 (22.2%)	6 (33.3%)	0.457
Peripheral arteriopathy (%)	2 (11.2%)	4 (22.2%)	0.371
T2D duration (years)	18.2 ± 6.6	18.7 ± 8.3	0.827
Insulin use	8 (44.4%)	10 (55.6%)	0.505
Insulin dosed (UI/kg/day)	0.44 ± 0.2	0.62 ± 0.4	0.261
Glycosylated hemoglobin (HbA1C DCCT) (%)	7.18 ± 0.8	7.20 ± 1.1	0.9740
Severe hypoglycemia at last year (*n*)	0	2	NA
Type 2 diabetes related complications			
Retinopathy (%)	6 (33.3%)	12 (66.7%)	**0.046**
Nephropathy (%)	4 (22.2%)	4 (22.2%)	N.A.
Polyneuropathy (%)	4 (22.2%)	6 (33.3%)	0.457

NA= not applicable.

In addition, patients who converted to MCI had worse baseline MMSE, DSDRS, and MAIA scores (both retinal sensitivity and gaze fixation), as reflected by **Table 9.**

Table 9. Baseline MMSE, DSDRS and MAIA between NC-NC and NC-MCI.

	NC-NC	NC-MCI	*p* Value
***n* (%)**	18 (50%)	18 (50%)	NA
Mini-Mental State Examination (MMSE)	28.6 ± 0.9	27.6 ± 1.1	**0.006**
DSDRS mean±SD	4.77±2.2	7.11±1.7	**0.001**
Retinal sensitivity (dB)	23.8 ± 1.4	20.6 ± 3.8	**0.001**
Duration (min)	1.8 ± 0.5	2.9 ± 1.1	**0.001**
Fixation stability P1 (%)	86.3 ± 21.1	78.7 ± 25.0	**0.032**
Fixation stability P2 (%)	93.4 ± 12.7	90.1 ± 10.6	**0.044**
BCEA63 (^{o2}) (mean ± 1 standard deviation)	0.72 ± 0.6	2.4 ± 1.9	**0.001**
BCEA95 (^{o2})(mean ± 1 standard deviation)	6.04 ± 5.8	23.9 ± 18.1	**0.017**

BCEA63, bivariate contour ellipse area 63; BCEA95, bivariate contour ellipse area 95; MMSE, Mini-Mental State Evaluation; DSDRS, Diabetes Specific Dementia Risk Score.

The baseline and 3-year follow-up parameters of the MAIA by groups are shown in detail in **Table10.**

Table10. Microperimetry parameters at basal and 3-years follow-up

	Basal	3-years follow-up.	p value
Retinal sensitivity			
NC-NC	24.0±1.3	23.9±0.9	0.905
NC-MCI	20.6±3.9	16.9±2.9	**0.050**
MCI-MCI	22.8±0.6	22.9±0.8	0.317
Fixation stability P1 (%)			
NC-NC	86.1±21.7	89.5±8.9	0.222
NC-MCI	78.0±26.6	43.9±24.7	**0.001**
MCI-MCI	68.0±18.5	37.0±12.3	0.063
Fixation stability P2 (%)			
NC-NC	93.3±13.1	95.9±4.6	0.351
NC-MCI	88.8±11.2	66.5±16.9	**0.006**
MCI-MCI	72.5±23.7	63.5±24.8	0.063

	Basal	3-years follow-up.	p value
BCEA63 (^{o2}) (mean+/- 1 standard deviation)			
NC-NC	0.73± 0.4	0.9 ± 0.4	0.441
NC-MCI	0.7± 0.3	5.8 ± 2.8	**0.001**
MCI-MCI	6.5±2.1	8.5±2.8	0.063
BCEA95 (^{o2}) (mean+/- 1 standard deviation)			
NC-NC	6.1± 0.1	7.7± 0.6	0.318
NC-MCI	6.8± 0.8	52.8± 0.9	**0.002**
MCI-MCI	56.9±34.8	76.8±54.1	0.063

BCEA63, bivariate contour ellipse area 63; BCEA95, bivariate contour ellipse area 95.

The progression of the MMSE score were reflected in the **Figure 17** and the progression of BCEA95 (the gaze fixation parameter) in the **Figure 18.**

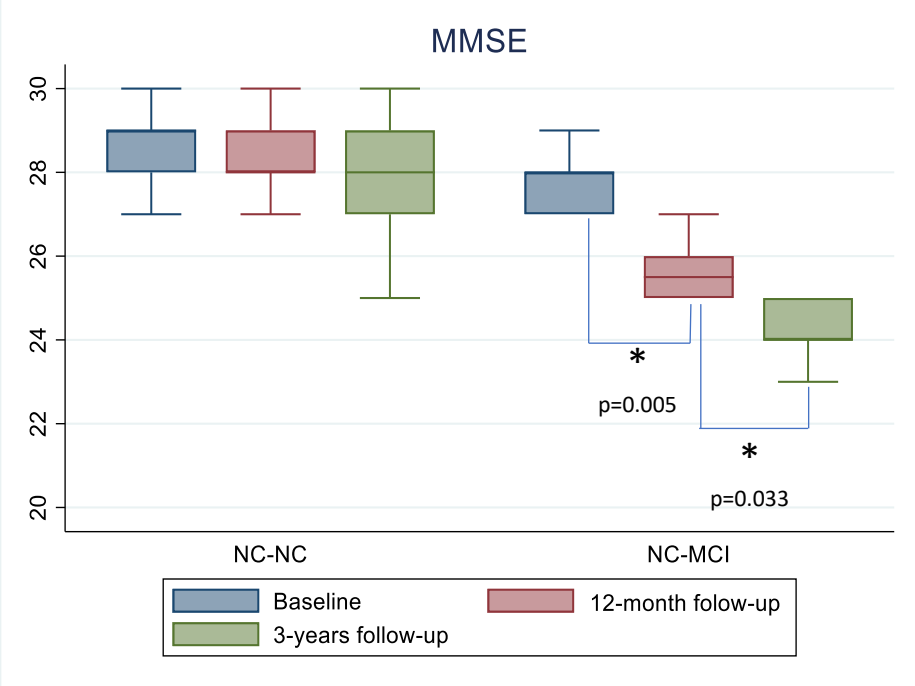


Figure 17. Progression of the MMSE score by NC-NC and NC-MCI.

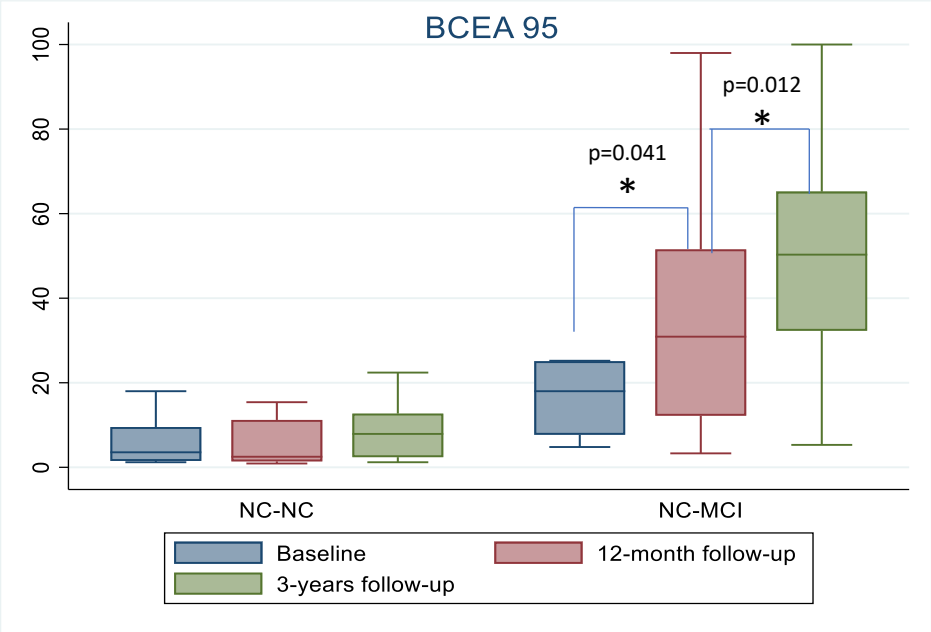


Figure 18. Progression of the BCEA95 by NC-NC and NC-MCI.

The results obtained by the MMSE at 3-years follow-up, significant differences were observed in NC-MCI in the domains of temporal orientation (27.5±1.2 vs 23.9±1.8, p=0.001), Attention calculation (4.4±0.7 vs 3.6±1.4, p=0.029), and Delay memory (2.6±0.5 vs 1.1±0.8, p=0.001). These differences were not found in the other groups. All domains evaluated by the MMSE are reflected by groups in **Table 11.**

Table 11. MMSE parameters at basal and 3-years follow-up.

	Basal	**3-years follow-up.**	**p value**
Total Score			
NC-NC	28.6±0.9	28.1±1.4	0.195
NC-MCI	27.5±1.2	23.9±1.8	**0.001**
MCI-MCI	26.5±1.4	14.1±1.6	0.157
Temporal orientation			
NC-NC	4.89±0.3	4.5±0.5	0.225
NC-MCI	4.8±0.4	4.1±0.7	**0.006**
MCI-MCI	4.7±0.6	4.2±0.2	0.054
Spatial orientation			
NC-NC	4.88±0.3	5.0±0	0.157
NC-MCI	4.7±0.7	4.6±0.5	0.416
MCI-MCI	4.5±0.5	4.6±0.4	0.514
Memory fixation			
NC-NC	3	2.9±0.3	0.157

	Basal	**3-years follow-up.**	**p value**
NC-MCI	2.8±0.4	2.9±0.4	0.157
MCI-MCI	2.8±0.3	2.8±0.4	0.351
Attention calculation			
NC-NC	4.6±0.6	4.1±0.8	0.116
NC-MCI	4.4±0.7	3.6±1.4	**0.029**
MCI-MCI	3.6±0.5	3.6±1.2	0.145
Delay memory			
NC-NC	2.76±0.7	2.6±0.6	0.362
NC-MCI	2.6±0.5	1.1±0.8	**0.001**
MCI-MCI	1.2±0.5	1.1±0.8	0.157
Nomination			
NC-NC	2	2	NA
NC-MCI	2	2	NA
MCI-MCI	2	2	NA
Repetition			
NC-NC	1	1	NA
NC-MCI	1	1	NA
MCI-MCI	1	1	NA
Understanding			
NC-NC	3	3	NA
NC-MCI	3	2.6±0.4	0.055
MCI-MCI	2.6±0.5	2.6±1.2	0.136
Reading			
NC-NC	1	1	NA
NC-MCI	1	1	NA
MCI-MCI	1	1	NA
Writing			
NC-NC	1	1	NA
NC-MCI	1	1	NA
MCI-MCI	0.5±0.3	0.5±0.3	NA
Drawing			
NC-NC	0.58±0.2	0.62±0.3	0.116
NC-MCI	0.54±0.3	0.5±0.2	0.157
MCI-MCI	0.51±0.7	0.51±0.1	0.314

NA= not applicable.

