# Retinal and cerebrospinal fluid markers of neurodegeneration and inflammationin clinically isolated syndromes

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## Introduction

Neuroretinal changes measured with optical coherence tomography (OCT) can be detected in clinically isolated syndromes (CIS) suggestive of multiple sclerosis (MS) [Oberwahrenbrock, 2013; Martinez-Lapiscina, 2016]. Thinning of retinal nerve fibre layer (RNFL) and ganglion cell/inner plexiform layer (GCIPL) reflects neurodegenerative processes, while inner nuclear layer (INL) has been proposed as a marker of inflammation [Balk, 2017; Knier, 2016]. We aimed at investigating the relationship between OCT measures and other markers, e.g. cerebrospinal fluid (CSF) oligoclonal bands, microvesicles count (MVs/uL), cytokines and multimodal evoked potentials (mEPs) in CIS.

## Methods

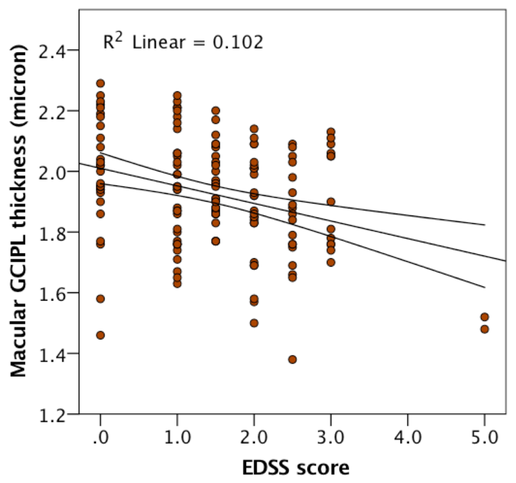
We enrolled 111 consecutive patients hospitalized for a first neurological episode suggestive of MS (65% diagnosed with MS according to McDonald 2010 criteria).
Patients underwent contrast-enhanced 1.5 Tesla brain MRI, EDSS, high and low-contrast visual acuity-VA (1,25% and 2,5%), mEPs (MEP, SEP, VEP) with score calculation [Leocani et al 2006], OCT with peripapillary RNFL and macular GCIPL and INL segmentation. All patients had CSF sampling with oligoclonal bands (OCB) and microvesicles count (MVs/uL) and, in a subgroup (N=91), cytokine and chemokine dosage was obtained (IL1b, IL2, IL4, IL5, IL6, IL7, IL8, IL10, IL12, IL13, IL17, IFNg, TNFa, CCL4, CCL2, GM-CSF, G-CSF).
Patients presenting with acute optic neuritis (n= 34, 28%) were excluded from the analyses. Spearman's rho was used to detect significant correlations with OCT variables (pRNFL, mGCIPL, INL). Stepwise multivariate regression analysis with forward selection was used to define independent determinants of GCIPL and INL thickness.

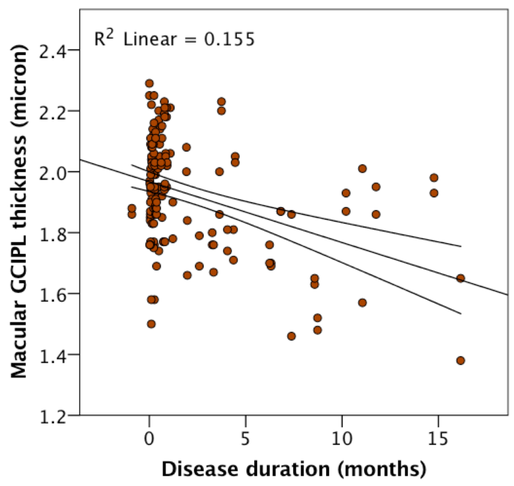
## Results

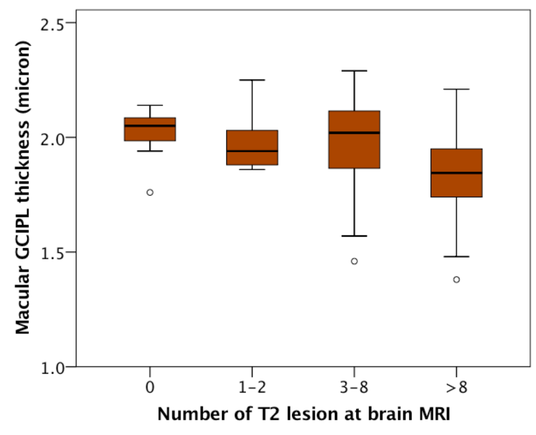
### Peripapillary RNFL and macular GCIPL thickness:

