Interpretation of longitudinal changes of the inner nuclear layer in MS

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With great interest we read the contributions of Sotirchos *et al*[1] and Cordona *et al* [2] on the inner nuclear layer (INL) in multiple sclerosis (MS). Both groups demonstrated descending slopes of INL thickness with age, but interpret these data different. Does progressive MS cause INL atrophy[1] or does early disease related INL edema resolve?[2]

We would like to add to this discussion by contrasting the extend of physiological variation of the INL thickness in one study[3, 4] with longitudinal INL changes over a 4-year follow-up period from our MS cohort.[5]

The physiological variation of the INL thickness in 26 healthy control subjects[3, 4] (mean age 41 ±12 years, 7 females) at two different visits is visualized in Fig 1A. The mean smallest detectable change of re-testing after one and two hours at these two visits was 5.2 µm. The maximum upper and lower limits of the 95% confidence intervals were 5.6 and -4.2 µm respectively (Fig 1A).

The mean change of the INL thickness from baseline in 27 healthy controls of our MS cohort[5] (52.1 ±5.5 years, 11 females) was 0.1 ±1.3 µm after two years and 0.2 ±1.2 µm after four years (Fig 1B, baseline INL 39.9 ±2.7 µm). In 92 individuals with MS[5] (54.5 ±8.9 years, 59 females) the pattern was similar ($p>0.05$) with a change of 0.1 ±1.3 µm after two years and 0.4 ±1.5 µm after four years (Fig 1C, baseline INL 40.5 ±3.1 µm). The mean MS disease duration was 21.1 ±6.3 years, with a median EDSS of 4.0 (IQR 2.5). Linear regression analyses of the annualized INL change (based on the difference between baseline and 4-year follow-up, mean change of left and right eye) demonstrated a relationship with age (Fig 1E, $p<0.05$) in MS, but not in controls (Fig 1D). This was most marked in individuals with secondary progressive disease ($p<0.01$, Fig 1F). Disease duration and EDSS at baseline were not associated with the rate of INL thinning. We observed increase (younger patients)

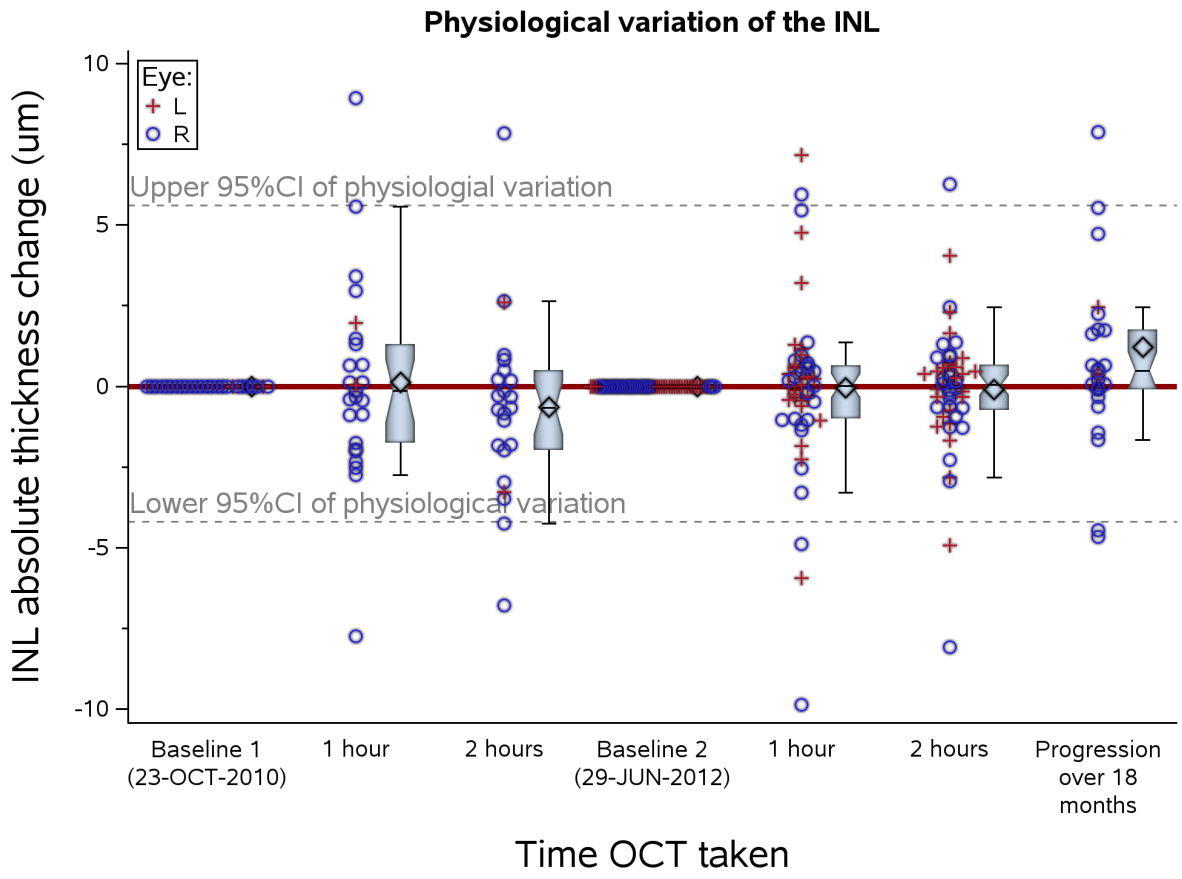
and a decrease (older patients) of the INL thickness over time with the observed range falling within the physiological variation (Fig 1A to C). Additionally, the magnitude of the annualized decrease in older MS patients was similar to the decrease in older healthy controls (Fig 1D to F). In view of these data we respectfully suggest that the effect size of INL thinning in MS, related to pathology or not, is very small. If however included in a study, we suggest assessment of physiological variation of the INL by, for example, two measurements at least one hour apart at each time point.

