Serum concentrations of interleukin-6 (IL-6) in the general adult population: possible implications for anti-IL-6 therapy in SARS-Cov-2 infection and IL-6-related diseases

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Interleukin-6 (IL-6) is an important cytokine in health and disease [1]. Serum IL-6 is a well-known inflammatory marker [2]. Moreover, IL-6-neutralizing therapies are used in a variety of inflammatory diseases, including rheumatoid arthritis, polyarticular and systemic juvenile idiopathic arthritis, giant cell arteritis, Castleman's disease and, chimeric antigen receptor T cell therapy (CAR-T)-induced cytokine release syndrome [3], and some forms of severe asthma [4]. Serum IL-6 concentrations have gained attention during the ongoing Coronavirus (SARS-Cov-2) pandemic [5] because infection may be followed by severe, life-threatening immune responses that include greatly increased serum IL-6 concentrations [6-9]. Furthermore, serum IL-6 concentrations may have prognostic value [7] and may be used as a criterion for initiating anti-IL-6 therapy [9]. Specifically, tocilizumab is an humanized anti-IL-6 receptor monoclonal antibody that is being tested in patients with severe SARS-Cov2 infection [8,9] because it was used with some success in analogous severe cytokine release syndrome after CAR-T therapy [3]. This study was aimed to describe serum IL-6 concentrations in adults. There is paucity of reports of the normal distribution of serum IL-6 concentrations and studies in general populations may help to interpret abnormal values of laboratory determinations in disease.

This study is a part of a cross-sectional survey of a general adult population in NW Spain, as described elsewhere [10,11]. Briefly, an age-stratified random sample from a single municipality was included in the *A-Estrada Glycation and Inflammation Study* (AEGIS), which was reviewed and approved by the Regional Ethics Committee (code 2010-315). A total of 1516 individuals (68% of those who were eligible) agreed to participate. A total of 17 patients with active inflammatory disease were excluded from the present study. The median age of the 1499 remaining participants was 52 years (range 18-91 years) and 669 (44.6%) were men. Serum IL-6 was measured by immuno-chemiluminiscence (Immulite 2000 System, Siemens Healthcare Diagnostics) immediately after blood sampling in all participants. According to the

manufacturer's instructions, the analytical sensitivity of the assay is 2 pg/mL and the upper reference value in adults is 5.9 pg/mL.

Serum IL-6 concentrations in relation to demographic factors (age and sex), lifestyle variables (alcohol consumption and smoking), body mass index, history of asthma, and atopy in the study population are represented in Table 1. Serum concentrations were undetectable (<2 pg/mL) in 736 individuals (49.1%). These cases with undetectable concentrations were attributed an arbitrary value of 2 pg/mL for statistical calculations. Serum IL-6 concentrations were higher than the upper reference value (>5.9 pg/mL) in 145 individuals (9.7%). Moreover, they were higher than 40 pg/mL (a potential threshold for initiating anti-IL-6 therapy in some settings) in 12 individuals (0.8%). Five of these 12 individuals were males. Their median age was 50 years (range, 20-79 years). A 34-year old male with a baseline IL-6 concentration of 44.9 pg/mL developed a Hodgkin's lymphoma two years later. A 79-year old male with baseline IL-6 concentration of 104.0 pg/mL developed a rectal adenocarcinoma 2 years later. The clinical records of these 12 outliers and their 5-year follow-up were otherwise unremarkable. Median serum IL-6 concentration in the study population was 2.1 pg/mL (interquartile range, <2-3.4 pg/mL; absolute range <2-107 pg/mL). After excluding outliers with IL-6 >40 pg/mL, median and interquartile range remained unchanged.

Serum IL-6 concentrations increased with age (Table1). Individuals older than 60 years showed a 97.5th percentile which was twice as high as that of individuals younger than 60 years (Table 1). In the multivariate analyses (logistic regression), age was the strongest factor associated with high (>4.0 pg/mL, top quintile) IL-6 levels (Table 1). Serum IL-6 concentrations were higher in males than in females, although the 97.5th percentile was similar for both sexes (Table 1). Light-to-moderate alcohol consumption tended to be negatively associated with IL-6 concentrations (Table1), which is consistent with an overall anti-inflammatory effect of alcohol consumption at that doses [10]. Smoking tended to be associated with higher IL-6 concentrations after adjusting for covariates (Table1), which is consistent its proinflammatory effect [10]. Overweight and, particularly, obesity were associated with higher IL-6 concentrations (Table 1). Adipose tissue produces and releases a variety of proinflammatory mediators including IL-6 [12]. Obesity has been found associated with higher IL-6 concentrations in some patients with asthma [13]. In fact, the endotype of non-type-2 neutrophilic asthma is associated to both obesity and high serum IL-6 concentrations [13-15]. Furthermore, elevated serum IL-6 concentration in patients with adult-onset asthma is associated with worse asthma outcome [15]. In our experience, a history of asthma (as defined

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by a positive patient response to the question "*Have you ever been diagnosed as having asthma*?") was not associated with IL-6 concentrations in the general population (Table 1). Atopy (as defined by positive skin prick tests [SPT] to a panel of common aeroallergens in the area) was associated with lower IL-6 concentrations but this effect was largely attenuated after adjusting for age (Table 1). Among those with a history of asthma, SPT-negative individuals (n= 55) showed higher IL-6 concentrations than SPT-negative individuals (n=65) (median 2.4 pg/mL and interquartile range <2-3.7 pg/mL, versus median <2 pg/mL and interquartile range <2-2.4 pg/mL, respectively; P=0.013, Mann-Whitney test), but that difference was also attenuated after adjusting for age (data not shown).

