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Clinical study

Autonomic nervous system assessment by pupillary response as a potential biomarker for cardiovascular risk: A pilot study

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ABSTRACT

Background: Cardiovascular risk (CVR) biomarkers are of increasing interest because of their potential utility in management of cardiovascular diseases. The activity of the autonomic nervous system (ANS) is known to be highly correlated with CVR and therefore, is a putative biomarker. Common ANS measurement tools have several technological limitations and high-variance signals. The pupillary responses (PR) is controlled by both components of the ANS, and recent advances in pupillometry are making this measurement, easy and reliable. Thus, PR assessment could become a useful clinical tool to measure the ANS modulation and its relation to CVR. Here, we aimed to evaluate differences in PR between low CVR and moderate/high CVR individuals.

Methods: We performed a cross-sectional study. We recruited voluntaries with low CVR (group 1, n = 12) and patients with moderate/high CVR (group 2, n = 7). An eye tracker was used to measure PR to different visual stimulus that included colors (white, black, gray) and images with known emotional valence (pleasant, unpleasant and neutrals), which were intercalated by pink "noise" images. Differences in PR between both CVR groups were assessed by Mann Whitney *U* test of different epochs of the PR.

Results: PR was significantly different between both CVR groups (p-value < 0,05) when the observed images were unpleasant, neutral, and pink noise, for different epochs of the PR.

Conclusions: This is the first study that demonstrates that PR is different according to CVR. Thus, PR could be considered as a novel biomarker of CVR to be tested in prospective studies.

1. Introduction

Cardiovascular diseases (CVD) are an important public health problem worldwide [1]. Because the pathophysiological process begins several years before its diagnosis, CVD is preventable with early diagnosis and aggressive treatment of its risk factors, such as diabetes, hypertension, smoking, obesity, sedentarism, or lifestyle, among others [2,3]. Although early diagnosis and aggressive treatment is the current standard of care [4], traditional CVR factors only explain approximately 75% of cardiovascular risk (CVR), which is far from the ideal of efficiency in the clinical setting [3,5]. Due to this limitation, an essential aim of the current research in cardiology has focused on the search for new CVR biomarkers, that would allow a more accurate evaluation of this condition. This diagnostic improvement will result in preventing adverse outcomes, such as myocardial infarction, stroke, and death [3–6]. However, the evidence that these biomarkers actually improve the prediction of CVR is surprisingly limited [3], which, added to the high cost and usual reduced availability, restricts clinical utility [4].

The balance of the autonomic nervous system (ANS) is associated with different CVR factors and is considered as a predictor of cardiovascular events [7–9] and therefore, could be a useful CVR biomarker. Heart rate variability is the most studied measure of ANS balance in epidemiological researches, however is not usually measured in clinical medicine because of practical and technical challenges, as well as the high variance of its measures [10,11], among other complications. On the other hand, the pupillary response (PR) to visual stimuli has gained interest because of advances in eye tracking methodology. Additionally, PR is under the control of both ANS branches [12]: pupillary contraction is mediated mainly by the parasympathetic ANS, while dilation is regulated mainly by the sympathetic ANS [13]. Given that automatic pupillometry is a clinically validated method to measure

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PR [14,15] exhibits a fast response time (<4 s), is economical, safe, with good "bed-side" results, and is characterized by a high level of agreement among operators [16], we conjecture that PR dynamics could be associated with different CVR factors, allowing the prediction of cardiovascular events. Thus, pupillometry could be applied to evaluate the functioning of the ANS and therefore, CVR in cardiology patients.

Despite the technological progress in pupillometry, there is very little evidence on the application of PR in other clinical areas other than neurology and ophthalmology. Hence, due to the limited available evidence on PR and CVR factors, and the need to have new clinical tools to help assess CVR more accurately, quickly, simply and economically, this study aimed to evaluate if there are differences in the PR of individuals with low and moderate/high CVR. These results are the starting point in the investigation of PR as a novel biomarker of CVR.