In addition, at 3-years follow-up significant differences were observed in some domains obtained by the RBANS in NC-MCI, theses domains were: Immediate memory index (95.0±13.3 vs 73.0±16.4, p=0.004); Language index (96.3±7.4 vs 89.6±5.4, p=0.012); Attention index (87.8±12.0 vs 65.4±23.2, p=0.001); Delayed memory (92.0±9.6 vs 71.7±12.3, p=0.001). All the domains evaluated by the RBANS are reflected by group in the **Table 12.**

Table 12. RBANS parameters at basal and 3-years follow-up.

RBANS	Basal	3-years follow-up.	p value
i_Immediate memory index			
NC-NC	97.2±7.9	97.7±16.3	0.508
NC-MCI	95.0±13.3	73.0±16.4	**0.004**
MCI-MCI	75.5±16.7	75.1±20.8	0.157
1_List Learning			
NC-NC	10.3±2.3	11.4±3.9	0.378
NC-MCI	9.1±2.3	5.6±2.8	**0.001**
MCI-MCI	5.1±4.6	5.5±4.0	0.157
2_History Memory			
NC-NC	6.4±1.2	5.8±1.9	0.264
NC-MCI	5.6±0.9	5.2±3.1	0.171
MCI-MCI	5.5±1.2	5.3±2.2	0.157
ii_Visuospatial / Constructional Index			
NC-NC	79.5±14.1	85.8±19.9	0.079
NC-MCI	82.7±17.6	82.6±17.2	0.810
MCI-MCI	83.5±16.6	81.5±15.7	0.157
3_Figure Copy			
NC-NC	6.4±1.5	6.4±3.2	0.338
NC-MCI	6.5±3.5	6.2±3.2	0.661
MCI-MCI	6.5±4.1	6.1±1.1	0.157
4_Line orientation			
NC-NC	7.6±1.5	7.6±3.1	0.826
NC-MCI	7.2±1.8	6.9±3.2	0.117
MCI-MCI	7.1±1.2	6.9±3.0	0.157
iii_Language index			
NC-NC	106.5±10.3	101.7±4.5	0.055
NC-MCI	96.3±7.4	89.6±5.4	**0.012**
MCI-MCI	91.2±9.3	89.1±11.6	0.157

RBANS	Basal	3-years follow-up.	p value
5_Picture Naming			
NC-NC	8.1±1.4	9.1±1.1	0.039
NC-MCI	8.6±2.0	9.5±1.3	0.091
MCI-MCI	8.6±1.2	8.6±1.2	NA
6_Semantic Fluency			
NC-NC	8.5±2.8	8.0±2.0	0.234
NC-MCI	7.3±2.1	5.6±1.8	**0.041**
MCI-MCI	5.5±1.2	5.1±1.2	0.157
iv_Attention index			
NC-NC	82.2±8.3	82.1±18.2	0.469
NC-MCI	87.8±12.0	65.4±23.2	**0.001**
MCI-MCI	63.1±15.0	62.5±19.1	0.457
7_Digit Spam			
NC-NC	5.5±2.0	6.0±1.2	0.303
NC-MCI	5.4±2.8	5.2±1.8	0.800
MCI-MCI	4.0±1.5	5.0±2.3	0.157
8_Coding			
NC-NC	6.4±3.2	6.6±3.4	0.596
NC-MCI	5.4±3.2	3.3±3.1	**0.007**
MCI-MCI	3.5±2.8	3.5±2.8	NA
v_Delayed memory			
NC-NC	96.4±6.8	93.5±7.5	0.174
NC-MCI	92.0±9.6	71.7±12.3	**0.001**
MCI-MCI	73.4±7.2	71.5±15.5	0.157
9_ Recall			
NC-NC	9.7±1.8	9.7±1.9	0.846
NC-MCI	8.5±1.6	5.2±2.3	**0.003**
MCI-MCI	5.0±1.7	5.1±1.7	0.157
10_List Recognition			
NC-NC	8.1±1.2	7.7±2.5	0.546
NC-MCI	6.8±2.2	4.2±2.6	**0.007**
MCI-MCI	4.5±0.6	4.5±1.2	0.157
11_History Recall			
NC-NC	8.0±1.9	8.1±1.9	0.414
NC-MCI	7.7±1.8	5.7±2.7	**0.023**
MCI-MCI	5.8±2.8	5.7±0.5	0.157

RBANS	Basal	3-years follow-up.	p value
12_Figure Recall			
NC-NC	8.2±2.6	8.0±1.3	0.280
NC-MCI	8.2±2.3	8.3±3.3	0.982
MCI-MCI	8	8	NA
RBANS Total			
NC-NC	86.3±6.7	88.8±11.4	0.154
NC-MCI	86.7±10.7	70.1±14.7	**0.005**
MCI-MCI	68.0±11.5	69.5±13.27	0.157

NA= not applicable

NC-MCI patients statistically significant correlations were observed between the difference in the fixation parameters (P1, P2, BCEA63 and BCEA 95) obtained by MAIA with the differences obtained by RBANS. For example, BCEA 95 has a correlation with total score of the RBANS (r = -0.6174; CI (-0.4060 – -0.7734) p = 0.006), but these differences were not statistically significant with total score of the MMSE.

In **Figure 19,** we can see box plot of the differences (baseline – 3-years follow-up) between RBANS, MMSE, BCEA95 and RS by Groups.

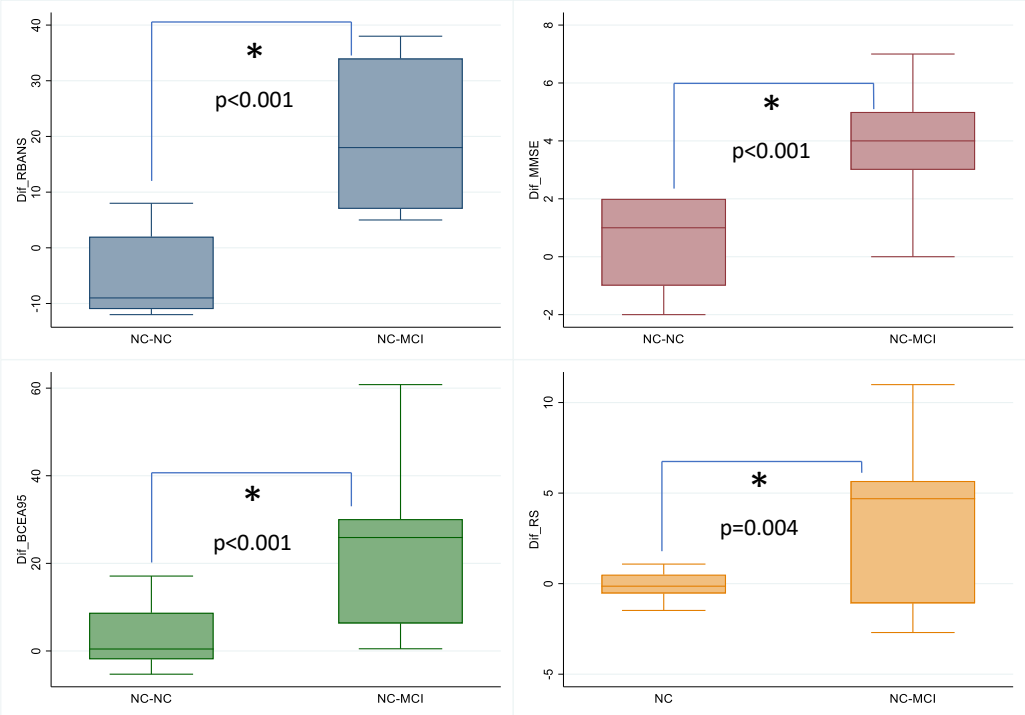


Figure 19. Box plot of the differences (baseline – 3-years follow-up) between RBANS, MMSE, BCEA95 and RS by Groups

The registers of gaze fixation in a representative patient in whom a worsening of RBANS is displayed in **Figure 20.** In addition, in the **Figure 21** the correlation between the difference in BCEA95 and the difference in the RBANS.

In addition, the difference in the retinal sensitivity obtained by MAIA has a correlation with a total score of RBANS (r = -0.4573; CI (-0.3504 – -0.61372) p = 0.048).

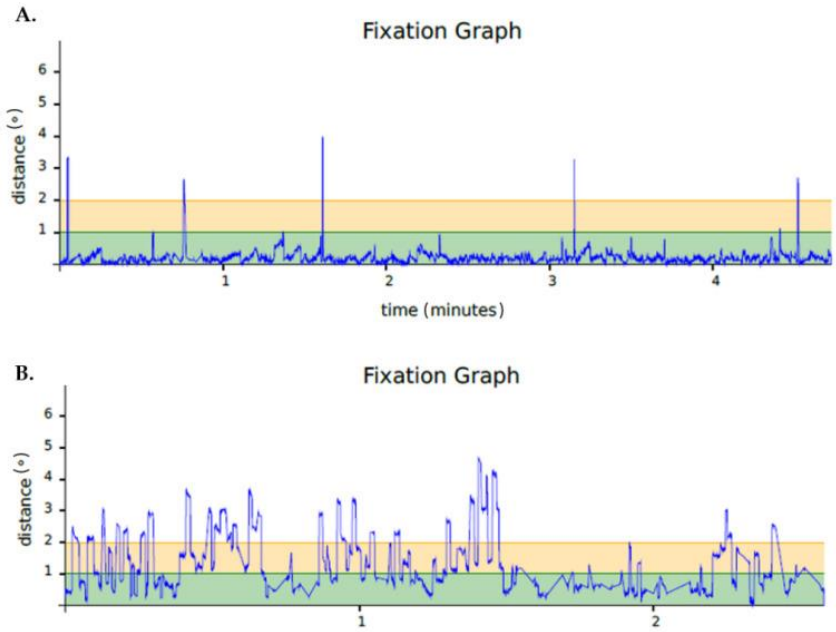


Figure 20. (**A**)**.** Fixation graph of a MCI patient at the beginning of the study. (**B**) Fixation graph of the same patients with follow-up at 12-months and 3-years

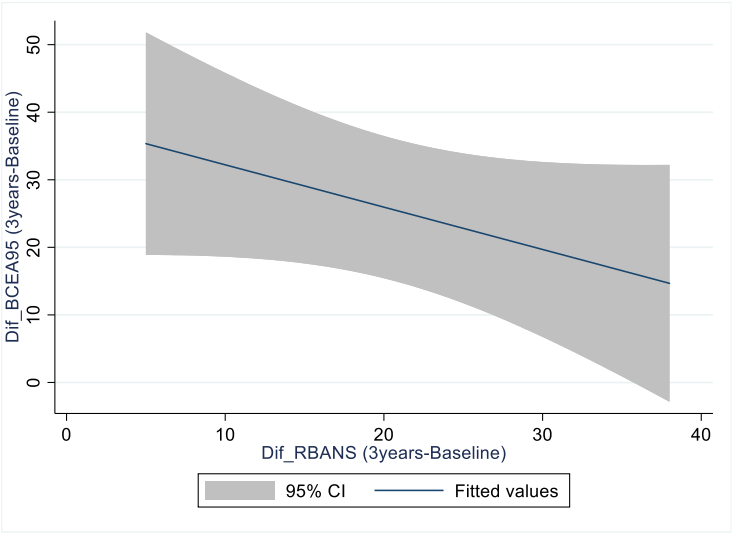


Figure 21. Correlation between differences in BCEA95 and differences in RBANS.

