Journal of Neurochemistry JNC

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Journal:	*Journal of Neurochemistry*
Manuscript ID:	JNC-2015-0354.R1
Manuscript Type:	Original Article
Date Submitted by the Author:	n/a
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Area/Section:	Molecular Basis of Disease
Keywords:	Paraoxonase-1, Paraoxonase activity, Arylesterase activity, Alzheimer's disease, vascular dementia, mild cognitive impairment

Serum Paraoxonase and Arylesterase activities of paraoxonase-1 (PON-1), mild cognitive impairment, and 2-year conversion to dementia. A pilot study.

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Keywords: Paraoxonase-1; Paraoxonase activity; Arylesterase activity; Alzheimer's disease; vascular dementia; mild cognitive impairment.

List of Abbreviations:

PON-1, Paraoxonase-1; LOAD, late onset Alzheimer's disease; VAD, vascular dementia; MCI, mild cognitive impairment; OxS, Oxidative Stress; HDL, High Density Lipoprotein; LDL, Low Density Lipoprotein; CSF, Cerebrospinal Fluid; MMSE, mini mental state examination; MCI/MCI, stable MCI patients; MCI/LOAD: MCI patients converted to LOAD; MCI/VAD: MCI patients converted to VAD; CVD: cardiovascular disease; OR, Odds Ratio; 95% CI, 95% Confidence Interval; B, unstandardized regression coefficient; SE, standard error; U/L, Units/Liter; kU/L, kilo Units/Liter.

Word count:

words in the manuscript (including figure legends): 2,995

number of figures: 1

number of references: 43

number of Tables: 3

words in the abstract: 195

Running title: paraoxonase and arylesterase activities in dementia

Abstract

Background: Converging lines of evidence suggest that paraoxonase-1 (PON-1) may confer protection against inflammatory and oxidative challenge which, in turn, plays a key-role in the onset and progression of dementia.

Objectives: To evaluate whether serum PON-1 paraoxonase/arylesterase activities might predict the clinical conversion of mild cognitive impairment (MCI) to late onset Alzheimer's disease (LOAD) or vascular dementia (VAD).

Methods: Serum paraoxonase and arylesterase activities were measured by spectrophotometric assays at baseline in 141 MCI patients (median age: 77 years; interquartile range 71-81) and in 78 healthy controls (median age: 76 years; interquartile range 73-79)

Results: After 2 years of follow-up, 86 MCI remained stable (MCI/MCI), 34 converted to LOAD (MCI/LOAD), while 21 converted to VAD (MCI/VAD). Baseline arylesterase activity was lower in all MCI groups compared with controls (all $p<0.01$), while paraoxonase activity was lower in MCI/VAD group compared to Controls ($p<0.05$) and MCI/MCI patients ($p=0.009$). Low paraoxonase and arylesterase activities (I quartile) were associated to higher risk of conversion to VAD (O.R.:3.74, 95% C.I.: 1.37-10.25 and OR: 3.16, 95% CI: 1.17-8.56, respectively).

Conclusions: Our results suggest that in MCI patients low PON-1 activity might contribute to identify individuals susceptible to develop vascular dementia.

Introduction

Dementia is one of the major causes of disability among elderly people, affecting 35.6 million people in 2010 worldwide, with doubling numbers by 2030 (Prince *et al.* 2013). Among all causes of dementia, late onset Alzheimer's disease (LOAD) and vascular dementia (VAD) accounts for 50-80% and 20-30%, respectively. The lack of well-recognized distinctive neuropsychological parameters makes it difficult to draw a differential of the two types of dementia especially in older patients (Graham *et al.* 2004). Moreover, it is well known that some markers of vascular lesion, typical of VAD, might be also altered in LOAD (Zuliani *et al.* 2008); likewise, amyloid β (Aβ) plaques, *i.e.* a classical pathological hallmark of Alzheimer disease, have been found, at a minor extent, also in patients with cerebrovascular disease (De La Torre 2004).

