

Serum interleukin-6 levels are higher in old age subjects with Alzheimer's dementia

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Abstract

Elevated interleukin-6 (IL-6) levels have been found positively associated with markers of physical frailty as well as identified as a potential biomarker of Alzheimer's disease (AD). Thus, we explored the levels of plasma IL-6 at baseline in a cohort of older subjects with or without cognitive impairment, which results may have also implications for coronavirus disease 2019 (COVID-19) clinical management. This is a retrospective study including a cohort of over 60 years old-age subjects, 72 healthy controls, 95 mild cognitive impairment, and 73 AD were included in the study. Plasma IL-6 was measured in all subjects. The sample population included 240 subjects, mostly women with a mean age of 78.61±6.30 (range: 60-93) years. Age significantly correlated with IL-6 plasma levels ($r=0.204$, $P=0.002$) even after controlling by gender. No difference was found in body mass index (BMI), nutritional status (assessed by mini nutritional assessment), and comorbidity indices (cumulative illness rating scale-severity and comorbidity index) among groups. Instead, IL-6 significantly differed, having patients affected by AD higher levels compared to the other groups. Final linear regression analysis showed that independently of age, gender, BMI, nutritional status, number of clinically relevant concomitant diseases, the diagnosis of AD was associated with higher IL-6 plasma levels. These data indicate that serum IL-6 is more elevated in AD, supporting that IL-6 may have also a potential role in response to COVID-19 in old age subjects with cognitive impairment.

Introduction

Aging per se is associated with a dysregulation of the immune and inflammatory responses, including changes in the produc-

tion and regulation of cytokines. One of them is the interleukin-6 (IL-6), which is produced by both lymphoid and nonlymphoid cells. IL-6 production usually is low, and its serum levels are often undetectable in the absence of disease. Despite controversial results,¹ there is strong evidence that IL-6 serum concentration increases with age.² High levels of IL-6 have been reported in the plasma and serum of aged mice and humans.³ Thus, it has been hypothesized that many of the changes in immune function seen with advanced age may be the consequence of increased IL-6 production. Interestingly, elevated serum IL-6 has been found positively associated with markers of physical frailty, even after adjustment for confounders. Indeed, older with higher plasma IL-6 levels had reduced hippocampal volumes,⁴ and IL-6 has been identified as a potential biomarker of Alzheimer's disease (AD) at an early stage.⁵

In December 2019, coronavirus disease 2019 (COVID-19) emerged in Wuhan and rapidly spread throughout the world. The dysregulation of the immune response has been hypothesized as the main feature in severe phenotype presentation.⁶ A study enrolling 48 subjects with COVID-19 admitted to the General Hospital of Central Theater in Wuhan showed that the inflammatory cytokine IL-6 plasma level was significantly elevated in critically ill patients, which is almost 10-folds higher than those in other patients. They concluded that serum severe acute respiratory syndrome-related coronavirus 2 viral load (RNAemia) was strongly associated with cytokine storm and can be used to predict the poor prognosis of COVID-19 patients.⁷ According to data from the outbreaks in Italy and China,⁸ mortality rates rise significantly among older patients, and in particular, in the presence of comorbidities. With the present study, we explored the levels of plasma IL-6 in a cohort of older subjects with or without cognitive impairment, which results may have implications for clinical management.

Materials and Methods

Subjects and study design

This retrospective study focused on cognitive impairment and dementia in old age subjects. The general inclusion criteria were as follows:

Healthy controls

Age and education-adjusted the mini-mental state examination (MMSE) score ≥ 27 ; no active neurological or psychiatric

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Contributions: VB planned the project, analyzed data, interpreted results and wrote the manuscript; MB contributed to data discussion; RC, MS, PB contributed to data generation; PM discussed results and edited the manuscript.

Conflict of interests: the authors declare no potential conflict of interests.

Ethical approval: the study is carried in accordance with the 1964 Helsinki Declaration and its later amendments and with the ethical standards of the institutional ethics committee, and was approved by the ethics committee of the University of Perugia, Perugia, Italy.

Informed consent: all subjects provided written informed consent to participate in the study.

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disorder; no ongoing medical problems or related treatments interfering with cognitive function; a normal neurological exam; no psychoactive medications; the ability to live and function independently in the community.

(Amnestic) mild cognitive impairment

Mild cognitive impairment (MCI) can be classified into two main categories: amnestic MCI (aMCI) if performance on neuropsychological tests of episodic memory was poor, and non-amnestic MCI (naMCI) in the case of poor performance on neuropsychological tests covering cognitive domains other than memory, such as executive functions, language or visuospatial abilities. Considering that aMCI is highly associated with progression to AD,⁹ in this study, we included only this category of subjects. aMCI was diagnosed according to Petersen's criteria:¹⁰ memory complaint usually corroborated by an informant;

objective memory impairment for age; mostly preserved general cognitive function; largely intact functional activities; not demented.