When we analyzed the RBANS results in NC-MCI by domains, we did not obtain statistically significant correlations between the difference in the RBANS domains with the difference in retinal sensitivity obtained by MAIA.

Regarding to NC-MCI, when we analyzed the correlations between the differences in the fixation parameters (P1 P2, BCEA63 and BCEA95) with the differences obtained by the different domains of the RBANS battery, we found statistically significant differences between the delayed memory and the parameters fixation. For example, Fixation stability P2 (%) has a correlation with differences between the delayed memory of the RBANS (r = -0.8436; CI (-0.7723 – -0.8942) p = 0.001), **Figure 22.** Nevertheless, these differences were not statistically significant with total score of the MMSE**.**

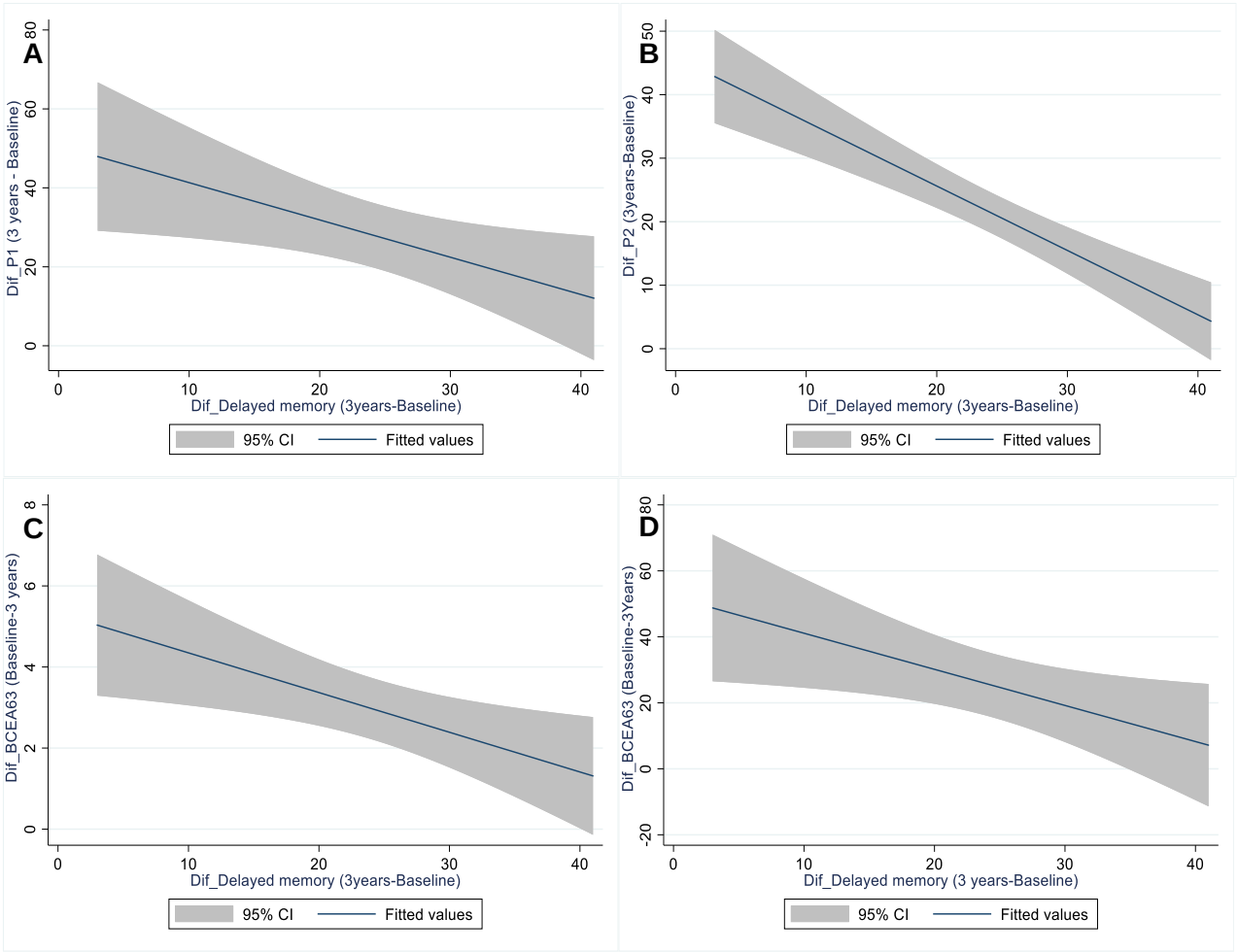


Figure 22. Correlation between differences in Delayed memory obtained by RBANS and. **A**. Differences in P1 obtained by MAIA. **B.** Differences in P2 obtained by MAIA. **C.** Differences in BCEA63 obtained by MAIA. **D.** Differences in BCEA95 obtained by MAIA.

Finally, in our sample, we obtained the cut-off point of 21.15 dB in the RS parameter to predict the change from NC to MCI, with 100% sensitivity and specificity. The gaze fixation parameters (P1%) had a cut-off 37.18±2.6% IC (35.6%-38.7%) with sensitivity and specificity of 88.89%.

5.3. UNDERLYING MECHANISMS BY WHICH RETINAL MICROPERIMETRY IS USEFUL FOR THE SCREENING AND MONITORING OF THE COGNITIVE FUNCTION:

A total of 33 patients with T2D (45% women, mean age 72.1±4.6 years) were recruited fulfilling inclusion/exclusion criteria. The characteristics of the patients are shown in **Table 13**.

Table 13. Baseline characteristics of the patients.

n	33
Age (years) mean±SD	72.1±4.6
Gender (women) %	45%
BMI (kg/m^2) mean±SD	29.74±0.9
Smoker (%)	59.9%
Hypertension (%)	84.8%
Dyslipemia (%)	87.9%
Sleep apnea syndrome (%)	16.6%
Ischemic heart disease (%)	18.1%
Peripheral arteriopathy (%)	6.0%
T2D duration (years, mean±SD)	15.55±7.4
HbA1C (%) (DCCT mean±SD)	7.38±0.8
Diabetic Retinopathy (%)	36.3%
Diabetic Nephropathy (%)	27.2%
Diabetic Polyneuropathy (%)	18.1%
Visual acuity (20/20)	0.85
Retinal microperimetry (right eye)	
Sensitivity (mean±SD)	20.75±0.9
Duration (min) (mean±SD)	2.55±0.4
Reliability index % (mean±SD)	98.1±1.1
BCEA63%(º^2) (mean±SD)	3.67±1.6
BCEA95%(º^2) (mean±SD)	32.62±9.3
Evoked ophthalmic potentials (right eye)	
Latency P100 (ms)12x16 (mean±SD)	106.43 ±11.4
Latency P100 (ms)24x34 (mean±SD)	111.97 ± 10.8
Amplitude 75-100 (uV) 12x16 (mean±SD)	6.17 ± 2.8
Amplitude 75-100 (uV) 24x34 (mean±SD)	5.94 ± 2.81

BMI: body mass index, T2D type 2 diabetes, BCEA63 Bivariate contour ellipse area 63% (º^2), BCEA95 Bivariate contour ellipse area 95% (º^2).

VEP parameters significantly correlated with RS evaluated by microperimetry: Latency P100 (ms)-RS: R 0.728, p<0.01; Amplitude 75-100uV (12x16)-RS: R 0.517, p 0.021.). We found no significant correlations between the GF parameter BCEA95% and VEP: Latency P100 (ms)-BCEA95% R 0.174, p 0.333; Amplitude 75-100uV (12x16)-BCEA95 R 0.201, p=0.154.

6. Discussion

6.1. PREVALENCE OF MCI IN THE T2D POPULATION >65 YEARS IN A TERTIARY CARE SETTING AND THE APPLICABILITY OF DSDRS AS A SCREENING TOOL FOR PATIENTS WITH T2D >65 YEARS WITH COGNITIVE IMPAIRMENT.

In our study, we found a high prevalence of cognitive impairment in the T2D patients older than 65 who attended a third-level hospital, as part of the MOPEAD protocol in Spain. The suspected diagnostic was confirmed in a reference memory clinic, and the clinical DSDRS score showed a significant predictive value of cognitive impairment.

Although the DSDRS was not designed as a screening tool, our results suggest that it might eventually be used as a complementary test for this purpose. Most of the developed countries are using electronic medical history, thus making largely available reliable data in the medical records regarding age, level of formal education, history of acute metabolic events, history of diabetic foot, history of microvascular disease, history of cerebrovascular disease, history of cardiovascular disease, diagnosis of depression. This information permits us to calculate DSDRS easily.

An interesting proposal could be the automatic calculation of the DSDRS score during or even before the patient's visit. In this regard, it could be extremely useful in clinical practice to receive a warning alarm in the event that the diabetic patients present a score ≥7 in order to facilitate the identification of patients at higher risk of presenting cognitive impairment. This strategy would allow selecting those patients in whom a specific assessment of cognitive status should be implemented. However, specific studies to confirm these preliminary results in larger cohorts are needed.

Unrecognized cognitive dysfunction can affect treatment adherence and diabetes self-management resulting in poor glycemic control, an increased frequency of severe hypoglycemic episodes, and hospital admissions.