Although the pathogenesis of LOAD and VAD is far to be completely elucidated, there are plenty of evidence suggesting that oxidative stress (OxS) and inflammation might be mutually implicated in the development of both diseases (Bennett *et al.* 2009; Cervellati *et al.* 2014c; Cervellati *et al.* 2013). These two patho-physiological conditions result from a derangement of a pre-existing balance between contrasting forces, with pro- (oxidant and inflammatory) agents prevailing against the protective ones (Bergamini *et al.* 2004).

Paraoxonase- 1 (PON-1) is an enzyme present on the surface of high density lipoprotein (HDL) cholesterol (James 2006), which possesses three distinct catalytic activities: 1. paraoxonase, also named organophosphatase, hydrolyzing organophosphates and pesticides; 2. arylesterase, catalyzing the hydrolysis of non-phosphorous arylesters; and 3. lactonase, directed towards lactones which are considered as its primary substrates (Khersonsky and Tawfik 2006). It is well recognized that this ester hydrolase is one of the main responsible of antioxidant and anti-inflammatory properties of this lipoprotein. Indeed, several lines of evidence suggest that PON-1 protects low density lipoprotein (LDL) (Mackness *et al.* 1991), HDL, endothelium, and macrophages against OxS, and also stimulate HDL-mediated cholesterol efflux from macrophages (Rosenblat *et al.* 2006b). Accordingly, low serum level of PON-1 enzymatic activity has been found to be associated with a

risk of adverse cardiovascular events in several population-based studies (Bhattacharyya *et al.* 2008; Martinelli *et al.* 2009; Tang *et al.* 2012). More generally, subjects with an impaired arylesterase/paraoxonase activity appear to be more susceptible to develop diseases in which oxidative damage and lipid peroxidation are involved compared with subjects with higher PON1 activity (Mastorikou *et al.* 2008).

The above considerations might account for our previous findings of a significant decrease in PON-1 serum activity in patients with LOAD or VAD (Cervellati *et al.* 2014a). Noteworthy, a reduction of serum arylesterase also emerged in patients with mild cognitive impairment (MCI), the symptomatic stage often preceding the onset of dementia (Cervellati *et al.* 2014a). It is intriguing to hypothesize that in MCI patients decreased levels of PON-1 may result in a weaker defense against reactive species, which in turn lead to the formation of high amount of lipoperoxidation by-products.

Thus far, most studies dealing with PON-1 and dementia focused on cross-sectional comparisons between demented patients and controls, thus failing to prove a possible causative role of this HDL-bound enzyme in dementia development. The aim of this pilot longitudinal study was to evaluate the potential of Paraoxonase and Arylesterase activities of PON-1 to predict the conversion from MCI to LOAD or VAD.

Methods

Patients selection

One hundred forty one patients referring to the Day Service for Cognitive Decline (University of Ferrara, Italy) or to the Geriatric Unit of the IRCCS "Casa Sollievo della Sofferenza" (San Giovanni Rotondo, Italy) were enrolled into the study from 2009 to 2014 and followed for a mean period of 2 years (2.0±0.7years). The diagnosis of MCI was made by trained geriatricians based on the presence of short/long-term memory impairment, with/without impairment in other

single or multiple cognitive domains, in an individual who didn't meet the standardized criteria for dementia (Petersen *et al.* 2001). Mini mental state examination (MMSE) along with a battery of neuropsychological tests was used to evaluate the degree of cognitive impairment (Zuliani *et al.* 2007). Patients with MCI due to known causes (*e.g.* depression, extensive white matter pathology, vitamin B_{12} deficiency) were excluded. Subjects affected by severe congestive heart failure (New York Heart Association class III-IV), severe liver or kidney disease, severe chronic obstructive pulmonary disease, and cancer, and those taking NSAIDS, antibiotics or steroids at the time of recruitment were also excluded. Based on the clinical evolution at follow-up, MCI patients were categorized into MCI/MCI (n: 86, 47 women and 39 men), if cognitive performance of the patient remained stable or improved; MCI/LOAD (n: 34, 19 women and 15 men), if patient converted to LOAD according to the NINCDS-ADRDA criteria (McKhann *et al.* 1984); MCI/VAD (n: 21, 10 women and 11 men), if patient was diagnosed as having a VAD by the NINDS-AIREN criteria (Román *et al.* 1993).

