



## Prognostic value of serum interleukin-6 (IL-6) levels in long term care

Stéphane Maurel<sup>a</sup>, Brigitte Hamon<sup>b</sup>, Jean Taillandier<sup>b</sup>,  
Eric Rudant<sup>a</sup>, Laurence Bonhomme-Faivre<sup>a</sup>,  
Christophe Trivalle<sup>b,\*</sup>

<sup>a</sup> *Service de Pharmacie, AP-HP, Hôpital Paul Brousse, 14 Avenue Paul Vaillant Couturier, F-94800 Villejuif, France*

<sup>b</sup> *Pôle Gériatologique, AP-HP, Hôpital Paul Brousse, 14 Avenue Paul Vaillant Couturier, F-94800 Villejuif, France*

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### Abstract

The aim of this prospective study was to examine the relationship between IL-6 levels and survival in an elderly population in a long term hospital care ward. All of the 184 women and 65 men hospitalized in the geriatric unit regardless of their health status were included. The plasma levels of interleukin-6 were measured at baseline and deaths were assessed over a 2-year period. IL-6 levels of at least 3 pg/ml in men and 5.6 pg/ml in women were respectively associated with a relative risk of death of 2.28 (CI<sub>95</sub>: 1.04–4.95) and 1.52 (CI<sub>95</sub>: 1.06–2.18). After adjustment for age class, the reduced survival observed with these thresholds only remained unchanged in men, the difference in survival in women was not significant. Our conclusion is that even in an elderly hospitalized population, high IL-6 levels were associated with poor survival. The lower survival rate after adjustment for class of age found in men but not in women suggests a gender-related specificity.  
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### 1. Introduction

Aging is associated with disorders of the immune system (Miller, 1996) and inflammatory response (Ballou and Kushner, 1997). Many immune functions are altered

\* Corresponding author. Tel.: +33 1 45 59 38 43; fax: +33 1 45 59 36 91.  
E-mail address: [christophe.trivalle@pbr.ap-hop-paris.fr](mailto:christophe.trivalle@pbr.ap-hop-paris.fr) (C. Trivalle).

with age, such as impairment of T-cell functions, in particular a decline in T-cell proliferative capacity and a decrease in IL-2 production (Miller, 1996). These changes, termed as “immunosenescence”, may result in a greater incidence of infections, cancer or autoimmune diseases and are responsible for a shorter immune response after vaccination (Ershler, 1993a; Ginaldi et al., 2001). On the other hand, aging is associated with a low-grade inflammatory activity reflected by an enhanced level of circulating acute phase proteins, such as C-reactive protein (CRP) or haptoglobin (Ballou and Kushner, 1997), and cytokines such as IL-6 (Cohen et al., 1997; Mysliwska et al., 1998). Most studies have focused on IL-6 that plays an important role in a wide range of immunological, inflammatory and metabolic functions (reviewed by Krabbe et al., 2004; Morley and Baumgartner, 2004). Although increased serum levels of IL-6 in the elderly may be explained by the incidence of age-related diseases, healthy elderly individuals also seem to have higher concentrations (Wei et al., 1992). Several factors contribute to IL-6 release, including stress (Kiecolt-Glaser et al., 2003), smoking, obesity or age-associated endocrine modifications such as a decrease in testosterone, estrogen and dehydroepiandrosterone (Daynes et al., 1993; Ershler and Keller, 2000). These findings suggest that the normal aging process is associated with IL-6 production dysfunction which may be exacerbated by age-related diseases. Thus it may be difficult to separate an effect linked to normal physiological ageing, from the effect of a clinical or subclinical disease on IL-6 levels.

In any case, IL-6 has been shown to be a good overall predictor of mortality (Harris et al., 1999; Bruunsgaard et al., 2003) most probably because IL-6 reflects the presence of diseases that increase the risk of death. On the other hand it has been suggested that IL-6 plays a pathogenetic role in age-related diseases such as osteoporosis, Alzheimer’s disease, multiple myeloma or atherosclerosis (Ershler, 1993b; Krabbe et al., 2004).