Regarding hypoglycemia, two independent studies (26)(25), showed that three or more severe hypoglycemic episodes increased by almost two-fold the risk of dementia in T2D patients. Despite of the role of hypoglycemia in the cognitive function, the frequency and severity of hypoglycemia does not appear

in current questionnaires for the evaluation of the risk of developing dementia, and therefore, this crucial point should be tackled urgently. In order to fill this gap, we have designed a hypoglycemia survey which was administered to all the patients included in the present study. You can access in the following link https://docs.google.com/forms/d/e/1FAIpQLSecipJ6gH94-ZpvEQpZcGfpr2ff6KWB2IfnBgVJy73hcZIvkw/viewform.

Notably, 6 patients who fulfilled the criteria of cognitive impairment presented a history of severe hypoglycemia while no cognitively healthy participant presented hypoglycemia.

Other diabetes-specific dementia predictors that are not taken into account these scoring systems are diabetes duration, glucose lowering treatment (86) (87).

In our study, the prevalence of MCI was 87.2% and for AD 7.7% in the group of patients that attended the memory clinic. The prevalence of MCI in our sample was calculated based on the 39 patients that attended the memory clinic. However, it should be noted that more than half of the patients that met criteria for referral to the memory clinic declined to continue the study, mainly due to associated comorbidities and lack of interest. At present, the real prevalence of MCI in T2D patients is unclear. Gao et al. reported a prevalence of MCI in T2D of 62.2% and AD 11.9% in Chinese population (88), while Albai et al. (89) reported 42.03% MCI in T2D patients having a mean age of 63 years (range 57–71).

The mean age of patients included in our study was 75 years, all of them were patients recruited from a tertiary care setting and most of them presented co-morbidities. These factors should be taken into account when comparing the different series reported in the literature. It should be noted that the neuropsychological battery and the data reported was not homogeneous between the studies. Gao et al. (88) and Albai et al. (89) did not report data on the MMSE score or details on the neuropsychological battery that was used. Additionally, Albai et al (89), did not report data on the education level (one of the most important variables that influences cognitive function scoring), and both cited studies lack data on the cardiovascular risk factors, complications of T2D and hypoglycemia. The comparison between the results from our study and the

main recent studies that reported prevalence of MCI in T2D patients are show at the **Table 14.**

Table 14. Comparison between the results from our study and the main recent studies that reported prevalence of MCI in T2D patients

	HVH	Albai et al[19]	Gao et al[18]
N (%)	39	207	1109
Race	Caucasian	Caucasian	Asian
Age (years) median [min-max]	75 [68-84]	58 [47-65]	72.4 (mean)
Education level <9 years %	83.87	NR	84.2
Sex (women) %	44.1	46.9	56.3
BMI (kg/m2) mean±SD	28.51±4.01	28±5.1	24.5±3.4
Smoker (%)	54.83	30.09	26.1
Hypertension (%)	87.09	69.6	NR
Dyslipidemia (%)	96	NR	NR
Ischemic heart disease (%)	25.80	41.5	NR
Peripheral arteriopathy (%)	19.2	14	NR
T2D duration (mean±SD) years	19.1±6.2	10 [8-13]	11.2±3.7
Insulin use%	67.74	NR	35.3
HbA1C (%) mean±SD	7.69±0.76	8.2	7.21±1.34
Severe hypoglycaemia (n)	64	NR	NR
Diabetic retinopathy (%)	54.83	18.8	29.3
Diabetic nephropathy (%)	38.70	NR	25.3
Diabetic polyneuropathy (%)	22.58	NR	13
MMSE (mean±SD)	26.72±2.14	NR	NR
Prevalence of MCI%	87.2	42.03	62.2

NR: not reported, BMI: body mass index, MCI: mild cognitive impairment, MMSE: Mini-Mental State Examination, DSDRS: diabetes specific dementia risk score

Furthermore, the population included in our study was selected from the patients attended in a tertiary setting, showing a high prevalence of MCI among this population, while the other two studies included patients from general population. At present, there is no data regarding the prevalence of cognitive impairment in T2D patients attended in a third level hospital, which represents a

strength of our study, even if we have preliminary results that need to be validated.

Patients attended in a third-level hospital usually are plurimedicated. They usually are prescribed complex treatment regimes, some of them including insulin. As reflected by Table 3, almost 70% of the patients were using insulin. The importance of early detection of cognitive impairment in this population comes from the need to adapt and adjust the specific treatment for T2D to the capacity of management of the patient, in order to avoid worsening of T2D associated complications and to limit possible errors in the administration of the medication, which may lead to hypoglycemia or other unfavorable and potentially life-threatening events.

Several neuropsychological questionnaires have been proposed for the screening of cognitive decline in T2D population (90). However, the number of patients whose cognitive function needs evaluating by the general practitioner or the endocrinologist/diabetologist is potentially enormous, and a simpler and more cost-effective case-finding strategy to detect undiagnosed cognitive impairment is needed. In our study, we showed that a simple score (DSDRS) calculated based on clinical variables is useful as a screening tool for cognitive impairment (AUROC 0.739, p 0.024, CI 95% (0.609–0.825)) (50).

No differences were seen between the predictive values of MMSE and DSDRS separately as screening tools for cognitive impairment. Nevertheless, as mentioned, the novelty of the DSDRS as a screening tool is that it can be calculated using existing data in the medical history of the patient. Patients with DSDRS ≥ 7 can be candidates for a more specific study of cognitive function. This score consists of several clinical and demographic variables (age, gender, education, history of diabetic foot, acute metabolic events, depression, microvascular disease, cardiovascular disease and cerebrovascular disease). It should be noted that in our study, as per MOPEAD protocol, patients with stroke or depression were excluded.

We admit that in the real world, the DSDRS could be higher than the obtained in the present study. Nevertheless, even with this bias, our results showed that the DSDRS was a useful tool for identifying patients with diabetes at

risk of dementia. The DSDRS was designed as a risk score of dementia at 10 years, ranging from a 5% of dementia risk for those with the lowest score up to a 73% risk for the highest score. The DSDRS was not designed as a screening tool, but it might eventually be used as a complementary test for this purpose, in particular, if more detailed information regarding diabetic complications (i.e., degree of diabetic retinopathy) and glycemic control (i.e., hypoglycemic events and glycemic variability) was added.

When the DSDRS score was added to the MMSE, the predictive value for cognitive impairment significantly improved, supporting the hypothesis that DSDRS can be an useful complementary screening tool (AUROC 0.902 (p 0.003, CI 95% (0.840–0.992)), significantly higher than MMSE (AUROC 0.785, p 0.007, CI 95% (0.814–0.9648)) or DSDRS separately (AUROC 0.739, p 0.024, CI 95% (0.609–0.825)).

One limitation of our study is the small sample size (as per protocol 100 patients/country (78)) and the fact that most of the patients that met criteria for referral to the memory clinic declined further participation in the study due to associated comorbidities and lack of interest. This observation deserves a comment: The study of cognitive status is not a current priority for the patients and health care providers. This is a significant gap that should urgently be filled due to the importance of the early detection of cognitive impairment and the implications in the management of the complex treatment regimes.

Our results are preliminary, and a specific study to confirm that a refined DSDRS score could be a useful complementary test for identifying T2D patients who should be referred to a memory clinic is needed. If our results are confirmed in a further study, the DSDRS could be a screening tool that might easily be implemented and automatically calculated by the electronic medical records of the patients, as part of the daily clinical practice.

6.2. USEFULNESS OF RETINAL MICROPERIMETRY FOR SCREENING AND MONITORING MCI IN PATIENTS WITH TYPE 2 DIABETES.

As far as we know, this is the first prospective study aimed at evaluating the usefulness of retinal microperimetry not only as a tool for screening but also for the annual monitoring of cognitive function in patients with T2D > 65 years old.

The baseline evaluation confirmed and extended our previous results that retinal sensitivity and gaze-fixation parameters measured by microperimetry are useful for the detection of cognitive impairment in patients with T2D > 65 years (84) (71).

For the complete cognitive diagnosis and inclusion in the study, we used a complete neuropsychological evaluation performed by a trained neuropsychologist using the repeatable battery for the assessment of neuropsychological status (RBANS). RBANS takes about 30-60 min to assess a variety of cognitive domains and to classify patients as normo-cognitive (NC), MCI, or having dementia (80).

For the prospective study we chose MMSE, which is one of the tests recommended in the ADA guidelines (29) to be used in clinical practice in patients with T2D > 65 years both for screening and for annual monitoring. ADA guidelines also recommend for this purpose the Montreal cognitive assessment (MoCA) test (40). However, MoCA needs previous training and periodic certification, making MMSE the most accessible and widely used score for the evaluation of cognitive function in daily clinical practice.

At 3-years follow-up, all the patients included in the study performed retinal microperimetry, MMSE and the neuropsychological test RBANS. The patients were reclassified into 3 groups according to the results obtained by the RBANS at 3-years of follow-up. Being of special interest those patients belonging to NC-MCI who changed from a normo-cognitive baseline situation to mild cognitive impairment diagnosed by the neuropsychological battery RBANS.

In our study, the MMSE was able to diagnose 16/18 (88.8%) patients with MCI diagnosed by RBANS.

A very important finding of our work was that normo-cognitive patients who converted to MCI at the end of the study had a greater presence of HTA and diabetic retinopathy. Probably the greater presence of retinopathy translates greater presence of neurodegeneration in our sample. Furthermore, data from our previous studies showed that retinal sensitivity was correlated with brain neurodegeneration (grey matter volumes on brain MRI and glucose uptake on PET) (84). This previous data, together with data from the present study, supports the hypothesis that NC patients with type 2 diabetes>65 years that present lower levels of retinal sensitivity at baseline might have more brain neurodegeneration and are at higher risk of progression to MCI during follow-up.

We found significant correlations between MMSE and MAIA parameters (retinal sensitivity and gaze-fixation) at baseline. However, only gaze-fixation parameters (P1, P2, BCEA63, BCEA95) showed significant worsening at the 12-months of follow-up and were correlated to the global score of RBANS at 3-years follow-up. Furthermore, the worsening of RBANS was parallel to the worsening in MAIA parameters, especially gaze fixation, in that sense we found significant differences between the fixation parameters (P1 P2, BCEA63 and BCEA95) with the differences obtained delayed memory by RBANS as reflected by **Figure 20,** suggesting that retinal microperimetry, can be a reliable and more subtle tool for the monitoring of the cognitive function in patients with type 2 diabetes. Particularly, such cognitive domains as attention and delayed recall significantly correlated with all gaze-fixation parameters during follow-up.

