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CIRCULATING CYTOKINES AND LOWER BODY MUSCLE PERFORMANCE IN OLDER ADULTS AT HOSPITAL ADMISSION

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Abstract: *Background:* Aging-related traits, including gradual loss of skeletal muscle mass and chronic inflammation, are linked to altered body composition and impaired physical functionality, which are important contributing factors to the disabling process. We sought to explore the potential relationship between lower-body muscle strength decline and inflammatory mediators in older adults. *Methods:* We performed a cross-sectional analysis in 38 older adults admitted to an acute care of the elderly unit (57.9% women, mean age=87.9±4.9 years; mean body mass index [BMI]=26.5±4.7 kg/m^2). Clinical and functional outcomes including weight, height, BMI, dependence, physical and cognitive performance, and muscle strength measured by one-repetition maximum (1RM) for leg-extension, leg-press, chest-press and handgrip strength, were assessed. Blood serum content of 59 cytokines, chemokines and growth factors was assessed by protein arrays. Multivariate linear regression analyses were used to examine the relationship between cytokine concentrations and muscle strength parameters. *Results:* After controlling for confounding factors (age, sex, BMI, cumulative illness rating score and physical performance score), 1RM leg-press had a significant negative relationship with GRO (CXCL2) (β= −18.13, p=0.049), MIG (CXCL9) (β= −13.94, p=0.004), IGF-1 (β= −19.63, p=0.003), CK-BETA 8 (CCL23) (β= −28.31, p=0.018) and GCP-2 (CXCL6) (β= −25.78, p=0.004). Likewise, 1RM leg-extension had a significant negative relationship with IGFBP-1 (β= −11.49, p=0.023). *Conclusions:* Thus, several serum cytokines/chemokines and growth factors are negatively associated with lower muscle strength in older patients. Further investigation is required to elucidate the mechanism of elevated inflammatory mediators leading to lower muscle strength.

Key words: Hospitalization, inflammation, elderly, physical function, muscle function.

Introduction

Aging is associated with a decline in immune competence and is often characterized by the reduced production of immune cells, and by elevated blood levels of pro-inflammatory mediators (1). The deterioration of the immune system with age correlates with age-related changes to the cardiovascular system in this vulnerable population, and is a risk factor for cardiovascular diseases and other chronic conditions (2). For instance, chronic low-grade inflammation contributes to the pathogenesis of sarcopenia (defined as age-related loss of muscle mass and function), and can trigger oxidative stress, endothelial dysfunction and insulin resistance through the indirect action of inflammatory mediators (3). In this sense, peripheral blood-based biomarker analysis might provide crucial information on causal mechanisms of healthy (or successful) versus unhealthy aging, as it reflects both inherent genetic and environmental factors. It could also help to unravel the protein networks and individual protein candidates involved in disease or aging processes.

Acute medical illnesses and subsequent hospitalization have devastating consequences on functional capacity, especially in the elderly, and are often sentinel events (4). Low level of mobility and complete bedrest episodes are common in acutely hospitalized older persons, and more than half of this population do not recover preadmission functional status (5, 6), increasing the risk for higher resource use, caregiver burden, institutionalization, and death (7). Previous studies have shown a rapid decline (range 4% to 10%) of total lean leg mass in healthy older adults after only seven days of in-hospital inactivity (8), and lower muscle mass has been associated with a lower likelihood of survival after hospitalization in older patients (9). The chronic inflammation and changes in body composition associated with declining physical function makes older adults even more vulnerable to the negative impact of hospitalization (9).

On the other hand, sarcopenia and dynapenia (defined as age-related loss of muscle strength and power) are associated with an increase in all-cause morbidity and mortality risk in older adults, as well as with a wide range of acute and chronic diseases that affect millions of people worldwide (10, 11). Both age-related conditions and gradual loss in skeletal muscle mass are linked to altered body composition (12) and poorer functionality, which are important contributing factors to the

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disabling process (13). Additionally, low muscle strength has a crucial role in other chronic diseases such as sarcopenic obesity and frailty, creating a spectrum of phenotypes and several clinical conditions (14). There is evidence to indicate that inflammation could be the bridging link between sarcopenia and dynapenia (8, 14, 15).

There is probably no single, ideal biomarker to measure normal physiological functions, but it is possible that a panel of complementary bio/markers (imaging, serum biomarkers, and functional tests) could be used to gain a better understanding of a person's functional status. Some biomarkers have been tested in community-dwelling older people, including serum cytokines, chemokines and growth factors, and other lymphokine markers, but these have not been explored during hospitalization in older medical patients (14). The integration of various biomarkers measured at different stages during hospitalization might have utility for predicting functional phenotypes.

