MOLECULAR IMAGING OF THE RETINA IN PATIENTS WITH MULTIPLE SCLEROSIS BY RAMAN SPECTROSCOPY

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1. Summary of the project

Multiple sclerosis (MS) is an inflammatory neurodegenerative disease that produces significant damage of the retina. At present, clinical course and response to therapy are difficult to define and many aspects of its pathogenesis are unknown. For this reason, it is extremely important to elucidate markers of disease prognosis and to improve understanding of how CNS tissue is damaged. Moreover, the development of neuroprotective therapies is hampered because technical difficulties for studying the brain, especially for addressing molecular changes that would favor a better understanding of MS pathology and development of new therapies.

The retina is part of the CNS and retinal inflammation and neurodegeneration is found in MS. Raman spectroscopy (RS) is a technique that identifies chemical properties of samples due to its ability to detect molecular vibration frequencies that characterize molecular species. We have previously demonstrated the ability of RS to reveal molecular changes in rats in response to neuroinflammation. Our proposal aims to evaluate molecular changes in retina in vitro and patients with MS by RS. Identifying molecular signatures associated with the disease and with different MS phenotypes (relapsing-remitting vs primary progressive, etc) will allow to improve the understanding about the molecular changes associated with the disease and to develop imaging markers as prognostic tools, as well as obtaining information that will be used for developing new neuroprotective therapies.

2. Results from Hospital Clinic of Barcelona

We recruited patients with multiple sclerosis, acute optic neuritis and healthy subjects. All of these participants were imaged using a Raman spectrophotometer coupled with a scanning laser ophthalmoscope (RS-SLO). Additionally, patients with multiple sclerosis underwent neurological examination, retinal structural imaging using optical coherence tomography (OCT) and functional evaluation of afferent visual pathway using multifocal visual evoked potentials (mfVEP).

By using RS-SLO, we quantified the following metabolites: N-acetyl-aspartate (NAA), NADH, flavin adenine dinucleotide (FAD), cytochrome C, glutamate and glutamic acid, L-alpha-phosphatidylcholine (PhosCol) in all subjects. By using SD-OCT, we quantified peripapillary retinal nerve fiber layer (pRNFL) and ganglion cell plus inner plexiform layer (GCIPL) thicknesses. Using mfVEP, we estimated latency (ms) of nerve conduction from retina to primary visual cortex.

We evaluated molecular Raman signature of the following MS phenotypes:

1. CIS/RRMS vs PMS (PPMS and SPMS). CIS/RRMS: patients with clinically isolated syndrome and patients with relapsing remitting multiple sclerosis. PPMS/SPMS: patients with primary progressive or secondary progressive multiple sclerosis
2. Eyes with previous optic neuritis vs eyes without previous optic neuritis
3. RRMS with active MS (based in the prospective follow-up of the previous 2 years and defined as presence of > relapses and > 1 gadolinium enhancing lesion) vs stable patients (no relapses and no gad+ lesions previous 2 years)
4. RRMS with disability (EDSS>2.0) compared with patients without high disability (EDSS<2)

We compared molecular concentrations by Raman spectroscopy with other well-known markers of axonal injury (pRNFL and GCIPL by OCT) and myelin damage (latency in mfVEP)

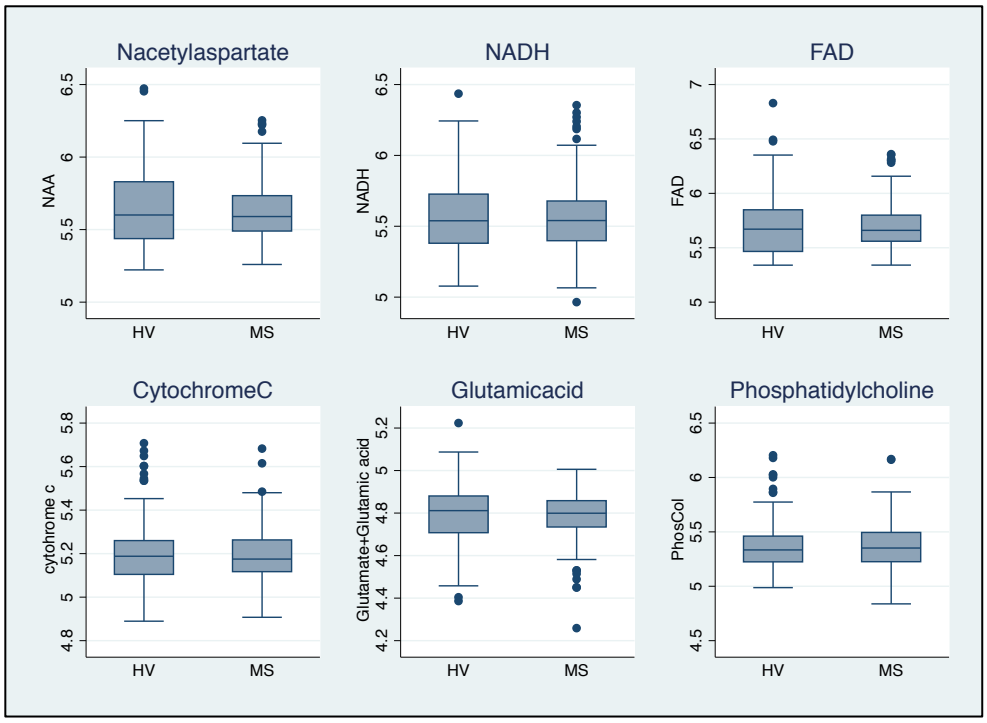
2.1 Metabolites in patients with multiple sclerosis and healthy subjects