In addition, 78 older subjects (50 women and 28 men) without symptoms of dementia and without any functional disability attributable to cognitive issues were enrolled as controls.

Diagnosis of diabetes, hypertension and cardiovascular disease (CVD), as well as current smoking (yes/no) were performed as described elsewhere (Zuliani *et al.* 2007).

All patients (controls and MCI) underwent a brain Computer Tomography (CT) scan at baseline performed with a third-generation SIEMENS SOMATONHQ (10 mm thickness), and the CT information was used to support the clinical diagnosis and to diagnose possible brain pathologies associated with secondary cognitive impairment.

This study conforms to The Code of Ethics of the World Medical Association (Declaration of Helsinki) and was conducted accordingly to the guidelines for Good Clinical Practice (European Medicines Agency). Written informed consent was obtained from each patient during the first office visit at baseline before the possible inclusion in the study. No identifying information were available to the Authors of the study in order to protect the anonymity of the patients.

Serum sampling and biochemical assays

Venus blood samples from patients was collected after overnight fasting and centrifuged at 1500g for 10 minutes. Serum was aliquoted and stored at -80°C until analysis.

Serum paraoxonase and arylesterase activities were measured by UV-VIS spectrophotometric assays in a 96-well plate format by using a Tecan Infinite M200 microplate reader (Tecan group Ltd, Switzerland).

Briefly, paraoxonase activity was determined by continuous monitoring the increase in the absorbance at 412 nm caused by 4-nitrophenol formation after addition of 5 µl of serum, diluted 49 times, in 245 µl of reaction mixture consisting in 1.5 mM paraoxon (Cat. No. 855790, Sigma-Aldrich, Milan, Italy), 0.9 M NaCl, and 2 mM $CaCl_2$ dissolved in 10 mM Tris-HCl, pH 8. A molar extinction coefficient of 17000 M^{-1} cm^{-1} was used for the calculation of enzyme activity, expressed in units per liter. One unit of paraoxonase activity is defined as 1 µmol of 4-nitrophenol formed per minute under the given conditions.

The arylesterase activity was measured by adding 10 µL of serum, diluted 24 times, to 240 µL of reactions mixture composed by 1 mM phenylacetate and 0.9 mM $CaCl_2$ dissolved in 9 mM Tris-HCl, pH 8. A molar extinction coefficient of 1310 M^{-1} cm^{-1} was used for the calculation of enzyme activity, expressed in kilo unit per liter. One unit of arylesterase activity accounts for 1 µmol of phenol produced in a minute under the conditions of the assay.

Statistical analysis

The normality distribution of the variables was checked by the Kolmogorov-Smirnov test. Variables not normally distributed were presented as median (interquartile range) and group comparisons were performed using Kruskall-Wallis followed by Mann-Whitney U tests (Bonferroni correction for multiple comparisons). Categorical variables are presented as numbers and percentages, and differences in frequency distributions were examined using the Chi-square test.

Multinomial logistic regression analysis was used to assess the effects of low levels of paraoxonase activity (<44.16 U/L = first quartile in MCI patients) or arylesterase activity (<56.44 kU/L = first

quartile in MCI patients) on the risk of 2-year conversion from MCI to LOAD or VAD. The analysis was adjusted for possible confounders including age, gender, smoking habit, hypertension, CVD, and diabetes. All analyses were conducted using SPSS 21.0 for windows (SPSS Inc., Chicago, Illinois, USA). A $p<0.05$ was considered statistically significant.

Results

The clinical characteristics of patients and the levels of paraoxonase and arylesterase activity in MCI/MCI, MCI/LOAD, MCI/VAD, and in non-demented controls are summarized in Table 1. No significant difference emerged as regards to mean age, gender, MMSE score or the prevalence of hypertension, smoking habit, diabetes, and CVD between the groups examined. The years of formal education were lower in MCI/VAD and MCI/MCI patients compared to controls (Kruskal-Wallis p:0.001). On the whole, MCI patients had lower median arylesterase and paraoxonase levels compared with controls but only for arylesterase the difference was significant ($p<0.0001$).

