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The Pupil Diameter as a Possible Indicator of Neurodegenerative Diseases and Response to Acetylcholinesterase Inhibitors Therapy: In-Depth Measurements Following Topical Administration of Tropicamide and Pilocarpine

Raffaele Nuzzi, MD[1], Alessandro Bojino, MD[2], Maria Sole Polito, MD[1], Chiara Luppi, MD[3], Federico Tridico, MD[1], and Massimiliano Massaia, MD, PhD[3]

Abstract

Background: The aim of this study is to assess whether pupillary modifications following ocular anticholinergic and cholinergic drugs can identify subjects with neurodegenerative diseases from early stages. **Methods:** 51 subjects were divided into 3 groups, according to different neurodegenerative diseases, and compared with a control group of 10 patients. Pupil diameter has been measured at different times after topical administration of tropicamide 0.01% in the right eye. Then, topical administration of pilocarpine 0.06% has been performed, followed by pupillary constriction measurement. Pupillary response rates were stratified according to acetylcholinesterase inhibitors intake. **Results:** Observed mydriasis and pupillary constriction was similar in all study groups at all evaluation times. Patients without acetylcholinesterase inhibitors intake presented greater mydriasis. **Conclusions:** Although it was not possible to observe significant differences among groups in terms of pupillary response, the analysis of pupillary features may become an useful tool to detect efficacy of acetylcholinesterase inhibitors.

Keywords

Alzheimer's disease, mydriatic agents, acetylcholinesterase inhibitors, vascular dementia

Background

Reduction of cholinergic activity in the central nervous system correlates with the severity of Alzheimer's disease (AD). Pilot studies, conducted by Shinto et al in 1994, proposed a new method for AD diagnosis. In these studies, it is stated that patients with Alzheimer's disease presented an exaggerated pupillary response to highly-diluted solution of tropicamide,[1] a M4 receptor antagonist that competes with acetylcholine. This hypersensitivity has been attributed to a neuronal loss in the Edinger-Westphal nucleus (parasympathetic oculomotor nucleus) which seems to be a selective target in the early stages of the disease.[2-6] However, more recent studies questioned this hypersensitivity as a diagnostic test[7-11] showing a potential involvement of other factors, mainly related to individual variability of pupillary behavior. Even so, pupillary light reflexes (PLR) have been recently taken into consideration as potential markers of AD.[12-15] The purpose of this study is to evaluate whether the analysis of pupillary reflexes (both direct and consensual) and pupillary diameter, following topical ocular administration of highly-diluted solutions of ocular anticholinergic (tropicamide 0.01%) and cholinergic agents (pilocarpine 0.06%), can accurately identify subjects with neurodegenerative diseases from early stages (mild cognitive impairment), when the risk for AD is increased.[12]

[1] S.C.U. Ophthalmology—A.O.U. Città della Salute e della Scienza, Turin, Italy
[2] Department of Surgical Sciences, University of Turin, Turin, Italy
[3] Alzheimer Evaluation Unit, S.C.U. Geriatrics and Bone-Metabolic Disease—A.O.U. Città della Salute e della Scienza, Turin, Italy

Corresponding Author:
Raffaele Nuzzi, SCU. Ophthalmology—A.O.U. Città della Salute e della Scienza, Via Cherasco 23, 10126, Turin, Italy.
Email: prof.nuzzi_raffaele@hotmail.it

Methods

51 subjects evaluated at the Alzheimer Assessment Unit (AAU) of our institution (University Clinic "City of Health and Science of Turin") have been enrolled in this study. The age of the study population ranged between 60 and 84 years. The subjects under evaluation were divided into 4 groups:

1. 17 patients diagnosed with mild cognitive impairment due to primary degenerative onset (7 men, 10 women, mean age [± SEM], 73.12 ± 6.18; Age range, 60-81; Mini Mental State Score 25.5 ± 3.2) according to the National Institute on Aging-Alzheimer's Association criteria of 2011 (NIA-AA).
2. 11 patients diagnosed with AD (7 men, 4 women, mean age [± SEM], 73.7 ± 5.0, age range, 67-82, Mini Mental State Score 18.9 ± 4.7) according to the National Institute on Aging -Alzheimer's Association criteria of 2011 (NIA-AA).
3. 13 patients diagnosed with vascular dementia (7 men, 5 women, mean age [± SEM], 79.5 ± 6.0, age range, 67-84, Mini Mental State Score 22.1 ± 3.3) according to the NINDS AIREN criteria.
4. 10 unaffected subjects as control group. This group featured subjects enrolled among the patients' caregivers. (4 men, 6 women, mean age [± SEM], 72.8 ± 5.6; Age range, 61-80;)