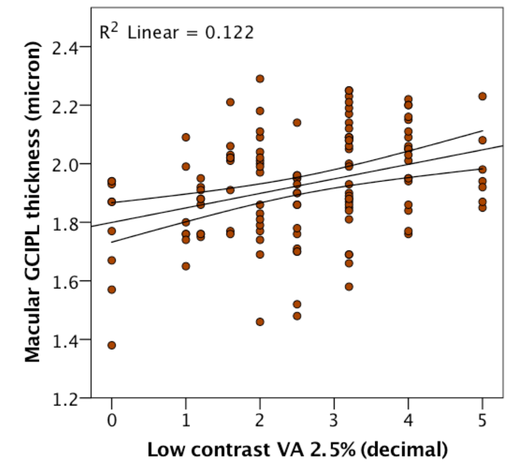
Both pRNFL and mGCIPL significantly correlated with measures of global (EDSS) and visual (LCVA) disability. Inner retinal layers were correlated with disease duration, number of T2 brain MRI lesion and with multimodal evoked potentials score (multimodal EPs score; VEP score; MEP-SSEP score). No correlation was instead found with CSF light chain neurofilaments, oligoclonal bands, IgG Index or presence of gadolinium enhancing lesion at brain MRI.

| | | mGCIPL (micron) | pRNFL (micron) | Age (years) | Disease duration (years) | EDSS | Multimodal EPs (VEP, SSEP, MEP score) | SSEP - MEP (score) |
|---|---|---|---|---|---|---|---|---|
| Spearman's rho | **mGCIPL** | / | **.701**** | .023 | **-.225**** | **-.270**** | **-.291**** | **-.280**** |
| Sig. (2-tailed) | | / | .000 | .779 | .006 | .001 | .001 | .001 |
| N | | / | 147 | 147 | 147 | 147 | 135 | 142 |
| pRNFL_Globale | **pRNFL** | **.701**** | / | .023 | **-.274**** | **-.282**** | **-.296**** | **-.306**** |
| Sig. (2-tailed) | | .000 | / | .770 | .000 | .000 | .000 | .000 |
| N | | 147 | / | 158 | 158 | 158 | 135 | 142 |
| | | **HCVA (decimal)** | **LCVA 2.5% (decimal)** | **Brain T2 lesions (classes 0 to 3)** | **Link index (ratio)** | **BOG (presence/absence)** | **Enhancing brain lesions (presence/absence)** | **CSF NfL (pg/mL)** |
| Spearman's rho | **mGCIPL** | **.187*** | **.344**** | **-.412**** | .046 | -.131 | -.159 | -.061 |
| Sig. (2-tailed) | | .035 | .000 | .000 | .582 | .113 | .060 | .522 |
| N | | 127 | 127 | 147 | 147 | 147 | 141 | 113 |
| pRNFL_Globale | **pRNFL** | **.243**** | **.318**** | **-.409**** | .137 | .006 | -.046 | -.115 |
| Sig. (2-tailed) | | .005 | .000 | .000 | .086 | .943 | .574 | .210 |
| N | | 134 | 134 | 158 | 158 | 158 | 152 | 120 |



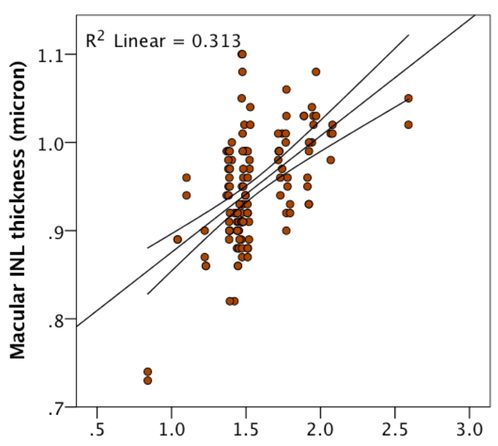



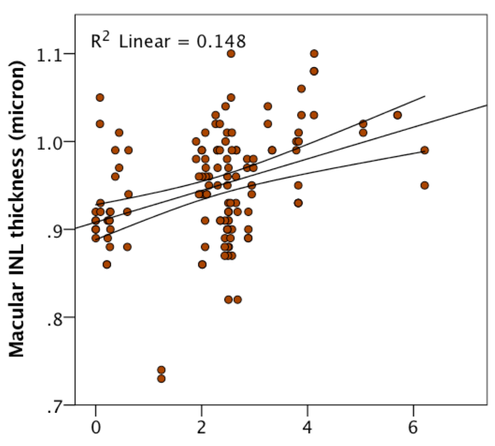




At multivariate regression analysis only disease duration, lesion load, EDSS and LCVA significantly contributed to the model. We also added in the regression the cytokines/chemokines that were associated with GCIPL and we found that CSF IFN-g was a independent predictor of GCIPL thickness (higher IFN-g levels predicted thinner GCIPL).

