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Interleukin-6 and amyotrophic lateral sclerosis

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ABSTRACT

Background: IL-6 is an inflammatory cytokine that is a possible factor in progression of the disease. We have investigated venous blood levels of IL-6 in controls and ALS patients in relation to clinical staging and respiratory function.

Methods: We studied 82 patients with ALS and 43 age and gender-matched healthy control subjects. Blood was drawn at the same time of day in the mornings to avoid diurnal variation. IL-6 levels were estimated according to a fixed protocol. Clinical measures included ALSFRS-R, vital capacity, and mean bilateral phrenic nerve CMAP amplitude. A multi-regression data analysis was used in addition to conventional statistical methods.

Results: IL-6 levels were positively correlated with increasing age in the control group. In ALS patients mean IL-6 levels were raised but the levels were markedly variable from case to case and did not reach significance (p 0.1). In addition to age effects reduction in phrenic nerve CMAP amplitude was correlated with increased IL-6 levels (p 0.026).

Conclusions: IL-6 levels were physiologically influenced by aging in controls and by respiratory dysfunction in ALS. There was marked variability in levels from case to case, which might be related to respiratory factors, which cause pulmonary inflammation.

1. Introduction

Interleukin-6 (IL-6) is multifunctional cytokine involved in the regulation of the immune response, inflammation, metabolism and hematopoiesis, produced by immune and blood cells, endothelial cells, and myocytes on contraction [1], which can cross the blood-brain barrier [2] and has been extensively investigated in neurodegenerative disorders.

A number of previous studies have reported increased serum and CSF levels of IL-6 in patients with ALS, probably related to the well-described role of inflammatory processes in motor neuron degeneration [3–7]. Studies of skin biopsies in ALS patients have demonstrated higher IL-6 immunoreactivity as compared with diseased control subjects. IL-6 immunoreactivity was markedly positive in the epidermis and dermal blood vessels, in particular in patient with longer disease duration, showing correlation with serum levels [8]. However, abnormal levels were not consistently found, some authors reporting values similar to controls [9]. In a study of ALS patients tested over a 4 year period, IL-6 plasma levels were strongly associated with C-reactive protein levels [10]. Moreover, from the large set of neuroinflammatory markers investigated, it was the only marker undergoing a late-stage upregulation [6]. There was no correlation between ALSFRS-R change and IL-6 levels [6] although other investigators noted higher levels of IL-6 at disease onset and decreased levels later [10]. Hypoxia is a factor that is possibly associated with greater IL-6 release in ALS and other medical conditions [4], such as occurs in obstructive sleep apnea [11]. Serum and CSF levels of IL-6 were found to correlate with PaO2 in ALS [4]. This may be explained by the release of proinflammatory cytokines by hypoxia-induced microglia activation [12].

2. Material and methods

2.1. Patients and controls

We assayed IL-6 in plasma samples from 82 patients with ALS observed in our center and in 43 gender and age-matched healthy controls. Controls and patients older than 80 years were excluded. Patients with other medical or neurological conditions, eg diabetes, peripheral neuropathy and dementia were excluded. None required ventilator assistance.

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Table 1
Results.

	IL-6 levels (pg/ml) mean (SD)	M/F ratio	Mean age (SD)	Mean disease duration (SD)	Region onset (bulbar and spinal)	Mean ALSFRS-R (SD)	Mean predicted FVC (SD)	Mean phrenic ampl (SD)
Controls (43)	2.23 (1.61)	1.42	57.7 (8.9)	–	–	–	–	–
ALS patients (82)	5.53 (12.94)	1.43	60.5 (9.9)	27.5 months (43.7)	17 bulbar 65 spinal	39.2 (7.7)	85–4 (22.9)	0.53 (0.22)
P values	0.1	0.9	0.22	–	–	–	–	–

FVC – forced vital capacity; Phrenic ampl – mean value of bilateral phrenic nerve peak-to-peak amplitude of the motor response (normal ≥0.4 mV).

All these patients had probable or definite ALS according to the revised El Escorial [13], and had neurophysiological changes according to Awaji criteria [14]. The patients with ALS were evaluated clinically (see below) at the same visit as the blood samples were taken.