The aim of this study was to evaluate the prognostic importance of IL-6 serum levels in a geriatric population hospitalized in a long-term care ward.

## 2. Subjects and methods

We conducted a cross-sectional study with a prospective follow-up for mortality. The study concerned all patients  $\geq 65$  years old who were cared for in three geriatric units of the Paul Brousse Hospital. A total of 287 subjects were hospitalized in these units. Patients younger than 65 or in palliative care were excluded ( $n = 38$ ). Finally, 249 subjects were included from January 2001 to March 2001 regardless of their health status. Blood samples were obtained during this period and stored at  $-80^{\circ}\text{C}$  until analysis. Follow-up data on mortality were reported until March 2003. In our long-term care unit, the mean duration of stay is about 3 years. Patients were divided into two groups according to plasma IL-6 levels, and differences in survival were tested for each group. Survival analysis was performed separately for men and women because gender could influence the levels of IL-6 and the mortality.

Blood samples were collected in the morning in EDTA-containing Vacutainer tubes and taken to the Pharmacology laboratory, between January and March 2001, when the patients were in stable clinical conditions. After centrifugation, plasma was immediately aliquoted, frozen and stored at  $-80^{\circ}\text{C}$ . Plasma IL-6 was measured by enzyme-linked immunosorbent

assay using a commercial kit (Immunotech, Coulter). The detectable limit was 3 pg/ml. Intra-assay and inter-assay coefficients of variation were respectively 6.8 and 14.6%. According to the instruction manual, no interference or cross-reactivity with other cytokines or receptors were found with this kit.

The data are presented as mean  $\pm$  S.D. Significant differences between the study groups were explored with the Student *t* test. When the application conditions of the *t*-test were not present, the non-parametric Mann–Whitney test was used. Linear relations between variables were evaluated by Pearson's correlation analyses. Categorical variables were compared by the  $\chi^2$ -test. Survival differences were evaluated by a log-rank test on the basis of IL-6 levels. A Cox regression model was used to compare survival according to baseline IL-6 adjusting for age class (defined by age median). Statistical analysis was performed with the open source statistical system "R" version 1.7.1. A value of  $p < 0.05$  was considered to be statistically significant.

### 3. Results

The investigated population included 184 women (73.9%) and 65 men (26.1%) with a mean age of  $85 \pm 8$  years. Age medians were 80.5 and 87.8 for men and women, respectively. Women were older than men ( $86.3 \pm 7.5$  versus  $79.6 \pm 8.5$ ,  $p < 0.001$ ). Cognitive decline or loss of autonomy was the main reason for hospitalization. At hospital admission, patients suffered from dementia (45.8%), cardiac diseases (35.4%), depression and other psychiatric diseases (23.5% and 17.6% respectively), COBP (15.4%), rheumatological diseases (13.8%), stroke (13.1%), cancer (10.4%) and Parkinson disease (4.5%) (Fig. 1).

At baseline, 87 patients (35%) had a detectable level of IL-6 ( $\geq 3$  pg/ml) and the mean level was  $26.3 \pm 41.9$  pg/ml. There were no differences for IL-6 means between men and women ( $5.99 \pm 13.09$  pg/ml versus  $9.9 \pm 30.6$  pg/ml,  $p = 0.930$ ). The proportion of detectable IL-6 was identical for men and women (35%,  $p = 0.930$ ).

The IL-6 levels were positively correlated with age for women ( $r = 0.17$ ,  $p = 0.025$ ) and for men ( $r = 0.32$ ,  $p = 0.010$ ). There was no correlation between diseases and IL6 levels.

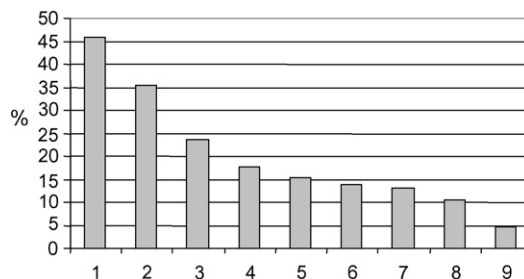


Fig. 1. Most prevalent medical problems in a long-term hospital-based population of frail patients aged 65 years and older. (1) Dementia; (2) cardiac diseases; (3) depression; (4) other psychiatric diseases; (5) chronic obstructive pulmonary disease; (6) rheumatological diseases; (7) stroke; (8) cancer, (9) Parkinson disease.