As regards to the single MCI sub-groups, lower levels of paraoxonase were found only in MCI/VAD compared to controls (Mann-Whitney $p<0.05$), while lower levels of arylesterase activity were observed in MCI/MCI, MCI/LOAD, and MCI/VAD compared to controls (Mann-Whitney all $p<0.001$). When we focused on possible differences in PON-1 activities between MCI sub-groups, we found a significant overall difference for paraoxonase (Kruskal-Wallis $p<0.05$), and a trend toward a statistical difference for arylesterase (Kruskal-Wallis $p=0.06$). As depicted in Figure 1, paraoxonase activity was significantly different only in MCI/VAD compared to MCI/MCI patients ($p=0.009$), while no differences emerged as regards to arylesterase.

As reported in Table 2, low arylesterase activity (<56.44 kU/L: I quartile) was more frequent in MCI/VAD patients compared with both MCI/MCI and MCI/LOAD (47.6% vs. 22.4% and 12.1%, respectively; $p<0.05$). As regards paraoxonase activity, a not significant trend toward a higher

prevalence of low activity levels (<44.16 U/L: I quartile) was observed in MCI/VAD compared to MCI/MCI (45.0% vs. 20.9%).

Finally, by multinomial logistic regression we tested whether low baseline levels of serum paraoxonase and arylesterase activity were useful in predicting the 2-year risk of conversion from MCI to LOAD or VAD. As reported in Table 3, after multivariate adjustment for age, gender, smoking habit, CVD, hypertension, and diabetes low levels of paraoxonase activity were significantly associated with an increased likelihood of converting to VAD (Model 1: MCI/VAD vs MCI/MCI, Odds Ratio (OR): 3.74, 95% Confidence Interval (CI): 1.37-10.25) but not LOAD. Likewise, low levels of arylesterase resulted associated with the risk of conversion to VAD (Model 2: MCI/VAD vs MCI/MCI, OR: 3.16, 95%CI: 1.17-8.56), but not LOAD.

Discussion

A large body of studies, although with some controversies (Pi *et al.* 2012), suggest that low PON-1 activity may be associated with an increased risk of developing LOAD or VAD (Wehr *et al.* 2009; Cervellati *et al.* 2014a; Alam *et al.* 2014; Zengi *et al.* 2012; Paragh *et al.* 2002; Erlich *et al.* 2012; Bednarska-Makaruk *et al.* 2013; Dantoine *et al.* 2002). However, it is important to underscore that this compelling indication arises from cross-sectional studies. Thus, the cumulating evidence makes still difficult to draw a firm conclusion regarding the possible causal nature of PON-1 impairment with respect to the onset of dementia. Nevertheless, our and others' recent finding of a trend toward lower levels of PON-1 activity in MCI compared to controls (Dantoine *et al.* 2002; Wehr *et al.* 2009; Cervellati *et al.* 2014a) was interpreted as a meaningful clue in favor of a precocious role of this enzyme in the pathogenic mechanisms of LOAD or VAD. The above considerations sparked the present investigation, which aimed to evaluate whether low levels of PON-1 arylesterase and/or paraoxonase activity might be related to a worse clinical course in MCI subjects.

From the comparison of the median values, it already emerged that low arylesterase activity was associated with MCI diagnosis, independently of the subgroup considered, while paraoxonase was

reduced only in those MCI that converted to VAD during the 2-year follow-up. Most importantly, in MCI patients low serum levels of both PON-1 activities were independently associated with a higher likelihood of developing VAD, but not LOAD.