Alzheimer's disease

AD was diagnosed according to standard research criteria.¹¹ AD diagnosis was confirmed by a combination of clinical and neuropsychological evaluation (assessing different cognitive areas such as memory, language, and constructional praxis), and brain imaging (3T magnetic resonance imaging and, if needed, positron emission computed tomography).

From 635 enrolled from January 2016 to September 2018, according to inclusion and exclusion criteria, 240 subjects were included in the study. After a clear explanation of the study, all subjects provided written informed consent to participate in the research. In all groups individuals with elevated inflammatory markers - as serum C-reactive protein, erythrocyte sedimentation rate and white blood cell count - or evidence of acute inflammatory or infectious diseases, diabetes, malignancies, immunologic or hematologic disorders or treatment with anti-inflammatory drugs (including aspirin or non-steroidal anti-inflammatory drugs in the last three months) were excluded from the study.

Cognitive, functional and neuropsychological assessment

Cognitive performances were assessed with a neuropsychological battery that included MMSE^{12,13} and a large battery of specific tests evaluating different cognitive areas, as previously reported.^{14,15} The clinical dementia rating scale¹³ was used to score dementia severity, while the geriatric depression scale¹⁶ assessed current depressive symptoms. An informant-based rating of functional status was carried out using the basic activity of daily living¹⁶ and instrumental activity of daily living.¹⁷ A higher score indicates a preserved ability to perform basic and instrumental activities of daily living. The nutritional status was assessed by the administration of the mini nutritional assessment (MNA).¹⁸

Comorbidity

Comorbidity was evaluated with the cumulative illness rating scale (CIRS).¹⁹ This rating scale consists of 14 items covering heart, hypertension, vascular and respiratory disorders, a combined eye-ear nose-throat item, upper and lower gastrointestinal systems, hepatobiliary system, kidney, genitourinary diseases, musculoskeletal diseases, endocrine/metabolic disorders, neurological system, and behavioral-psychi-

atric disorders. Severity in each single item is rated according to the following algorithm: 1 = no, 2 = mild, 3 = moderate, 4 = severe, 5 = life-threatening. After completion of CIRS, by a medical doctor, two summary measures can be constructed: the illness severity index (CIRS-SI) -that reflects the overall severity of diseases and the average rating of the 14 CIRS items-, and the comorbidity index (CIRS-CI) -computed by counting the number of items with a score ≥ 3 (moderate to severe pathology). As a result, the CIRS-CI can be considered as the number of clinically relevant concomitant diseases.

Analytical methods

Anthropometric determinations [weight, height, and body mass index (BMI)] were measured by standard technique. BMI was calculated as weight in kilograms divided by square of height expresses in meters (kg/m^2). Plasma samples were collected using EDTA coated tubes and centrifuged at 3000 rpm for 15 minutes at 4°C before being aliquoted and then frozen at -80°C. Plasma samples were analyzed with ELISA (Bio-Rad Laboratories, Hercules, CA, USA) for the assessment of peripheral IL-6. The samples were prepared according to the manufacturer's instructions. All samples and standards were run in duplicate and were measured as pg/ml. The system running protocol was set according to the manufacturer's guidelines (Bio-Rad Laboratories).

Statistical analysis

The observed data were normally distributed (Shapiro-Wilk W-Test) and are presented as mean \pm standard deviation, analy-

sis of variance (with Tukey's *post hoc* analyses) or Pearson's Chi-squared (χ^2) test were used, as appropriate, to assess differences among groups. Simple and partial Pearson's correlation analyses were also performed, as indicated. The independent effect of AD presence on IL-6 (dependent variable) was tested by a linear regression analysis controlling by multiple covariates. The minimal sample size was estimated according to a global effect size of 25% with type I error of 0.05 and a power of 95%, resulting in 210 subjects (GPower 3.1.7). All p values are 2-tailed, and the level of significance was set at $P \leq 0.05$. Statistical analyses were performed using the SPSS 20 software package (SPSS, Inc., Chicago, IL, USA).

Results

The sample population includes 240 subjects, mostly women (150; 62.5%), with a mean age of 78.61 ± 6.30 (range: 60-93) years. The population was slightly overweight, with an average BMI of 26.48 ± 4.12 kg/m^2 (93 subjects, 39% had a BMI over 25 kg/m^2). The IL-6 levels were 1.96 ± 2.41 pg/mL with no difference between genders ($P=0.135$) and no correlation with BMI ($r=0.038$, $P=0.638$). Age instead significantly correlated with IL-6 plasma levels ($r=0.204$, $P=0.002$) as shown in Figure 1, even after controlling by gender ($r=0.211$, $P=0.001$).