2. Methods

2.1. Design and recruitment of subjects

We performed a cross-sectional and analytical proof of concept study. We included volunteers with low CVR, which were undergraduate and graduate students of the Faculty of Medicine of Universidad de Chile (group 1, n = 12). The participants were invited to join the study through posters published in the University's boards. We also included patients, older than 18 years, with moderate or high CVR treated in the cardiology clinic at the Clinical Hospital of Universidad de Chile, (group 2, n = 7). We contacted these patients by telephone to invite them to participate in our study. Once the volunteers agreed to participate in this research, a meeting was arranged in order to carry out the informed consent process and, subsequently, the evaluations. Individuals with reduced mobility, language disorders, retinal diseases and cognitive impairment – according to a self-report of clinical problems – were excluded from the study.

2.2. Measurement of pupillary reactivity (PR)

In a dim-lit room (11 lx), pupillary diameter was measured using an eye tracker system (EyeLink 1000). The participants were subjected to an observation task of visual stimuli that were presented on a 27-inch LED monitor (ViewSonic VW2753 MH-Led), at a fixed distance of 70 cm from the subject's eyes. During the execution of the task, the individuals remained seated with the head resting in a chin-support. Subjects freely viewed the sequence projection of 60 different images with a known emotional valence (20 pleasant, 20 neutral and 20 unpleasant), taken from the IAPS database of the University of Florida [17]. Each image was projected for 4 s and was followed by pink noise (image in gray tones, without content), which was presented for the same 4-s duration. These pink noise images served to lead the pupillary diameter to a steady state and to diminish the previous-trial effect. Additionally, three screens with a different luminance (black, white, and medium gray) were presented interspersed with a pink noise image before, at the middle and at the end of the sequence of images with emotional content. Each one of these images was also shown during 4 s.

2.3. Data recording and analysis

The pupillary diameter signal was recorded using a video-oculagraphic commercial system (EyeLink 1000, SR Research Ltd.). Pupil size was measured in arbitrary units at 500 Hz and 16 bits of precision, with a system resolution of 0.1% of the pupillary diameter. Calibration and drift correction was performed 6 times across each task. Maximal eye-positioning error accepted during calibration was 0.3 degrees, using a spatial grid of 9 points.

Data analysis was carried out using Matlab (The Mathworks, Inc.). Data visualization, artifact detection/correction and exclusion of single epochs were done by means of semi-automated algorithms implemented in a custom graphical user-interface.

After elimination of artifacts, for each type of visual stimulus we measured: basal pupillary diameter (BD), maximum contraction diameter (CD), maximum dilatation diameter (DD), pupillary diameter at 800 ms (D800), contraction latency (CL), dilatation latency (DL), contraction velocity (CV), dilatation velocity (DV), contraction amplitude [(CA) = CD-BD], dilatation amplitude [(DA) = DD-CD], pupillary change [(PC) = DD-BD], in addition to the "ratios" between CV and DV (See Fig. 1).

2.4. Clinical evaluation and estimation of cardiovascular risk

A trained physician performed an anamnesis and abbreviated physical examination in order to obtain the sociodemographic and medical history of the individual, and to measure blood pressure, pulse, weight, height, and waist circumference. With this information, the CVR (according to Framingham) was later estimated with the D 'Agostino equations [18].

2.5. Statistical analysis

Due to small sample sizes, the results of the continuous variables were expressed as median (25th percentile–75th percentile), while qualitative variables were reported as absolute frequency and percentage. The Mann-Whitney *U* test was used to compare continuous variables corresponding to subjects with low and moderate or high CVR, and the Fisher exact test was used for qualitative variables. All the analyzes were carried out with the Stata 12 Software, considering as significant, a p-value <0.05.

2.6. Bioethics aspects

All subjects were invited to voluntarily participate in the study after acceptance and signing of an informed consent. The study protocol was approved by the Ethics Committee in Research on Human Beings of the Faculty of Medicine of the University of Chile, which follows the Declaration of Helsinki.

