Aging Clinical and Experimental Research

Hydroperoxides serum levels and multimorbidity among older patients with mild cognitive impairment or Late Onset Alzheimer's Disease

--Manuscript Draft--

Manuscript Number:	ACER-D-14-00184R2
Full Title:	Hydroperoxides serum levels and multimorbidity among older patients with mild cognitive impairment or Late Onset Alzheimer's Disease
Article Type:	Original Paper
Abstract:	Background: Oxidative stress (OxS) might be involved in the pathogenesis of late onset Alzheimer disease (LOAD); noteworthy, the presence of multimorbidity is regarded as a common denominator of OxS and dementia. Aim: to evaluate the contribution of multimorbidity to OxS in LOAD and mild cognitive impairment (MCI). Methods: serum hydroperoxides and multimorbidity (CIRS-CI scale) were evaluated in 46 Controls, 104 MCI and 75 LOAD. Results: a trend toward an increase of hydroperoxides from Controls to MCI to LOAD was observed (LOAD vs Controls p=0.01). This OxS marker was positively and significantly correlated with CIRS-CI in Controls (p= 0.002) and patients with MCI (p= 0.005) but not in those with LOAD (p= 0.104). Conclusions: multimorbidity is associated with systemic OxS but only in elderly people with either no or mild cognitive impairment. Although OxS is elevated in LOAD patients, its association with multimorbidity seems to be negligible, confirming the existence of strong disease-specific pro-oxidant mechanisms.
Corresponding Author:	Giovanni Zuliani ITALY
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	
Corresponding Author's Secondary Institution:	
First Author:	Carlo Cervellati
First Author Secondary Information:	
Order of Authors:	Carlo Cervellati
	Arianna Romani
	Cristina Bosi
	Stefania Magon
	Angelina Passaro
	Carlo M Bergamini
	Giovanni Zuliani
Order of Authors Secondary Information:	
Author Comments:	Ferrara, January 13, 2015 Dear Editor: We are submitting the revised version of the manuscript entitled "Hydroperoxides serum levels and multimorbidity among older patients with mild cognitive impairment or Late Onset Alzheimer's Disease". The manuscript has been checked for typesetting and grammar errors as requested by

	the reviewer. Best regards, Giovanni Zuliani
Response to Reviewers:	AS requested by the reviewer, we checked and corrected typos and grammar errors throughout the manuscript

Hydroperoxides serum levels and multimorbidity among older patients with mild cognitive impairment or Late Onset Alzheimer's Disease

Carlo Cervellati PhD[1], Arianna Romani BSc[1], Cristina Bosi MLT[2], Stefania Magon MD[2], Angelina Passaro MD[2], Carlo M. Bergamini MD, PhD[1], and Giovanni Zuliani MD, PhD[2*]

[1] Department of Biomedical and Specialist Surgical Sciences, Section of Medical Biochemistry, Molecular Biology and Genetics University of Ferrara, 44121, Ferrara, Italy

[2] Department of Medical Science, Section of Internal Medicine, Gerontology, and Clinical Nutrition, University of Ferrara, 44100, Ferrara, Italy

***Corresponding Author:**

Prof. Giovanni Zuliani MD PhD

Section of Internal and CardioRespiratory Medicine,

Department of Medical Sciences,

University of Ferrara, Via Aldo Moro 8, 44124, Ferrara, ITALY

Tel: 39-0532-237018; Fax: 39-0532-210884

e-mail: zlngnn@unife.it gzuliani@hotmail.com

ABSTRACT

Background: Oxidative stress (OxS) might be involved in the pathogenesis of late onset Alzheimer disease (LOAD); noteworthy, the presence of multimorbidity is regarded as a common denominator of OxS and dementia.

Aim: to evaluate the contribution of multimorbidity to OxS in LOAD and mild cognitive impairment (MCI).

Methods: serum hydroperoxides and multimorbidity (CIRS-CI scale) were evaluated in 46 Controls, 104 MCI and 75 LOAD.

Results: a trend toward an increase of hydroperoxides from Controls to MCI to LOAD was observed (LOAD vs Controls p=0.01). This OxS marker was positively and significantly correlated with CIRS-CI in Controls (p= 0.002) and patients with MCI (p= 0.005) but not in those with LOAD (p= 0.104).