There are strong associations between chronic systemic inflammation pathways and aging processes, which in turn impact on muscle mass and functional capacity/disability (16). We hypothesized that different cell types including macrophages, adipocytes and muscle cells communicate via metabolic hormones, cytokines/chemokines, adipokines and growth factors to regulate skeletal muscle metabolic and functional properties. Dysregulation of signaling molecule homeostasis due to health state, environmental or genetic changes, may contribute to age-related pre-disability conditions defined by reduced physical performance and low muscle mass. To better define the possible relationship between muscle strength decline and inflammatory mediators, we performed a cross-sectional analysis to examine for potential associations between maximal muscular strength and circulating cytokines in hospitalized older patients.

Materials and methods

Design and clinical setting

The study was conducted in the Acute Care of the Elderly (ACE) unit of the Department of Geriatrics in a tertiary public hospital (Complejo Hospitalario de Navarra, Spain). The department has 35 allocated beds with a staff of 11 geriatricians (distributed in the ACE unit, orthogeriatrics and outpatient consultations). Admissions to the ACE unit derive mainly from the Accident and Emergency Department, with heart failure, pulmonary and infectious diseases as the main causes of admissions. The study followed the principles of the Declaration of Helsinki and was approved by the local Research Ethics Committee (ID Pyto2018/7, N°264; 15 May 2018). All patients or their legal representatives provided written consent.

Geriatricians evaluated patients admitted to the ACE unit. A trained research assistant conducted a screening interview to determine whether potentially eligible patients met the following inclusion criteria: age ≥75 years, Barthel Index score ≥60 points, able to ambulate (with/without assistance) and to communicate and collaborate with the research team. Exclusion criteria included expected length of stay <3 days, very severe cognitive decline (a score of 7 on the Global Deterioration Scale), terminal illness, uncontrolled arrhythmias, acute pulmonary embolism or myocardial infarction, or extremity bone fracture in the past 3 months.

Clinical and functional parameters

Height was measured to the nearest 0.1 cm and body mass was measured to the nearest 100 g. Body mass index (BMI) was computed as weight in kilograms divided by height in meters squared. Maximal dynamic strength was measured using a onerepetition maximum (1RM) test for the bilateral leg-extension, leg-press and chest- press exercise (Exercycle S.L., BH Group, Vitoria, Spain). The participants were instructed to perform each repetition as fast as possible during the assessment (17). Handgrip strength was measured in the seated position using a Takei dynamometer (Takei Scientific Instruments Co., Tokyo, Japan). Participants were asked to perform 2 maximum force trials for each hand, with the dynamometer beside but not against their body, and the measurements were recorded in kilograms. The maximum value attained during the four trials was used as the final score (11). Functional capacity was assessed by the Short Physical Performance Battery (SPPB) (16), which combines balance, gait velocity, and leg strength as a single score on a 0 (worst) to 12 (best) scale. Gait speed was calculated for each participant using distance in meters and time in seconds, and was obtained by dividing the distance travelled (4 m) on a flat and unobstructed path by the time to cover that distance. Cognitive function as assessed with the Mini-Mental State Examination (MMSE, 30-point questionnaire; scale of 0 [worst] to 30 [best]) (18); mood status with the 15-item Yesavage Geriatric Depression Scale (GDS, Spanish version; scale of 0 [best] to 15 [worst]) (19); and activities of daily living (ADLs) with the Barthel Index of independence, with a scale of 0 (severe functional dependence) to 100 (functional independence) (20). Data related to number of diseases, cumulative illness rating scale score (CIRS) and length of hospital stay were also collected from clinical records.

Cytokine measures

Fasting venous blood samples were collected from the antecubital vein into EDTA vials in the morning after an overnight fast (08:00 to 09:00 am). Samples were centrifuged at 1500 × for 10 minutes at 4°C and the serum was collected and stored at -80°C until analysis. Quantification of 80 analytes in serum samples was performed using the Abcam Human Cytokine Antibody Array (80 targets; #ab133998) (Myriad RBM, Austin, TX). Briefly, dot-blot protein arrays were blocked with the manufacturer's blocking buffer at room temperature for 30 minutes, and then incubated overnight with 200–250 μg of serum. After washing, a biotinylated anti-cytokine antibody mixture was added to the membranes, followed by incubation with horseradish peroxidase-conjugated

streptavidin and then exposure to the manufacturer's peroxidase substrate. Chemiluminescence signals were quantified with the ImageQuant ECL system (Bio-Rad, Madrid, Spain) and normalized to the positive control signals. Perseus software (version 1.5.6.0) was used for statistical analysis (21).