We found that aged and sex-adjusted NAA concentration was lower in eyes from patients with MS than in HV [beta= -0.0181194 95% CI (-0.0349757 to -0.0012632); p-value= 0.035] using mixed effect models for accounting for inter-eye correlation. No other molecule was significantly different across groups:

Table 1: Description of the distribution of metabolites across healthy subjects and MS patients

	Multiple sclerosis n=270 eyes	**Healthy volunteers n=148 eyes**
N-acetyl-aspartate (NAA)	5.62 (0.19)	5.65 (0.27)
NADH	5.55 (0.23)	5.58 (0.28)
Flavin adenine dinucleotide (FAD)	5.70 (0.19)	5.71 (0.28)
Cytochrome C	5.19 (0.12)	5.20 (0.15)
Glutamate and glutamic acid	4.79 (0.10)	4.79 (0.13)
L-alpha-phosphatidylcholine	5.37 (0.21)	5.37 (0.22)

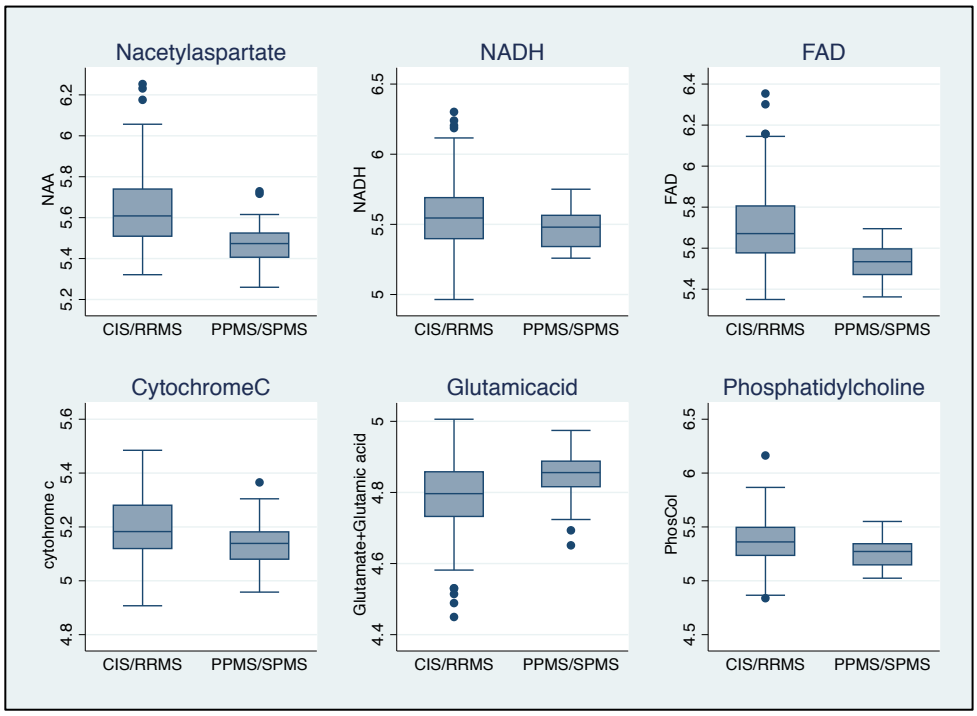
Figure 1: Graphic description of the distribution of metabolites across healthy subjects and MS patients



2.2 Metabolites in patients with multiple sclerosis with different phenotypes

We did not find any significant difference in metabolites in the proposed phenotypes in mixed effect models including age and sex as covariates. The only significant variable was age, which was highly significant in all models.