Conclusions: multimorbidity is associated with systemic OxS but only in elderly people with either no or mild cognitive impairment. Although OxS is elevated in LOAD patients, its association with multimorbidity seems to be negligible, confirming the existence of strong disease-specific pro-oxidant mechanisms.

Keywords

Alzheimer's disease; hydroperoxides; mild cognitive impairment; multimorbidity; oxidative stress.

Introduction

The most important risk factor for the incidence of dementia is aging [1,2], and oxidative stress (OxS) is widely regarded as one of the most important biological features of the ageing process [3,4]. Although it has not been yet elucidated the real nature of its involvement, a pivotal role of OxS in the pathogenesis of most of the diseases affecting older people is well-recognized [4]. Recent data from our and other's studies have demonstrated that OxS is strictly associated with late onset Alzheimer disease (LOAD)[3,5]. Notably, OxS has been also related to the presence of mild cognitive impairment (MCI), an intermediate stage between the expected cognitive decline of normal aging and dementia [6]. Moreover, in separate studies, higher levels of peripheral OxS markers have been found to be associated with a decreased physical performance in older people [7].

Because of their advanced age, the most demented patients are affected by multimorbidity, that is the co-occurrence of multiple diseases or medical conditions within a single person [8]. Noteworthy, multimorbidity might be involved not only in the development of OxS condition [9], but also in the pathogenesis and clinical presentation of dementia, by damaging central nervous system and increasing the rate of cognitive/physical decline [10]. Of consequence, although previous studies have considered the possible confounding effects of some specific diseases (e.g. diabetes and hypertension), it is not clear whether the systemic OxS observed in demented older subjects might actually result from dementia itself, from the effect of multimorbidity, or from both of these conditions.

In the present study we tried to evaluate the possible contribution of multimorbidity to systemic OxS in older individual affected by dementia. To this aim we evaluated a large sample of older individuals affected by LOAD or MCI, and compared them to a sample of cognitively normal older adults.

Materials and methods

2.1. Subjects

Two hundred twenty five outpatients referring to the Day Service for Cognitive Decline (University of Ferrara, Italy) were enrolled into this study from 2006 to 2013, including:

1) Forty-six Patients with LOAD by the National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria [11]. Only patients with "probable" Alzheimer's disease were included to increase specificity.
2) One hundred and four older subjects with MCI. This condition was defined as 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 [12].
3) Seventy-five older cognitively normal older adults individuals (Controls) without evidence of cognitive impairment nor of functional impairment attributable to cognitive deficits.

This study was carried out accordingly to the Declaration of Helsinki (World Medical Association, http://www.wma.net), the guidelines for Good Clinical Practice (European Medicines Agency, http://www.ema. europa.eu). All the participants (and/or their caregiver if demented) were informed about the research project and signed an informed consent.

Personal data and medical history were collected by a structured interview from patients and caregivers. All patients underwent a general and neurological examination. For neuropsychological assessment, all patients were given a battery of tests as previously described [4]. Clinical-chemistry analyses (serum B-12 vitamin, serum folate, etc.) were routinely performed to exclude other causes of cognitive impairment. Subjects with diagnosis of severe congestive heart failure, severe liver or kidney disease, severe chronic obstructive pulmonary and cancer or taking non-steroidal anti-Inflammatory drug (NSAIDS), antibiotics or steroids were excluded.

2.2. Functional status, cognitive status, and comorbidities index assessments

Functional status was measured at two levels:

- Basic activity daily living (BADLs) [13], assessed by considering the following six tasks: bathing, dressing, toileting, maintaining continence, feeding, and transferring (Maximum score 6/6).
- Instrumental activities of daily living (IADLs) [13], evaluated by assessing six tasks as follows: use of telephone, managing shopping, medications, finances, and use of transportation. (Maximum

score 19/19). The global cognitive status was assessed by the mini-mental state examination (MMSE).