Additionally, we found that the cognitive domains that changed at 3-years of follow-up evaluated by the RBANS in our sample were: immediate memory index, visuospatial / constructional (due to a worsening in Language and semantic Fluency), attention and especially the delayed memory (due to the worsening of the Word recall, word recognition and memory of history). As reflected by **Table 12.**

This is the first study that manages to correlate differences between fixation parameters obtained by retinal microperimetry with cognitive domains in people with type 2 diabetes over 65 years of age who change from a normo-cognitive situation to present mild cognitive impairment in the follow-up to 3-years.

As previously explained (71), this finding could be attributed to the fact that the areas of the brain involved in gaze fixation are different from those of retinal sensitivity.

In the case of Retinal sensitivity, the anatomical region involved in the processing of the information that goes through the optic pathway from the retinal ganglion cells, is the lateral geniculate body of the thalamus. This region, which relays information to the primary visual cortex. The involvement of the lateral geniculated body has already been demonstrated in Alzheimer's disease (91). In additional our group showed previously that retinal sensitivity correlated with grey matter volumes in brain MRI in T2D patients with MCI and AD but not white matter (84), suggesting that retinal sensitivity is a marker of neurodegeneration. One can hypothesize that neurodegeneration does not significantly change over 12-months.

On the other hand, gaze fixation depends on the complex white matter network. The brain structures that play an essential role in gaze fixation are the superior colliculus and parietal and frontal cortex. (92). It should be noted that the retina has several types of ganglion cells, most of them directly connected to the geniculate body of the visual pathway, but others are connected directly to the superior colliculus (93). The superior colliculus and the parietal and frontal cortex play an essential role in gaze fixation (72) (73).The later ones respond best to small stimuli and abrupt changes in light intensity.

In support of this data, our group previously showed that gaze fixation was impaired in young patients with obesity and significantly correlated with cognitive function, in particular with attention span, while retinal sensitivity was not altered and did not correlate with the neurocognitive scores (92). These results suggest that alterations in white matter connectivity are more subtle and occur earlier than neurodegeneration in the visual pathway, thus explaining why the alterations in both gaze fixation and cognitive domains (attention and delayed recall) could already be seen at 12-month follow-up, while retinal sensitivity remained unchanged.

Gaze fixation and not Retinal sensitivity were correlated with attention and delayed recall, which depend mainly on the default brain network and the white matter connections between the dorsolateral and prefrontal cortex and inferior and superior parietal lobules (94) (95). In support of the fact that Gaze fixation depends on the white matter network, such as frontal eye fields and the dorsomedial prefrontal cortex, with the involvement of the superior colliculus (95). **Figure 23.**

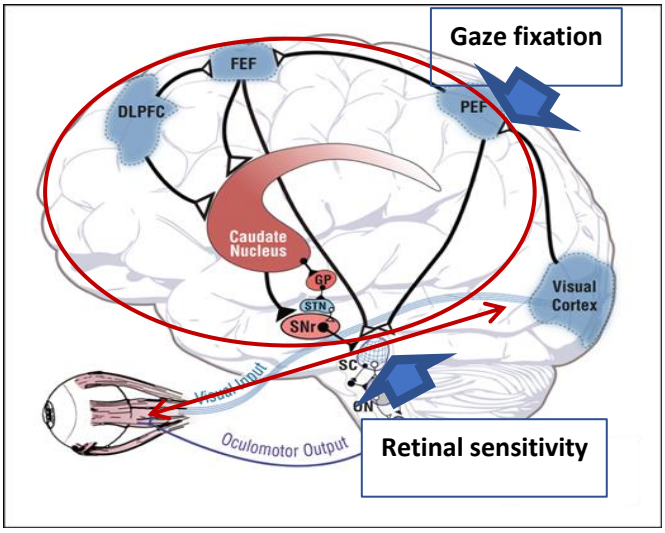


Figure 23. Retinal sensitivity and gaze fixation evaluated by microperimetry is correlated with different visual pathway.

Therefore, as expected, in our study Gaze fixation parameters did not correlate with visual evoke potentials. The presence of mild non-proliferative diabetic retinopathy in 36.3% of the patients that were included in the study do not have a significant role in the results, since the same patient was performed both tests in the same conditions and time.

Our study we showed first evidence that Retinal sensitivity and not Gaze fixation evaluated by microperimetry is correlated with visual pathway explored by visual evoke potentials. Our findings are of interest in the context of proposing the microperimetry a useful tool for screening and monitoring of the cognitive function and not as an ophthalmological test. These results are the first piece of evidence towards proving our hypothesis that Gaze fixation and Retinal sensitivity are independent parameters and can be considered complementary measurements of cognitive function, thus enhancing the reliability of the results obtained by microperimetry aimed at identifying subjects with cognitive impairment.

Interestingly, the baseline retinal sensitivity parameter evaluated by microperimetry with a cutoff point of <21.15 dB was able to predict the conversion from NC to MCI in 100% of patients (better than MMSE). With the fixation parameters, it was not possible to establish a single cut-off point with such a high sensitivity and specificity, probably due to sample size.

Our study has limitations, such as presenting a small sample of patients. However, this study provides more scientific evidence regarding the use of retinal microperimetry as a screening and monitoring test, because it is able to detect small cognitive changes that detected only by neurocognitive batteries that are difficult to implement in routine clinical practice regardless of psychological status or educational level.

Nevertheless, it should be noted that we aimed to explore the utility of retinal microperimetry as a simple tool for the annual monitoring of the cognitive function in patients with T2D > 65 years, replicating the recommendations of the actual ADA guidelines and daily clinical practice. At present, it is impossible to implement a complete battery of neuropsychological tests for all T2D patients > 65 years, and even MMSE would be time consuming if we used it for the evaluation of all patients with T2D > 65 years. Another important limitation of the neuropsychological tests, including MMSE, is that the patient´s performance depends on both emotional mood and educational level. Finally, by repeating the same test each year there is a learning phenomenon that could bias the total score

The associated anxiety-depression that happen in a significant proportion of ageing patients with T2D is not generally considered (91). Is should be noted that the prevalence of depression is two-fold higher in patients with T2D compared with the general population worldwide (21), and it has recently been reported as 27.5% among T2D patients in the Mediterranean population (96). Retinal microperimetry, is a simple, objective, and rapid test that can be largely used for the monitoring of cognitive performance regardless of psychological status or educational level.

These results suggest that since retinal sensitivity is a reliable screening tool for diagnosis, the evaluation of gaze fixation could represent a better biomarker for annual follow-up.

7. Conclusions

7.1. T2D patients in a tertiary setting have high prevalence of MCI and cognitive impairment.

7.2. DSDRS is useful for screening cognitive impairment in T2D patients >65 years.

7.3. Both retinal microperimetry parameters (retinal sensitivity and gaze fixation) are useful for the screening of cognitive impairment in patients > 65 years withT2D. Gaze fixation evaluated by microperimetry is a more sensitive parameter for annual monitoring of the cognitive function in patients with T2D>65 years.

7.4 Retinal sensitivity evaluated by microperimetry is a useful tool to predict the risk of progression to MCI after 3 years in normo-cognitive patients with T2D>65 years.

7.5 Retinal sensitivity and gaze fixation explore different neural circuits and are complementary diagnostic tools.

8. New projects and future perspectives

The results of the present work support the concept of establishing reliable, non-invasive, reproducible, and easily applicable tests in routine clinical practice, such as retinal microperimetry and the DSDRS score for screening for cognitive impairment in people over 65 years of age with diabetes.

In order to explore in a more specific way and to be able to clarify the physio-pathological pathways of cognitive deterioration in people with diabetes, revalidating the use of retinal microperimetry in this population, the following prospective, multicenter studies are in progress:

1. **Retinal and cognitive dysfunction in type 2 diabetes: unraveling the common pathways and identification of patients at risk of dementia (RECOGNISED).**

   No: ECR-RET-2019-14.

   ACRONYM: RECOGNISED.

   ClinicalTrials.gov No: NCT04281186.

   Funding Agency: EC-Horizon 2020 (Grant Agreement: 847749).

   Coordinator: Rafael Simó.

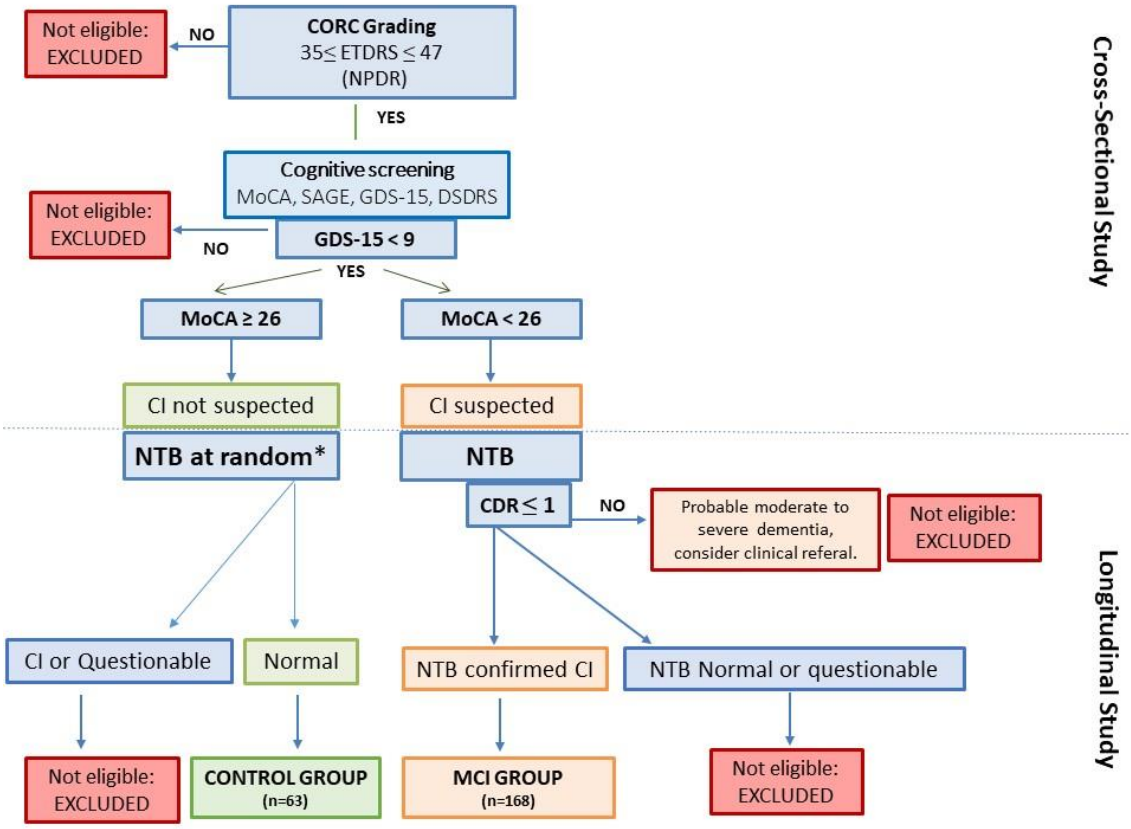
   Work Package Leaders: Andreea Ciudin and Cristina Hernández.