In summary, serum IL-6 concentrations in the general adult population show wide variation. A small number of individuals without apparent inflammatory disease show markedly abnormal serum IL-6 concentrations. Metabolic factors (obesity) and lifestyle factors (alcohol consumption and smoking) may have some influence on serum IL-6 concentrations. Serum IL-6 concentrations are slightly higher in males than in females and they significantly increase with age. The latter is particularly important for diseases that are either more frequent or clinically more severe in older patients, such as some systemic inflammatory diseases, some forms of asthma, or SARS-Cov-2-induced disease [3-5,15]. The meaning of a certain high concentration of IL-6 may be different depending on the age considered. These results may help to interpret the magnitude of IL-6 elevation in such SARS-Cov2 and IL-6-related inflammatory diseases.

Conflicts of interest:

The authors declare that they have no conflicts of interest.

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Table 1. Serum concentrations of serum IL-6 in the adult population (stratified by demographic factors, lifestyle factors, body mass index, history of asthma and atopy), and multivariate analysis (logistic regression) of covariates associated with high (>4.0 pg/mL [top quintile]) IL-6 concentrations.

	No.	Univariate analysis[a]			Multivariate analysis[b]	
		Median (IQR range)	**97.5th percentile**	**P-value**	**Odds ratio** (95% CI)	**P-value**
Sex						
Females	830	<2 (<2-3.2)	14.3	Ref.	1	Ref.
Males	669	2.4 (<2-3.8)	14.9	<0.001	1.58 (1.14-1.20)	0.006
Age (years)						
18-40	424	<2 (<2-2.6)	11.8	Ref.	1	Ref.
>40-60	528	2.1 (<2-3.1)	10.8	<0.001	1.21 (0.80-1.85)	0.353
>60-80	473	2.7 (<2-4.5)	21.8	<0.001	3.05 (2.00-4.66)	<0.001
>80	74	3.2 (<2-5.1)	24.5	<0.001	6.10 (3.33-11.1)	<0.001
Alcohol consumption (units/w) [c]						
Abstainers (<1)	539	2.2 (<2-3.5)	15.2	Ref.	1	Ref.
Light drinkers (1-13)	591	<2 (<2-3.3)	12.6	0.038	0.78 (0.56-1.08)	0.140
Moderate drinkers (14-27)	239	2.3 (<2-3.6)	14.4	0.362	0.61 (0.39-0.93)	0.024
Heavy drinkers (>27)	130	2.5 (<2-4.4)	24.1	0.087	0.92 (0.55-1.52)	0.747
Smoking						
Never smokers	820	<2 (<2-3.4)	13.7	Ref.	1	Ref.
Ex-smokers	388	2.4 (<2-3.7)	18.0	0.008	1.20 (0.85-1.70)	0.297
Current smokers	291	<2 (<2-3.2)	12.8	0.039	1.78 (1.19-2.67)	0.005
Body mass index (kg/m^2) [d]						
Normal weight (≤25)	421	<2 (<2-2.7)	11.4	Ref.	1	Ref.
Overweight (>25-30)	567	2.2 (<2-3.5)	12.0	<0.001	1.37 (0.93-2.01)	0.107
Obese (>30)	511	2.4 (<2-4.2)	18.0	<0.001	1.89 (1.28-2.78)	0.001
History of asthma [e]						
No	1342	2.2 (<2-3.5)	14.4	Ref.	1	Ref.
Yes	120	<2 (<2-3.0)	14.7	0.149	0.97 (0.57-1.65)	0.920
Atopy (SPT-positivity) [f]						
No	1166	2.2 (<2-3.6)	14.7	Ref.	1	Ref.
Yes	331	<2 (<2-3.0)	14.3	<0.001	0.83 (0.57-1.20)	0.328

[a]Comparison with the reference category was performed by means of the Mann-Whitney test.
[b]Logistic regression. Dependent variable: high (>4.0 pg/mL, top quintile) serum IL-6 concentrations. All covariates were forced to enter the equation. Complete data were available for 1460 individuals.
[c]Alcohol consumption was calculated by the sum of units regularly consumed per week (1 glass of wine or 1 beer are approximately equivalent to 10 g of alcohol or 1 unit; 1 cup of spirits is approximately equivalent to 20 g of alcohol or 2 units).
[d]Body mass index was calculated as the weight (in kg) divided by the square of the height (in meters).
[e]Positive answer to the question "*Have you ever been diagnosed as having asthma?"* (data were not available for 37 individuals).
[f]At least 1 positive skin prick test (SPT, >3mm) to house dust mites *(Dermatophagoides pteronyssinus* and *Lepidoglyphus destructor*), pollens (*Phleum pratense, Plantago lanceolata, Betula alba,* and *Parietaria judaica*), vegetable panallergens (profilin and peach lipid transfer protein), moulds (*Alternaria alternata* and *Aspergillus spp*), and animal dander (dog and cat) (data were not available for two individuals).
Ref., reference category. IQR, interquartile range. CI, confidence interval.