Statistical analysis

Clinical, functional and cytokine values (a.u) are expressed as mean, standard deviation (SD) or frequencies. Unpaired Student's t-test was used to test the differences between the sex groups. Interactions by sex were explored including interaction terms into the models, as there were no significant interactions (Ps > 0.1), the analyses were performed for men and women together. Spearman's (rho) rank correlation coefficient was used to assess relationships between clinical/functional parameters and cytokines, adjusted for age and sex. Rho values <0.30 were considered low or weak correlations, 0.30–0.70 modest or moderate correlations and 0.70–9.0 strong or high correlations, with Rho coefficients >0.90 denoting a very high correlation. To explore the association between muscle strength parameters and serum cytokines, multiple linear regression analysis and analysis of covariance (ANCOVA) were used simultaneously. The value of muscle strength parameters was used as the dependent variable and cytokine concentration levels were used as the independent variables, controlled for age, sex, BMI, CIRS score, and SPPB score confounders. The covariates included in the adjusted analyses were based on a conceptual model according to the literature and association between clinical/functional outcomes and cytokine concentration (Rho >0.30).

Preprocessing of cytokine expression levels was applied as described by González-Morales et al. (22). Additionally, the log2-transformed values were normalized using quantile normalization to remove unwanted technical variation and to obtain an approximately normal distribution. The kit can detect 80 cytokines simultaneously in a single sample; however, 31 cytokines were below the limit of detection. All the analyzed cytokines were searched against a human protein database to detect the main biological processes related to the overexpressed proteins (23).

A protein-protein interaction (PPI) network analysis was performed using the STRING 10.0 database (Search Tool for the Retrieval of Interacting Genes/Proteins), accessible at https://string-db.org (24). STRING analysis was performed using the medium confidence score (0.400) to compare our filtered data with homologous proteins in the *Homo sapiens* database. This tool includes interactions from published literature describing experimentally-studied interactions, as well as those from genome analysis using several well established methods based on domain fusion, phylogenetic profiling and gene neighbourhood concepts. Statistical analyses were performed with SPSS v24.0 for Windows (SPSS Inc., Chicago, IL) as well as the statistics software R, version 3.6.2, and the packages *limma* and *corrplot* (25). Statistical significance was considered at a two-tailed p-value of 0.05.

Results

Analyses were conducted in a convenience sample of 38 older adults (57.9% women), mean (SD) age 87.9 (4.9) years (range, 78–100 years). The mean length of hospital stay was 7.7 days (min and max, 3 and 11 days, respectively). The two groups were comparable for age, sex distribution, and number of comorbid conditions and functional characteristics by SPPB, Barthel Index, and MMSE, score. In total, 80 proteins were measured and 59 were detected and quantified with the Image-Quant ECL system (Table 1).

The association between cytokines concentrations and clinical data was investigated using Spearman rank (Rho) correlation. This analysis revealed the following significant negative relationships between the levels (a.u) of the cytokines ENA-78 (CXCL5), GRO (CXCL2), MIG (CXCL9), IGF-1, CK-BETA 8 (CCL23), GCP-2 (CXCL6), GNDF, IGFBP-1 and the 1RM leg-extension: rho= –0.31 (p=0.049), rho= –0.40 (p=0.013), rho= –0.42 (p=0.008), rho= –0.41 (p=0.010), rho= –0.32 (p=0.045), rho= –0.39 (p=0.014), rho= –0.36 (p=0.025) and rho= –0.34 (p=0.040), respectively. Similarly, IGFBP-1 and GCP-2 (CXCL6) were significantly associated with the 1RM leg-press: rho= –0.34 (p=0.032) and rho= –0.34 (p=0.033) respectively. Finally, leptin levels were significantly associated with BMI, rho= 0.40 (p=0.011), and osteopontin levels were significantly associated with age, rho= 0.54 (p<0.001) (Figure 1A).

Functional enrichments and representative genes are summarized in Figure 1B, and a complete list of module genes can be found in File S1. Gene ontology (GO) was used to assign related gene categories into their associated pathways through an enrichment analysis with multiple testing corrections. As shown in Figure 1B, PPI network analysis showed that the subset composed of GRO (CXCL2), MIG (CXCL9), CK-BETA 8 (CCL23), GCP-2 (CXCL6), IGF-1, and IGFBP-1 were involved in chemokine-mediated signaling pathway (false discovery rate [FDR]: 2.38×10-6), regulation of signaling receptor activity (FDR: 1.94×10-5), inflammatory response (FDR: 0.00027), regulation of insulin-like growth factor receptor signaling pathway (FDR: 0.00049), G proteincoupled receptor signaling pathway (FDR: 0.0021), positive regulation of cellular process (FDR: 0.0023), and immune response (FDR: 0.0042). Figure 1B summarizes and table S1 the pathway and functional themes of the genes encoding each protein, obtained by STRING analysis (Electronic supplementary material).