Exclusion criteria included concomitant ocular treatments that may influence pupillary response, concurrent ocular diseases (pupillary alterations, iris or corneal abnormalities, glaucoma, previous iridectomy, ischemic optic neuropathy, occlusion of retinal veins or central artery), third cranial nerve paralysis, multiple sclerosis, autonomic nervous system diseases, anisocoria of more than 0.4 mm, autonomic diabetic neuropathy, daily alcohol intake of more than 3 alcoholic units for men and 2 alcohol units for women.

For horizontal pupil diameter assessment, a photographic method has been applied through the use of lensless spectacles featuring a millimetric scale paper, as a dimensional reference. Photographic acquisition was carried out with a Nikon D5100 SLR camera, with an 18-55 mm objective. All acquired photos were then analyzed for dimensional assessment with a graphic software (Adobe Illustrator, Adobe Systems Inc., United States).

During the image acquisition process subjects were advised not to look into the camera lens. Instead, they were asked to stare at a remote point placed in front of them, in order to avoid miosis due to accommodating convergence (which occurs typically during near objects fixation), since it could potentially interfere with all measurements. Afterward, various PLR (ipsilateral and contralateral, direct and consensual) were assessed with repeated light stimulations (3 for each eye) carried out with a white light flashlight featuring a fixed luminous flux of 3 lumens, for every enrolled participant.

After the pupil diameter evaluation at baseline, a single drop of a highly-diluted tropicamide solution (0.01%) was instilled into the conjunctival sac of the right eye, while a drop of sterile water was administered into the left eye. Then, pupillary diameter was evaluated at 10, 20, 30 and 40 minutes after the drug administration. At the 40th minute, a single drop of pilocarpine 0.06% was instilled into the conjunctival sac of the right eye to evaluate miosis recovery. Pupillary diameter in constriction was then evaluated at 50, 60 and 70 minutes.

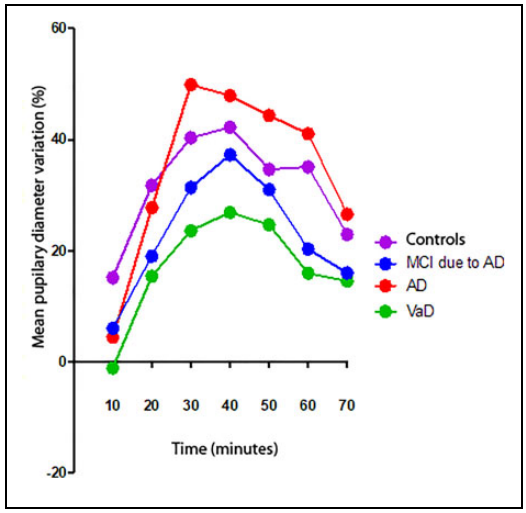

Figure 1. Temporal progression of pupillary data in the 4 study groups. Changes from baseline in different times are expressed in percentages.

Statistical data analysis was performed with one-way ANOVA, Mann-Whitney test, mixed effects logistic regression model and Spearman rank correlation test. Statistical significance was defined at a p-value <0.05.

Results

At baseline evaluation, no significant differences were found between the groups under study (control group: 2.30 mm ± 0.20; MCI due to AD: 2.89 mm ± 0.85; AD: 2.68 mm ± 0.51; VaD: 2.89 mm ± 0.74). Similarly, analysis of the pupillary constriction width (PCW), expressed as the difference in mm between the basal pupil diameter and the diameter following the exposure to the light stimulus, did not show statistically significant results, with overlapping data among the study groups for all types of reflexes considered (ipsilateral/contralateral, direct/consensual reflex). The pupillary diameter variation in the right eye was evaluated in terms of differences (in mm and in percentage) from the baseline diameter and the contralateral pupil (Figures 1 and 2).

