ELSEVIER

Urologic Oncology: Seminars and Original Investigations 37 (2019) 976–987

UROLOGIC ONCOLOGY

Clinical-Prostate cancer

# Frailty syndrome is associated with changes in peripheral inflammatory markers in prostate cancer patients undergoing androgen deprivation therapy

Rut Navarro-Martínez, RN, PhD[a,b], María Serrano-Carrascosa, RN[c], Cristina Buigues, RN, PhD[a], Julio Fernández-Garrido, RN, PhD[a], Vanessa Sánchez-Martínez, RN, PhD[a], Ana B. Castelló-Domenech, RN[a], Laura García-Villodre, RN[a], Augusto Wong-Gutiérrez, MD, PhD[c], José Rubio-Briones, MD, PhD[c], Omar Cauli, MD, PhD[a,*]

[a] *Department of Nursing, University of Valencia, Valencia, Spain*
[b] *Department of Haematology, Hospital General Universitario, Valencia, Spain*
[c] *Department of Urology, Fundación IVO, Valencia, Spain*

Received 5 April 2019; received in revised form 28 June 2019; accepted 12 August 2019

**Abstract**

**Objective:** To evaluate the role of peripheral inflammation (leukocyte differential count, the proinflammatory cytokines IL-beta, TNF-$\alpha$, IL-6, IL-8, and the inflammatory markers fibrinogen and C-reactive protein [CRP]) in frailty syndrome in patients with prostate cancer (CaP) undergoing antiandrogen therapy (ADT).

**Methods:** A total of 46 men between 51 and 92 years of age with CaP and receiving ADT were classified as frail, prefrail or robust according to the Fried scale. A geriatric assessment was performed, based on the Minimental State Examination for cognitive function, the Barthel index for basic activities of daily living, the Yesavage scale for geriatric depression, and the Athens insomnia scale. In addition, blood samples were collected to assess peripheral inflammation biomarkers including proinflammatory cytokines, fibrinogen, CRP and leukocyte differential count, as well as other biochemical and hematological parameters.

**Results:** A significant negative correlation between the severity of frailty syndrome and lymphocyte count was observed ($P < 0.01$). The concentration of IL-6 ($P < 0.05$), CRP ($P < 0.05$), and fibrinogen ($P < 0.01$) were significantly associated with frailty syndrome, but not of TNF-$\alpha$, IL-beta, or IL-8. The severity of frailty syndrome was not dependent upon the clinical disease stage at diagnosis, the time elapsed since CaP diagnosis, the presence of metastases, or prostatectomy.

**Conclusions:** Further research into the role of leukocyte subtypes and peripheral inflammation and the associated adverse outcomes in patients with CaP under ADT is warranted in order to tailor interventions aimed at reducing symptoms of frailty syndrome, such as loss of muscle strength and low physical activity. 

*Keywords:* Interleukin-6; Fibrinogen; Prostate; Biomarker; Geriatric assessment

## 1. Introduction

Androgen deprivation therapy (ADT) is the standard treatment for prostate cancer (CaP) in many patients, affording numerous clinical benefits ranging from improved survival in the case of locally advanced CaP associated with radiotherapy, to improvements in symptoms control for individuals with the recurrent and/or metastatic disease [1]. However, ADT is also associated with serious adverse effects including lean weight loss or sarcopenia, muscle weakness, fatigue, and inactivity, which overlap closely with the physical changes associated with frailty syndrome [2–5]. In men with CaP—many of whom already present a risk of frailty, since the disease fundamentally affects elderly people—the toxicity of ADT could therefore

*Corresponding author. Tel.: +34 963 86 41 82; fax: +3496388140.
*E-mail address:* Omar.Cauli@uv.es (O. Cauli).

https://doi.org/10.1016/j.urolonc.2019.08.005
1078-1439/

plausibly accelerate the development of frailty and its adverse consequences [3,6].