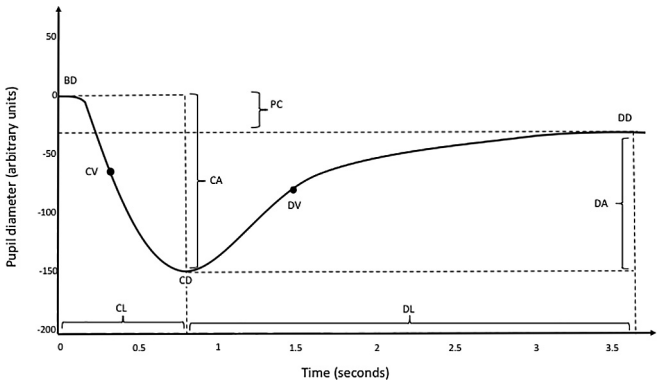

Fig. 1. Pupillary Response diagram and measurements. A typical pupil response to a visual stimulus is drawn (i.e: pupillary size during observation time); BD: Basal diameter, CD: Constriction diameter, DD: Dilation diameter, CL: Constriction latency, DL: Dilation latency, CV: Constriction velocity, DV: Dilation velocity, CA: Constriction amplitude = CD – BD, DA: Dilation amplitude = DD – CD, PC: Pupillary change = DD – BD; CV and DV mean and max were also measured, and CV/DV ratios were calculated.

3. Results

Baseline characteristics of the patients are shown in Table 1. Significant differences were found in CVR-related characteristics, such as: age, 28 (IQR 25.5–29.5) vs 70 (IQR 63–71) years; systolic blood pressure, 113 (IQR 109–125) vs 134 (IQR 130–136) mmHg; body mass index, 23.35 (IQR 20.79–24.95) vs 32.64 (IQR 27.64–33.95) cm/m^2; and waist circumference 76.45 (IQR 67.5–82.25) vs 97.5 (IQR 93–117) cm. Five patients from group 2 had high CVR (71.43%), and two had moderate CVR (28.57%). Group 2 presented several simultaneous non-cardiovascular comorbidities, especially neurologic, endocrine, rheumatologic, and digestive. In group 1, 4 subjects (33.33%) had comorbidities not under pharmacological treatment at the time of evaluation (2 asthma, 1 allergic rhinitis and 1 insulin resistance).

Fig. 2 shows PR in both CVR groups for each type of image, and includes mean pupillary size variability with its 95% confidence interval for each group, during the observation time. Specific PR results are displayed in Table 2, and they show significant differences between low-CVR and moderate/high-CVR, in PR parameters that differ according to image type: unpleasant images (DD, CL, mean and maximum DV, DA), neutral images (BD, DL, DA), pink noise (DD, D800, DL, DA, PC, peak DV, mean CV/DV ratio, peak CV/DV ratio) and gray color (BD). No difference was observed between both groups in images with pleasant emotional valence, nor in black and white images.

4. Discussion

This study demonstrates for the first time that PR exhibits contrastive values among individuals with different levels of CVR (low vs moderate/high). This agrees with differences in baseline characteristics among both groups, as they differed in age, body mass index, waist circumference, systolic blood pressure, and CVR-related diseases.

To date, there is a lack of literature about the use of PR in fields other than neurology and ophthalmology. In cardiology, regarding the specific relationship between PR and CVR, one study proved that pupillary diameter and re-dilation time were negatively correlated to body mass index in children and adolescents [19]. Other study found that in elderly subjects watching a video, those with elevated blood pressure and glycaemia showed greater variation in many PR parameters compared to subjects without those two disturbances [20]. Most evidence about PR is available in diabetics, a group of patients with a well-stablished high CVR [21]; they have showed a smaller basal pupillary diameter and constriction velocity than healthy controls in response to a light stimulus [22–27]. Furthermore, these alterations described in PR of diabetics increase in the presence of diabetic neuropathy [23,24,27–29], a condition that increases CVR of diabetic patients [21]. Despite this, so far there are no studies in the literature that have evaluated PR according to CVR in a direct way.