There are several lines of *in vitro* and *in vivo* evidence (Kasprzak *et al.* 2009; Rosenblat *et al.* 2006a; Aviram *et al.* 1998; Bhattacharyya *et al.* 2008; Tang *et al.* 2012) demonstrating that arylesterase and paraoxonase are two distinct and poorly, although significantly, related catalytic functions of PON-1 (Spearman's r in our sample: 0.30). Although our previous work was, to the best of our knowledge, the unique to measure both serum activities in relation to the diagnosis of dementia, we found an overall consistency of the results with other large studies dealing with this topic (Wehr *et al.* 2009; Bednarska-Makaruk *et al.* 2013; Erlich *et al.* 2012). While arylesterase was repeatedly found to be associated with a LOAD diagnosis, paraoxonase resulted to be related with VAD in other two works (Alam *et al.* 2014; Paragh *et al.* 2002). Consistently, the most extensively explored PON-1 coding single nucleotide polymorphisms (i.e. rs662 and rs854560), which strongly influence paraoxonase rather than arylesterase activity (Tang *et al.* 2012), failed to be associated with susceptibility to develop LOAD based on the available published studies (Pi *et al.* 2012). This discrepancy is not completely surprising since, as shown by Rosemblat's study on recombinant PON-1 (Rosenblat *et al.* 2006a), these two biochemical activities appear to be disconnected to each other, with arylesterase, but not paraoxonase, being correlated to lactonase function. Although the in vivo substrate(s) of this enzyme is still unclear, lipolactonase activity has recently emerged as the native catalytic activity of PON-1 which, in turn, help HDL particles to prevent the accumulation of lipid peroxides in oxidized LDLs (Rosenblat *et al.* 2006a). In line with this reasoning, it may be speculated that the impairment of arylesterase in MCI patients might play a role in the derangement of systemic oxidative balance that affect these individuals regardless of their progression to dementia (Baldeiras *et al.* 2010; Cervellati *et al.* 2014b).

Nonetheless, it must be underlined that also paraoxonase may contribute, to a smaller extent, to the antioxidant mechanisms of HDLs (Zengi *et al.* 2012; Bhattacharyya *et al.* 2008). This is an

important point, since the ability to protect LDLs and HDLs from oxidative challenge seems to partially mediate by other anti-atherogenic functions of PON-1 including stimulation of eNOS-dependent NO production (Besler *et al.* 2011) with subsequent endothelial anti-inflammatory effects, and enhancement of cholesterol efflux from cholesterol-laden macrophages (Tang *et al.* 2012). These biological functions rather than the mere concentration of HDLs, might be the effective player in the protection afforded by these lipoproteins against CVD (Rohatgi *et al.* 2014; Holzer *et al.* 2013). As a matter of fact, the role of PON-1 in atherosclerosis was demonstrated in mice lacking (Shih *et al.* 1998) or over-expressing PON-1 (Tward *et al.* 2002). Successively it was demonstrated that both activities were independently related to a lower risk of major cardiac event in humans (Bhattacharyya *et al.* 2008; Tang *et al.* 2012). All these athero-protective activities of PON 1 might account for our finding of arylesterase and, mostly, paraoxonase as predictive marker of conversion from MCI to VAD.

At present, although the existence of overlaps are becoming increasingly clear, LOAD and VAD are still regarded as two separate diseases. By definition, VAD is referred as "a disease with a cognitive impairment resulting from cerebrovascular disease and ischemic or hemorrhagic brain injury" (Iemolo *et al.* 2009), with the deprivation of oxygen and nutrients that eventually leads to neurons death. Atherosclerosis is the most important cause of cerebrovascular damage leading to VAD. Combining our results with these notions, it is tempting to hypothesize that a low level of PON-1 activity may reflect in a weaker physiological defense against pro-atherogenic processes, thus increasing the risk of VAD onset.

Finally, this study has important limitations that must be acknowledged.