References

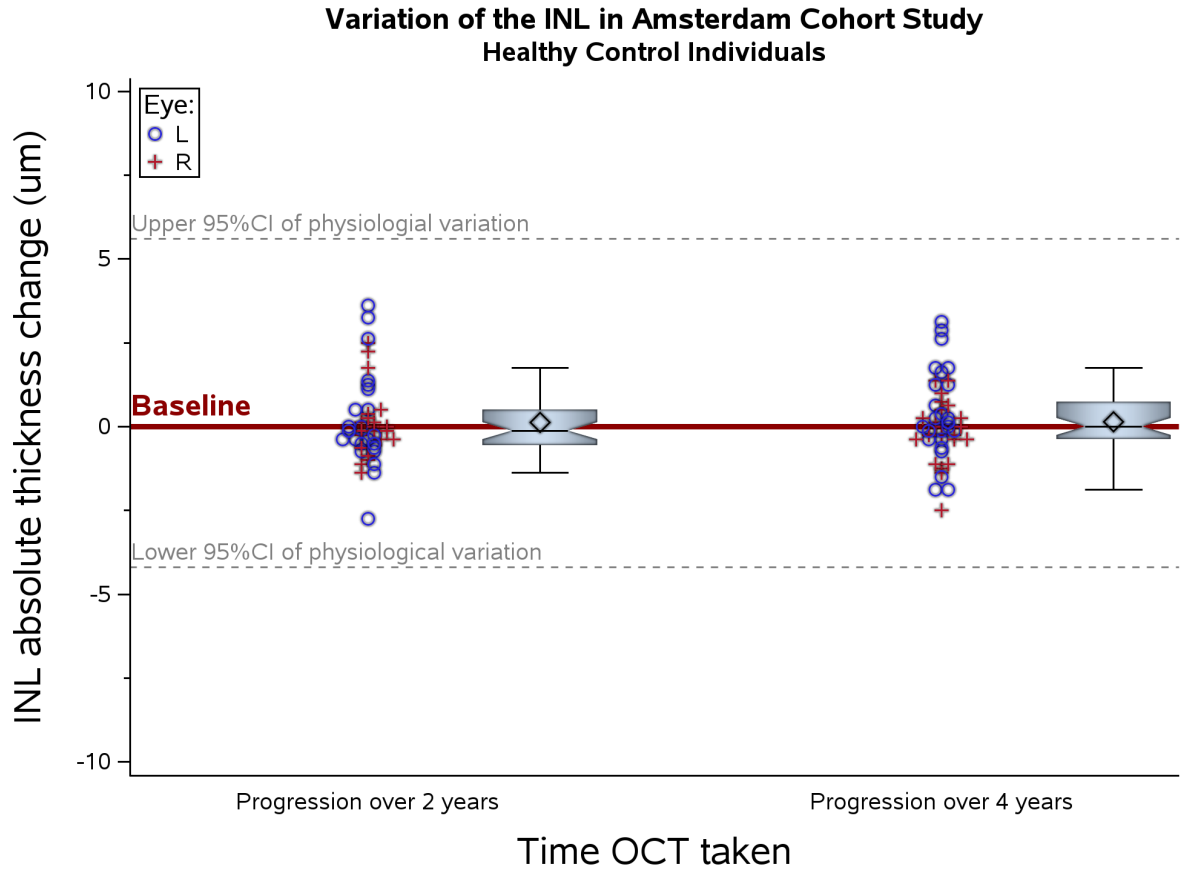
1. Sotirchos ES, Caldito NG, Filippatou A, et al. Progressive Multiple Sclerosis Is Associated with Faster and Specific Retinal Layer Atrophy. Annals of Neurology 2020;87:885-896.
2. Cordano C, Yiu HH, Oertel FC, et al. Retinal INL Thickness in Multiple Sclerosis: A Mere Marker of Neurodegeneration? Ann Neurol 2021;89:192-193.
3. Balk LJ, Oberwahrenbrock T, Uitdehaag BM, Petzold A. Physiological variation of retinal layer thickness is not caused by hydration: a randomised trial. J Neurol Sci 2014;344:88-93.
4. Balk LJ, Sonder JM, Strijbis EM, et al. The physiological variation of the retinal nerve fiber layer thickness and macular volume in humans as assessed by spectral domain-optical coherence tomography. Invest Ophthalmol Vis Sci 2012;53:1251-1257.
5. Coric D, Balk LJ, Verrijp M, et al. Cognitive impairment in patients with multiple sclerosis is associated with atrophy of the inner retinal layers. Mult Scler 2018;24:158-166.