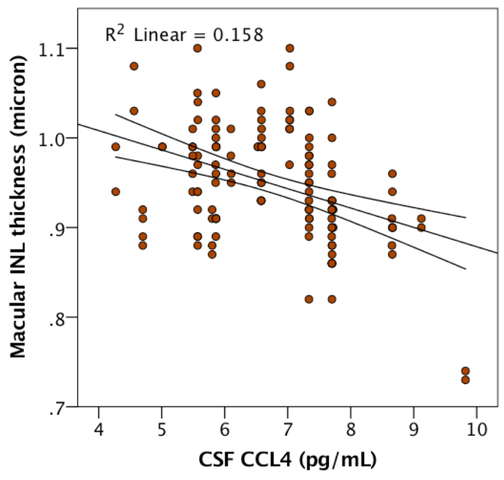
### Macular inner nuclear layer thickness:

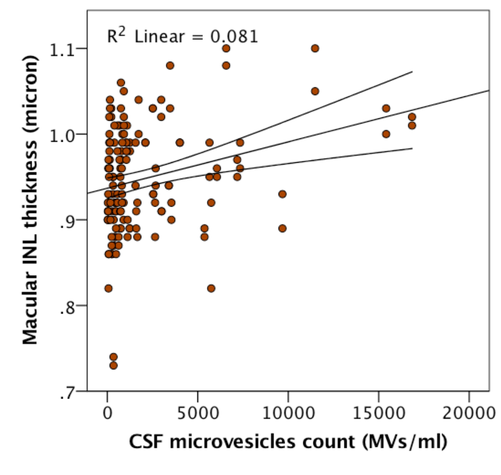
Macular INL thickness was not correlated with any of the previously analyzed clinical, neuroradiological or neurophysiological variables. We collected CSF samples of 91/111 subjects for cytokine, chemokine and growth factors dosage and microvesicles count (MVs).
INL thickness was directly correlated with the anti-inflammatory cytokines IL10, IL13, IL5 but also with IL6, IL7, IL12, GM CSF, G CSF and MVs while the pro-inflammatory IL1b, CCL2, CCL4, IL 8 and IL 4 were inversely correlated with INL.
At multivariate regression analysis only IL10, IL7 and CCL4 significantly contributed to the model. In particular, peripapillary RNFL and GCIPL did not significantly contributed to the model.




| MULTIVARIATE LINEAR REGRESSION ANALYSIS (dependent variable "macular INL thickness") | | | | | |
|---|---|---|---|---|---|
| Model | R (R square) | Unstd. Coefficients | Std error | Beta | Sig. |
| **1** | **.560 (.313)** | **.743** | .027 | | **.000** |
| CSF IL-10 (pg/mL) | | .132 | .017 | .560 | .000 |
| **2** | **.646 (.417)** | **.723** | .026 | | **.000** |
| CSF IL-10 (pg/mL) | | .123 | .016 | .522 | .000 |
| CSF IL7 (pg/mL) | | .015 | .003 | .325 | .000 |
| **3** | **.689 (.474)** | **.844** | .041 | | **.000** |
| CSF IL-10 (pg/mL) | | .104 | .016 | .441 | .000 |
| CSF IL7 (pg/mL) | | .016 | .003 | .331 | .000 |
| CSF CCL4 (pg/mL) | | -.014 | .004 | -.253 | .000 |




## Discussion and conclusions

The present study demonstrates that, even in early MS and in eyes never affected by optic neuritis, inner retinal thickness (pRNFL or mGCIPL) is associated with the accumulation of damage within CNS revealed by visual pathway damage (VEP score and visual acuity), spinal cord damage (MEP-SSEP and EDSS), and brain lesion load. Therefore, inner retina reflects parallel processes occurring both in the optic pathways and global neuroaxonal loss and should be considered as an indicator of previous structural damage accumulation.
Retinal INL thickening is not associated with clinical or instrumental indicators of structural damage in this cohort of early MS without previous ON but it reflects the anti-inflammatory activity following MS relapses. The present findings support its role as a marker of concurrent immunological activation.

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