First, although statistically significant, the small sample size limits the overall reliability of our results; of consequence, a replication of the results in a larger sample is warranted. Second, the lack of complete data on lipid profile also represent a caveat of this study. However, in our previous large cross-sectional study HDL-C did not interfere with PON 1 in multivariate analysis (Cervellati *et al.* 2014a). Moreover, several studies failed to find a significant correlation between PON-1

activity and plasma lipid concentration (Ayub *et al.* 1999; Kasprzak *et al.* 2009), most probably cause of the structural and functional modifications occurring in HDL particles during diseases (Ferretti *et al.* 2006). Third, we did not assay the lactonase activity of PON-1, which represents the physiological activity of the enzyme (Khersonsky and Tawfik 2006), considering also the new evidence for a protective role of PON-1 against neurodegeneration led by homocysteine thiolactone and hyperhomocysteinemia (Borowczyk K *et al.* 2012), a condition present also in VAD (Cervellati *et al.* 2014c). Finally, the lack of MRI data, i.e. of a precise measure of the burden of cerebrovascular lesions did not allow us to identify possible relationships between PON-1 activities and the severity of cerebrovascular disease. However, the results from the CT scan, although much less sensitive compared to MRI, did not highlight any difference between subjects with or without cerebrovascular lesions (data not shown).

In conclusion, our study although limited by the relatively small sample size, adds to the current literature since it is the first study showing that low serum arylesterase and, mostly, paraoxonase activity of PON-1 may be helpful in identifying, among MCI patents, those individuals with a higher risk to progress to VAD.

Acknowledgements and conflict of interest disclosure

The authors thank Ilaria Crivellari for her valuable contribution in making the summarizing schematic for the "In this issue" section.

The Study was supported by “Local Research Project” grant from University of Ferrara, Italy. The authors have no conflict of interest to declare.

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Figure Legend

Figure 1. Box plot analysis for serum Paraoxonase (panel A) and Arylesterase (panel B) activity in 86 MCI not converting to dementia (MCI/MCI), in 34 MCI converting to LOAD (MCI/LOAD), and in 21 MCI converting to VAD (MCI/VAD) after 2 years follow-up. The boundaries of the box represent the 25^{th}-75^{th} percentile. The line within the box indicates the median. The whiskers above and below the box correspond to the highest and lowest values, excluding outliers.

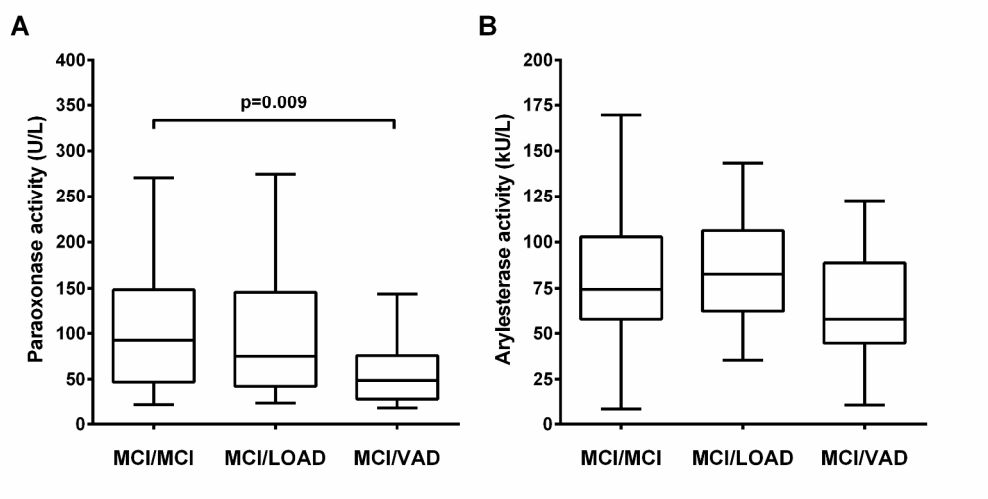


Figure 1. Box plot analysis for serum Paraoxonase (panel A) and Arylesterase (panel B) activity in 86 MCI not converting to dementia (MCI/MCI), in 34 MCI converting to LOAD (MCI/LOAD), and in 21 MCI converting to VAD (MCI/VAD) after 2 years follow-up. The boundaries of the box represent the 25th-75th percentile. The line within the box indicates the median. The whiskers above and below the box correspond to the highest and lowest values, excluding outliers.
260x131mm (300 x 300 DPI)

Table 1: Principal characteristics of non-demented healthy controls, MCI/MCI, MCI/LOAD and MCI/VAD patients.