During the follow-up period, 89 patients died (35.7%). There was no significant difference in mortality between men and women (27.7% versus 38.6%,  $p = 0.115$ ).

### 3.1. Survival of men

There was no correlation between the length of survival and age ( $r = -0.15$ ,  $p = 0.223$ ). Baseline IL-6 levels were higher in non-survivors than in survivors ( $11.8 \pm 20.4$  pg/ml versus  $3.8 \pm 8.1$  pg/ml,  $p = 0.031$ ). There were significant differences in mortality rate according to IL-6 levels: patients with IL-6 values  $\geq 3$  pg/ml at the beginning of the study had a 43.5% mortality rate, whereas patients with IL-6  $< 3$  pg/ml had a 19% mortality rate ( $p = 0.035$ ). IL-6  $\geq 3$  pg/ml was associated with a relative risk of 2.28 (CI<sub>95</sub>: 1.04–4.95).

Univariate analysis by Log-rank test showed a significantly lower survival rate in patients with IL-6  $\geq 3$  pg/ml (Fig. 2). These results remained unchanged after adjustment for age class by multivariate Cox analysis.

### 3.2. Survival of women

There was a negative correlation between the length of survival and age ( $r = -0.25$ ,  $p < 0.001$ ). IL-6 levels of non-survivors and survivors were not different at the beginning of the study ( $12.4 \pm 29.4$  pg/ml versus  $8.2 \pm 31.3$  pg/ml,  $p = 0.135$ ). Furthermore, there was no significant difference in mortality rate between the two groups according to IL-6  $<$  or  $\geq 3$  pg/ml (35.8% versus 43.8%,  $p = 0.293$ ).

Univariate analysis by the Log-rank test and multivariate Cox analysis adjusted for age class showed no difference in survival for patients according to the IL-6 levels. In contrast, differences in the mortality rate were observed when the groups were differentiated at a threshold of 5.6 pg/ml. Patients with IL-6  $\geq 5.6$  pg/ml had a higher mortality rate than patients with IL-6  $< 5.6$  pg/ml (50.9% versus 33.6%,  $p = 0.028$ ). The relative risk for IL-6  $\geq 5.6$  pg/ml was 1.52 (CI<sub>95</sub>: 1.06–2.18). Univariate analysis by Log-rank test showed a significantly lower survival rate in patients with IL-6  $\geq 5.6$  pg/ml (Fig. 3) but this finding was not statistically significant after inclusion of age class with the Cox model.

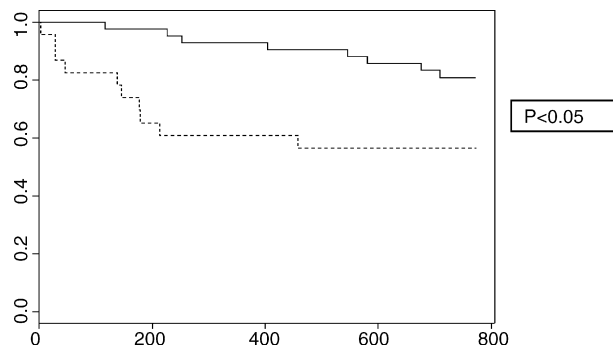


Fig. 2. Kaplan–Meyer analysis of cumulative rates of survival stratified in two groups on the basis of IL-6 levels for men. Continuous line: IL-6  $< 3$  pg/ml, broken line: IL-6  $\geq 3$  pg/ml.