Overall, the maximal pupillary dilation excursion achieved after application of tropicamide 0.01% was 47.02% (±18.43) in the control group, 37.29% (±29.97) in MCI due to AD patients, 54.39% (±30.15) in AD group and 30.23% (±18.68) in patients diagnosed with VaD. However, the difference among groups was not statistically significant (*P value: 0.061*). However, the comparison between controls and the VaD group showed a statistically significant difference (*P value*: <0.05). The time required to reach the peak of mydriasis is variable from

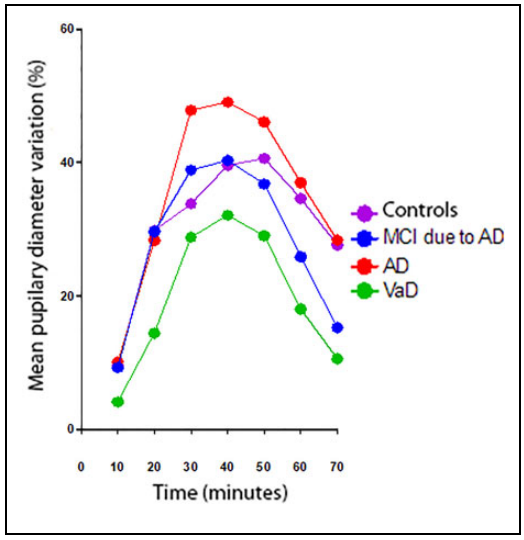

Figure 2. Temporal progression of pupillary data in the 4 study groups. The degree of anisocoria in different times is expressed in percentages.

one individual to another, but usually it's been observed at the thirtieth minute after mydriatic instillation. As shown in Figure 3, the value of pupillary diameter at baseline is inversely related to the maximal dilation width obtained after mydriatic instillation (r = -0 384, p = 0.0059). This phenomenon occurs in all study groups, showing an overlapping behavior.

Following the application of low-dose pilocarpine 0.06%, the maximal pupillary constriction width achieved, in relation to the pupillary diameter measured at the 40th minute, was 14.12% (±4.10) in the control group, 18.50% (±5.14) in the MCI group, 16.86% (±0.38) in the AD group and 15.05% (±1.38) in patients with the diagnosis of VaD, with no statistically significant differences (*P value: 0.7624*). The time required to reach the miosis peak was predominantly 30 minutes, 20 minutes in few cases. As shown in Figure 4, the value of pupillary diameter at the 40th minute is directly proportional to the maximal pupillary constriction obtained after application of the miotic drug ($r = 0.3714$, $p = 0.0079$) and this behavior is observed in every group under study.

Affected patients were then divided according to the presence of acetylcholinesterase inhibitors intake (donepezil, rivastigmine or galantamine, ACheI) (Figure 5). All data were analyzed through Mann-Whitney Test. Patients without AChel therapy presented a greater mydriasis than those under treatment with AChel (Figure 6). This trend occurs at all observation times and becomes statistically significant at the 20th and 30th minute (Table 1).

Discussion

In our study there is a high individual variability in pupillary response, especially in affected patients, but also in the control group (a trend that probably reflects physiological differences