	CONTROLS (n=78)	MCI (n=141)	MCI/MCI (n=86)	MCI/LOAD (n=34)	MCI/VAD (n=21)
Age (years)	76 (73-79)	77 (73-82)	77 (71-81)	78 (74-83)	79 (75-82)
Gender (females, %)	64	54	55	56	48
Education (years)[c]	8 (5-13)	5 (4-8)	5 (4-8)	5 (5-8)	5 (3-8)
MMSE score	26.4 (25.0-28.0)	25.9 (24.0-27.0)	26.3 (25.0-27.0)	25.0 (23.2-25.6)	25.1 (24.7-25.9)
Hypertension (%)	64	58	58	56	62
Smoking (%)	5	5	5	9	0
Diabetes (%)	16	21	19	21	33
CVD (%)[a]	15	30	32	21	33
PON-1 (U/L)[b]	77.2 (46.3-161.9)	74.0 (43.8-141.2)	92.5 (46.8-148.5)	75.0 (42.24-145.9)	48.7 (28.3-75.0)
Arylesterase (kU/L)[c, d]	104.1 (84.9-122.8)	72.6 (56.4-103.1)	74.4 (57.8-102.7)	82.7 (61.1-106.1)	57.8 (44.7-88.7)

Not-normally distributed variables are expressed as median (interquartile range), discrete variables as percentage. MCI/MCI: stable MCI patients; MCI/LOAD: MCI patients converted to LOAD; MCI/VAD: MCI patients converted to VAD; CVD: cardiovascular disease; MMSE: Mini Mental State Examination.

[a] $p<0.05$ χ^2, controls vs. MCI

[b] p<0.05 Kruskal-Wallis between controls, MCI/MCI, MCI/LOAD and MCI/VAD

[c] p<0.001 Kruskal-Wallis between controls, MCI/MCI, MCI/LOAD and MCI/VAD

[d] p<0.01 Mann-Whitney, controls vs. MCI

Table 2: Prevalence of low levels (first quartile) of Paraoxonase and Arylesterase activity in MCI/MCI, MCI/LOAD, and MCI/VAD patients.

	MCI/MCI	MCI/LOAD	MCI/VAD	Chi-square (df); *p*
Paraoxonase activity	20.9%	30.3%	45.0%	5.65 (2); ***0.05***
Arylesterase activity	22.4%*	12.1%*	47.6%	9.17 (2); ***0.01***

* $p<0.05$ vs MCI/VAD

MCI/MCI: stable MCI; MCI/LOAD: MCI to LOAD; MCI/VAD: MCI to VAD.

Cut-off value for Paraoxonase activity: 44.16 U/L. Cut-off value for Arylesterase activity: 56.44 kU/L.

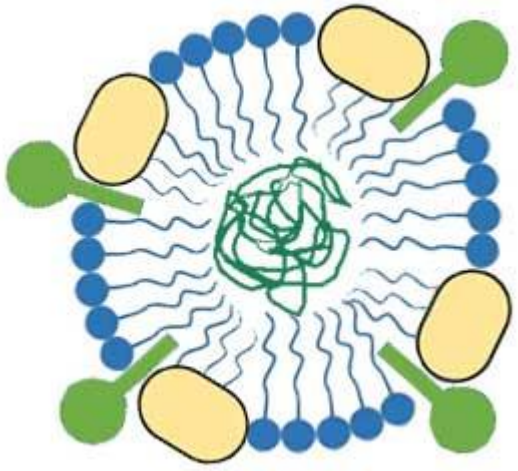
Table 3. Multinomial logistic regression for the risk of conversion from MCI to late onset Alzheimer's disease (LOAD) or vascular dementia (VAD) in subjects with low levels (first quartile) of paraoxonase or arylesterase activity.