   Associated investigator: Ángel M Ortíz Zúñiga.

   AIMS OF THE PROJECT:

   1) To determine whether functional and/or structural retinal biomarkers or circulating biomarkers can differentiate people with mild cognitive impairment (MCI) within the type 2 diabetes mellitus (T2D) population.
   2) To determine whether functional and/or structural retinal biomarkers or circulating biomarkers can be used to determine the rate of cognitive decline in people with T2D and MCI and those at increased risk of developing dementia.

SCHEMATIC FLOWCHART OF THE STUDY:



Start Date: 01-2021.

End Date: 01-2024.

2. Structural and functional brain changes as a proxy for Virtual Reality cognitive training efficacy in Type 2 Diabetes Mellitus elderly patients (T2CogVR).

ACRONYM: T2CogVR.

Principal Investigator: Rafael Simó.

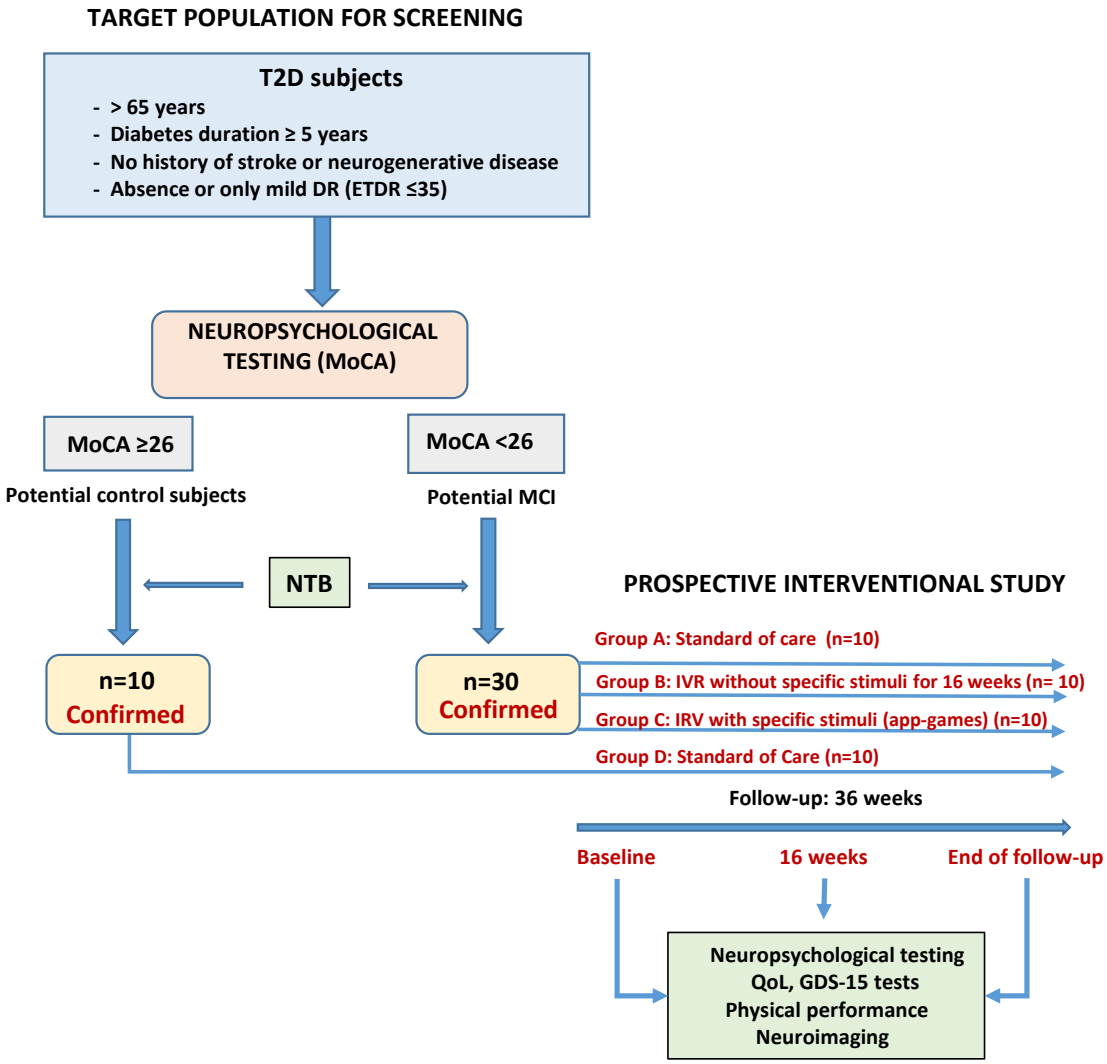
Associated Investigator: Ángel M Ortíz Zúñiga

AIMS OF THE PROJECT:

1) To determine whether an IVR cognitive training program can arrest the cognitive decline that occurs in patients with T2D diagnosed with MCI.
2) To examine whether an IVR cognitive training program is able to modify brain function assessed by resting state functional MRI.

3) To assess whether functional changes are associated with structural MRI measurements.

SCHEMATIC FLOWCHART OF THE STUDY:



START DATE: 09-2021.

END DATE: 01-2024.

9. BIBLIOGRAPHIC REFERENCES.

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract. 2019 Nov;157:107843.

2. Spauwen PJJ, Köhler S, Verhey FRJ, Stehouwer CDA, van Boxtel MPJ. Effects of type 2 diabetes on 12-year cognitive change: results from the Maastricht Aging Study. Diabetes Care. 2013 Jun;36(6):1554–61.

3. Ritchie K, Lovestone S. The dementias. Lancet. 2002 Nov 30;360(9347):1759–66.

4. Dubois B, Feldman HH, Jacova C, Cummings JL, Dekosky ST, Barberger-Gateau P, et al. Revising the definition of Alzheimer's disease: a new lexicon. Lancet Neurol. 2010 Nov;9(11):1118–27.

5. Kopf D, Frölich L. Risk of incident Alzheimer's disease in diabetic patients: a systematic review of prospective trials. J Alzheimers Dis. 2009;16(4):677–85.

6. Soriguer F, Goday A, Bosch-Comas A, Bordiú E, Calle-Pascual A, Carmena R, et al. Prevalence of diabetes mellitus and impaired glucose regulation in Spain: the Di@bet.es Study. Diabetologia. 2012 Jan;55(1):88–93.

7. Hebert LE, Scherr PA, Bienias JL, Bennett DA, Evans DA. Alzheimer disease in the US population: prevalence estimates using the 2000 census. Arch Neurol. 2003 Aug;60(8):1119–22.

8. Biessels GJ, Staekenborg S, Brunner E, Brayne C, Scheltens P. Risk of dementia in diabetes mellitus: a systematic review. Lancet Neurol. 2006 Jan;5(1):64–74.

9. Huang C-C, Chung C-M, Leu H-B, Lin L-Y, Chiu C-C, Hsu C-Y, et al. Diabetes mellitus and the risk of Alzheimer's disease: a nationwide population-based study. PLoS One. 2014;9(1):e87095.

10. Xu W, Qiu C, Gatz M, Pedersen NL, Johansson B, Fratiglioni L. Mid- and late-life diabetes in relation to the risk of dementia: a population-based twin study. Diabetes. 2009 Jan;58(1):71–7.

11. Xu WL, von Strauss E, Qiu CX, Winblad B, Fratiglioni L. Uncontrolled diabetes increases the risk of Alzheimer's disease: a population-based cohort study. Diabetologia. 2009 Jun;52(6):1031–9.

12. Tuligenga RH, Dugravot A, Tabák AG, Elbaz A, Brunner EJ, Kivimäki M, et al. Midlife type 2 diabetes and poor glycaemic control as risk factors for cognitive decline in early old age: a post-hoc analysis of the Whitehall II cohort study. Lancet Diabetes Endocrinol. 2014 Mar;2(3):228–35.

13. Crane PK, Walker R, Larson EB. Glucose levels and risk of dementia. N Engl J Med. 2013 Nov 7;369(19):1863–4.

14. Reisberg B, Ferris SH, de Leon MJ, Crook T. The Global Deterioration Scale for assessment of primary degenerative dementia. Am J Psychiatry. 1982 Sep;139(9):1136–9.

15. Petersen RC, Doody R, Kurz A, Mohs RC, Morris JC, Rabins PV, et al. Current concepts in mild cognitive impairment. Arch Neurol. 2001 Dec;58(12):1985–92.

16. Winblad B, Palmer K, Kivipelto M, Jelic V, Fratiglioni L, Wahlund L-O, et al. Mild cognitive impairment--beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. J Intern Med. 2004 Sep;256(3):240–6.

17. Petersen RC. Mild Cognitive Impairment. Continuum (Minneap Minn). 2016 Apr;22(2 Dementia):404–18.

18. Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011 May;7(3):270–9.

19. Petersen RC. Mild cognitive impairment as a diagnostic entity. J Intern Med. 2004 Sep;256(3):183–94.

20. Espinosa A, Alegret M, Valero S, Vinyes-Junqué G, Hernández I, Mauleón A, et al. A longitudinal follow-up of 550 mild cognitive impairment patients:

evidence for large conversion to dementia rates and detection of major risk factors involved. J Alzheimers Dis. 2013;34(3):769–80.

21. Steiner ABQ, Jacinto AF, Mayoral VF de S, Brucki SMD, Citero V de A. Mild cognitive impairment and progression to dementia of Alzheimer's disease. Rev Assoc Med Bras (1992). 2017 Jul;63(7):651–5.

22. Boustani M, Callahan CM, Unverzagt FW, Austrom MG, Perkins AJ, Fultz BA, et al. Implementing a screening and diagnosis program for dementia in primary care. J Gen Intern Med. 2005 Jul;20(7):572–7.

23. Ross GW, Abbott RD, Petrovitch H, Masaki KH, Murdaugh C, Trockman C, et al. Frequency and characteristics of silent dementia among elderly Japanese-American men. The Honolulu-Asia Aging Study. JAMA. 1997 Mar 12;277(10):800–5.