***Figure 1**. A-C: Scatterplots and box- and whisker plots of the absolute change of the inner nuclear layer (INL) in µm. The median (horizontal line in the box), 25-75 percentiles (box), 5-95 percentiles (whiskers) and mean (diamond in the box) are shown, as well as scatters (circles) of all individual observations for the right eye (circles) and left eye (crosses). Each individual's baseline value was set as reference which is indicated by the horizontal solid line.*
*(**A**): physiological variation in 26 healthy controls after 1 hour and 2 hours re-testing at two different visits 18 months apart. The maximum upper and lower 95% confidence intervals of the INL change are indicated with the dashes lines. The exact same lines are also drawn in figure (B) and (C), to evaluate these results in comparison with the physiological variation of the INL. The INL change between the baseline measurements of the two visits is shown in the rightmost scatter and box- and whisker plot as 'Progression over 18 months'. **(B)**: This plot shows the INL change in 25 healthy controls of the Amsterdam MS cohort from baseline (solid reference line) to two and four years follow-up (one measurement per visit). **(C)**: Likewise the INL change in 91 individuals with MS of the Amsterdam cohort at two and four years follow-up is shown normalized to each individual's own baseline (solid reference line). **D-F**: Data of Amsterdam MS cohort. Scatterplots of the associations between age and annualized INL change (mean change of left and right eye) over 4-year follow up. Data are shown for **(D)** healthy controls; **(E)** a pooled cohort of individuals with MS, and **(F)** individuals with a secondary progressive disease course. The linear fit is shown (solid line), with the 95% confidence curves (dashed lines). The unstandardized regression coefficient and level of significance are shown.*