Multiple linear regression analyses were performed to confirm the relationship between cytokines and muscle strength parameters. After controlling for confounders, 1RM leg-press showed a significant negative relationship with GRO (β= –18.13, p=0.049), MIG (β= –13.94, p=0.004), IGF-1 (β= –19.63, p=0.003), CK-BETA 8 (β= –28.31, p=0.018), and GCP-2 (β= –25.78, p=0.004). Likewise, there was a significant

Table 1
Clinical and functional characteristics and plasma cytokine concentrations of the sample

Variable	Full sample n = 38		Women n = 22		Men n = 16		P for sex
	Mean	SD	Mean	SD	Mean	SD	
Clinical and functional characteristics							
Age, years	87.9	4.9	88.0	5.5	87.9	4.5	0.934
Body mass, kg	67.1	15.6	58.8	10.9	73.8	15.8	0.003
BMI, kg/m^2	26.5	4.7	25.7	4.5	27.1	4.9	0.371
No. of diseases (%)[a*]	21.1/31.6/5.3/13.2/28.9		12.5/37.5/12.5/6.3/31.3		27.3/27.3/18.2/27.3		0.280
CIRS score[b]	13.3	6.8	9.5	4.0	16.0	7.1	0.002
Length of hospital stay, days	7.7	2.1	7.4	2.4	7.8	1.8	0.583
SPPB, score[c]	4.4	2.5	4.9	2.6	4.0	2.4	0.242
Barthel Index (ADLs), score[f]	75.3	17.3	75.2	21.5	75.3	19.6	0.990
Depression (GDS), scoree	3.9	4.0	3.1	2.8	3.5	3.3	0.479
MMSE, score[d]	23.3	4.0	23.5	3.9	23.1	4.1	0.773
Gait speed, m/s	0.52	0.26	0.51	0.22	0.52	0.29	0.914
1RM Leg extension exercise, kg	64.1	28.3	54.5	20.2	71.1	31.6	0.029
1RM Leg press exercise, kg	134.6	42.7	117.1	47.1	147.3	35.0	0.074
1RM Chess press exercise, kg	29.5	10.4	24.1	7.6	33.4	10.6	0.005
Handgrip strength, kg	16.6	5.6	13.9	4.4	18.4	5.7	0.013
Cytokines concentrations (a.u)							
ENA-78 (CXCL5)	15.8	0.9	16.1	0.7	15.5	1.0	0.041
GRO (CXCL2)	15.9	0.6	16.1	0.6	15.7	0.7	0.151
GRO-alpha (CXCL1)	13.2	0.6	13.3	0.7	13.2	0.6	0.651
IL-1beta	14.0	0.5	14.0	0.6	14.1	0.5	0.857
IL-2	12.6	1.2	12.6	1.3	12.6	1.1	0.893
IL-3	13.8	0.6	13.8	0.6	13.8	0.7	0.725
IL-4	12.4	0.8	12.3	1.0	12.4	0.7	0.647
IL-8	12.5	0.9	12.2	0.9	12.8	0.8	0.052
IL-10	13.9	0.6	13.9	0.6	13.9	0.7	0.757
IL-15	13.7	0.4	13.6	0.4	13.7	0.5	0.463
INF-delta	13.2	1.0	13.3	1.0	13.2	1.1	0.723
MCP-1 (CCL2)	15.7	1.1	15.4	1.0	15.5	1.1	0.474
MCP-2 (CCL8)	12.7	0.8	12.7	0.8	12.6	0.9	0.671
MCP-3 (CCL7)	12.4	0.7	12.3	0.8	12.6	0.6	0.222
MCSF (CSF-1)	12.9	0.6	12.8	0.4	12.9	0.7	0.802
MIG (CXCL9)	14.1	1.3	14.1	0.9	14.1	1.6	0.923
MIP-1beta (CCL4)	14.1	0.6	13.8	0.7	14.2	0.5	0.015
MIP-1delta (CCL15)	17.0	0.6	17.1	0.6	17.0	0.6	0.651
RANTES (CCL5)	20.0	0.5	20.3	0.5	19.9	0.5	0.022
SCF (sKITLG)	13.6	0.6	13.7	0.5	13.6	0.6	0.506
TARC (CCL17)	14.6	0.6	14.7	0.6	14.6	0.6	0.565
EGF	19.0	0.6	19.1	0.5	18.9	0.6	0.213
IGF-I	14.6	0.9	14.8	0.8	14.4	1.0	0.265
Angiogenin	18.8	0.4	18.8	0.4	18.7	0.3	0.936
Oncostatin M	13.5	0.4	13.6	0.4	13.5	0.4	0.413
PDGF-BB	17.1	0.7	17.2	0.5	17.1	0.8	0.651
Leptin (LEP)	17.2	2.2	17.5	2.2	17.0	2.2	0.527

Table 1 (Continued)
Clinical and functional characteristics and plasma cytokine concentrations of the sample

