The correlation between inflammation and aging has not yet been 100% understood. Chronic inflammation differs widely from its acute form in its biochemical pattern and in its biological purpose. There is a large body of evidence that already characterized chronic inflammation in detail and that proved that many age-related diseases interact with a simultaneous subjacent chronic inflammation state. Yet, it is still not clear whether chronic inflammation plays a major role in ageing itself (cause) or if it is the expression of already triggered harmful processes (consequence), acting as another intermediate link in the age-related-damage chain to which tissues are exposed with the years. In any case, chronic inflammation accomplishes its biological effect through specific cellular action. This activity is conditioned and regulated by certain molecules, like inflammatory cytokines. It is logical to think that if the concentration of these mediators can be reduced, its action and the inflammatory harm they produce can be slowed down too, at least up to some extent. We obtained an autologous serum (AS) with high concentrations of growth factors and anti-inflammatory cytokines that in former studies proved local action when applied in dermis. A systemic effect of this serum could be claimed if the reduction of the blood concentration of some inflammatory cytokines after its application could be proven. This sole measurement was the aim of this pilot study, leaving the impact and clinical effect of the accomplished cytokine reduction out of its scope.

KEYWORDS: chronic inflammation, cytokines, autologous serum, aging.

its biological purpose but also in its biochemical pattern. Aging is a complicated, multi-factorial, unavoidable, deleterious process. There are many biological theories that claim to be the full explanation of the aging process, though today it is well accepted that none of them provides full evidence to comprehend the aging process completely [5]. In general, they can be divided into two groups: a) stochastic theories, that accept the damage as a fortuitous consequence of the action of external agents and b) deterministic theories that consider the deterioration of ageing as an inherent process of human nature [6]. Among these major theories, there is the “Inflammation Theory of Ageing”, sometimes considered as a sub-theory [7] of the “Free Radical Theory [8]”. Briefly, it states that constantly increasing pro-inflammatory cytokines are a consequence of bystander damage and the cause of cell deterioration and aging. Thus, inflammation can be considered a core process of aging [9] and vice versa, aging is sometimes referred as a chronic inflammatory state condition. There is a large body of evidence that has already successfully related: a) aging and chronic inflammation [9], b) aging and age-related diseases [10] -that interact within a simultaneous subjacent chronic inflammatory state- and c) aging with both kinds of processes. Yet, it is still not clear whether chronic inflammation plays a major role in ageing itself (cause) or if it is the expression of already triggered harmful processes (consequence) that act as another intermediate link in the age-related-damage chain to which tissues are exposed with the years.

In any case, chronic inflammation involves persistent acute inflammation due to the mismanagement of the well orchestrated inflammation resolution phase [11]. A normal inflammatory onset starts with tissue damage or pathogen invasion and it aims not only to eliminate the causative agent, but also to restore tissue structure and function [12]. When any of these occur, pro-inflammatory mediators such as interleukin 1 beta (IL-1b), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-a) or histamine are released. Their action will quickly result in: vasodilation, endothelium enhanced permeability, complement activation and neutrophil chemotaxis. Inflammation will last until its biological effect is accomplished. Then, it should resolve. Many mechanisms are involved in inflammation triggering, maintenance and resolution. The problem with chronic inflammation is not how often it starts, but how often it fails to resolve [13]. It may be the consequence of: a) failure to remove acute inflammation stimuli, b) leukocyte persistent production of pro-inflammatory mediators and reactive oxygen species (ROS) or c) constant chemotactic stimuli [11]. As any other human complex organic process, chronic inflammation achieves its biological purpose through specific cellular action and modulation. This activity is conditioned and regulated by certain molecules. The whole inflammatory/anti-inflammatory regulation process is governed by specific cytokine stimuli.
When getting older, physiological cytokine patterns change. Chronic and acute inflammation, diseases, traumas and other events may trigger these changes. With the years, blood plasma content of some specific cytokines is naturally, slowly and regularly increased [14]. Other cytokines may just remain partially elevated after acute stress. Mechanisms that regulate these processes are complex. Independently of how they occur (beyond the scope of this study), there is certain knowledge about what cytokines will be found in elevated concentrations in the blood of aged people. The human body gains content of pro-inflammatory cytokines like: TNF-α, C reactive protein (CRP), IL-6 and IL-1 [14], as well as of remnant products of their molecular interaction, such as amyloids (CRP-tissue aggregation). Data of NHANES III survey showed that chronically elevated acute-phase markers have proven to be indicators of higher human mortality.