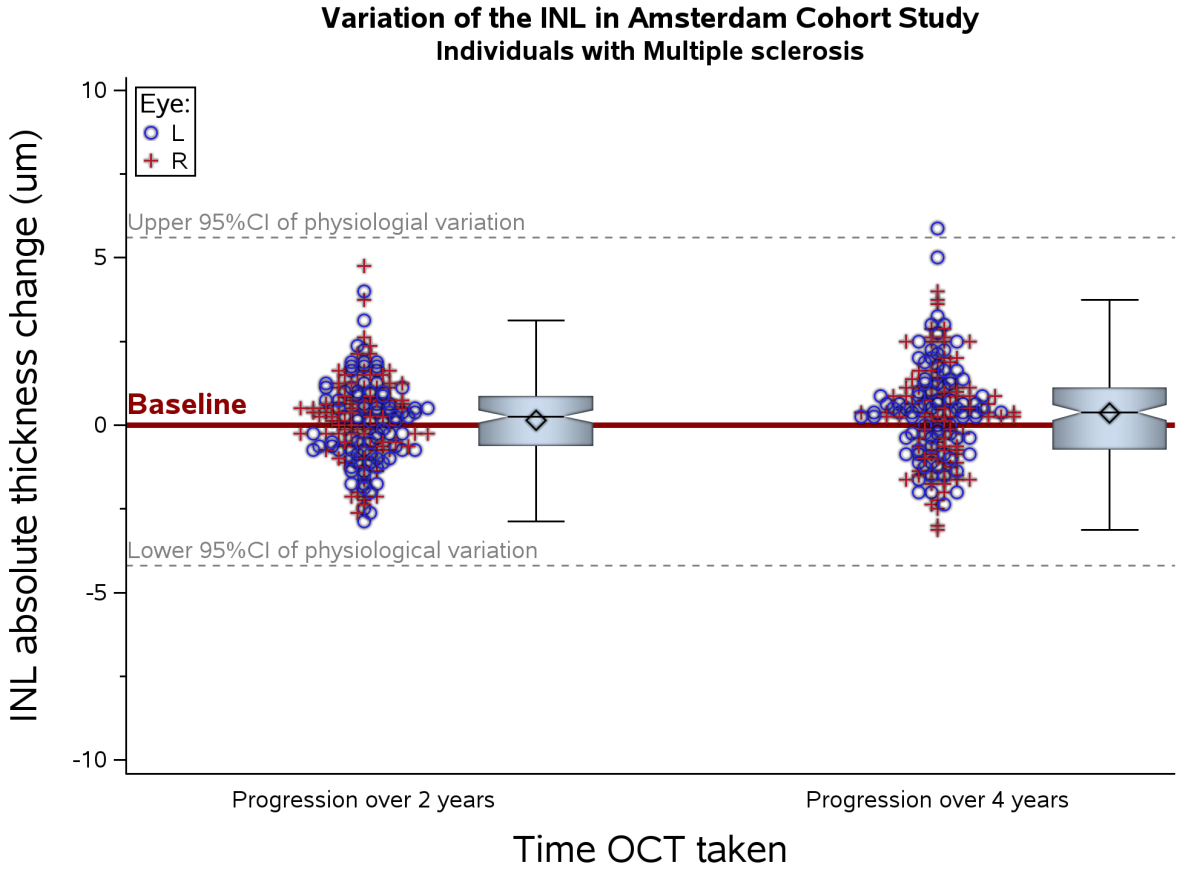
(A)



