INTERLEUKIN 6, SOLUBLE TUMOR NECROSIS FACTOR RECEPTOR I AND RED BLOOD CELL DISTRIBUTION WIDTH AS BIOLOGICAL MARKERS OF FUNCTIONAL DEPENDENCE IN AN ELDERLY POPULATION: A TRANSLATIONAL APPROACH

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**Running title:** Inflammation and functional dependence.
ABSTRACT

In present investigation we have analyzed the association between functional dependence and inflammatory biomarkers using the Barthel Index (BI) and the Katz Index (KI). This analysis may contribute to translational medicine by incorporating the clinical and laboratory data to better understand the relationship between chronic inflammation and functional dependence in the elderly population. The ultimate goal of this study was to identify possible useful biomarkers of functional dependence in the elderly. Participants in this study consisted of 120 older subjects (90 women and 30 men; range 68-105 years) who were selected from the Santa Teresa nursing home (Oviedo, Spain). We studied functional status using the following tools to diagnose the functional dependence by clinicians: BI and KI for activities of daily living. We analyzed morbidity, sociodemographic characteristics and a panel of inflammatory and inflammatory-related markers. In linear regression models adjusted by age, sex, anti-inflammatory drug use and morbid conditions high levels of interleukin 6 (IL-6) and soluble TNF receptor-I (sTNF-RI) were associated with functional dependence as measured using BI and KI. Elevated levels of red blood cell distribution width (RDW) were also associated with functional dependence measured using the KI after adjusting for the same potential confounders. The current results suggest that high IL-6, sTNF-RI and RDW levels are independently associated with the functional dependence in the elderly population. The results are consistent with the presumed underlying biological mechanism, in which the up-regulation of inflammatory mediators is associated with functional dependence in elderly subjects.

Keywords: Functional dependence; Interleukin 6; soluble TNF receptor-I; Red blood cell distribution width; Chronic Inflammation.
1. INTRODUCTION

Aging is characterized by a subclinical inflammatory process defined as two- to four-fold elevations in circulating levels of inflammatory mediators such as cytokines, their soluble receptors, chemokines and acute-phase proteins as well as minor increases in specific types of immune cells [1-2]. The molecular and physiological significance of systemic low-grade inflammation in chronic diseases is not yet fully understood; however, several studies have proposed chronic inflammation as a risk factor for major deleterious health-related events in older persons [3-4].

Physical disability is associated with poor quality of life, increased risk for hospitalization, mortality, the need for long-term care and higher health care costs [5]. This situation highlights the importance of developing effective screening measures to analyze functional dependence. The functional decline of elderly individuals has been associated with age-related alterations in their immune functions and stress response [6]. In fact, several studies have shown that the subclinical inflammatory process is implicated in the functional decline among the elderly population [4, 7-9]. However, most studies have investigated specific functional execution tests (e.g., grip strength, knee strength, chair stand, walking time or walking speed), rather than a whole spectrum of functional characteristics associated with basic functional performance and with relevant clinical significance [10]. Additional data are required to elucidate the relationship between subclinical inflammation and functional status in elderly individuals. Therefore, we have analyzed functional dependence using the Barthel Index (BI) and the Katz Index (KI). BI and KI are assessment instruments used by clinicians to determine a patient’s level of independence in basic daily activities using a large panel of functional variables [11-12]. BI and KI are more operational instruments and more
related to dependence in activities necessary of daily-life that other methods exclusively based on force, such as hand grip. Furthermore, it is a methodology with high reproducibility, and it does not add special tasks to daily work. Analysis of chronic inflammation using BI and KI may contribute to translational medicine by incorporating the clinical and laboratory data to better understand the relationship between chronic inflammation and functional status in the elderly population.