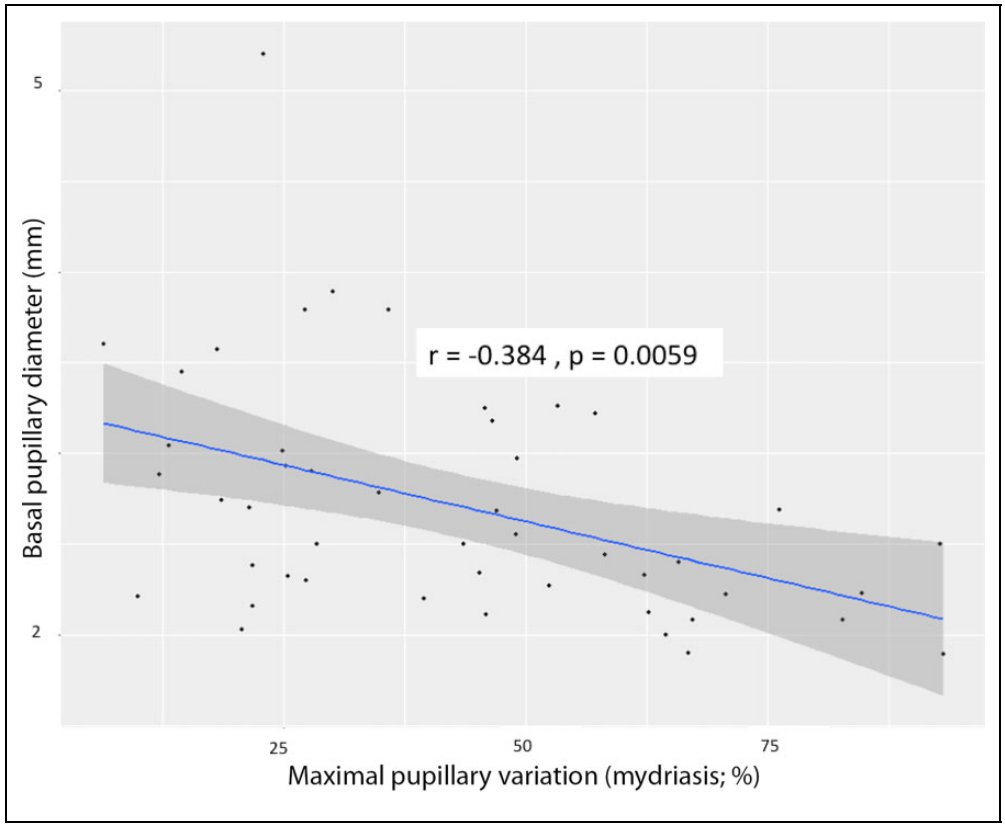

Figure 3. Correlation between basal pupillary diameter (mm) and maximal pupillary dilation (%).

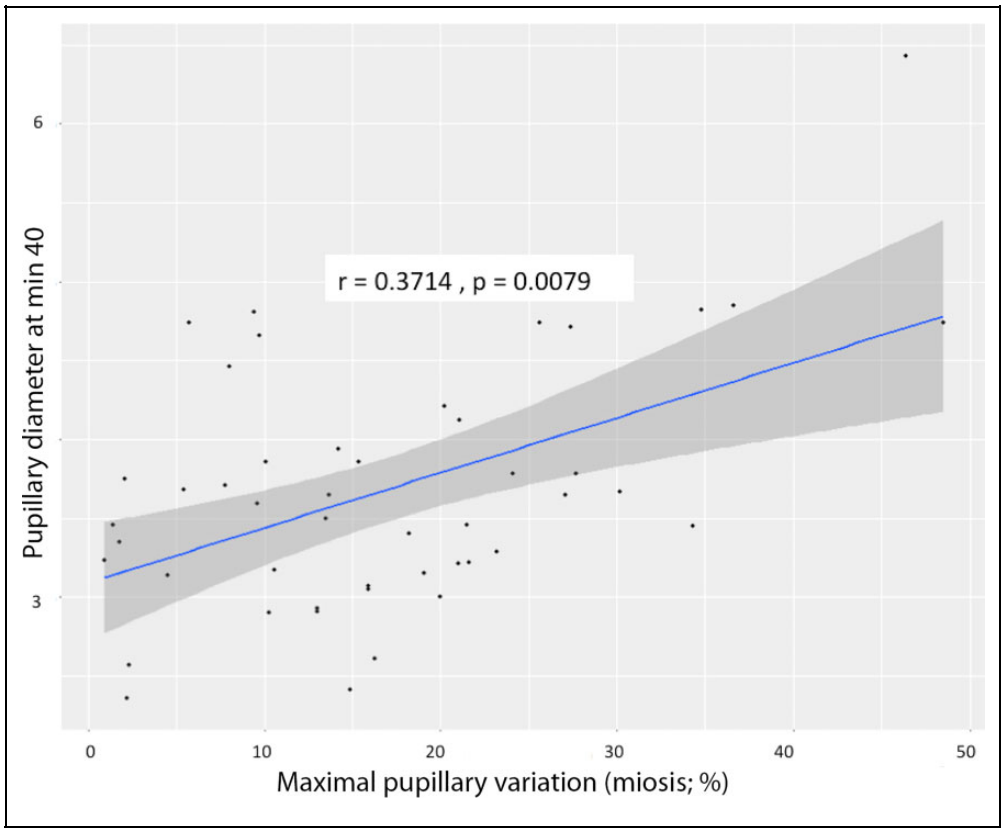

Figure 4. Correlation between pupil diameter at minute 40 (mm) and maximal pupillary constriction (%).