24. Simó R, Ciudin A, Simó-Servat O, Hernández C. Cognitive impairment and dementia: a new emerging complication of type 2 diabetes-The diabetologist's perspective. Acta Diabetol. 2017 May;54(5):417–24.

25. Sheen Y-J, Sheu WHH. Association between hypoglycemia and dementia in patients with type 2 diabetes. Diabetes Res Clin Pract. 2016 Jun;116:279–87.

26. Whitmer RA, Karter AJ, Yaffe K, Quesenberry CP, Selby JV. Hypoglycemic episodes and risk of dementia in older patients with type 2 diabetes mellitus. JAMA. 2009 Apr 15;301(15):1565–72.

27. Marseglia A, Fratiglioni L, Laukka EJ, Santoni G, Pedersen NL, Bäckman L, et al. Early Cognitive Deficits in Type 2 Diabetes: A Population-Based Study. J Alzheimers Dis. 2016 Jun 15;53(3):1069–78.

28. Tripathi M, Vibha D. Reversible dementias. Indian J Psychiatry. 2009 Jan;51 Suppl 1:S52-55.

29. American Diabetes Association. 12. Older Adults: Standards of Medical Care in Diabetes-2021. Diabetes Care. 2021 Jan;44(Suppl 1):S168–79.

30. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975 Nov;12(3):189–98.

31. Wojtyńska R, Szcześniak D. DemTect®--effective to asses MCI and dementia--validation study of the Polish language version. Aging Ment Health. 2016;20(5):510–6.

32. Sanford AM. Mild Cognitive Impairment. Clin Geriatr Med. 2017 Aug;33(3):325–37.

33. Ciesielska N, Sokołowski R, Mazur E, Podhorecka M, Polak-Szabela A, Kędziora-Kornatowska K. Is the Montreal Cognitive Assessment (MoCA) test better suited than the Mini-Mental State Examination (MMSE) in mild cognitive impairment (MCI) detection among people aged over 60? Meta-analysis. Psychiatr Pol. 2016 Oct 31;50(5):1039–52.

34. Milne A, Culverwell A, Guss R, Tuppen J, Whelton R. Screening for dementia in primary care: a review of the use, efficacy and quality of measures. Int Psychogeriatr. 2008 Oct;20(5):911–26.

35. Davey RJ, Jamieson S. The validity of using the mini mental state examination in NICE dementia guidelines. J Neurol Neurosurg Psychiatry. 2004 Feb;75(2):343–4.

36. Mitchell AJ. A meta-analysis of the accuracy of the mini-mental state examination in the detection of dementia and mild cognitive impairment. J Psychiatr Res. 2009 Jan;43(4):411–31.

37. Petersen RC, Stevens JC, Ganguli M, Tangalos EG, Cummings JL, DeKosky ST. Practice parameter: early detection of dementia: mild cognitive impairment (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology. 2001 May 8;56(9):1133–42.

38. Butler SM, Ashford JW, Snowdon DA. Age, education, and changes in the Mini-Mental State Exam scores of older women: findings from the Nun Study. J Am Geriatr Soc. 1996 Jun;44(6):675–81.

39. Espino DV, Lichtenstein MJ, Palmer RF, Hazuda HP. Ethnic differences in mini-mental state examination (MMSE) scores: where you live makes a difference. J Am Geriatr Soc. 2001 May;49(5):538–48.

40. Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc. 2005 Apr;53(4):695–9.

41. Magierska J, Magierski R, Fendler W, Kłoszewska I, Sobów TM. Clinical application of the Polish adaptation of the Montreal Cognitive Assessment (MoCA) test in screening for cognitive impairment. Neurol Neurochir Pol. 2012 Apr;46(2):130–9.

42. Carrillo MC, Brashear HR, Logovinsky V, Ryan JM, Feldman HH, Siemers ER, et al. Can we prevent Alzheimer's disease? Secondary "prevention" trials in Alzheimer's disease. Alzheimers Dement. 2013 Mar;9(2):123-131.e1.

43. Dehnel T. The European Dementia Prevention Initiative. Lancet Neurol. 2013 Mar;12(3):227–8.

44. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation. 1998 May 12;97(18):1837–47.

45. Gage BF, van Walraven C, Pearce L, Hart RG, Koudstaal PJ, Boode BSP, et al. Selecting patients with atrial fibrillation for anticoagulation: stroke risk stratification in patients taking aspirin. Circulation. 2004 Oct 19;110(16):2287–92.

46. Barnes DE, Covinsky KE, Whitmer RA, Kuller LH, Lopez OL, Yaffe K. Predicting risk of dementia in older adults: The late-life dementia risk index. Neurology. 2009 Jul 21;73(3):173–9.

47. Jessen F, Wiese B, Bickel H, Eiffländer-Gorfer S, Fuchs A, Kaduszkiewicz H, et al. Prediction of dementia in primary care patients. PLoS One. 2011 Feb 18;6(2):e16852.

48. Reitz C, Tang M-X, Schupf N, Manly JJ, Mayeux R, Luchsinger JA. A summary risk score for the prediction of Alzheimer disease in elderly persons. Arch Neurol. 2010 Jul;67(7):835–41.

49. Kivipelto M, Ngandu T, Laatikainen T, Winblad B, Soininen H, Tuomilehto J. Risk score for the prediction of dementia risk in 20 years among middle aged

people: a longitudinal, population-based study. Lancet Neurol. 2006 Sep;5(9):735–41.

50. Exalto LG, Biessels GJ, Karter AJ, Huang ES, Katon WJ, Minkoff JR, et al. Risk score for prediction of 10 year dementia risk in individuals with type 2 diabetes: a cohort study. Lancet Diabetes Endocrinol. 2013 Nov;1(3):183–90.

51. Cook NR. Statistical evaluation of prognostic versus diagnostic models: beyond the ROC curve. Clin Chem. 2008 Jan;54(1):17–23.

52. Deckers K, Schievink SHJ, Rodriquez MMF, van Oostenbrugge RJ, van Boxtel MPJ, Verhey FRJ, et al. Coronary heart disease and risk for cognitive impairment or dementia: Systematic review and meta-analysis. PLoS One. 2017;12(9):e0184244.

53. Norton S, Matthews FE, Barnes DE, Yaffe K, Brayne C. Potential for primary prevention of Alzheimer's disease: an analysis of population-based data. Lancet Neurol. 2014 Aug;13(8):788–94.

54. Plassman BL, Williams JW, Burke JR, Holsinger T, Benjamin S. Systematic review: factors associated with risk for and possible prevention of cognitive decline in later life. Ann Intern Med. 2010 Aug 3;153(3):182–93.

55. Deckers K, van Boxtel MPJ, Schiepers OJG, de Vugt M, Muñoz Sánchez JL, Anstey KJ, et al. Target risk factors for dementia prevention: a systematic review and Delphi consensus study on the evidence from observational studies. Int J Geriatr Psychiatry. 2015 Mar;30(3):234–46.

56. Chou C-H, Feng I-J, Chen Y-C, Chen J-H, Lin H-J, Wang J-J, et al. Risk of Dementia in Diabetic Patients with Hyperglycemic Crisis: A Nationwide Taiwanese Population-Based Cohort Study. Neuroepidemiology. 2020;54(5):419–26.

57. London A, Benhar I, Schwartz M. The retina as a window to the brain-from eye research to CNS disorders. Nat Rev Neurol. 2013 Jan;9(1):44–53.

58. Yau JWY, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, et al. Global prevalence and major risk factors of diabetic retinopathy. Diabetes Care. 2012 Mar;35(3):556–64.

59. Simó R, Stitt AW, Gardner TW. Neurodegeneration in diabetic retinopathy: does it really matter? Diabetologia. 2018 Sep;61(9):1902–12.

60. Launer LJ, Miller ME, Williamson JD, Lazar RM, Gerstein HC, Murray AM, et al. Effects of intensive glucose lowering on brain structure and function in people with type 2 diabetes (ACCORD MIND): a randomised open-label substudy. Lancet Neurol. 2011 Nov;10(11):969–77.

61. Exalto LG, Biessels GJ, Karter AJ, Huang ES, Quesenberry CP, Whitmer RA. Severe diabetic retinal disease and dementia risk in type 2 diabetes. J Alzheimers Dis. 2014;42 Suppl 3:S109-117.

62. Simó R, Hernández C. Novel approaches for treating diabetic retinopathy based on recent pathogenic evidence. Prog Retin Eye Res. 2015 Sep;48:160–80.

63. Cheung CY-L, Ikram MK, Chen C, Wong TY. Imaging retina to study dementia and stroke. Prog Retin Eye Res. 2017 Mar;57:89–107.

64. Stitt AW, Curtis TM, Chen M, Medina RJ, McKay GJ, Jenkins A, et al. The progress in understanding and treatment of diabetic retinopathy. Prog Retin Eye Res. 2016 Mar;51:156–86.

65. Solomon SD, Chew E, Duh EJ, Sobrin L, Sun JK, VanderBeek BL, et al. Diabetic Retinopathy: A Position Statement by the American Diabetes Association. Diabetes Care. 2017 Mar;40(3):412–8.

66. Little K, Llorián-Salvador M, Scullion S, Hernández C, Simó-Servat O, Del Marco A, et al. Common pathways in dementia and diabetic retinopathy: understanding the mechanisms of diabetes-related cognitive decline. Trends Endocrinol Metab. 2022 Jan;33(1):50–71.

67. Liao H, Zhu Z, Peng Y. Potential Utility of Retinal Imaging for Alzheimer's Disease: A Review. Front Aging Neurosci. 2018;10:188.

68. Acton JH, Greenstein VC. Fundus-driven perimetry (microperimetry) compared to conventional static automated perimetry: similarities, differences, and clinical applications. Can J Ophthalmol. 2013 Oct;48(5):358–63.

69. Rohrschneider K, Bültmann S, Springer C. Use of fundus perimetry (microperimetry) to quantify macular sensitivity. Prog Retin Eye Res. 2008 Sep;27(5):536–48.

70. Wu Z, Ayton LN, Guymer RH, Luu CD. Comparison between multifocal electroretinography and microperimetry in age-related macular degeneration. Invest Ophthalmol Vis Sci. 2014 Aug 26;55(10):6431–9.

71. Simó-Servat O, Ciudin A, Ortiz-Zúñiga ÁM, Hernández C, Simó R. Usefulness of Eye Fixation Assessment for Identifying Type 2 Diabetic Subjects at Risk of Dementia. J Clin Med. 2019 Jan 8;8(1):E59.