Figure 2: Graphic description of the distribution of metabolites across different MS clinical phenotypes



CIS/RRMS: patients with clinically isolated syndrome and patients with relapsing remitting multiple sclerosis.

PPMS/SPMS: patients with primary progressive or secondary progressive multiple sclerosis

Figure 3: Graphic description of the distribution of metabolites across eyes with and without ON

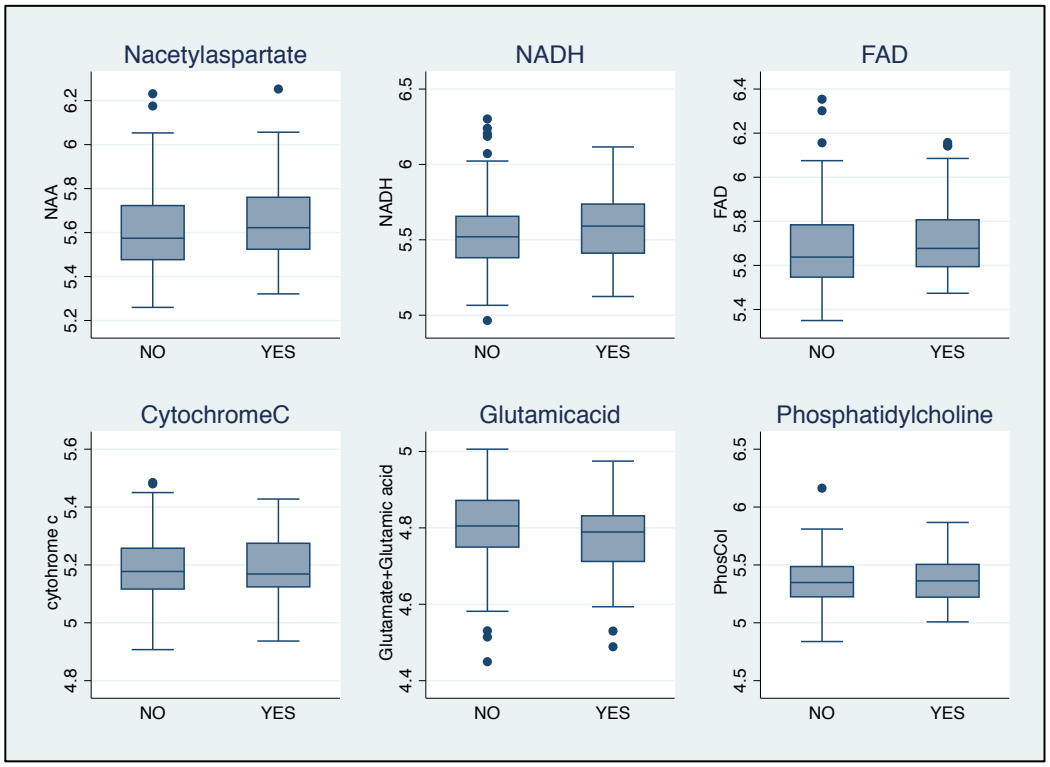


Figure 4: Graphic description of the distribution of metabolites across eyes according to disease activity