2.2. Tests and measurements

To quantify the IL-6 plasma levels, whole venous blood was collected in vacutainer tubes (lithium heparin tubes) and immediately centrifuged. The plasma was collected and stored at −80 °C until analyzed. Human plasma Il-6 concentrations were measured using Bioplex Pro Chemokine assay (Biorad Bio-Rad® Bio-Plex Pro™) performed according to the manufacturer's instructions using a Bio-Plex 200 system and the Bio-Plex Manager 5 software (all from Bio-Rad). For the measurement of IL-6 plasma levels, whole venous blood was collected into vacutainer tubes (lithium heparin tubes) and immediately centrifuged. The plasma was collected and stored at −80 °C until analyzed. Human plasma Il-6 concentrations were measured using the Bio-Plex Pro™ Human Chemokine assay performed according to the manufacturer's instructions. Measurement of IL-6 was performed in duplicate for each sample and the mean concentration was calculated. The concentration of IL-6 was obtained by interpolating fluorescence intensity to the 7-point standard curve generated using recombinant protein supplied in the kit and calculated using the software Bio-Plex Manager 5.

a) Clinical evaluation

All the ALS patients were evaluated with the ALS functional rating scale, revised (ALSFRS-R).

b) Respiratory function tests

For each patient respiratory function tests were performed using the same technique and by the same technicians, always using nose clips for nose occlusion. Forced Vital Capacity (FVC) was determined with patients in the sitting position, using a computer-based spirometer (microQuark®, Cosmed®). The best of three satisfactory and consistent expiratory maneouvres, each obtained after a maximal inspiratory effort, was used to determine the values of FVC. The predicted FVC% was used for statistical analysis.

Phrenic nerve conduction was evaluated bilaterally with surface recordings in all subjects. Motor responses were elicited by percutaneous electrical supramaximal stimulation in the neck, and recorded at the homolateral costosternal angle (active electrode), with the reference electrode at the costal margin 16 cm from the active electrode [16].

We recorded the time of non-invasive ventilation adaption (NIV) in the population of ALS patients, as decided by the team of specialists involved in the respiratory care and independent from this study.

2.3. Statistical methods

Control and ALS patient data was compared using the Student *t*-test or Chi square test for discrete variables. Since IL-6 levels were not normally distributed we performed a log transformation for comparisons, correlations (Pearson correlation coefficient) and multiple regression analysis. A p value lower than 0.05 was considered significant.

2.4. Ethics

All subjects gave their written informed consent. The study conformed to the standards defined in the latest revision of the Declaration of Helsinki. The protocol was approved by the Local Ethics Committee.

3. Results

The results are summarised in Table 1. In control subjects there was no difference in IL-6 levels between genders (p 0.8). However, there was a positive correlation between age and IL-6 levels (r = 0.45, p 0.003): 30% of the increase in IL-6 levels in the healthy controls was dependent on increasing age. In the ALS patients there was a trend for IL-6 levels to be increased compared with healthy controls, despite the same age distribution in the two groups, but this did not reach statistical significance (Table 1; Fig. 1). In the ALS patients, there was no difference between bulbar-onset (17 subjects, mean 2.90 pg/ml, SD 1.79) vs spinal-onset patients (65 subjects, mean 2.13 pg/ml, SD 10.87, p 0.33), the IL-6 level was not correlated with disease duration (p 0.78) or ALSFRS-R (p 0.09), but it was negatively correlated with FVC (r = 0.35, p 0.04), and with mean bilateral phrenic nerve evoked potential amplitude (phrenic ampl) (r 0.33, p 0.006), and positively correlated with age (r = 0.4, p < 0.001). Using a multiple regression model the only independent factor in addition to age was phrenic nerve amplitude (p 0.026) (Fig. 2).

In this ALS population, the time of NIV initiation was recorded in 77 out the 82 patients included, it was uncertain in the remaining 5. Within the time interval of 6 months after blood sampling, 33 patients required NIV, but 44 did not receive this medical indication. The mean IL-6 value was 8.93 pg/ml (SD = 2.17) in the former group, and

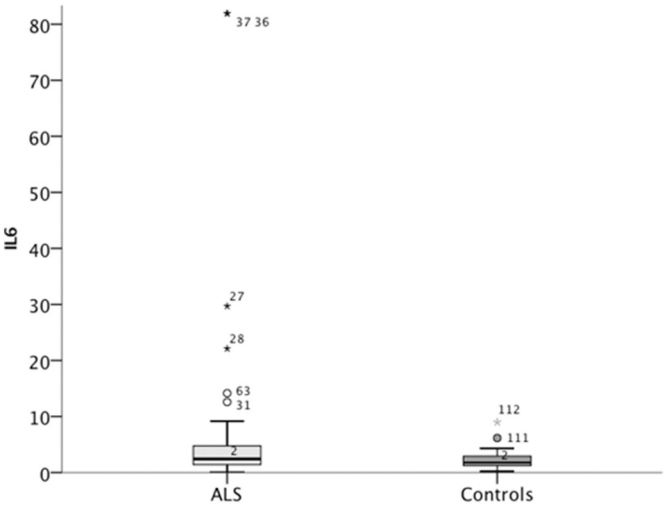

Fig. 1. Comparison between IL-6 levels in controls and ALS patients. We observe a non-significant trend for greater values in ALS population.