Variable	Full sample n = 38		Women n = 22		Men n = 16		P for sex
	Mean	SD	Mean	SD	Mean	SD	
BDNF	18.2	0.7	18.5	0.6	18.0	0.8	0.066
BLC	15.5	2.1	15.2	2.0	15.7	2.2	0.427
CK-BETA 8 (CCL23)	13.3	0.5	13.3	0.6	13.3	0.5	0.808
Eotaxin-1	15.0	1.3	14.8	1.2	15.1	1.5	0.478
Eotaxin-2	14.4	0.5	14.4	0.7	14.4	0.4	0.859
FGF-6	14.7	1.6	14.4	1.6	14.9	1.6	0.338
FGF-7	13.0	0.6	12.8	0.6	13.2	0.6	0.083
FGF-9	13.5	0.5	13.7	0.5	13.4	0.4	0.143
Flt-3 Ligand	13.3	0.7	13.5	0.7	13.2	0.7	0.336
Fractalkine	14.2	0.5	14.2	0.5	14.1	0.6	0.554
GCP-2 (CXCL6)	14.0	0.7	14.0	0.8	13.9	0.6	0.613
GDNF (hGDNF)	14.4	0.5	14.5	0.5	14.4	0.5	0.637
IGFBP-1	15.1	0.9	15.0	0.8	15.1	0.9	0.728
IGFBP-2	18.4	0.4	18.4	0.4	18.4	0.4	0.980
IGFBP-3	13.0	0.5	13.0	0.6	13.1	0.5	0.584
IGFBP-4	13.8	0.8	13.6	0.9	13.9	0.8	0.311
IL-16	14.4	0.5	14.5	0.4	14.4	0.5	0.542
IP-10 (CXCL10)	15.6	0.6	15.6	0.4	15.6	0.8	0.991
LIF (MLPLI)	14.2	0.3	14.2	0.3	14.2	0.4	0.831
LIGHT (HVEM-L)	14.2	0.5	14.3	0.4	14.1	0.6	0.434
MCP-4 (CCL13)	15.0	2.0	14.7	1.9	15.2	2.2	0.463
MIF	14.3	0.7	14.5	0.7	14.3	0.6	0.387
NAP-2 (CXCL7)	17.8	0.4	17.9	0.4	17.7	0.4	0.330
NT-4	14.2	0.6	14.2	0.5	14.2	0.6	0.737
Osteopontin	17.7	0.9	17.6	1.0	17.8	0.8	0.407
Osteoprotegerin	13.6	0.5	13.7	0.5	13.5	0.5	0.175
PARC (CUL9)	14.4	1.8	14.1	1.7	14.6	1.9	0.404
PLGF	13.5	0.6	13.5	0.6	13.5	0.7	0.890
TGF-beta2	14.7	0.5	14.7	0.6	14.6	0.3	0.575
TGF-beta3	13.7	0.9	14.0	0.7	13.6	1.1	0.221
TIMP-1	16.9	0.4	17.0	0.4	16.9	0.5	0.314
TIMP-2	19.4	0.4	19.4	0.4	19.3	0.4	0.612