72 (30%) were HC, 95 (39.6%) MCI, and 73 (30.4%) were affected by AD. Table 1 shows the clinical characteristics of the sample population stratified by

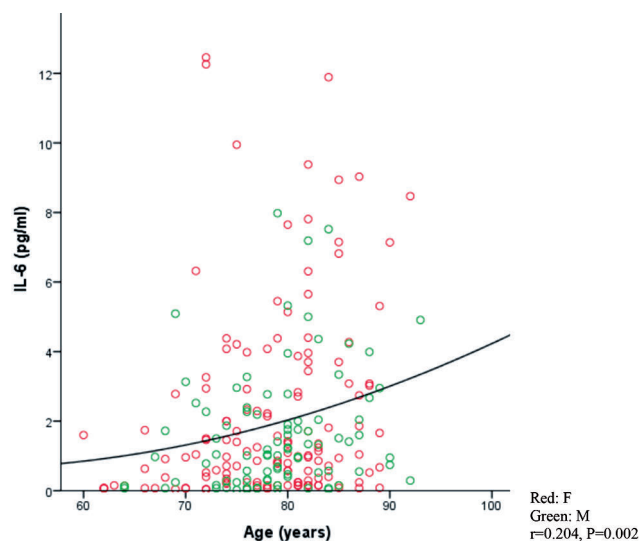


Figure 1. Simple correlation between age and interleukin (IL)-6 in all population.

groups. A higher proportion of females were present in all groups. No difference was found in BMI, nutritional status (assessed by MNA), and comorbidity indices (CIRS-SI and CIRS-CI) among groups. IL-6 significantly differed among groups having patients affected by AD higher levels compared to other groups, as shown in Figure 2. Indeed a linear regression analysis by the general linear model showed that independently of age, gender, BMI, nutritional status, number of clinically relevant concomitant diseases, the diagnosis of AD was associated with higher IL-6 plasma levels (Table 2).

Discussion

With around 23 percent of over 65-years subjects, Italy has one of the oldest populations in the world, and the COVID-19 infection is now taking a concern among the older persons. Current analyses from the National Institute of Health, researchers determined that the average age of patients who tested positive and died was 81 years old. They were mostly men affected by mul-

tiple diseases, with AD presence in the 15% of cases. The dramatic rise in mortality rates among the older population can be mainly attributed to two factors. First, the dysregu-

lation of the immune and inflammatory responses. Second, older people are more likely to have underlying health concerns. Current management of COVID-19 is sup-

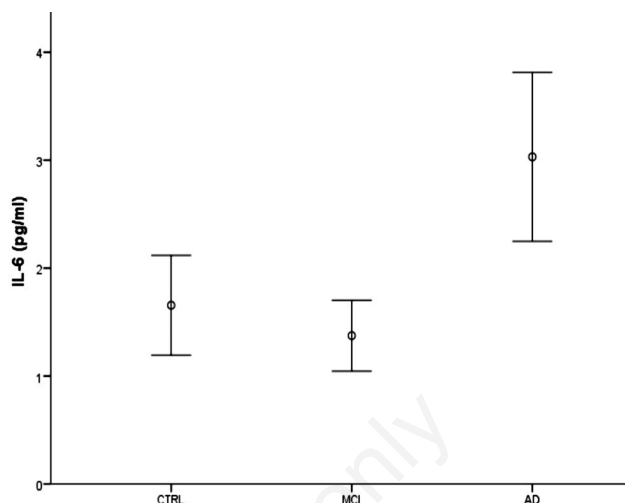


Figure 2. Mean plasma interleukin (IL)-6 levels among groups. Error bars represent the 95% confidence interval of a mean. Healthy controls (CTRL)= 1.65 ± 1.93 , mild cognitive impairment (MCI)= 1.37 ± 1.57 , Alzheimer's disease (AD)= 3.03 ± 3.28 ; $P<0.0001$ by analysis of variance. CTRL vs AD $P=0.002$; MCI vs AD $P<0.0001$ by Tukey's *post hoc*.

Table 1. Clinical characteristics of all sample population stratified by groups (n=240).