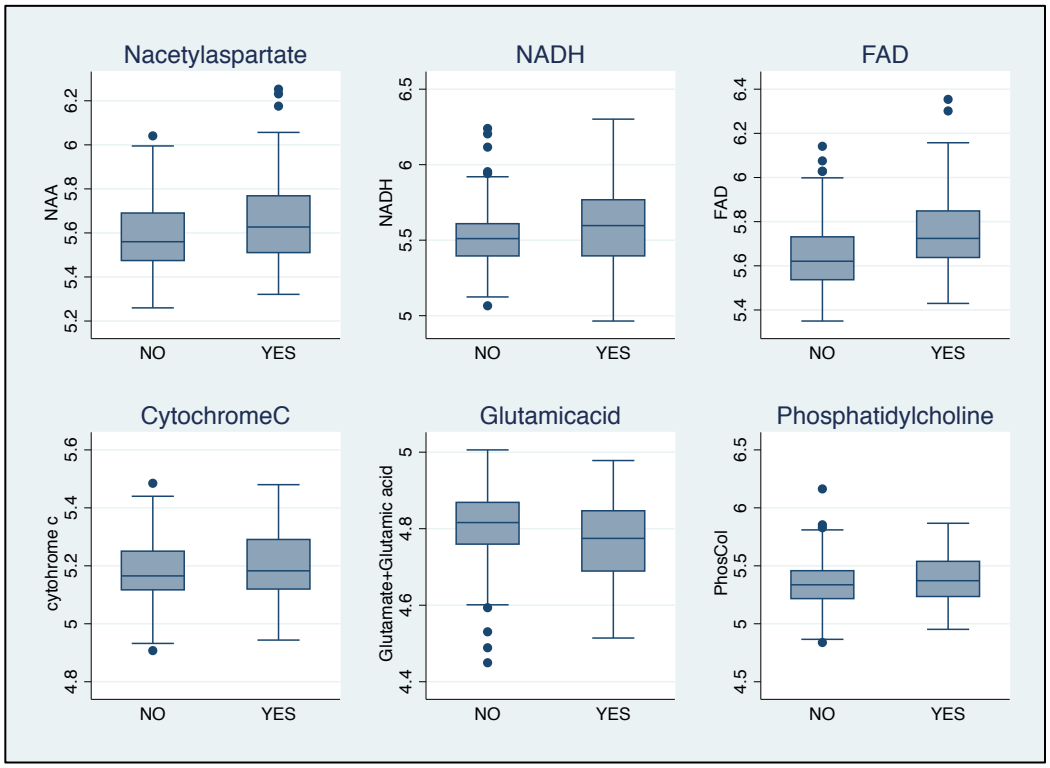
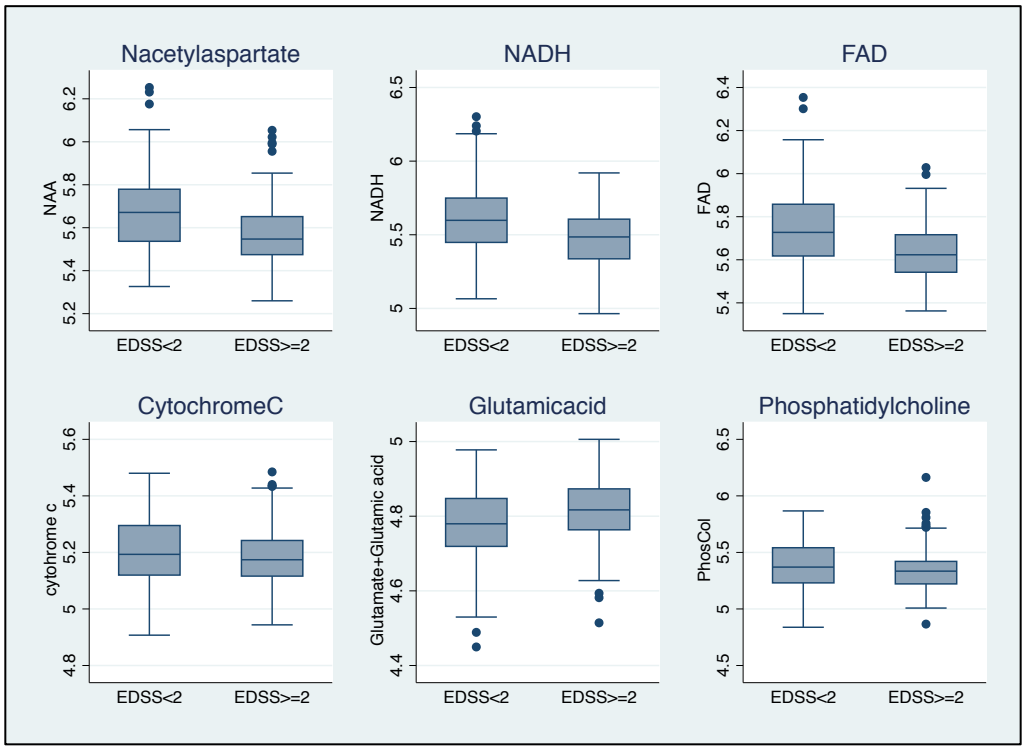


Figure 5: Graphic description of the distribution of metabolites across eyes according to disease activity



2.3 Relationship between molecular imaging in retina and other markers (OCT and mfVEP)

We did not find any association between NAA and pRNFL and GCIPL (validated markers for axonal injury) or between L-alpha-phosphatidylcholine and mean latency of multifocal visual evoked potentials (marker of nerve conduction, remyelination)

Figure 6: Graphic description of the association between pRNFL and GCIPL and NAA levels