Frailty syndrome is characterized by a loss of functional reserve in multiple physiological systems, causing increased vulnerability to stressful events and predisposing to an increased risk of severe adverse health outcomes, including disability, hospitalization, and premature death [7,8]. Data derived from large cohorts of elderly people led Fried et al. [9], to propose a widely accepted operational definition that considers frailty to be a clinical syndrome which includes the presence of 3 or more of the following clinical criteria: involuntary weight loss in the last year, muscle weakness, slow walking, self-reported fatigue, and low physical activity. Immune system alterations, impacting both adaptive, and innate immune responses, have emerged as one of the most relevant "hallmarks of aging" processes, and immunological factors were among the biomarkers described for frailty [10]. The role of systemic inflammation as a key element of functional decline during aging is based on the hypothesis that the aging process is related to a systemic increase in proinflammatory mediators from various sources [11,12]. This increase is either directly related to sustained exposure to infectious agents throughout life, to age-related changes in gut microbiota, to metabolic dysfunction, or to secretion of antigens generated as a consequence of cell death and the subsequent accumulation of cell debris. Although the physiopathology of frailty is not fully understood, chronic systemic inflammation has been considered one of the most important components contributing to its development [13]. Proinflammatory cytokines are the most reported biomarkers of inflammation that have been repeatedly associated having a causal relationship a role in frailty syndrome [14–18], in community-dwelling individuals in particular [13,19]. In terms of cellular markers of inflammation, alterations in leukocyte counts have also been associated with frailty syndrome [13,20]. Several studies have reported increased levels of proinflammatory cytokines and inflammatory markers as well as a high neutrophil-lymphocyte index in frail older oncological patients [21–23], suggesting that these inflammatory markers can trigger or foster the progression of frailty syndrome in oncology patients. The relationship between inflammatory biomarkers and the severity of frailty syndrome in patients with CaP under ADT has not been investigated, and we sought to identify peripheral biomarkers that would provide useful information for clarifying the links between the physical changes and major physiopathological mechanisms responsible for the development of frailty in CaP under ADT.

In this context, the main objectives of our study were to evaluate the relationship between:

- Peripheral inflammatory markers (blood white cells and inflammatory markers) and the severity of frailty syndrome in patients with CaP.
- Frailty syndrome and complete geriatric assessment.
- Frailty syndrome and other hematological parameters and clinical variables.

## 2. Methods

### 2.1. Study population

A cross-sectional clinical trial was carried out in patients with CaP ($N = 46$) followed up by the Department of Urology of an oncological center (Urology Oncology Department, Fundación IVO, Valencia, Spain). The inclusion criteria were a diagnosis of CaP (all stages) and the prescription of ADT. In order to compare the selectivity of biomarkers for frailty syndrome in CaP patients, we measured the same inflammatory mediators in blood samples of a control group of men ($N = 46$) living in nursing homes in Valencia province characterized by a similar age and level of frailty syndrome. The exclusion criteria for all groups were severe cognitive impairment (Mini-Mental State Examination [MMSE] score $< 21$), severe psychiatric disorders or blindness, and acute infections. The trial was carried out in compliance with the guidelines of the Declaration of Helsinki, and the study protocol was approved by the local Ethics Committee (University of Valencia, Reference number: H1511682610849). All participants gave written informed consent before being enrolled in the study.

### 2.2. Study variables

The variables included sociodemographic characteristics (age, body mass index [BMI], and smoking status), clinical variables of CaP (clinical stage at diagnosis, time since diagnosis, type of drug for ADT, previous prostatectomy, and bone metastases). We measured 5 frailty criteria (involuntary weight loss, low energy or exhaustion, slow mobility, muscle weakness, and low physical activity) according to Fried et al [9]. Geriatric assessment was based on the MMSE for cognitive function, the Barthel index for basic activities of daily living, the Yesavage scale for geriatric depression, and the Athens insomnia scale as previously described [20]. The Charlson Comorbidity Index adjusted for age was calculated with a free online toll (https://www.mdcalc.com/charlson-comorbidity-index-cci).