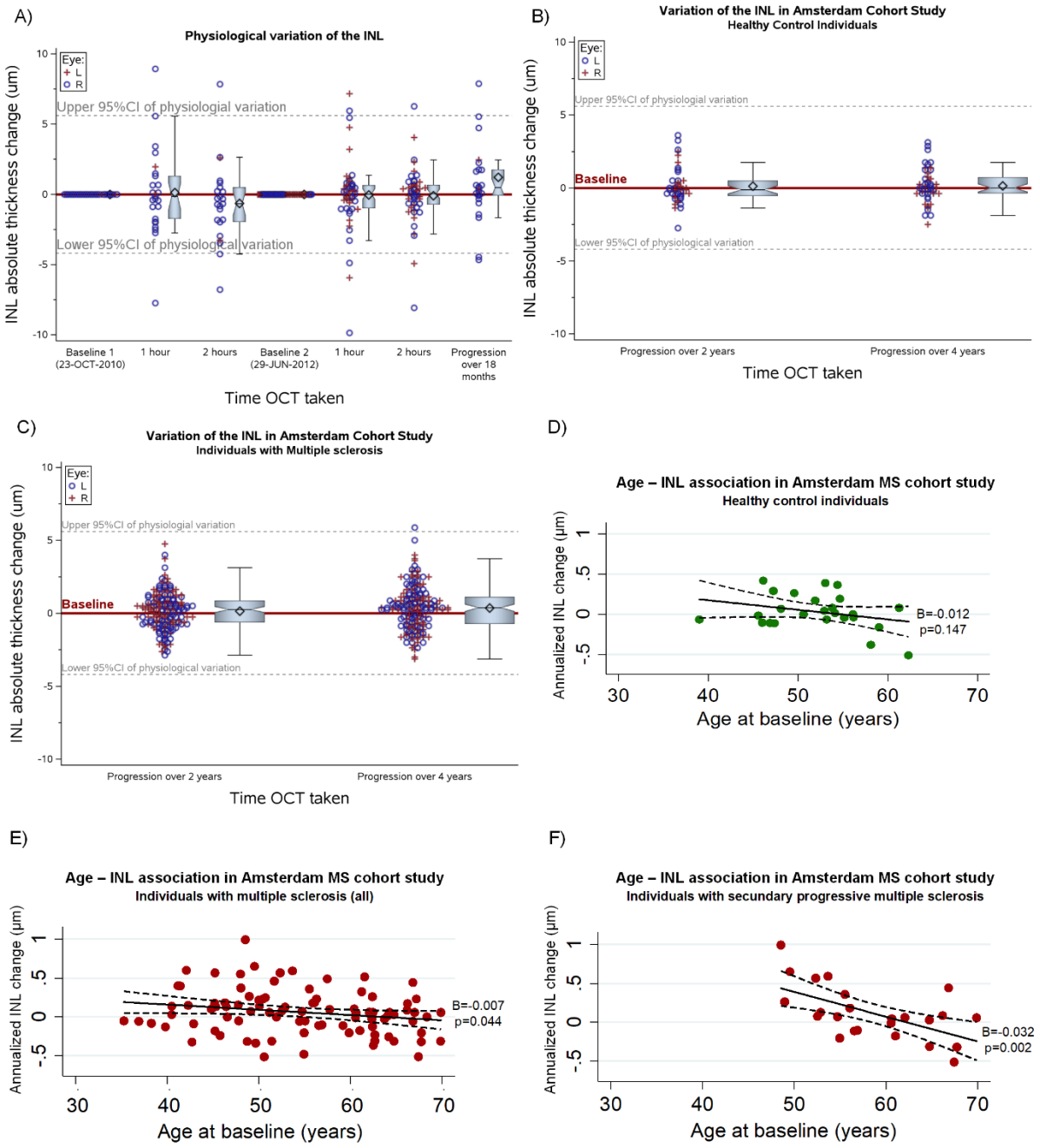
(B)


Variation of the INL in Amsterdam Cohort Study
Healthy Control Individuals
Eye:
L
R
Upper 95%CI of physiologial variation
Baseline
Lower 95%CI of physiological variation
10
5
0
-5
-10
INL absolute thickness change (um)
Progression over 2 years
Progression over 4 years
Time OCT taken

(C)


Variation of the INL in Amsterdam Cohort Study
Individuals with Multiple sclerosis
Eye:
L
R
Upper 95%CI of physiologial variation
Baseline
Lower 95%CI of physiological variation
10
5
0
-5
-10
INL absolute thickness change (um)
Progression over 2 years
Progression over 4 years
Time OCT taken

(D)


A)
Physiological variation of the INL
Eye:
L
R
Upper 95%CI of physiologial variation
Lower 95%CI of physiological variation
INL absolute thickness change (um)
Baseline 1 (23-OCT-2010)
1 hour
2 hours
Baseline 2 (29-JUN-2012)
1 hour
2 hours
Progression over 18 months
Time OCT taken
B)
Variation of the INL in Amsterdam Cohort Study
Healthy Control Individuals
Baseline
Progression over 2 years
Progression over 4 years
C)
Variation of the INL in Amsterdam Cohort Study
Individuals with Multiple sclerosis
D)
Age – INL association in Amsterdam MS cohort study
Healthy control individuals
Annualized INL change (µm)
Age at baseline (years)
B=-0.012
p=0.147
E)
Age – INL association in Amsterdam MS cohort study
Individuals with multiple sclerosis (all)
B=-0.007
p=0.044
F)
Age – INL association in Amsterdam MS cohort study
Individuals with secundary progressive multiple sclerosis
B=-0.032
p=0.002