Growth factors and anti-inflammatory cytokines can be obtained and concentrated with this serum. The products released by mononuclear cells and platelets during the serum production are derived from intracellular reservoirs and also by de novo synthesis. The list of factors present in this serum is probably larger than what has already been published: IL-1Ra 11 ng/mL, EGF 1 pg/ml, TGF-B1 39,5 ng/ml, IGF-1 108,9 ng/ml, PDGF-AB 27,1 pg/ml, after 24 hours/37°C incubation, 3000g/10m centrifugation and -20°C freezing (numbers express means of measurements with specific ELISA Kits by R&D Systems, Minneapolis, MN, USA, from blood samples from 10 volunteers) [4]. It is coherent to think that if the concentration of these mediators can be reduced, then its action and the inflammatory harm can be slowed down too, at least up to some extent.

**MATERIALS AND METHODS**

8 women between 30 and 60 years old with no history of systemic pathologies, cancer or any malign lesions were included in this pilot study. They were not undergoing any chronic treatment, not receiving any daily medication and they were not pregnant nor breast feeding. Every subject had blood samples extracted the day before the first session (Sample 1 – day 0) and 30 days after the second session (Sample 2 – day 45). Many markers could have been useful to demonstrate the hypothesis of this study, but ultra-sensible C reactive protein (usCRP) and IL-6 were chosen due to: a) high prevalence of increased concentrations among aged people, b) initial role in inflammation cascade, c) synergistic biological effect. usCRP was measured by turbidimetric quantification (0,05 mg/L limit of detection) and interleukin-6 (IL-6) with an Immunoassay (0,005 pg/mL limit of detection).

Five sequential steps [4] were followed to obtain the serum: 1) **Extraction**: left arm venous blood was obtained using a Vacutainer™ system. 30 ml were collected in the special tube/syringes were they were expose to 2 mm borosilicate glass spheres; 2) **Incubation**: blood extracted was incubated at 37°C for 24 hours; 3) **Centrifugation**: single cycle of 10 m/ 5000
4) **Preparation**: serum was extracted individually from each device and concentrated in one syringe. 20G x ¾ needles were used; 5) **Storage**: for the second application session, two 3.6 ml vials were kept 15 days at -20°C until taken out 40 minutes before application. Subjects had two serum vials (3.6 ml each) injected in the right gluteus every session (Session 1 – day 1 and Session 2 day 15). 19G/40 mm needles and 0.2 µm biological filters were used and manual standard intramuscular technique was performed. Since group size was small (n=8), parametric tests seemed not suitable for these analysis. The assumption of normal distributions was assessed for both variables with a Shapiro-Wilk test and was rejected. Mean concentrations of IL-6 and CRP before and after serum application were compared. The non-parametric Mann-Whitney U test was used. Statistical Product and Service Solutions (SPSS) Ibérica, S.L.U., España, 17.0 for Windows® was the software used for statistical analysis.

**RESULTS**

IL-6 mean pre-treatment concentration: 1,150 (SD 0.272). IL-6 mean post-treatment concentration: 0.063 (SD 0.042). p = 0.002. CRP mean pre-treatment concentration: 0.113 (SD 0.005). CRP mean post-treatment concentration: 0.098 (SD 0.019). p = 0.426.

**DISCUSSION**

The difference between pre and post treatment usCRP blood concentrations was not statistically significant and the difference between pre and post treatment IL-6 blood concentrations was statistically significant. But although the reduction of the mean concentration of IL-6 after the injection of this autologous serum under the described protocol was important, this fact must be analyzed with caution and taking the following points under consideration: Although non-parametric analysis brought-up significant data, this was a pilot study.
with a very limited number of subjects (n=8). Inferences should not be done regarding general female population. Further investigations should provide sufficient evidence to evaluate and build-up the full distribution model of the variable. The clinical relevance of this finding has not been assessed yet. The impact of this concentration reduction should be thoroughly studied and solid evidence must be provided in order to decide whether the statistically significant differences found in this pilot study are worthy of continuing this research line: these reductions must be certified, their usefulness should be proved and an administration protocol must be settled down. It is very important to have in mind that IL-6 blood concentration may fluctuate according to many variables not taken into account in this pilot study. It is not likely to be observing biased results for these 8 cases, but still, multivariable models and further analysis will be mandatory to isolate and understand the serum effect. Among the samples there are some extreme values that because of the very small number of subjects included in this pilot study may mislead conclusions and add complexity to the interpretation of the results.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Alejandro Garcia-Larrosa for his contribution to this work.

REFERENCES