The illness burden was measured by using the cumulative illness rating scale - comorbidity index (CIRS- CI) [14]. In this scale, diseases are scored by organ systems and categorized into 14 items: heart, vascular, hematopoietic, respiratory; eyes, ears, nose; throat and larynx; upper gastrointestinal, lower gastrointestinal, liver, renal, genitourinary, musculoskeletal, neurological, endocrine-metabolic, and behavioral-psychiatric. Each item was rated based upon the clinical data available according to the following scores: 0, no problem; 1, mild problem; 2, moderate disability or morbidity; 3, severe/constant significant disability/chronic problems; 4, life-threatening problems. We specifically considered the comorbidity index (CIRS-CI), computed by counting the number of items (excluding the two referring to psychiatric and neurological system) for which moderate to life-threatening disorders was reported.

2.3. Assessment of serum hydroperoxides

Venous blood was collected from subjects upon an overnight fast. Blood samples were immediately centrifuged and then divided in serum aliquots which were stored at -80°C until analysis.

Hydroperoxides (HY) were assessed by colorimetric assay based on the reaction between these lipid peroxidation by-products and N,N-diethyl-para-phenylendiamine from Sigma-Aldrich, (St. Louis, MO, USA) [4]. Briefly, for each subject, 5 μL of serum or standard (H_2O_2) was added to a solution containing 190 μL of acetate buffer (pH 4.8) and 5 μL of chromogen (0.0028M). The solution was incubated at 37∘C and then read for optical density after 1 and 4minutes by Tecan Sunrise-96 well microplate spectrophotometer (Tecan group Ltd., UK). The concentration of hydroperoxides was obtained by the average ΔA_{505}/min and expressed as U Carr, where 1 UC corresponds to 0.023mM of H_2O_2 [4]. The intra-assay coefficient of variation (CV) was 3.7%, whereas the inter-assay CV was 4.3%.

2.4. Statistical analysis

Mean values were compared by analysis of variance (ANOVA) (Fisher's least significant difference as *post-hoc* test) or Kruskall-Wallis for variable with not normal distribution. The covariates included in the analysis of covariance (ANCOVA) were: age (years), sex (M/F), hypertension (Y/N), cardiovascular diseases (Y/N), diabetes (yes/no), and smoking habit (current/never). Prevalence

was compared by the χ^2 test. The relationship between variables of interest was checked by either Pearson's (if variables were normally distributed) or Spearman's (in case of non-normally distribution) correlation analysis. Adjusted Odds Ratios (O.R.; 95% confidence interval - 95%CI) were calculated in order to check the independence of the association between CIRS-CI and serum hydroperoxides levels. In this analysis, hydroperoxides were considered the dependent (dichotomous) variable, with the cut-off equal to the median value (296 UC) calculated in the control group. SPSS 17.00 for Windows (Chicago, Illinois, USA) was used for statistical analysis.

Results

[insert Table 1]

In Table 1 are reported the principal characteristics of the subjects enrolled into the study. Individuals included in the Control group were younger ($p<0.01$) and had a higher mean educational level compared with other two groups. The prevalence of female gender was lower in MCI group compared to others ($p<0.05$), while the prevalence of hypertension, CVD, and diabetes did not vary significantly among the groups. As expected by selection criteria, the mean MMSE score was pathological in LOAD, while it was within normal limits both in MCI and Controls. Likewise, compared with not-demented subjects, patients with LOAD displayed higher scores for CIRS-CI, and lower scores for IADLs ($p<0.05$). A trend toward a progressive increase in serum levels of hydroperoxides from Controls to MCI to LOAD was observed, but the difference was significant only in LOAD vs Controls, even after adjustment (ANCOVA).

[insert Table 2]

The possible link between hydroperoxides serum levels and multimorbidity, as well as clinical scores of cognitive/functional status was checked within the three groups examined by simple correlation analysis (Table 2). This OxS marker was positively and significantly correlated with CIRS-CI in Controls ($p=0.002$) and in MCI ($p=0.005$) but not in LOAD patients ($p=0.104$); on the contrary, no significant correlations emerged between serum hydroperoxides and MMSE, BADLs, and IADLs score. Finally, by multivariate logistic regression analysis (covariates: age, sex and smoking) we demonstrated that CIRS-CI was independently associated with high serum hydroperoxides levels in Controls (O.R. = 1.37; 95% CI = 1.18-1.59), and MCI patients (O.R. = 1.25; 95% CI = 1.01-1.54), but not in LOAD (O.R. = 1.22; 95% CI = 0.96-1.58).