Considering that other autonomic tone evaluation techniques, such as heart rate variability, have shown a strong association with mortality after acute myocardial infarction, CVR factors, and CVR [7–11], it is reasonable to think that PR may have a similar behavior. Thus, our promissory results, opens the door to new prospective studies to evaluate CVR according to PR, which is an easy, fast, inexpensive, and highly replicable measure. Also, new researches could be conducted to test the utility of PR to guide medical treatment of patients with high CVR or a stablished CVD.

Although this research presents preliminary results that must be taken with caution and needs to be confirmed in bigger studies, there are some interesting findings that are consistent with other measure of ANS balance as HRV [7–9]. Many PR parameters showed a higher sympathetic activity in subjects with moderate/high CVR than in controls (BD in neutral and gray images, DL in neutral images, peak DV in pink noise images). Also, other PR parameters showed a lower parasympathetic activity in subjects with moderate/high CVR than in controls (CL in unpleasant images, 800 ms diameter and CV/CD ratios in pink noise images). Finally, when the ANS was modulated by unpleasant images that stimulate the sympathetic branch, we observed that mean and peak DV were paradoxically higher in healthy subjects than in those with moderate/high CVR, which could be explained by a lower "system's gain ability" in the last group.

Experimental research is helpful to understand the biological relationship between CVR and ANS. Classical CVR factors such as

Table 1
Basal characteristics of patients.

Characteristic	Low CVR (n = 12)	Moderate/High CVR (n = 7)	p-value*
Age, years	28 (25.5–29.5)	70 (63–71)	0,000
Males, %	6 (50%)	4 (57,14%)	0,500
Systolic blood pressure, mm Hg	113 (109–125)	134 (130–136)	0,001
Diastolic blood pressure, mm Hg	78 (70–84)	82 (79–86)	0,581
Heart rate, bpm	66 (60–72)	68 (58–77)	0,966
Body mass index, kg/m^2	23.35 (20.79–24.95)	32.64 (27.64–33.95)	0,001
Waist circumference, cm	76.45 (67.5–82.25)	97.5 (93–117)	0,003
Coronary heart disease, %	–	4 (57,14%)	–
Non-coronary atherosclerosis, %	–	4 (57,14%)	–
Hypertension, %	–	5 (71,43%)	–
Diabetes mellitus, %	–	1 (14,29%)	–
Arrhythmias, %	–	2 (28,57%)	–
Non-CV comorbidity, %	4 (33,33%)	4 (57,14%)	0,356
Chronic medication			
Beta blockers	–	4 (57,14%)	–
ACEI/ARB	–	4 (57,14%)	–
Calcium channel blockers	–	2 (28,57%)	–
Statins	–	2 (28,57%)	–
Antiplatelets	–	3 (42,86%)	–
Diuretics	–	4 (57,14%)	–
Oral antidiabetics	–	2 (28,57%)	–
Insulin	–	0 (0%)	–
Others	–	5 (71,43%)	

CV: Cardiovascular, ACEI: Angiotensin Converting Enzyme Inhibitors, ARB: Aldosterone Receptor Blockers, CVR: Cardiovascular Risk.
* p-value for two-tailed tests are shown.