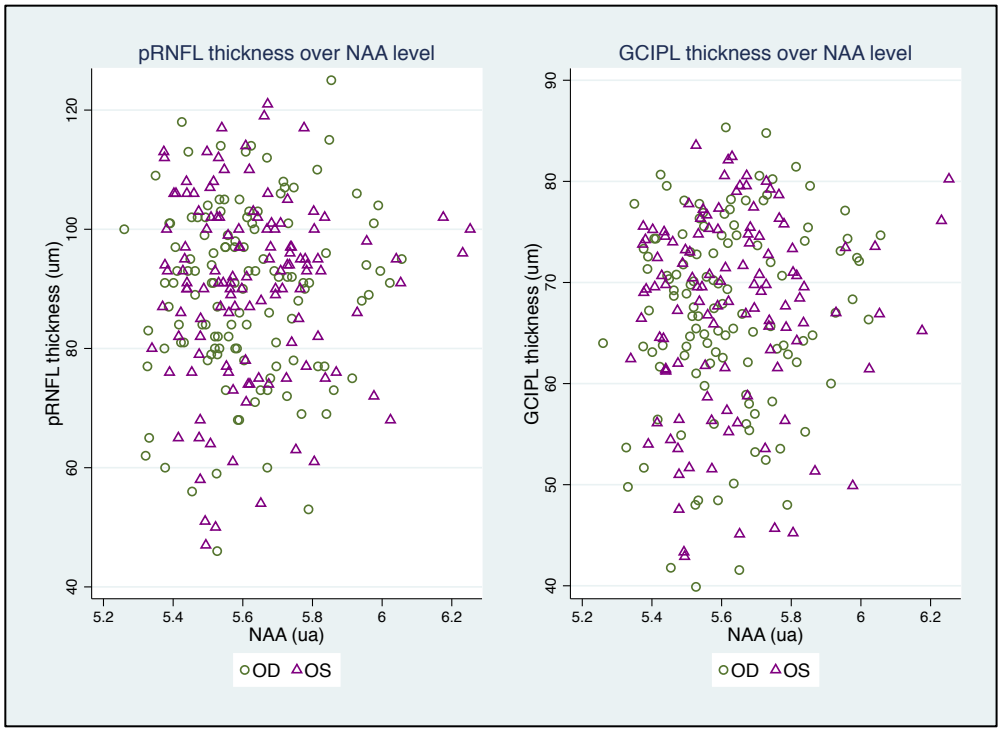
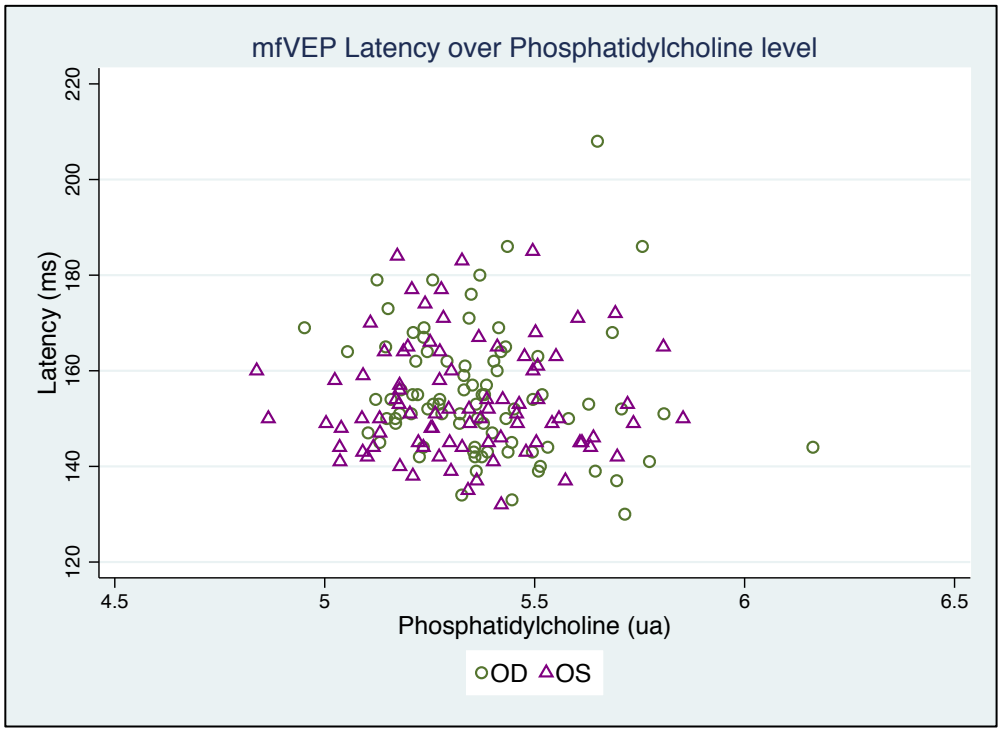