### 2.3. Measurement of frailty syndrome

Frailty level was measured according to the 5 Fried criteria [9] as previously reported by our group [2]. Briefly, the criteria were assessed as follows: (1) unintentional body weight loss (5% or 4.5 kg or more in the last year); (2) self-reported chronic fatigue: participants met the criteria if they answered "A few times", "Often", or "Most of the time" to the question "How often in the last week did you feel that everything you did was an effort?", included in the Center

for Epidemiologic Studies depression scale; (3) a low level of physical activity was measured using the International Physical Activity Questionnaire, validated for the Spanish language. The total amount of energy spent on activities during 1 week was calculated and divided into quintiles. The individuals in the lowest quintile (20.0% less active) received a positive score for this frailty criterion; (4) according to the standards of the short physical performance battery [24], participants who walked 4.6 m in a longer time than the worst quintile of the sex- and height-adjusted sample fulfilled the reduced walking speed criterion; our values were: men taller than 173 cm: ≥6 seconds, height < 173 cm: ≥7 seconds; women taller than 159 cm: ≥6 seconds, height < 159 cm: ≥7 seconds; and (5) to measure muscle weakness, grip strength (Kg) was measured 3 times in each hand alternately with a hydraulic dynamometer (Jaymar, J.A. Preston, Corp., Jackson, MS, USA) according to the standards for Hispanic populations established for epidemiologic studies of the elderly [25]. Participants were considered frail if they met at least 3 criteria, and prefrail if they met 1 or 2. All measurements were performed by trained members of the Department of Nursing at the University of Valencia, using a questionnaire with detailed instructions.

### 2.4. Measurement of inflammatory markers

The serum concentrations of the proinflammatory cytokines TNF-$\alpha$ and IL-6 were measured using commercial enzyme-linked immunosorbent assay kits according to the manufacturer's instructions (TNF-$\alpha$ [ab100654], IL-6 [ab46042], IL-1 beta [ab46042], IL-8 [ab46032] Human ELISA Kit, Abcam). We also determined the leukocyte differential count and acute phase reactant proteins such as fibrinogen and CRP in order to better characterize the analysis of inflammatory biomarkers.

### 2.5. Measurement of hematological and biochemical markers

Blood serum (5 ml) was obtained by collecting blood in BD Vacutainer tubes and centrifuging them at 500 g for 10 minutes at room temperature. All samples were kept at 4°C to 6°C, and processed within 2 hours of collection. Hemoglobin concentration and leukocyte, erythrocyte, and platelet counts were measured on automated instruments in hospital hematology laboratories. Biochemical and hematological analyses were also performed.

### 2.6. Statistical analysis

Descriptive statistics, including a measurement of central tendency (mean), standard error of the mean, and range values were used to describe all the quantitative variables. The normal distribution of each variable was assessed with the Shapiro-Wilk test in order to determine whether a parametric or nonparametric test should be applied. The differences between 2 groups were analyzed with the nonparametric Mann-Whitney $U$ test or the parametric Student $t$ test. The differences between the 3 groups were analyzed using the nonparametric Kruskal-Wallis test or parametric analysis of variance (ANOVA), followed by posthoc testing where appropriate. The correlations between 2 quantitative variables were evaluated with the nonparametric Spearman test or the parametric Pearson test. Multinomial logistic regression models were used to assess effects of the variables found to be significant in the bivariate analysis (serum fibrinogen, CRP, IL-6, and lymphocyte levels) for the risk of being frail or prefrail vs. nonfrail. Logs (of each biomarker) were initially analyzed as continuous covariables for maximum efficiency; the estimated effects were expressed as odds ratios (ORs) of being frail vs. nonfrail or prefrail vs. nonfrail for each standard deviation unit increase of the log (of each biomarker), so that the relative contributions of each biomarker could be readily compared. Statistical significance was established at $P < 0.05$. The SPSS version 25.0 statistical package (SPSS Inc., Chicago, IL, USA) was used throughout the process.