BMI, body mass index; CIRS, Cumulative Illness Rating Scale; 1RM, 1 repetition maximum; SPPB, Short Physical Performance Battery. a. The most prevalent diseases were coronary, pulmonary, genitourinary, neurologic and other diseases. b. The CIRS scale evaluates individual body systems, ranging from 0 (best) to 56 (worst). c. The SPPB scale ranges from 0 (worst) to 12 (best). d. MMSE, The Mini-Mental State Examination ranges from 0 (worst) to 30 (best). e. The Yesavage Geriatric Depression Scale ranges from 0 (best) to 15 (worst). f. The Barthel Index ranges from 0 (severe functional dependence) to 100 (functional independence). *Difference by Chi-squared. ENA-78 (CXCL5): Epithelial-derived neutrophil-activating peptide 78, GRO (CXCL2): Growth-regulated protein, GRO-alpha (CXCL1): Growth-regulated alpha protein, IL-1beta: Interleukin 1 beta, IL-2: Interleukin 2, IL-3: Interleukin 3, IL-4: Interleukin 4, IL-8: Interleukin 8, IL-10: Interleukin 10, IL-15: Interleukin 15, MCP-1 (CCL2): Monocyte Chemoattractant Protein-1, MCP-2 (CCL8): Monocyte Chemoattractant Protein-2, MCP-3 (CCL7): Monocyte Chemoattractant Protein-3, MCSF (CSF-1): Macrophage colony-stimulating factor, MIG (CXCL9): Monokine induced by interferon-gamma, MIP-1beta (CCL4): Macrophage Inflammatory Proteins-1beta, MIP-1delta (CCL15): Macrophage Inflammatory Proteins-1delta, RANTES (CCL5): Regulated on activation, normal T cell expressed and secreted, SCF (sKITLG): Mast cell growth factor or C-kit ligand, TARC (CCL17): Thymus and activation regulated chemokine, EGF: Epidermal growth factor, IGF-I: Insulin-like growth factor I, Ang: Angiogenin, OSM: Oncostatin M, PDGF-BB: Platelet-derived growth factor-BB, Leptin (LEP), BDNF: Brain-derived neurotrophic factor, BLC: B lymphocyte chemoattractant, CK-BETA 8 (CCL23): CC chemokine (CCL23), Eotaxin-1: CC chemokine (CCL11), Eotaxin-2: CC chemokine (CCL24), FGF-6: Fibroblast growth factor 6, FGF-7: Fibroblast growth factor 7, FGF-9: Fibroblast growth factor 9, Flt-3 Ligand: Fms-related tyrosine kinase 3 ligand, Fractalkine: Chemokine (C-X3-C motif) ligand 1, GCP-2 (CXCL6): Granulocyte chemotactic protein-2, GDNF (hGDNF): Glial cell line derived neurotrophic factor, IGFBP-1: Insulin like growth factor binding protein 1, IGFBP-2: Insulin like growth factor binding protein 2, IGFBP-3: Insulin like growth factor binding protein 3, IGFBP-4: Insulin like growth factor binding protein 4, IL-16: Interleukin-16, IP-10 (CXCL10): Interferon gamma-induced protein 10, LIF (MLPLI): Leukemia inhibitory factor, LIGHT (HVEM-L): Tumor necrosis factor ligand superfamily member 14, MCP-4 (CCL13): Potential role for monocyte chemotactic protein-4, MIF: Macrophage migration inhibitory factor, NAP-2 (CXCL7): Neutrophil-activating peptide, NT-4: Neurotrophin-4, Osteopontin, Osteoprotegerin, PARC (CUL9): Cytoplasmic anchor protein in p53, PLGF: Placental growth factor, TGF-beta2: Transforming growth factor beta 2, TGF-beta3: Transforming growth factor beta 3, TIMP-1: Tissue inhibitor of metalloproteinases-1, TIMP-2: Tissue inhibitor of metalloproteinases-2.

Figure 1
Correlation patterns

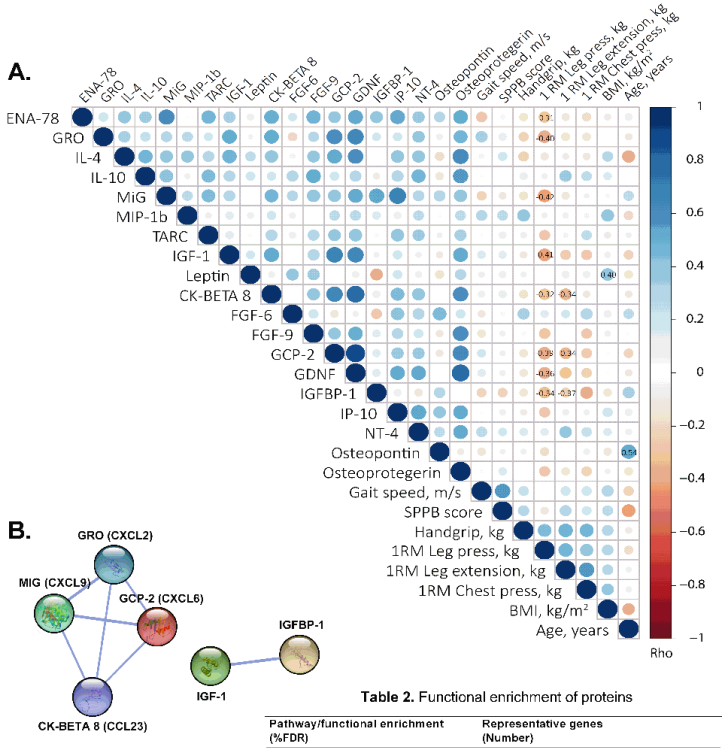

Table 2. Functional enrichment of proteins

Pathway/functional enrichment (%FDR)	Representative genes (Number)
Chemokine-mediated signaling pathway (2.38e-06)	CCL23,CXCL2,CXCL6,CXCL9 (4)
Regulation of signaling receptor activity (1.94e-05)	CCL23,CXCL2,CXCL6,CXCL9,IGF1 (5)
Inflammatory response (0.00027)	CCL23,CXCL2,CXCL6,CXCL9 (4)
Regulation of insulin-like growth factor receptor signaling pathway (0.00049)	IGF1,IGFBP1 (2)
G proteincoupled receptor signaling pathway (0.0021)	CCL23,CXCL2,CXCL6,CXCL9 (4)
Positive regulation of cellular process (0.0023)	CCL23,CXCL2,CXCL6,CXCL9,IGF1,IGFBP1 (6)
Immune response (0.0042)	CCL23,CXCL2,CXCL6,CXCL9 (4)