Due to the complexity and ubiquitous nature of low-grade inflammation [13-14], a single type of inflammatory biomarker may not provide adequate specificity or sensitivity to correlate to the clinical information. A profile or specific panel of inflammatory and inflammatory-related parameters would allow for further biomarker development. Furthermore, cross-sectional single inflammatory mediator measurements may not reflect the true complexity of the relevant inflammatory processes in vivo [15]. The analysis of multiple inflammatory biomarkers may provide a reliable general view of clinical status [9]. It seems that the clinical-practical approach may be headed in this direction [16]. Therefore, we conducted an observational cross-sectional study that evaluated the relationship between functional dependence and chronic inflammation through the analysis of a large panel of inflammatory and inflammatory-related parameters. The ultimate objective of the present study was to identify potential differential inflammatory biomarkers of functional dependence in the aged population.

2. METHOD

2.1. Participants
Participants in this study were 120 institutionalized subjects who were older than 65 years (90 women and 30 men; age range 68-105 years) and were selected from the Santa Teresa nursing home (Oviedo, Spain). The subjects were not rigorously selected according to their morbidity characteristics to obtain a representative sample from the population. Participants were recruited from November 2008 to February 2009. Exclusion criteria were recent or current infection, malignant disease, malnutrition and pharmacological interference (immunosuppressive and anti-neoplastic drugs and testosterone). Malnutrition was defined as a serum albumin less than 3 g/dl in a recent determination (month prior to enrollment in the study). Blood was drawn from the participants. Initial evaluations were carried out by experienced geriatricians and included an exhaustive review of the subjects’ medical and pharmacology history, a physical examination and a physical activity questionnaire. The presence of disease was based on explicit diagnosis in the medical history. Diseases that were considered in the current analysis were cognitive impairment, dementia, osteoporosis, hypertension, chronic obstructive pulmonary disease, osteoarthritis, depression, heart failure, ischemic heart disease, rheumatoid arthritis, hyperthyroidism, cancer, dyslipidemia and type 2 diabetes.

The functional abilities of the subjects were assessed using BI and KI [11-12]. BI is a 10-item scale with the following items: feeding, grooming, bathing, toilet use, dressing, walking, transfers, climbing stairs, fecal incontinence and urinary incontinence. The highest score is 100 (independence), and the lowest score is 0 (total dependence). KI ranks the adequacy of performance in the following six functions: bathing, dressing, toileting, transferring, continence, and feeding. Subjects were scored using ‘yes’ or ‘no’ for independence in each of the six functions. A score of 6 indicated
functional independence, and a score of 2 or less indicated severe functional impairment. BI and KI were assessed by nursing aides who live with the patient daily, and therefore, can evaluated in an objective and effortless way the individual performance. The functional situation of each subject was assessed the week prior to inclusion in the study. This must eliminate subjective bias related to the interrogation of a particular subject.

Each participant or the participant's guardian received information about the purposes and objectives of the study and signed an informed consent form. The study was approved by the Hospital Central de Asturias (Oviedo, Spain) Ethics Committee.

2.2. Blood Collection

Blood samples were obtained by venipuncture following an overnight fast and 15-minute rest in the morning. All venous blood samples were obtained in the morning before 10:00 AM to preclude circadian variation. Blood samples were drawn into vacutainer tubes with a gel separator for serum or containing EDTA for plasma (BD, NJ, USA). After processing, the serum and plasma were divided into aliquots and stored at -80°C pending analysis.

2.3. Biochemical Analysis

The hematological parameters, including hemoglobin concentration (HGB), red blood cell distribution width (RDW) and total leucocyte (WBC), neutrophil (NEU) and monocyte (MONO) counts, were measured using an automated hematology analyzer
SYSMEX SF-3000 (GMI Inc., MI, USA) in a well-standardized hematology laboratory at the Monte Naranco Hospital (Oviedo, Spain).