72. Bergeron A, Guitton D. Fixation neurons in the superior colliculus encode distance between current and desired gaze positions. Nat Neurosci. 2000 Sep;3(9):932–9.

73. Ortiz-Zúñiga ÁM, Simó-Servat O, Rojano-Toimil A, Vázquez-de Sebastian J, Castellano-Tejedor C, Hernández C, et al. The Gaze Fixation Assessed by Microperimetry: A Useful Tool for the Monitoring of the Cognitive Function in Patients with Type 2 Diabetes. J Pers Med. 2021 Jul 22;11(8):698.

74. Randolph C, Tierney MC, Mohr E, Chase TN. The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS): preliminary clinical validity. J Clin Exp Neuropsychol. 1998 Jun;20(3):310–9.

75. Duff K, Patton D, Schoenberg MR, Mold J, Scott JG, Adams RL. Age- and education-corrected independent normative data for the RBANS in a community dwelling elderly sample. Clin Neuropsychol. 2003 Aug;17(3):351–66.

76. Duff K, Ramezani A. Regression-Based Normative Formulae for the Repeatable Battery for the Assessment of Neuropsychological Status for Older Adults. Arch Clin Neuropsychol. 2015 Nov;30(7):600–4.

77. Hammers DB, Suhrie KR, Porter SM, Dixon AM, Duff K. Validation of one-year reliable change in the RBANS for community-dwelling older adults with amnestic mild cognitive impairment. Clin Neuropsychol. 2020 Aug 20;1–24.

78. Rodríguez-Gómez O, Rodrigo A, Iradier F, Santos-Santos MA, Hundemer H, Ciudin A, et al. The MOPEAD project: Advancing patient engagement for the

detection of “hidden” undiagnosed cases of Alzheimer’s disease in the community. Alzheimers Dement. 2019 Jun;15(6):828–39.

79. Jack CR, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer’s disease. Alzheimers Dement. 2018 Apr;14(4):535–62.

80. Shura RD, Brearly TW, Rowland JA, Martindale SL, Miskey HM, Duff K. RBANS Validity Indices: a Systematic Review and Meta-Analysis. Neuropsychol Rev. 2018 Sep;28(3):269–84.

81. Rovner BW, Folstein MF. Mini-mental state exam in clinical practice. Hosp Pract (Off Ed). 1987 Jan 30;22(1A):99, 103, 106, 110.

82. Parvizi J, Van Hoesen GW, Damasio A. The selective vulnerability of brainstem nuclei to Alzheimer’s disease. Ann Neurol. 2001 Jan;49(1):53–66.

83. Klemp K, Larsen M, Sander B, Vaag A, Brockhoff PB, Lund-Andersen H. Effect of short-term hyperglycemia on multifocal electroretinogram in diabetic patients without retinopathy. Invest Ophthalmol Vis Sci. 2004 Oct;45(10):3812–9.

84. Ciudin A, Simó-Servat O, Hernández C, Arcos G, Diego S, Sanabria Á, et al. Retinal Microperimetry: A New Tool for Identifying Patients With Type 2 Diabetes at Risk for Developing Alzheimer Disease. Diabetes. 2017 Dec;66(12):3098–104.

85. Aminoff MJ, Goodin DS. Visual evoked potentials. J Clin Neurophysiol. 1994 Sep;11(5):493–9.

86. Bruce DG, Davis WA, Casey GP, Starkstein SE, Clarnette RM, Almeida OP, et al. Predictors of cognitive decline in older individuals with diabetes. Diabetes Care. 2008 Nov;31(11):2103–7.

87. Irie F, Fitzpatrick AL, Lopez OL, Kuller LH, Peila R, Newman AB, et al. Enhanced risk for Alzheimer disease in persons with type 2 diabetes and APOE epsilon4: the Cardiovascular Health Study Cognition Study. Arch Neurol. 2008 Jan;65(1):89–93.

88. Gao Y, Xiao Y, Miao R, Zhao J, Cui M, Huang G, et al. The prevalence of mild cognitive impairment with type 2 diabetes mellitus among elderly people in China: A cross-sectional study. Arch Gerontol Geriatr. 2016 Feb;62:138–42.

89. Albai O, Frandes M, Timar R, Roman D, Timar B. Risk factors for developing dementia in type 2 diabetes mellitus patients with mild cognitive impairment. Neuropsychiatr Dis Treat. 2019;15:167–75.

90. Koekkoek PS, Janssen J, Kooistra M, Biesbroek JM, Groeneveld O, van den Berg E, et al. Case-finding for cognitive impairment among people with Type 2 diabetes in primary care using the Test Your Memory and Self-Administered Gerocognitive Examination questionnaires: the Cog-ID study. Diabet Med. 2016 Jun;33(6):812–9.

91. Erskine D, Taylor JP, Firbank MJ, Patterson L, Onofrj M, O'Brien JT, et al. Changes to the lateral geniculate nucleus in Alzheimer's disease but not dementia with Lewy bodies. Neuropathol Appl Neurobiol. 2016 Jun;42(4):366–76.

92. Ciudin A, Ortiz AM, Fidilio E, Romero D, Sánchez M, Comas M, et al. Retinal Microperimetry: A Useful Tool for Detecting Insulin Resistance-Related Cognitive Impairment in Morbid Obesity. J Clin Med. 2019 Dec 11;8(12):E2181.

93. Dugger BN, Tu M, Murray ME, Dickson DW. Disease specificity and pathologic progression of tau pathology in brainstem nuclei of Alzheimer's disease and progressive supranuclear palsy. Neurosci Lett. 2011 Mar 17;491(2):122–6.

94. Bartolomeo P, Thiebaut de Schotten M, Chica AB. Brain networks of visuospatial attention and their disruption in visual neglect. Front Hum Neurosci. 2012;6:110.

95. Sandrone S, Catani M. Journal Club. Default-mode network connectivity in cognitively unimpaired patients with Parkinson disease. Neurology. 2013 Dec 3;81(23):e172-175.

96. Nicolau J, Simó R, Sanchís P, Ayala L, Fortuny R, Rivera R, et al. Prevalence and Clinical Correlators of Undiagnosed Significant Depressive

Symptoms Among Individuals with Type 2 Diabetes In A Mediterranean Population. Exp Clin Endocrinol Diabetes. 2016 Nov;124(10):630–6.

9. Annexes

10.1. LIST OF PUBLICATIONS RELATED TO THIS DOCTORAL THESIS:

10.1.1 Clinical Applicability of the Specific Risk Score of Dementia in Type 2 Diabetes in the Identification of Patients with Early Cognitive Impairment: Results of the MOPEAD Study in Spain.

Ortiz Zuñiga AM, Simó R, Rodriguez-Gómez O, Hernández C, Rodrigo A, Jamilis L, Campo L, Alegret M, Boada M, Ciudin A. **Clinical Applicability of the Specific Risk Score of Dementia in Type 2 Diabetes in the Identification of Patients with Early Cognitive Impairment: Results of the MOPEAD Study in Spain.** J Clin Med. 2020 Aug 24;9(9):2726. doi: 10.3390/jcm9092726. PMID: 32847012; PMCID: PMC7565958.

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10.1.2 Usefulness of Eye Fixation Assessment for Identifying Type 2 Diabetic Subjects at Risk of Dementia.

Simó-Servat O, Ciudin A, Ortiz-Zúñiga ÁM, Hernández C, Simó R. **Usefulness of Eye Fixation Assessment for Identifying Type 2 Diabetic Subjects at Risk of Dementia**. J Clin Med. 2019 Jan 8;8(1):59. doi: 10.3390/jcm8010059. PMID: 30626106; PMCID: PMC6352169.

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doi: 10.3390/jcm8010059

10.1.3 A Useful Tool for Detecting Insulin Resistance-Related Cognitive Impairment in Morbid Obesity.

Ciudin A, Ortiz AM, Fidilio E, Romero D, Sánchez M, Comas M, Gonzalez O, Vilallonga R, Simó-Servat O, Hernández C, Simó R. **Retinal Microperimetry: A Useful Tool for Detecting Insulin Resistance-Related Cognitive Impairment in Morbid Obesity.** J Clin Med. 2019 Dec 11;8(12):2181. doi: 10.3390/jcm8122181. PMID: 31835729; PMCID: PMC6947364.

doi: 10.3390/jcm8122181.

doi: 10.3390/jcm8122181.

doi: 10.3390/jcm8122181.

doi: 10.3390/jcm8122181.

doi: 10.3390/jcm8122181.

doi: 10.3390/jcm8122181.

doi: 10.3390/jcm8122181.

doi: 10.3390/jcm8122181.

doi: 10.3390/jcm8122181.

doi: 10.3390/jcm8122181.

doi: 10.3390/jcm8122181.

10.1.4 The Gaze Fixation Assessed by Microperimetry: A Useful Tool for the Monitoring of the Cognitive Function in Patients with Type 2 Diabetes.

Ortiz-Zúñiga ÁM, Simó-Servat O, Rojano-Toimil A, Vázquez-de Sebastian J, Castellano-Tejedor C, Hernández C, Simó R, Ciudin A. **The Gaze Fixation Assessed by Microperimetry: A Useful Tool for the Monitoring of the Cognitive Function in Patients with Type 2 Diabetes.** J Pers Med. 2021 Jul 22;11(8):698. doi: 10.3390/jpm11080698. PMID: 34442342; PMCID: PMC8398405.

doi: 10.3390/jpm11080698.

doi: 10.3390/jpm11080698.

doi: 10.3390/jpm11080698.

doi: 10.3390/jpm11080698.

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10.2. PATIENTS REFERRED TO MEMORY CLINIC.

Table S1. Patients referred to the Memory Clinic

Criteria for referral to Memory Clinic, as per MOPEAD Protocol (as explained in the text): MMSE score ≤ 27 or DSDRS≥ 7 and positive answer for ≥ 2 of the initial questions or DSDRS ≥ 10

	YES (N=82)	NO (N=30)
MMSE≤27 + DSDRS<7	16	0
MMSE>27+DSDRS≥7	32	0
MMSE≤27+DSDRS≥7	34	0
MMSE>27+DSDRS<7	0	30
Total	82	30

Table S2. Confirmed cognitive impairment at the memory clinic: 39 patients attended of the 82 that were referred

	Cognitive impairment (37)	Normocognitive (2)
MMSE≤27 + DSDRS<7	4	1
MMSE>27+DSDRS≥7	14	1
MMSE≤27+DSDRS≥7	19	0
MMSE>27+DSDRS<7	0	0
Total	34	2