	HC (n=72)	MCI (n=95)	AD (n=73)	P
No.	65	95	72	
F/M	35/37	58/37	57/16	0.001*
Age (years)	76.36 ± 7.36	78.51 ± 5.66	81.01 ± 5.02	<0.0001
BMI (kg/m ²)	26.54 ± 4.03	26.89 ± 3.71	25.89 ± 4.68	0.428
MNA	21.46 ± 3.61	21.24 ± 4.44	21.01 ± 3.06	0.840
MMSE	28.69 ± 1.71	24.70 ± 2.96	18.13 ± 5.08	<0.0001
GDS	4.90 ± 3.13	4.24 ± 2.59	5.38 ± 3.17	0.050
ADL	5.53 ± 0.75	5.43 ± 0.87	5.15 ± 1.17	<0.0001
IADL	6.18 ± 1.80	5.46 ± 2.05	2.53 ± 1.89	<0.0001
CIRS-SI	0.65 ± 0.34	0.62 ± 0.31	0.66 ± 0.30	0.446
CIRS-CI	0.78 ± 1.01	0.58 ± 0.86	0.78 ± 0.86	0.787

Data are expressed as mean±standard deviation. * $\chi^2=13.573$. HC, healthy controls; MCI, mild cognitive impairment; AD, Alzheimer's disease; BMI, body mass index; MNA, mini nutritional assessment; MMSE, mini mental state examination; GDS, geriatric depression scale; ADL, activity of daily living; IADL, instrumental activity of daily living; CIRS-SI, cumulative illness rating scale-severity index; CIRS-CI, cumulative illness rating scale-comorbidity index.

Table 2. Linear regression analyses exploring the association of diagnosis of Alzheimer's disease with interleukin-6, controlling for multiple confounding factors (n=240).

	B	95% CI	P
Age	0.085	0.001-0.169	0.048
Gender F/M	-0.770	-1.846-0.307	0.158
BMI	0.082	-0.031-0.196	0.153
MNA	0.189	0.052-0.325	0.007
CIRS-CI	0.921	0.323-1.518	0.003
Diagnosis of AD	0.893	0.173-1.613	0.016

CI, confidence interval; Gender indicated as F=0 and M=1. BMI, body mass index; MNA, mini nutritional assessment. CIRS-CI, cumulative illness rating scale-comorbidity index; Diagnosis of Alzheimer's disease (AD): healthy controls are indicated as 0, mild cognitive impairment are indicated as 1, AD are indicated as 2.

portive, and respiratory failure from acute respiratory distress syndrome is the leading cause of mortality.²⁰ A cytokine profile resembling secondary hemophagocytic lymphohistiocytosis seems to be associated with COVID-19 disease severity, characterized by an increase of inflammatory markers and, in particular, IL-6 levels. Predictors of fatality from a recent retrospective, multicenter study of 150 confirmed COVID-19 cases in Wuhan, China, included elevated IL-6, suggesting that mortality might be due to virally driven hyper inflammation.²¹ In this context, immunosuppression is likely may be beneficial. Interestingly a multicenter, randomized controlled trial of tocilizumab (IL-6 receptor blockade, licensed for cytokine release syndrome) has been approved in patients with COVID-19 pneumonia and elevated IL-6 in China (ChiCTR2000029765). Also, in Italy, it has been approved in a clinical trial for the treatment of severely ill, hospitalized COVID-19 patients.

Our data in a cohort of old age subjects (over 60 years) show that plasma IL-6 levels increase along with aging and, particularly, in subjects affected by AD and independent of the number of clinically relevant concomitant diseases. Systemic inflammation gradually increases with age, commonly referred to as inflammation. Of the cytokines implemented in the inflammation process, IL-6 is regarded as one of the main inflammatory components resulting in the age-associated diseases.²² Physiologically, IL-6 is a hormone-like cytokine with pleiotropic capabilities, including roles in immunological homeostasis, such as upregulating acute phase response proteins and signaling within the central nervous system.²³ Previous meta-analyses have also reported associations between upregulated peripheral IL-6 with AD.²⁴ However, the role of baseline plasma IL-6 levels in the outcome prediction of COVID-19 infection is still unknown. According to previous data,²² it is reasonable to hypothesize that these persons may have a higher probability of being non-survivors during COVID-19 infection. In fact few studies have shown that the presence of AD is a significant parameters for predicting COVID-19 non-survival in older persons.^{25,26} The basal higher level of IL-6 in subjects affected by AD could be the missing link explaining the higher mortality in this population. The lack of subgroup of subjects with AD and experienced COVID-19 represent the main limitation of the study. Thus, as the number of cases of COVID-19 is increasing rapidly, there is an urgent need for more studies to test such a hypothesis.

Conclusions

These data support that patients with COVID-19 should be screened for hyperinflammation to identify the subgroup of patients for whom immunosuppression could improve prognosis and mortality. More reports are needed to understand the potential benefits of IL-6 receptor blockade in severe older COVID-19, and future researches should be encouraged to provide more data for this subset of patients. Lastly, clinicians should consider tracking IL-6 to monitor patient status and prognosis.

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