All inflammatory biomarkers were measured in duplicate using commercially available enzyme-linked immunosorbent assays according to the manufacturer’s recommendations from frozen plasma and serum samples. The average of the two measurements was used in the analysis. The levels of interleukin-6 (IL-6) (Gen-Probe Diaclone SAS, Besancon Cedex, France), interleukin-10 (IL-10), soluble IL-6 receptor (sIL-6R) (Invitrogen Corp., CA, USA) and soluble gp130 (sgp130) (R&D Systems Inc., MN, USA) were assayed in plasma samples. Interleukin 1 receptor antagonist (IL-1ra) and tumor necrosis factor-α (TNF-α) (Invitrogen Corp., CA, USA) and soluble TNF receptor-I (sTNF-RI) and interleukin 1β (IL-1 β) (Bender MedSystems Inc., VI, AUT) levels were measured in serum samples.

2.4. Statistical Analysis

Descriptive statistics were used to characterize the study population and to describe the studied parameters at baseline. The data were expressed as frequencies (percentages) for categorical variables and the mean ± standard deviation (SD) for continuous variables. The normality of the data was analyzed using the Kolmogorov-Smirnov test. RDW, TNF-α, sTNF-RI, IL-6, IL-1β and IL-10 were log-transformed to achieve a normal distribution. For clarity, the original values are used to describe the characteristics of study subjects.

The linear regression analysis for each measurement of the physical performance (BI and KI) was fitted against each of inflammatory and inflammatory-related markers.
after adjusting for age, sex and anti-inflammatory drug use (model 1) to account for potential confounding of these variables. To establish whether the association of biomarker levels and BI and KI were influenced by morbid conditions, sequential univariate linear regressions were also adjusted for age, sex, anti-inflammatory drug use and the total number of diseases per subject, including the incidence of cognitive impairment, dementia, osteoporosis, hypertension, chronic obstructive pulmonary disease, osteoarthritis, depression, heart failure, ischemic heart disease, rheumatoid arthritis, hyperthyroidism, cancer, dyslipidemia and type 2 diabetes (model 2).

The statistical software package SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Differences were considered statistically significant when \( p < 0.05 \).

3. RESULTS

Demographic and clinical characteristics for the sample population are shown in Table 1. The mean age of the sample population was 86 ± 7 years. The prevalence of women was 75.0%. The sample population displayed a wide range of functional statuses (BI score = 0-100; KI score = 0-6). Subjects did not follow the following global recommendations for physical activity that were provided by the World Health Organization (WHO) [17].

Table 2 displays the univariate linear regression models that were used to examine the relationship between the inflammatory and inflammatory-related biomarker
levels using BI outcome measurements. In univariate regressions that were adjusted by the confounders age, sex and anti-inflammatory drug use, high levels of sTNF-RI and IL-6 were directly associated with a low BI score. A higher KI score was associated with high levels of RDW, sTNF-RI and IL-6 in a model that was adjusted using the same potential confounders (Table 3). Because chronic inflammation was associated with morbid conditions, an additional analysis was conducted to determine whether the incidence of diseases altered the relationship between biological markers and BI or KI scores. When we applied univariate regressions that were adjusted by age, sex, anti-inflammatory drug use and morbid conditions, sTNF-RI and IL-6 for BI (Table 2) and RDW, sTNF-RI and IL-6 for KI remained statistically significant for KI (Table 3). Finally, HGB levels showed an association nearly significant with BI score for both models.

4. DISCUSSION

The current study showed that higher inflammation levels, indicated by the biological markers used in this study, were associated with poor index scores of daily living activities in an institutionalized elderly population with a wide range of age and functional statuses. Specifically, we report an intimate relationship between high levels of IL-6 and sTNF-RI and poor functional status, which was measured using BI and KI and was independent of age, sex, anti-inflammatory drug use and morbid conditions. Elevated levels of RDW were also associated with poor functional status that was measured using KI after adjusting for the same potential confounders. The results are consistent with the presumed underlying biological mechanism, in which the up-
regulation of inflammatory mediators is associated with the physiological decline in elderly subjects [13].