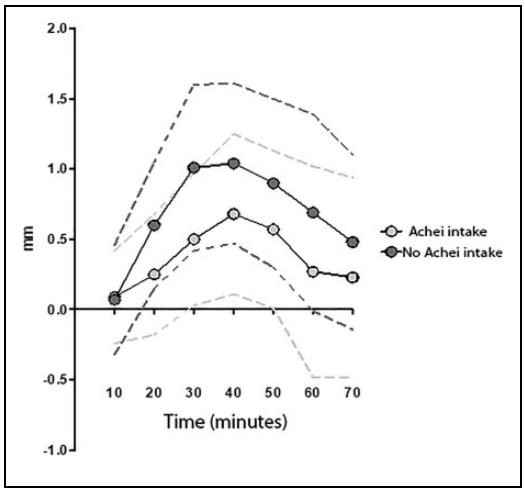

Figure 5. The graph shows the pupillary response to cholinergic agents with respect to baseline, in patients according to AChel therapy (Mean ± SD).

between individuals). For this reason, given the small sample size of each group, differences in pupillary response did not reach statistical significance, while variations in mean pupillary diameter appeared greater in the control group when compared with MCI patients. Even if differences of pupillary dilation slightly failed to reach statistically significance ($p = 0.061$), our analysis suggests that significant differences may be observed with larger samples. Our study, in agreement with E. Ferrario et al.,[11] outlined a statistically significant inverse correlation between the pupillary diameter at baseline and maximal pupillary dilatation after tropicamide administration ($r = -0.384$, $p = 0.0059$). This correlation is maintained in every group under study, revealing that results can be influenced more by differences in basal pupillary diameter and to a lesser extent by sensitivity to cholinergic agents. Similarly, the relation between the pupil diameter measured at the 40th minute and maximal pupillary constriction achieved with the instillation of low-dose pilocarpine showed a significant correlation between the 2 variables ($r = 0.3714$, $p = 0.0079$). Therefore, the smaller the diameter at baseline the greater the observed action of the drug. This behavior shows how the observed effect is influenced more by pupillary diameter before drug administration (in this case the diameter at the 40th minute), rather than pharmacological response. This might happen because narrow pupil diameters at baseline allow more "room" for pupil dilation after mydriatic drugs, followed by greater pupillary constriction when recovering to the initial state.[11]

Cholinergic neuronal loss occurring in Alzheimer's disease has been associated with memory loss and reduced learning ability. The progressive loss of cholinergic neurons leads to reduced acetylcholine availability with following deterioration of cognitive functions, worsening behavioral disorders and daily life activities. In this setting, acetylcholinesterase inhibitors have been recognized in the symptomatic treatment of

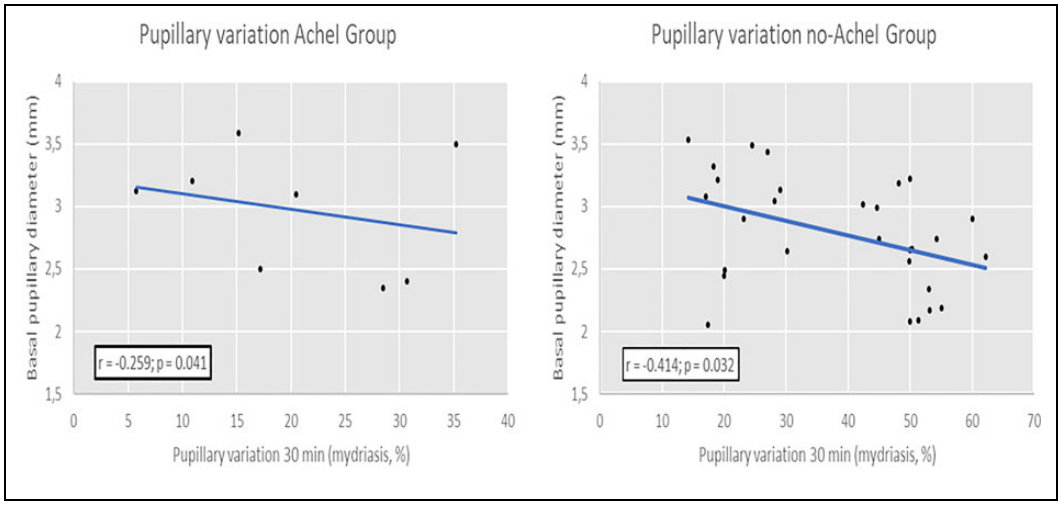

Figure 6. Correlation between basal pupil diameter (mm) and pupillary dilation at 30 minutes following the use of tropicamide (%), in patients taking Achel drugs and patients without Achel therapy.