Model 1:Paraoxonase	**B (SE)**	**Odds Ratio (95% CI)**
CONVERSION TO LOAD		
Intercept	-5.91 (2.88)*	
Low paraoxonase activity	0.49 (0.48)	1.63 (0.64-4.19)
CONVERSION TO VAD		
Intercept	-21.24 (3.32)**	
Low paraoxonase activity	1.26 (0.54)*	**3.51 (1.22-10.06)**
Model 2:Arylesterase	**B (SE)**	**Odds Ratio (95% CI)**
CONVERSION TO LOAD		
Intercept	-6.29 (2.93)*	
Low arylesterase activity	-0.85 (0.63)	0.42 (0.12-1.46)
CONVERSION TO VAD		
Intercept	-22.07 (3.39)**	
Low arylesterase activity	1.05 (0.54)*	**2.86 (1.12-8.18)**

For Model 1: R^2 = .125 (Cox & Snell), .148 (Nagelkerke).
For Model 2: R^2 = .139 (Cox & Snell), .165 (Nagelkerke).
* $p<0.05$, ** $p<0.01$.

Cut-off value for paraoxonase activity = 44.16 U/L; cut-off value for arylesterase activity = 56.44 kU/L. B: unstandardized regression coefficient; SE: standard error; 95% CI: 95% confidence interval.
Both models were corrected for possible confounders including: age, sex, smoking, hypertension, diabetes and cardiovascular diseases, with low paraoxonase or arylesterase activity as predictors and MCI/MCI, MCI/LOAD and MCI/VAD as dependent variable.

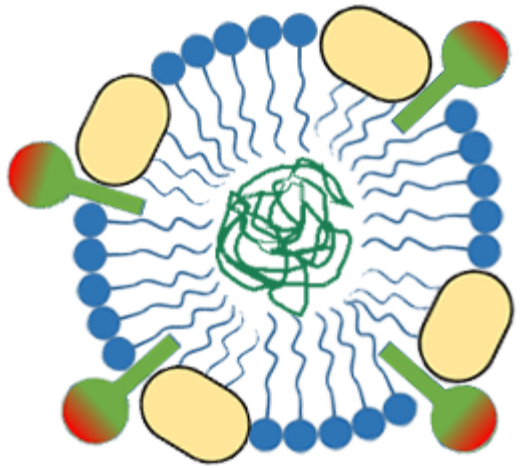
MCI patients with Normal PON-1 activity



- ↓ LDL oxidized
- ↓ Oxidative Stress
- ↓ Inflammation
- ↑ Cholesterol Efflux

stable MCI or Alzheimer's Disease

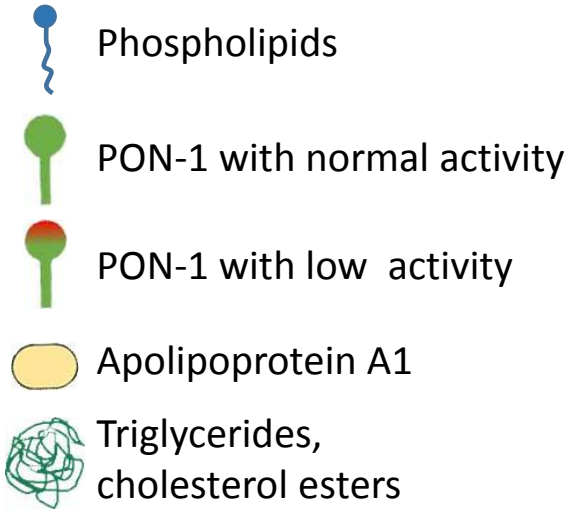
MCI patients with Low PON-1 activity



- ↑ LDL oxidized
- ↑ Oxidative Stress
- ↑ Inflammation
- ↓ Cholesterol Efflux

Vascular Dementia

Legend



Our study showed that in patients with mild cognitive impairment (MCI) low serum levels of PON-1 activitiy is associated with a higher likelihood of developing Vascular Dementia, but not Alzheimer's Disease. The observed connection might be explained by the ability of PON-1 to retard LDL oxidation, decrease oxidative stress, attenuate inflammation and increase cholesterol efflux.