We observed an association between higher circulating concentration of IL-6 and sTNF-RI and poor functional performance in models for both BI and KI scores even after adjusting for age, sex, anti-inflammatory drug use and morbid conditions. It should be noted that we used two functional dependence indices obtaining virtually identical results of both biological markers. These associations are consistent with several other reports. High levels of the sTNF-RI and sTNF-RII were associated with lower physical function [18] and with a decline in muscle mass and strength [7] in older populations. Hsu and colleagues [9] demonstrated that an inflammatory index containing sTNF-RI and sTNF-RII among others inflammatory parameters was inversely related to several measures of functional status: knee strength and grip strength. Furthermore, in recent investigation higher sTNF-R levels were associated with disability and physical dysfunction in a cohort of late middle-aged African Americans [19]. Because soluble receptors are more stable in the circulation over time compared with cytokines, soluble receptors might be more reliable markers of chronic inflammation [20]. In addition, several studies have proposed that high levels of soluble cytokine receptors may indicate the body’s attempt to control inflammation and may represent a prolonged or severe underlying inflammatory state [21]. Therefore, elevated sTNF-RI concentration may reflect a severe state of inflammation that is associated with low functional performance in the elderly population. IL-6 has also been associated with poor physical performance in elderly subjects. Stenholm and colleagues [22] reported that higher levels of IL-6 predict declines in muscle strength. In a study by Cesari and colleagues [23] using men and women aged from 65 to 102 years, a lower handgrip
strength was associated with higher levels of CRP and IL-6. Notably, high levels of IL-6 were significantly associated with a worse BI score after adjusting for diseases in a population-based sample consisting of 197 women and 65 men who were 90 years old [4]. Moreover, subjects with high levels of IL-6 display the greatest declines in functional dependence based on KI after 4 years of follow-up [24]. BI and KI are commonly employed diagnostic tools that are used to assess the functional dependence by clinicians. Therefore, the analysis of functional status using these indices integrates the experimental and clinical data to better understand the association between inflammatory biomarkers and functional dependence, which has the potential to ultimately improve translational research. In light of these findings, circulating IL-6 and sTNF-RI may constitute useful screening measures of functional dependence in the elderly population.

Inflammatory responses to specific insults involve a cascade of distinct cellular and molecular events. The first two cytokines in the classic inflammatory cascade are TNF-α and IL-1β, which are produced locally and are considered pro-inflammatory cytokines. TNF-α and IL-1β stimulate the production of IL-6. IL-6 inhibits the production of TNF-α and IL-1β and stimulates the release of sTNF-Rs and IL-1ra [25]. Cytokines work in networks, the effects of individual mediators on physiological functions depends on their place within the network [26]. Therefore, IL-6 and sTNF-RI could be considered as markers of the complex process of chronic inflammation that is associated with functional decline. Furthermore, it is relevant to note that we did not find association between neither sIL-6R nor sgp130 and functional dependence in our population. Our data support previous report according to which serum concentrations of sIL-6R and sgp130 show smaller differences between health and disease [27]. In the
circulation, IL-6 can combine with sIL-6R to form an IL-6/sIL-6R complex, which increases the half-life of IL-6 and allows signaling to occur in tissues devoid of membrane IL-6R, a process termed “trans-signaling” [27]. This complex can be rendered inactive by a soluble form of the gp130 receptor [28]. While there is abundance of research characterizing IL-6 levels and physiological decline [4, 22, 29], few studies have investigated the role of the sIL-6R and sgp130 [2]. This is somewhat surprising because sIL-6R and sgp130 are fundamental factors in the pathogenesis of diseases where IL-6 is considered as an important pathogenic factor [2, 27, 30].