Discussion

Free radicals become noxious for human organism when their potentially damaging actions are not adequately countered by antioxidants [9]. A defeat of this defense system inevitably leads to accumulation of by-products derived by oxidative damage to lipids and, in a less extent, to other molecules such as nucleic acid and proteins [9]. Consistent with our previous data, obtained in a larger sample [6], in the present study we found that serum concentration of hydroperoxides was significantly increased in LOAD compared with Controls, while MCI were in an "intermediate" condition. Nevertheless, a large body of evidence strongly suggest that OxS is also implicated in several aging-related disorders affecting organs and systems besides the brain [7,9]. Since the presence of multiple diseases is frequent in older individuals, including those affected by dementia [8], it was tempting to speculate that multimorbidity might partially account for the change in OxS observed in these patients.

The main finding of our study was that the number of clinically relevant concomitant diseases, as assessed by CIRS-CI, were significantly (and independently) associated with serum hydroperoxides level in Controls and MCI, but not in LOAD. Thus, while a trend toward an increase in serum hydroperoxides was observed from Controls to MCI to LOAD, a reverse trend was observed as regards the impact of multimorbidity on this phenomenon. Interestingly, although both OxS and multimorbidity were significantly increased in LOAD patients compared with Controls, we could demonstrate that CIRS-CI did not account at all for the increase in hydroperoxides in these patients. This finding strongly suggest that in LOAD the systemic OxS is primarily related to the disease itself and not secondary to concomitant conditions.

Consistently with our results, a solid body of evidence indicate that OxS is a critical, either upstream or downstream, event in the pathogenesis of AD [3, 15-18]. Indeed, signs of oxidative damage have been observed in hippocampal neurons that not yet show the neuropathological hallmarks of LOAD. i.e. amyloid-β (Aβ) aggregates or micro-fibrillary tangles [17]. Although the source of the shift in oxidative homeostasis has still to be fully understood, current evidence points to mitochondrial dysfunction [17] and metal dyshomeostasis [19-21]. More specifically, free copper and iron may act as catalysts in the Fenton reaction leading to the formation of the most reactive ROS (•OH) or lipoperoxidation by-products [20]. Furthermore, compelling evidence suggests that copper dysregulation might affect soluble Aβ aggregation [21] and increase ROS-mediated Aβ neurotoxicity [22]. Indeed, It has been shown that Aβ oligomers (mainly $A\beta_{1-42}$) bind Cu^{2+} with high affinity [22], forming a cuproenzyme-like protein able to generate H_2O_2. In turn, this

moderately reactive species (mostly upon the aforementioned metal-catalyzed conversion in hydroxyl radical) is able to activate the production of $\bullet O_2^-$ by microglial and neuronal NADPH oxidase [23]. The tight link between copper dyshomeostasis and LOAD has been proved by several clinical [24-26], meta-analysis [27], and genetic evidence [19]. Intriguingly, a recent work from Squitti et al. [19], suggests the existence of a "copper dysfunction" phenotype, characterized by high serum level of non-ceruloplasmin bound copper.

Metal dyshomeostasis could be also ascribed as one of factors accounting for the elevated level of systemic OxS observed in LOAD patients in the present and in many previous works by ourselves [4-6] and others [18, 28-30]. Indeed, regardless of some contrasting results [31], the serum/plasma concentration of different markers of proteins, DNA, and, mostly, lipids has been found to increase in patients affected by LOAD compared to older controls by several authors [4-6, 28-30]. In parallel with these data, there is abundant evidence showing that the increase of these markers of biomolecular oxidative damage is accompanied by a decrease in dietary-derived and/or endogenous antioxidants [4-6, 28, 32], leading to "full blown" OxS pattern.

Recent post-mortem evidence showed that, in MCI patients, the concentrations of tangles and plaques, as well as signs of oxidative damage in neocortex and hippocampus, are intermediate in amount between those occurring in normal aging and in LOAD [16,33]. In agreement with these observations, we found that in MCI patients the association between hydroperoxides and CIRS-CI was stronger compared with LOAD but much weaker compared with Controls. Therefore, it appears that the moderate increase in OxS we observed in MCI might be principally driven by the underlying brain pathology rather than by multimorbidity.