A. A clustered heatmap of Rho correlation coefficients over all clinical, functional and proteins pairs (using Rho distance, and average linkage). Dark red denotes high correlation (Rho→1), dark blue high anti-correlation (Rho→−1), and white a lack of correlation ($r \simeq 0$). Rho value 0.3 was set as threshold and significance was considered as $p < 0.05$. B. Interactome network for deregulated cytokines. Protein-protein interaction analysis based on STRING network analysis with an interaction confidence score of 0.4. Proteins are represented with nodes and edges (physical or functional interactions) are supported by at least a reference from the literature or from canonical information stored in the STRING database. All altered proteins are grouped according to their biologic process as noted in Gen Ontology (GO). FDR = false discovery rate (reported for STRING database).

negative relationship between 1RM leg-extension and IGFBP-1 (β= −11.49, p=0.023), Table 2.

Discussion

The relationship between muscle performance and pro/anti-inflammatory cytokines levels is poorly understood. The present study is the first, to our knowledge, to demonstrate an association between cytokine levels and muscle strength. The main finding is that the elevated plasma levels of six cytokines [GRO (CXCL2), MIG (CXCL9), IGF-1, CK-BETA 8 (CCL23), GCP-2 (CXCL6), and IGFBP-1] are negatively associated with lower extremity maximal muscle strength in older patients admitted to an ACE unit, after controlling for confounders such as age, sex, BMI, CIRS score and SPPB score. Although previous evidence has suggested an association between inflammatory markers and frailty in hospitalized older adults (26, 27), this is the first study to examine the relationship between lower dynamic maximal muscle strength with serum cytokines, chemokines, and growth factors markers.

There is some evidence that higher levels of inflammatory markers and low levels of anabolic hormones are, respectively, associated with muscle strength and physical performance decline in older people (28, 29). Aging is known to be characterized by quantitative and qualitative modifications of the immune system (30). This phenomenon – defined as immunosenescence – is marked by cytokine dysregulation, leading to elevations in pro-inflammatory cytokines and reductions in anti-inflammatory cytokines, triggering a chronic low-grade inflammatory state (31).

The role of the chemokines reported in the present study that are significantly associated with muscle performance has, as far as we know, not been previously investigated in older adults. In a prior study, elevated circulating levels of

Table 2

Multivariate linear regression models showing regression coefficients (β, unstandardized regression coefficient; standardized β regression coefficient; and R squared, coefficient of determination) with muscle strength (1RM leg-press exercise or 1RM leg-extension) as dependent variable[a], and listed cytokines concentration[b] as independent variables

Variables	β	Standard β (95%CI)	R squared	P value for β
1RM leg-press (dependent)[a]				
ENA-78 (CXCL5)[b]	–8.85	–0.19 (–23.9 to 6.2)	0.36	0.242
GRO (CXCL2)[b]	–18.13	–0.27 (–37.5 to –1.2)	0.40	0.049
MIG (CXCL9)[b]	–13.94	–0.42 (–23.2 to –4.6)	0.49	0.004
IGF-1[b]	–19.63	–0.42 (–32.3 to –7.0)	0.50	0.003
CK-BETA 8 (CCL23)[b]	–28.31	–0.33 (–51.3 to –5.2)	0.49	0.018
GCP-2 (CXCL6) [b]	–25.78	–0.41 (–42.9 to –8.6)	0.49	0.004
GNDF [b]	–19.46	–0.25 (–44.5 to 5.6)	0.38	0.125
IGFBP-1 [b]	–14.13	–0.28 (–28.7 to 0.56)	0.41	0.059
1RM leg-extension (dependent)[a]				
GCP-2 (CXCL6) [b]	–11.32	–0.27 (–24.1 to 1.4)	0.35	0.081
IGFBP-1 [b]	–11.49	–035 (–21.3 to –1.6)	0.39	0.023

a. ENA-78 (CXCL5), GRO (CXCL2), MIG (CXCL9), IGF-1, CK-BETA 8 (CCL23), GCP-2 (CXCL6), GNDF, and IGFBP-1. b. Adjusted for age, sex, BMI, CIRS score, and SPPB score. β=unstandardized regression coefficient.

MIG (CXCL9), CK-BETA 8 (CCL23), and GCP-2 (CXCL6) were associated with motor symptom severity, depression, and functional status in older adults (32). MIG/CXCL9 is a T-cell chemoattractant induced by IFN-γ and mostly produced by neutrophils, macrophages and endothelial cells. It is known to be a crucial chemokine in many inflammatory processes, particularly in those that are mediated by T-cells in patients with age-related macular degeneration (33). MIG/CXCL9 may participate in the induction of biomechanical stress within the joint cartilage and in the bony lesion in rheumatic illnesses (34), and it was also recently validated as an indicator of cardiovascular pathology (i.e. subclinical levels of arterial stiffness and cardiac remodeling) independent of age in a large cohort of 1001 subjects (the Stanford 1000 Immunones Project) (35).