Figure 7: Graphic description of the association between mfVEP [latency] and phosphatidylcholine levels



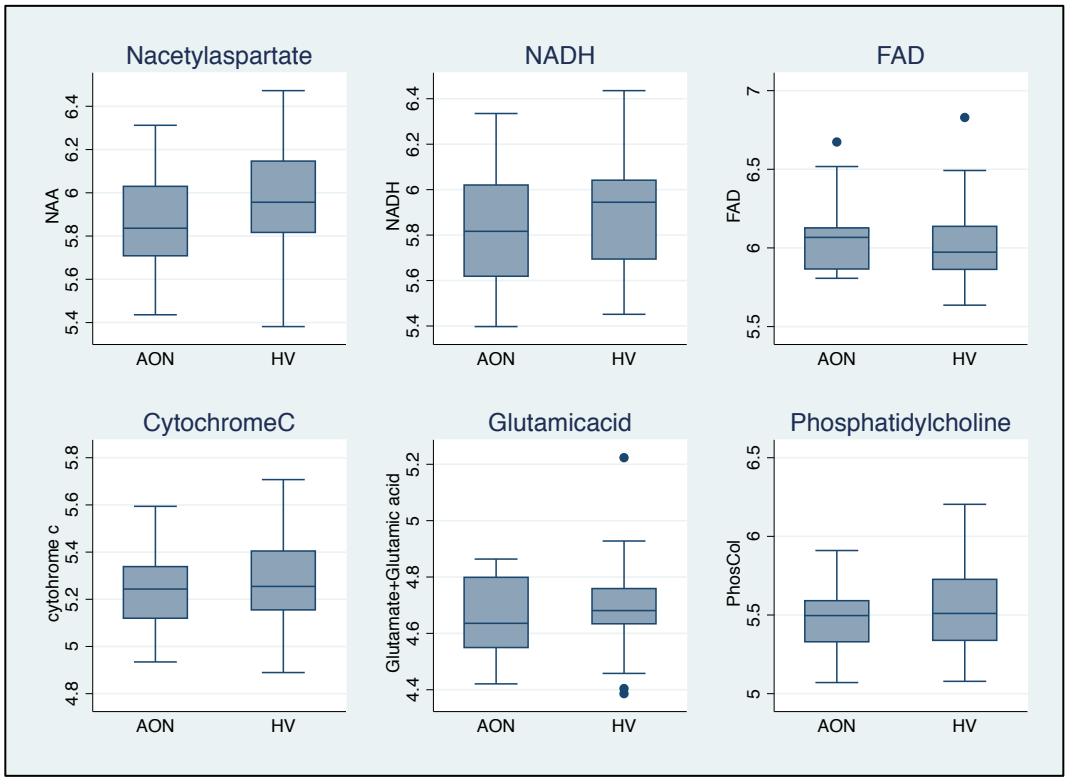
2.4 Metabolites in patients with incident acute optic neuritis

We did not find any significant difference in eyes with an acute damage and healthy volunteers.

Table 2: Description of the distribution of metabolites across healthy subjects and patients with acute optic neuritis

	Acute optic neuritis n=21 eyes	**Healthy volunteers n=44 eyes**
N-acetyl-aspartate (NAA)	5.89 (0.24)	5.95 (0.24)
NADH	5.83 (0.27)	5.88 (0.23)
Flavin adenine dinucleotide (FAD)	6.06 (0.24)	6.03 (0.23)
Cytochrome C	5.24 (0.15)	5.29 (0.20)
Glutamate and glutamic acid	4.65 (0.13)	4.69 (0.14)
L-alpha-p hosphatidylcholine	5.47 (0.24)	5.54 (0.28)

Figure 8: Graphic description of the distribution of metabolites across healthy subjects and AON patients



3. Relevance and future implications

Even though there was a mild difference in NAA between MS and healthy subjects, none of the metabolites was associated with validated clinical (EDSS, disease duration), OCT (GCIPL, pRNFL) or mfVEP (latency) markers. These results suggested that this technology is not useful for monitoring MS.

4. Publications

No publications.