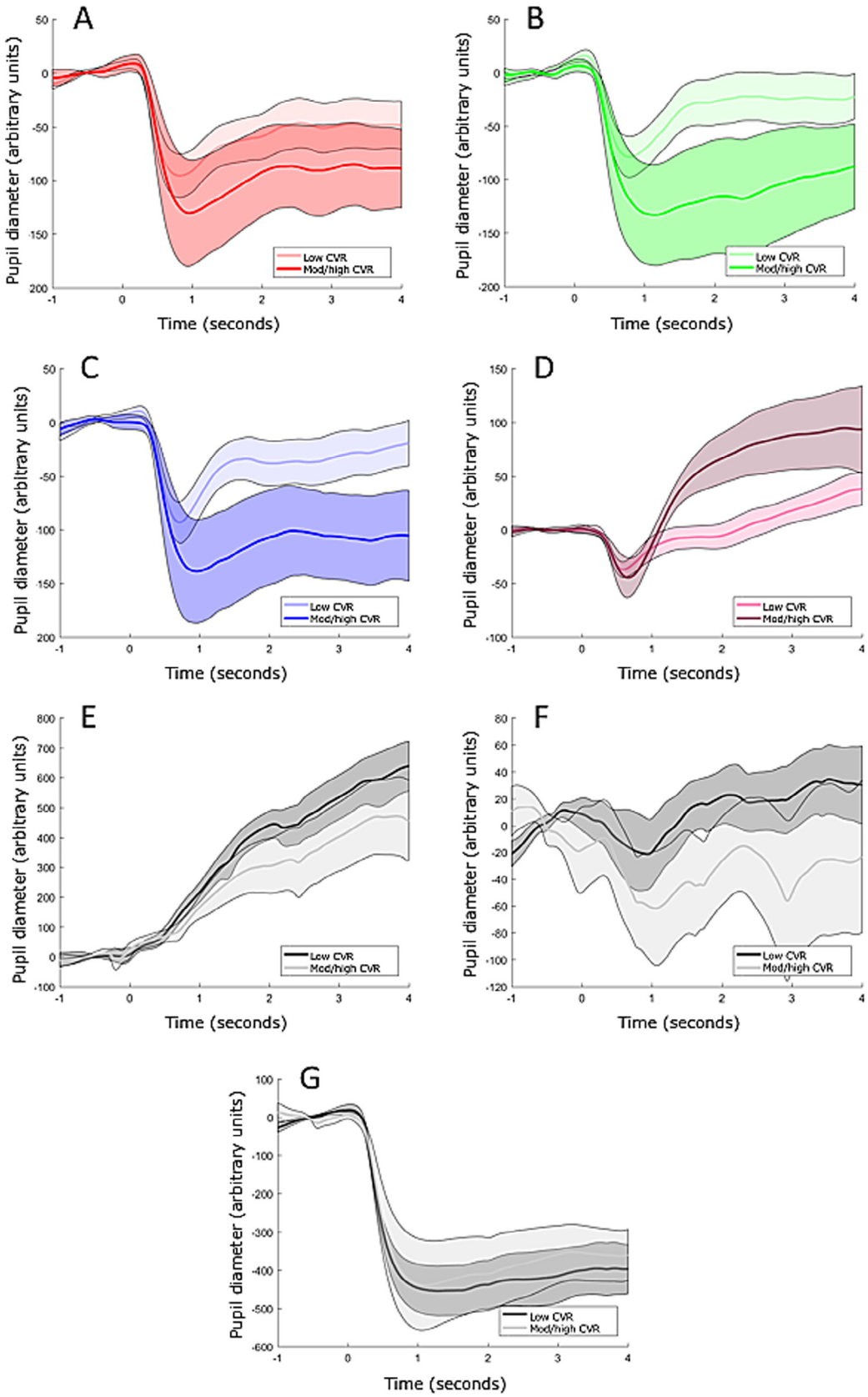

Fig. 2. Pupil Response according to cardiovascular risk group and image type**.** Mean (central solid line) and confidence interval (CI 95%) are shown for each cardiovascular risk group; Light color: low cardiovascular risk group, Dark color: moderate or high cardiovascular risk group; A. Pleasure image, B: Neutral image, C: Unpleasant image, D: Pink noise, E: Dark color, F: Grey color, G: White color. CVR: Cardiovascular Risk, Mod/high: Moderate or high.

Table 2
Pupillary response to different image types in low and moderate/high cardiovascular risk groups.