In the context of age-related diseases, data on the neutrophilspecific pro-inflammatory and chemoattractive cytokines/chemokines CK-BETA 8 (CCL23) and CXCL6 (GCP-2) are sparse. Both chemokines have pleitropic functions and belong to a group of muscle-secreted myokines whose levels are elevated in response to inflammatory stimuli (i.e., TNF-α treatment), as measured in conditioned medium of human skeletal muscle cells, and are implicated in β-cell survival (36). However, the extent to which these increases contribute to the pathogenesis and/or persistence of physical performance decline remains to be addressed.

IGF-1 and IGFBP-1 have an anabolic effect on skeletal muscle, which is related to the preservation of lean body mass (37). Along this line, our multiple linear regression analyses confirmed the negative relationship between IGF-1 and IGFBP-1 and muscle strength parameters. IGF-1 has been implicated in many anabolic pathways in skeletal muscle (38, 39). It has been demonstrated that high levels of IGFBP-1 in catabolic conditions, such as hospitalization, may reflect a pathophysiological state of inhibited IGF-1 bioactivity, leading to reduced muscle protein synthesis (40). In addition to (or possibly in interaction with) nutritional lifestyle, genetic predisposition has been shown to determine a large part (30%–60%) of variation in circulating IGF-I levels (41). Nevertheless, the literature on the relation between IGF-1 and functional decline as a result of prolonged bed-rest episodes is limited.

The results described here contribute to an improved understanding of the inflammatory changes and poor muscle performance that are present in hospitalized older patients, and are consistent with the biological role of these markers, which had not been reported previously as associated in older patients admitted to an ACE unit. The negative impact of hospitalization is considered to involve not only functional decline as a result of prolonged bed-rest episodes, but also functional abnormalities in the organs or tissues – including skeletal muscle – and other tissues with metabolic functions.

Another finding from this study was that leptin and osteopontin are significantly associated with BMI and age, respectively. Beyond its importance as a mediator of energy balance, leptin plays a major role in neuroendocrine, reproductive, and immune functions. Epidemiological studies on leptin are contentious, and have reported that it is reduced (42), unchanged (43), or even increased (44) independently of changes in body fat or sex during aging. Baumgartner et al. (44) reported that increases in serum leptin were significantly associated with decreases in serum testosterone but not with changes in BMI in men, whereas in women changes in leptin

were associated with changes in BMI but not with changes in serum estrone. The reason for these conflicting results is not known. Possibly, it might stem from the variability in the experimental design, different ages and/ or the different BMI of the study subjects, or the fact that often the age-leptin relationship was not the main focus of most of the studies.

Osteopontin, a pleiotropic cytokine, is expressed in a variety of tissues including macrophages and bodily fluids, and broadly regulates cell migration, adhesion, immune responses and inflammation (45). Skeletal muscle osteopontin has been shown to increase early after muscle injury and rapidly decline thereafter (45). Our findings are in accordance with previous reports indicating elevated circulating osteopontin levels in aging patients (46). Understanding how and why these changes occur will require further study.

Our study has some limitations that should be considered, including the small sample size, which limits our ability to further explore associations between cytokine proteins and muscle performance in acutely hospitalized older people. The cross-sectional nature of the study limits the interpretation of causality, and only associations can be drawn. Accordingly, longitudinal studies are needed to better understand the effects of hospitalization on cytokines in older patients admitted in an ACE unit, and to inform future interventional strategies that could reduce the acute inflammatory response related to hospital admission, such as intra-hospital physical exercise. In addition, the generalizability of our results is limited because of the inclusion of a selected population with relatively good functional capacity at preadmission (i.e., Barthel Index score ≥60 points), excluding those older adults with severe dementia, unstable hemodynamic condition or who were unable to walk at admission, which increases the possibility of selection bias. Nevertheless, our study has several strengths. We focused on a particularly vulnerable population of advanced age (mean age 87.9 years), and patients with multiple comorbidities and mild dementia/cognitive impairment were included in the study. Finally, the 1RM test was performed for measuring maximal muscle strength in acutely hospitalized older patients.

Conclusions and implications

The main finding of the present study is that several serum cytokines/chemokines and growth factors are negatively associated with lower muscle strength in older patients admitted to an ACE unit. Our findings suggest that the reduction in dynamic maximal muscle strength is directly associated with the aggravation of low-grade inflammation related to chronic diseases (47). Further investigation is required to establish the mechanism for elevated cytokine levels leading to lower muscle strength.

Ethical standards: Approved by the local Research Ethics Committee (ID Pyto2018/7, N°264; 15 May 2018).

Conflict of interest: None declared.

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