Table 1. Variation of the Right Pupil Diameter From Baseline in Patients at Each Time Interval in Which the Measurement (Mean ± SD in mm and %) Was Carried out.

	AChel therapy		
Min	Yes (n = 8)	No (n = 32)	*p-values*
10	0.09 ± 0.33 mm	0.07 ± 0.38 mm	*0. 8488*
	3.84 ± 11.17%	3.17 ± 13.1%	*0.9868*
20	0.25 ± 0.43 mm	0.55 ± 0.46 mm	*0.0382*
	11.79 ± 22.44%	21.36 ± 18.26%	*0.0353*
30	0.50 ± 0.47 mm	0.96 ± 0.58 mm	*0.0383*
	20.5 ± 24.74%	36.73 ± 23.45%	*0.0298*
40	0.68 ± 0.57 mm	0.98 ± 0.58 mm	*0.1509*
	26.98 ± 29.24%	37.99 ± 23.77%	*0.1743*
50	0.57 ± 0.56 mm	0.84 ± 0.61 mm	*0.2801*
	23.03 ± 27.28%	33.41 ± 24.55%	*0.2465*
60	0.27 ± 0.75 mm	0.65 ± 0.68 mm	*0.0708*
	14.49 ± 37.93%	26.59 ± 25.43%	*0.0806*
70	0.23 ± 0.71 mm	0.45 ± 0.60 mm	*0.3290*
	11.11 ± 28.51%	19.56 ± 22.38%	*0.2777*

Patients are divided into 2 categories according to whether or not they are treated with anticholinesterases (donepezil, rivastigmine or galantamine). At time 0 the diluted tropicamide solution (0.01%) is administered while at the 40th minute the low-dose pilocarpine (0.06%).

Alzheimer's disease, with donepezil, rivastigmine and galantamine as the main AChel currently in commerce in Europe. These drugs increase the availability of acetylcholine at the synaptic level by inhibiting the enzyme responsible for acetylcholine degradation. A greater acetylcholine amount at the synaptic level would induce a lower net effect of tropicamide with reduced mydriasis. In our study, this is observed at all times in which pupillometry was performed and assumes a statistical significance at 30 minutes after topical administration of highly-diluted solution of tropicamide, if compared to baseline values. Patients with no AChel intake showed greater mydriasis than regularly treated patients. However, the individual pupillary response variability may prevent the identification of changes induced by pharmacological therapy in the groups evaluated in this study. Moreover, given the fact that these results depend on a post-hoc analysis, the two compared groups feature unbalanced sample sizes, with presumably quite heterogeneous cholinergic system states between individuals.

Even so, in the light of these findings, it is possible to open new perspectives of clinical application aimed at evaluating the pupil response as an index of therapeutic efficacy, in association with clinical assessments based on common psychometric scales such as Mini-Mental State evaluation tests. In this sense, pupillary characteristics could provide additional information in order to differentiate patients into "responders" and "non-responders." This is still not completely possible with the data currently available in the literature, since findings in patients with AChel intake are still limited.[15-17] In addition, the drugs used in these studies are not homogeneous among all patients, they are not administered at the same dosage and for the same period of time. Therefore, it would be necessary to design future trials with larger samples and stratified study groups (with less heterogeneous populations, according to the above variables) performing clinical evaluations of pupillary characteristics in naïve patients, allowing the reevaluation of the same features once the treatment is introduced, eventually backing up the pupillary data with the analysis of AChel blood levels, thus avoiding biases induced by unbalanced individual pupillary behavior.

Authors' Note

The data generated and analyzed during this study are stored in the repository of the A.O.U. Città della Salute e della Scienza (City of Health and Science) of Turin, but are available from the corresponding author on reasonable request. Informed consent to participate has been collected in written form from all study participants. The study has been approved by the institutional Ethics Committee. All procedures in this study concerning his conduction and documentation were performed in conformity with the ethical principles set out in the Helsinki Declaration and its revisions.

Declaration of Conflicting Interests

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ORCID iD

Raffaele Nuzzi https://orcid.org/0000-0002-9213-2718

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