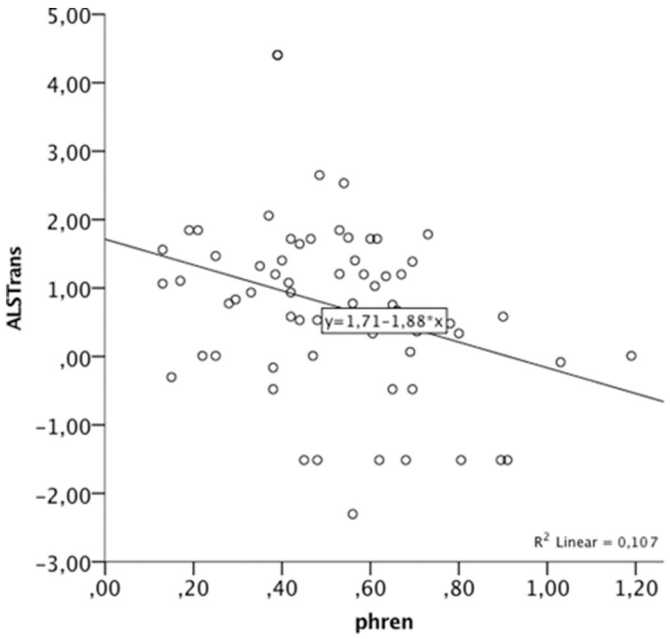

Fig. 2. Correlation between mean bilateral phrenic nerve motor evoked potential (CMAP) and IL-6 levels (the IL-6b values were log-transformed in order to normalize the data for regression analysis). A significant correlation was found.

2.17 pg/ml (SD = 1.58) in the latter group (p = 0.001). In the group 16 patients with IL-6 value above the 3rd interquartile (4.45 pg/ml), the time to NIV was shorter than 2 months after sampling in 9 patients, and uncertain in other 2 patients, in the remaining the time interval varied between 4 months (in 2) and 9 months.

4. Discussion

Several inflammatory biomarkers have been found to be elevated in ALS patients, in particular cytokines. This supports the concept that proinflammatory cytokines have a role in the pathogenesis or progression of ALS [15]. A recent meta-analysis has shown that blood tumour necrosis factor-α (TNF), TNF receptor 1, IL-6, interleukin-1β, IL-8 and vascular endothelial growth factor levels were significantly raised in patients with ALS compared with control subjects [17]. Abnormal numbers of inflammatory cells, and activated astrocytes and microglia have been identified in histological studies of spinal cord and brainstem in ALS [18]. Indeed, PET imaging studies revealed microglial activation in patients with ALS [19].

However, inflammation can have a neuroprotective role through involvement of T-regulatory (Treg)/Th2 immune system, leading to anti-inflammatory neuroprotective responses that block noxious pro-inflammatory mediators [20,21]. The progression rate in ALS seems to be modulated by endogenous Treg activity [22,23]. Elevated cytokine levels can favour either disease progression or neuroprotection depending on a complex interplay between immune cells and their mediators. In particular, IL-6 is a bi-functional cytokine, both performing as a pro-inflammatory mediator [24] and an anti-inflammatory myokine. [25] It has been suggested that increased IL-6 release could represent a neuroprotective reaction against excitotoxic damage [26], as observed in an ALS animal model. [6] IL-6 has a complex action on cell metabolism. It promotes glucose uptake and fatty acid oxidation [27] and increased IL-6 release can induce hypermetabolic imbalance as is associated with ALS [28]. IL-6 metabolism is of current interest as a factor in ALS. This cytokine has recently been proposed as a treatment target, leading to a multicenter, randomized, double-blind, placebo-controlled 16-week study evaluating the safety and tolerability of tocilizumab in subjects with ALS, a study that was completed in July 2018 [29]. We found an increased venous blood level of IL-6 with increasing age, a finding in agreement with Ershler [30], but this finding is not universal [31]. Since there is a diurnal variation in in IL-6 levels [32] all our blood samples were taken in the morning, but it is not clear whether this precaution was taken by all other investigators. The levels of IL-6 in our ALS patients were markedly variable (Table 1), and the mean value did not reach statistical significance in our study, as also shown in a meta-analysis by Hu et al. [17]. A further likely related factor is hypoxaemia, and associated pulmonary inflammation. Hypoxemia has been associated to higher IL-6 levels [4,11], probably consequent to hypoxia-activated vascular cells IL-6 induction [33]. In our patients there was a correlation with diaphragmatic CMAP amplitude, suggesting this was important. It would be relevant to have 24 h oxygen saturation studies at the time of blood sampling to definitively address this issue. However, analysing time to NIV initiation as determined by an independent medical team managing respiratory care, our results indicate that higher IL-6 levels were associated with impeding respiratory insufficiency.

In summary the significance of raised IL-6 levels in ALS is currently uncertain. A similar difficulty in understanding potential markers due to respiratory involvement in ALS has been noted, for example, in blood protein CC-16 levels [34].

We conclude that IL-6 should be explored as a marker of respiratory failure in ALS.

Disclosure of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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