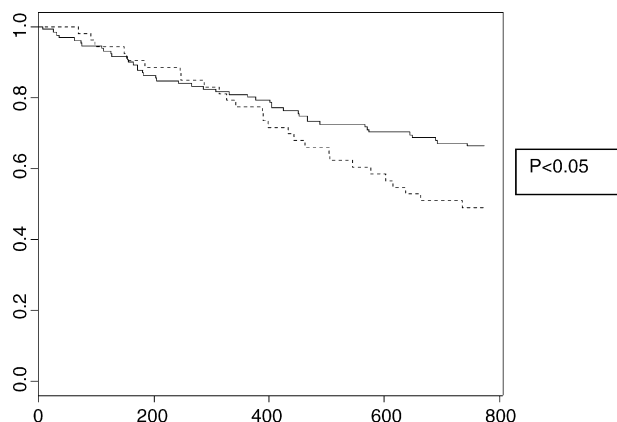


Fig. 3. Kaplan–Meyer analysis of cumulative rates of survival stratified in two groups on the basis of IL-6 levels for women. Continuous line: IL-6 < 5.6 pg/ml; broken line: IL-6  $\geq$  5.6 pg/ml.

#### 4. Discussion

This study showed that the IL-6 levels were elevated with increasing age, probably because of an increased prevalence and severity of diseases during aging. However, a dysfunction of IL-6 production linked to normal aging cannot be excluded. Although our study does not specifically distinguish the causes of increased IL-6 levels, the two above mechanisms are probably associated.

The main finding of our study is the poor survival prognosis at 2 years associated with high concentrations of IL-6. Considering the plury-pathologic state of our patients, the high IL-6 levels in this population were probably linked to the poor health status of the subjects, and this could explain the increased mortality. Moreover, IL-6 exerts deleterious effects on numerous physiological functions. Thus IL-6 seems to be involved in processes leading to functional decline and frailty in the elderly. For example, IL-6 plays a major role in the development of sarcopenia, anemia, osteoporosis, cognitive decline or hypoalbuminemia in the elderly (Morley and Baumgartner, 2004). High IL-6 levels are negatively associated with physical performance and muscle strength (Cesari et al., 2004) and predict the development of disabilities (Ferrucci et al., 1999). Thus, IL-6 may be both the cause and the consequence of morbidity. In any case, high IL-6 levels are associated with increased mortality (Harris et al., 1999; Bruunsgaard et al., 2003). For example, Reuben et al. (2000) found that elderly subjects with IL-6 levels in the highest quartile ( $\geq 3.20$  pg/ml) had a 43% 4-year mortality rate compared with the 19% mortality rate of those in the lowest quartile. To our knowledge, the main studies linking IL-6 concentrations and mortality dealt with relatively healthy or non-hospitalized elderly subjects. One study showed that the IL-6 concentrations represented a prognostic factor of low 1-year survival in a hospitalized elderly population (Thomas et al., 2001). Our study demonstrates in a frail elderly long-term care hospital population with multiple pathologies that high IL-6 levels were predictive of poor survival. Also, in a non-selected hospitalized population, determination of IL-6 concentrations could be helpful for identifying high-risk subjects.

In our population there was an important gender-associated difference in the association of IL-6 and survival. A higher mortality was observed for different IL-6 thresholds according to gender. Thus, higher IL-6 levels were required to be associated with a poor prognosis in women. Because the women in this study were older than the men, the age-related IL-6 concentration dysfunctions may have played a greater role in these women. We also found that increased IL-6 levels were associated with a poor prognosis unrelated to age only in men. Adjustment for age in men did not change the significant relationship between high IL-6 concentrations and poor survival. Thus, in women, increased mortality associated with increased IL-6 levels was probably age-related. This is suggested by the positive correlation between IL-6 concentrations and age on one hand, and the negative correlation between age and length of survival on the other hand. It is unclear why age is a confounding factor only in women.

A limitation of our study is the detection limit of our IL-6 assay that could explain why 65% of our subjects had an undetectable IL-6. However, our detection limit seems to have been adequate to identify a significant association between mortality and IL-6.

In conclusion, this study showed an increased risk of death associated with the detection of IL-6 in men and IL-6 concentration  $\geq 5.6$  pg/ml in women in frail elderly people in a long-term care ward. A gender-associated difference was found for the relationship between IL-6 and survival, because IL-6 was an age-independent poor prognostic factor only in men.

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