Image type and PR parameter	Low CVR (n = 12)	Moderate/High CVR (n = 7)	p-value*
Pleasant images			
–	–	–	ns
Neutral images			
Basal diameter, au	13.5 (7.2–20.9)	2.9 (−2.6 to 16.3)	0.038
Dilation Latency, sec	1.0 (0.5–1.2)	0.4 (0.2–0.7)	0.031
Dilation amplitude, au	78.6 (15.2–114.4)	14.7 (3.1–77)	0.045
Unpleasant images			
Dilation diameter, au	−13.5 (−56.9–37.9)	−67.3 (−196.3 to −12.4)	0.038
Constriction latency, sec	0.72 (0.4–0.8)	0.83 (0.76–1.13)	0.026
Mean dilation velocity, au/sec	0.1 (0.09–0.2)	0.08 (0.07–0.09)	0.021
Peak dilation velocity, au/sec	0.3 (0.2–0.4)	0.2 (0.1–0.2)	0.045
Dilation amplitude, au	82.0 (48.8–111.9)	38.2 (9.2–50.6)	0.045
Pink noise			
Dilation diameter, au	−15.6 (−28.2 to 36.2)	113.1 (18.5–143)	0.006
800 ms diameter, au	−6.8 (−29.4 to 8.0)	56.1 (18.5 to −60.6)	0.003
Dilation latency, sec	0.7 (0.3–1.0)	2.2 (0.8–3.1)	0.011
Peak dilation velocity, au/sec	0.2 (0.07–0.2)	0.3 (0.2–0.5)	0.038
Dilation amplitude	29.0 (9.6–73.8)	144.4 (35.9–190.2)	0.017
Pupillary change	−17.9 (−39.6 to 34.9)	107.0 (15.5–128.5)	0.004
CV mean/DV mean ratio	−2.4 (−5.7 to −1.9)	−0.9 (−1.1 to −0.8)	0.021
CV peak/DV peak ratio	−2.3 (−3.7 to −1.3)	−1.3 (−1.6 to −0.6)	0.002
Black color			
–	–	–	ns
Gray color			
Basal diameter	39.8 (11.3–50.2)	−3.9 (−12.7 to 19.8)	0.038
White color			
–	–	–	ns

To a better reading, only pupil response parameters that were statistically significant are shown as median (p25–p75). CV: Constriction Velocity, DV: Dilation Velocity, CVR: Cardiovascular Risk.
* p-value for one-tailed Mann Whitney *U* test.

aging, diabetes, hypertension, dyslipidemia, obesity, physical inactivity and smoking, among others [30], have a common final pathway: a chronic inflammatory state that drives to blood vessels into an atherosclerotic process and finally a cardiovascular event [31]. On the other hand, ANS balance modulate systemic inflammation, vascular function and atherosclerosis: patients with a higher sympathetic activity or decreased parasympathetic activity have higher levels of pro-inflammatory cytokines in plasma [32], endothelial dysfunction [33] and atherosclerosis progression [34]. Furthermore, a higher sympathetic activity induces hemodynamic stress and heart alterations that leads to different CVD and finally heart failure [35].

Despite the biological plausibility of our findings and the potential utility of PR as a new tool to asses and manage CVR in clinical practice, more researches are needed to confirm our results.

4.1. Study limitations

This pilot study has some limitations: a small sample size, a cross-sectional analysis, and the difficult to know if PR changes related to CVR are cause or consequence in this complex phenomenon. On the other hand, we did not include other known CVR biomarkers neither other equations to predict CVR. Thus, our early promising results must be taken with caution because PR needs to be tested in prospective epidemiological studies to test it utility to predict CVD-related outcomes.

5. Conclusion

In summary, the current study is the first to demonstrate that PR is different among subjects with different levels of CVR and this poses PR as a potential and novel tool for CVR assessment and treatment, that should be tested in prospective studies.

Conflict of interests

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jocn.2018.11.015.

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