We showed a close relationship between high levels of the hematological parameter RDW and a decline in functional status, which was measured using KI. The relationship between RDW and BI showed statistical differences that were nearly significant. Data on RDW and functional performance are scarce. However, the findings from the present study are consistent with those of other recently published studies that demonstrate an association between high levels of RDW and adverse clinical outcomes. RDW has been suggested as a strong, independent predictor of adverse outcome in chronic heart failure and a strong predictor of mortality in the general population of adults 45 years or older [31-32]. In a recent meta-analysis based on seven relevant studies of older subjects (11,827 community-dwelling older adults) with varied health and demographic compositions, Patel and colleagues [33] have reported that an elevated RDW level is a powerful predictor of mortality in older adults with and without age-associated disease and is independent of several risk factors for death. These results suggest an association between RDW and adverse outcomes in older subjects. RDW reflects the variability in the size of circulating red cells. The exact physiological mechanisms that underlie the association of RDW with functional decline are unknown.
Elevated RDW has been proposed to indicate inflammatory stress [31]. Cytokines inhibit erythropoietin-induced erythrocyte maturation, which is reflected in part by an increase in RDW [34]. Accordingly, rather than display a direct causal relationship with the functional decline, RDW should be considered a surrogate marker of chronic inflammation. Current findings are notable given that RDW is widely available to clinicians as part of the complete blood count and therefore incurs no additional costs [35]. Furthermore, we found a weak direct association between hemoglobin concentration and BI score. Current findings is consistent with previous investigations that reported mild and severe anemia as risk factor risk of functional and cognitive decline, frailty, hospitalization and all cause mortality in older subjects [36-37].

It is unclear whether the elevated levels of inflammatory markers are consequence of chronic diseases or serve as markers of chronic diseases that have an effect on functional performance. Alternatively, inflammatory markers may have their own effect upon the aging process. Previous studies have shown an independent association between disability and chronic disease. In a large community-based population that has been studied by Cohen and associates [38], higher levels of serum and mononuclear cell production of IL-6 are related to participants’ self-reports of fatigue and functional decline and were not necessarily associated with disease. Pennix and colleagues [21] have suggested that inflammation is a prognostic marker for incident mobility limitation over 30 months and is independent of cardiovascular disease events and incident severe illnesses. Previous studies have reported elevated levels of inflammatory parameters in debilitating conditions that are often associated with functional decline in elderly subjects as follows: anorexia, malnutrition, depression, weight loss, cachexia, emphysema and anemia [34, 39-40]. In our current

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study, we observed an intimate association of elevated levels of IL-6 and sTNF-RI with functional decline, even after adjusting for morbid conditions. One possible explanation is that the elevated levels of the inflammatory mediators that were observed in the current study reflect a biological deregulation due to aging, which is indicative of a pro-inflammatory state that may be associated with physical dependence independent of morbid conditions. However, we cannot exclude the effect of diseases or subclinical inflammatory diseases that were not recorded. Nevertheless, the findings from the present study suggest that chronic inflammation is a biological mechanism that may be associated with the decline in functional status in older adults. Results of the current investigation have important clinical implications and may be used to develop possible anti-inflammatory interventions to reduce chronic inflammation such as shifting sedentary lifestyles to low levels of aerobic and resistance physical activity [41-42], long-term training [43] or changes in lifestyle habits such as the cessation of smoking, eating a Mediterranean diet and reducing adipose [44-45].

Some limitations are noteworthy. First, a population sample greater than 120 participants would have been desirable. The low number of men should be noted. Second, because of cross-sectional characteristics of the present study, we have not been able to determine the causal directions of the observed association between inflammatory markers and physical function. Third, anti-inflammatory intake is a common feature in older adults. Therefore, data were adjusted to account for the impact of anti-inflammatory use on the results. Our statistical control for anti-inflammatory medications could be insufficient but is the best option currently available. Based on these limitations, the cautious interpretation of these results is warranted.
We conclude that elevated IL-6, sTNF-RI and RDW levels are independently associated with poor functional status in an institutionalized elderly population. Furthermore, we showed that objective measurements of chronic inflammation were indicators of functional status in an older population. The assessment of IL-6, sTNF-RI and RDW may represent a useful screening test and indicate potential targets of intervention. This topic requires a more detailed investigation that is of great interest in light of the present results.

5. ACKNOWLEDGMENTS

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6. REFERENCES


LEGEND

Figure 1. Associations between biomarkers and functional dependence in our population.