Challenging evidence, although based on small samples, suggest that OxS might be associated to the decline in functional and cognitive performance observed in older people [7]. In contrast with these findings, we didn't find any significant association between hydroperoxides and common indices used for the detection of physical and cognitive performance (i.e. IADLs, BADLs, and MMSE). Some important differences regarding the methodology of OxS detection, the different characteristics (e.g. age, lifestyle habits etc.) and size of the samples may, in our opinion, accounts for the discrepancies between ours and others' data.

Finally, the limitations as well as strengths of the study must be acknowledged. In one hand, the design of the study was cross-sectional, thereby precluding our ability to establish any temporal relationship between the variables considered in the analysis. On the other hand, to the best of

our knowledge, our study is one of the first exploring the potential association between a peripheral marker of OxS and multimorbidity in older subjects affected by LOAD.

In conclusion, our data suggest that multimorbidity, as measured by the CIRS-CI scale, might be related to systemic OxS level in elderly people. On the contrary, in LOAD patients a disease-specific pro-oxidant mechanisms seems to prevail, while the role of multimorbidity on systemic OxS seems to be negligible.

Conflict of interests statement

On behalf of all authors, the corresponding author states that there is no conflict of interest.

References:

[1] Benke T, Delazer M, Sanin G et al (2013) Cognition, gender, and functional abilities in Alzheimer's disease: how are they related?. J Alzheimers Dis 35:247-252

[2] van der Flier WM, Scheltens P (2005) Epidemiology and risk factors of dementia. J Neurol Neurosurg Psychiatry 76:v2-v7

[3] Lovell MA, Markesbery WR (2007) Oxidative damage in mild cognitive impairment and early Alzheimer's disease. J Neurosci Res 85:3036-3040

[4] Cervellati C, Cremonini E, Bosi C et al (2013) Systemic Oxidative Stress in Older Patients with Mild Cognitive Impairment or Late Onset Alzheimer's Disease. Curr Alzheimer Res 10:365-372

[5] Cervellati C, Romani A, Seripa D et al (2014) Oxidative balance, homocysteine, and uric acid levels in older patients with Late Onset Alzheimer's Disease or Vascular Dementia. J Neurol Sci 337:156-161

[6] Cervellati C, Romani A, Seripa D et al (2014) Systemic Oxidative Stress and Conversion to Dementia of Elderly Patients with Mild Cognitive Impairment. BioMed Res Int 2014:309507

[7] Mezzetti A, Lapenna D, Romano F et al (1996) Systemic oxidative stress and its relationship with age and illness. J Am Geriatr Soc 44:823-827

[8] Valderas JM, Starfield B, Sibbald B et al (2009) Defining comorbidity: implications for understanding health and health services. Ann Fam Med 7:357-363

[9] Dalle-Donne I, Rossi R, Colombo R et al (2006) Biomarkers of oxidative damage in human disease. Clin Chem 52:601-623

[10] Solomona A, Dobranci L, Kareholt I et al (1993) Comorbidity and the rate of cognitive decline in patients with Alzheimer dementia. Int J Geriatr Psychiatry 26:1244-1251

[11] McKhann G, Drachman D, Folstein M et al (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 34:939-944

[12] Petersen RC, Doody R, Kurz A et al (2001) Current concepts in mild cognitive impairment. Arch Neurol 58:1985-1992

[13] Katz S, Downs TD, Cash HR et al (1970) Progress in the development of the index of ADL. Gerontologist 1: 20-30

[14] Linn BS, Linn MW, Gurel L (1968) Cumulative illness rating scale. J Am Geriatr Soc 16:622-626

[15] Mao P, Reddy PH (2011) Aging and amyloid beta-induced oxidative DNA damage and mitochondrial dysfunction in Alzheimer's disease: implications for early intervention and therapeutics. Biochim Biophys Acta 1812:1359-1370

[16] Praticò D (2008) Evidence of oxidative stress in Alzheimer's disease brain and antioxidant therapy. Ann N Y Acad Sci 1147:70-78

[17] Wang X, Wang W, Li L et al (2013) Oxidative stress and mitochondrial dysfunction in Alzheimer's disease. Biochim Biophys Acta 1842:1240-1247

[18] Mangialasche F, Polidori MC, Monastero R et al (2009) Biomarkers of oxidative and nitrosative damage in Alzheimer's disease and mild cognitive impairment. Ageing Res Rev 8:285-305

[19] Squitti R, Polimanti R, Siotto M et al (2013) ATP7B variants as modulators of copper dyshomeostasis in Alzheimer's disease. Neuromolecular Med 15:515-522

[20] Squitti R, Polimanti R (2013) Copper phenotype in Alzheimer's disease: dissecting the pathway. Am J Neurodegener Dis 2:46-56

[21] Jin L, Wu WH, Li Q Y et al (2011) Copper inducing Abeta42 rather than Abeta40 nanoscale oligomer formation is the key process for Abeta neurotoxicity. Nanoscale 3:4746–4751

[22] Curtain CC, Ali F, Volitakis I et al (2001) Alzheimer's diseaseamyloid-β binds copper and zinc to generate an allosterically ordered membrane-penetrating structure containing superoxide dismutase-like subunits. J Biol Chem 276:20466–20473

[23] Wilkinson BL, Landreth GE (2006) The microglial NADPH oxidase complex as a source of oxidative stress in Alzheimer's disease. J neuroinflammation 3:30

[24] Squitti R, Barbati G, Rossi L et al (2006) Excess of nonceruloplasmin serum copper in AD correlates with MMSE, CSF [beta]-amyloid, and h-tau. Neurology 67:76-82

[25] Squitti R, Lupoi D, Pasqualetti P et al (2002) Elevation of serum copper levels in Alzheimer's disease. Neurology 59:1153-1161

[26] Squitti R, Ghidoni R, Scrascia F et al (2011) Free copper distinguishes mild cognitive impairment subjects from healthy elderly individuals. J Alzheimers Dis 23:239-248

[27] Ventriglia M, Bucossi S, Panetta V et al (2012) Copper in Alzheimer's disease: a meta-analysis of serum, plasma, and cerebrospinal fluid studies. J Alzheimers Dis 30:981-984

[28] Zito G, Polimanti R, Panetta V et al (2013) Antioxidant status and APOE genotype as susceptibility factors for neurodegeneration in Alzheimer's disease and vascular dementia. Rejuvenation Res 16:51-56

[29] Aldred S, Bennett S, Mecocci P (2010) Increased low-density lipoprotein oxidation, but not total plasma protein oxidation, in Alzheimer's disease. Clin Biochem 43:267–271

[30] Mecocci P, Polidori MC, Cherubini A et al (2002) Lymphocyte oxidative DNA damage and plasma antioxidants in Alzheimer disease. Arch Neurol 59:794–798

[31] Montine TJ, Quinn JF, Milatovic D et al (2002) Peripheral F2-isoprostanes and F4-neuroprostanes are not increased in Alzheimer's disease. Annals of neurology 52:175–179

[32] Cervellati C, Romani A, Bergamini CM et al (2014) PON-1 and ferroxidase activities in older patients with mild cognitive impairment, late onset Alzheimer's disease or vascular dementia. Clin Chem Lab Med (in press) (PMID: 25490030)

[33] Petersen RC, Parisi JE, Dickson DW et al (2006) Neuropathologic features of amnestic mild cognitive impairment. Arch Neurol 63:665-672

Table
Click here to download Table: tables.docx

Table 1: Principal characteristics of the sample according to clinical status.