Table 2. Association between biomarker levels and Barthel Index.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B (95% CI)</td>
<td>β</td>
</tr>
<tr>
<td>WBC (*10^9/µl)</td>
<td>-0.650 (-4.520, 3.220)</td>
<td>-0.030</td>
</tr>
<tr>
<td>NEU (*10^9/µl)</td>
<td>-0.345 (-5.317, 4.627)</td>
<td>-0.012</td>
</tr>
<tr>
<td>MONO (*10^9/µl)</td>
<td>10.967 (-7.936, 29.870)</td>
<td>0.143</td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>3.452 (-0.276, 7.180)</td>
<td>0.175</td>
</tr>
<tr>
<td>Variable</td>
<td>Model 1</td>
<td>Model 2</td>
</tr>
<tr>
<td>-------------------</td>
<td>------------------</td>
<td>------------------</td>
</tr>
<tr>
<td></td>
<td>B (95% CI)</td>
<td>β (p Value)</td>
</tr>
<tr>
<td>WBC (*10^3/µl)</td>
<td>0.105 (-0.137, 0.347)</td>
<td>0.077 0.393</td>
</tr>
<tr>
<td>NEU (*10^3/µl)</td>
<td>0.073 (-0.239, 0.384)</td>
<td>0.042 0.645</td>
</tr>
<tr>
<td>MONO (*10^3/µl)</td>
<td>-0.669 (-1.854, 0.517)</td>
<td>-0.114 0.216</td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>-0.141 (-0.377, 0.094)</td>
<td>-0.116 0.237</td>
</tr>
<tr>
<td>logRDW (%)</td>
<td>12.428 (0.844, 24.013)</td>
<td>0.185 0.036*</td>
</tr>
<tr>
<td>logTNF-α (pg/ml)</td>
<td>1.224 (-2.176, 4.623)</td>
<td>0.065 0.477</td>
</tr>
<tr>
<td>logTNF-RI (ng/ml)</td>
<td>2.792 (0.506, 5.079)</td>
<td>0.219 0.017*</td>
</tr>
<tr>
<td>logIL-6 (pg/ml)</td>
<td>1.419 (0.204, 2.635)</td>
<td>0.213 0.022*</td>
</tr>
<tr>
<td>sIL-6R (ng/ml)</td>
<td>-0.010 (-0.022, 0.001)</td>
<td>-0.152 0.086</td>
</tr>
<tr>
<td>sgp130 (ng/ml)</td>
<td>0.002 (-0.009, 0.013)</td>
<td>0.035 0.692</td>
</tr>
<tr>
<td>log IL-1β (pg/ml)</td>
<td>1.014 (-0.598, 2.626)</td>
<td>0.109 0.215</td>
</tr>
<tr>
<td>IL1-ra (pg/ml)</td>
<td>-0.001 (-0.007, 0.006)</td>
<td>-0.018 0.835</td>
</tr>
<tr>
<td>logIL-10 (pg/ml)</td>
<td>-1.166 (-5.524, 3.191)</td>
<td>-0.046 0.597</td>
</tr>
</tbody>
</table>

Model 1: Adjusted for age, sex and anti-inflammatory drug use.
Model 2: Adjusted for age, sex, anti-inflammatory drug use and morbid conditions.
B: Betas
CI: Confidence Intervals
β: Standardized betas

Table 3. Association between biomarker levels and Katz Index
*: Statistically significant.

WBC: white blood cell count, NEU: neutrophil cell count, MONO: monocyte cell count, HGB: hemoglobin concentration, RDW: red blood cell distribution width, TNF-α: tumor necrosis factor-α, sTNF-RI: soluble TNF receptor I, IL-6: interleukin-6, sIL-6R: soluble interleukin-6 receptor, sgp130: soluble gp130, IL-1β: interleukin-1β, IL-1ra: interleukin-1 receptor antagonist, IL-10: interleukin-10.