	CONTROLS (n=46)	MCI (n=104)	LOAD (n=75)
Age (years) §	70.9±8.6	75.4±7.1[a]	77.4±5.6[a]
Sex (females, %) *	76.5	58.3	77.0
MMSE score [§]	27.1±2.3	24.5±3.1[a]	21.0±4.0[a,b]
Education (years) [§]	9.5±4.4	6.3±3.9[a]	5.3±3.5[a]
Current smoking (%)*	4.0	10.5	6.8
Hypertension (%)	59.1	61.3	65.8
Diabetes (%)	11.8	13.2	17.7
CVD (%)	14.7	17.9	13.9
IADL *	8.0 (6.0-8.0)	7.0 (5.0-8.0)	4.0 (2.0-6.0)
BADL *	6.0 (5.0-6.0)	6.0 (5.0-6.0)	6.0 (4.0-6.0)
CIRS-CI *	1.0 (0.0-2.0)	1.0 (0.0-3.0)	2.0 (1-4.5)
Hydroperoxides, UC [#]	239.1±15.3	265.1±16.2	283.8±18.2[a]

Mean±SD for normally distributed variables; median (interquartile range) for not-normally distributed variables

MCI: mild cognitive impairment; LOAD: late onset Alzheimer's disease; CVD: cardiovascular disease; IADL: instrumental activities of daily living; BADL: basic activity daily living; CIRS-CI: cumulative illness rating scale - comorbidity index; UC: U Carr

* $p<0.05$ Kruskal-Wallis (median) or Chi-squared test (prevalence)

[§] $p<0.05$ ANOVA (post-hoc test: a: $p<0.05$ vs controls; b: $p<0.05$ vs MCI)

[#] $p<0.05$ with ANCOVA (covariates: age, sex, smoking, hypertension, diabetes, and CVD)

Table 2: Spearman's or Pearson's correlation coefficients for the relationship between hydroperoxides serum levels and indexes of multimorbidity, functional status, and cognitive status.

		CONTROLS	MCI	LOAD
CIRS-CI	r*	0.430 (p=0.002)	0.272 (p=0.005)	0.180 (p=0.104)
BADL	r	0.047 (p=0.682)	0.106 (p=0.232)	-0.155 (p=0.133)
IADL	r	0.209 (p=0.052)	0.122 (p=0.118)	0.034 (p=0.772)
MMSE	r§	0.202 (p=0.055)	0.043 (p=0.535)	0.083 (p=0.409)

MCI: mild cognitive impairment; LOAD: late onset Alzheimer's disease; IADL: instrumental activities of daily living; BADL: basic activity daily living; CIRS-CI: cumulative illness rating scale - comorbidity index

* Spearman's correlation coefficient

§ Pearson's correlation coefficients

Conflict of Interest Disclosure Form

It is the policy of the Journal *Aging Clinical and Experimental Research* to ensure balance, independence, objectivity, and scientific rigor in the Journal. All authors are expected to disclose to the readers any real or apparent conflict(s) ofinterest that may have a direct bearing on the subject matter of the article. This pertains to relationships with pharmaceutical companies, biomedical device manufacturers or other corporations whose products or services may be related to the subject matter of the article or who have sponsored the study.
The intent of the policy is not to prevent authors with a potential conflict of interest from publication. It is merely intended that any potential conflict should be identified openly so that the readers may form their own judgements about the article with the full disclosure of the facts. It is for the readers to determine whether the authors' outside interest may reflect a possible bias in either the exposition of the conclusions presented.
The corresponding author will complete and submit this form to the Editor-in-Chief on behalf of all authors listed below.

Article Title HYDROPEROXIDES SERUM LEVELS AND MULTIMORBIDITY AMONG OLDER PATIENTS WITH MILD COGNITIVE IMPAIRMENT OR LATE ONSET ALZHEIMER'S DISEASE

Authors
C. CERVELLATI, A. ROMANI, C. BOSI, S. TAGLIA, D. PASSARO, CM BERGAMINI and G. VALACCHI

I certify that there is no actual or potential conflict of interest in relation to this article. If any conflict exists, please define hereafter:

Conflict (if none, "None" or describe financial interest/arrangement with one or more organizations that could be perceived as a real or apparent conflict of interest in the context of the subject of this article):
NONE

Name GIOVANNI VALACCHI
Signature [signature] Date 08/07/2014

Please also insert a "conflict of interest statement" in the manuscript itself.
Please upload this form together with your manuscript at online submission.

Springer

http://www.springer.com/journal/40520

Aging Clinical and Experimental Research
Editor-in-Chief: Maggi, S.
ISSN: 1720-8319 (electronic version)
Journal no. 40520