## 3. Results

### 3.1. Study sample characteristics

A total of 46 men with CaP subjected to ADT were finally included in the study (Table 1). Their ages ranged from 51 to 92 years, with a mean age of 72.2 (±9.4) years. In addition, 56.5% ($n = 35$) of the patients were ≥65 years old. Almost all of the participants were married ($n = 40$), and only 10.9% lived alone. Regarding educational level, most patients reported having previous studies, and of these, 15.2% ($n = 7$) reported having completed university studies. The mean time since diagnosis was 106.27 (±13.0) months. According to international staging for CaP, at the time of diagnosis, 22 patients (47.7%) had stage II cancer, which according to the Gleason score (mean 7.2 [±0.1] points) was cancer with an intermediate risk of dissemination, 30.4% of the sample had stage III (locally advanced cancer), and 21.7% ($n = 10$) had metastatic disease (presence of bone metastases). At the time of the study, over half of the participants (65.2%) had undergone radical prostatectomy, and all were receiving ADT that included bicalutamide ($n = 1$) or leuprorelin ($n = 11$) or triptorelin ($n = 34$). As a result of ADT, the entire sample reached serum testosterone levels below castration levels (<50 ng/dl), although serum prostate-specific antigen levels were undetectable (<0.1 ng/ml). in only 10.8% ($n = 5$) of the sample. According to the BMI, 21.7% ($n = 10$) of the patients were classified as having "normal weight" (BMI = 18.5–24.9 kg/m$^2$), while 50.0% ($n = 23$) were "overweight" (BMI = 25–29.9 kg/m$^2$) and 28.3% ($n = 13$) presented "obesity" (BMI > 30 kg/m$^2$). None of the participants had BMI < 18.5 kg/m$^2$. Likewise, 56.5% of the patients ($n = 26$) had central or

android obesity with an abdominal perimeter ≥ 102 cm. Given that the mean systolic/diastolic blood pressure (BP) values were 135.8 (±2.1)/78.6 (±1.2) mmHg, most of the men had normal BP (i.e., <140/90 mmHg). The geriatric assessment scale and Charlson comorbidity index values are shown in Table 1.

### 3.2. Evaluation of frailty syndrome in the study sample

Based on the Fried criteria [9], 17.4% of the subjects were classified as frail (≥3 Fried criteria), 65.2% as prefrail (1 or 2 Fried criteria), and the remaining 17.4% as robust or nonfrail (no Fried criteria) (Fig. 1A). Furthermore, in terms of the number of criteria proposed by Fried, 17.4% of the participants did not present any of the 5 criteria, 43.5% presented only 1 criterion, 21.7% two, 13% three, 4.3% four, and none of the participants presented all 5 criteria (Fig. 1B). When we analyzed each of the 5 Fried criteria (Table 1), the most frequent criterion in the overall sample was low physical activity (58.7%), followed by slow gait speed (28.3%), self-reported fatigue (26.1%), weakness or low muscle strength (23.9%) and finally, involuntary weight loss in the last year (6.5%).

### 3.3. Evaluation of the relationship between frailty syndrome and clinical variables

There were no significant differences in age between the groups (frail (77.6 [±4.2] years), prefrail (71.2 (±1.6) years) and robust individuals (70.6 [±2.5] years), $P = 0.201$) or for the Charlson comorbidity index ($P = 0.521$). The only comorbidity associated with a higher level of frailty syndrome is diabetes ($P < 0.05$). Likewise, frailty syndrome was not associated with a specific characteristic of prostate cancer, as there were no significant differences between the 3 groups in terms of clinical stage ($P = 0.084$, chi-squared), total Gleason score ($P = 0.084$, Kruskal-Wallis test), the presence of metastasis ($P = 0.444$, chi-squared), the presence of radical prostatectomy ($P = 0.286$, chi-squared), or the type of drugs used for ADT ($P = 0.720$, chi-squared). For BMI and abdominal perimeter, although a higher percentage of patients had overweight or obesity among the prefrail and frail patients, the differences were not significant ($P = 0.733$ and $P = 0.432$, chi-squared, respectively). There were also no significant differences between the 3 groups in terms of BP (systolic BP: $P = 0.473$ and diastolic BP: $P = 0.758$, 1-way ANOVA). There were no significant differences in the scores obtained with each geriatric assessment scale between robust, prefrail, and frail individuals (Barthel index for basic activities of daily living [$P = 0.999$]; MMSE for cognitive impairment [$P = 0.092$]; Yesavage scale for geriatric depression [$P = 0.695$]; and Athens insomnia scale for sleep difficulty [$P = 0.906$].

### 3.4. Evaluation of the relationship between frailty syndrome and hematological parameters

There were no significant differences between the 3 groups in terms of the erythrocyte count ($P = 0.854$, Kruskal-Wallis test), haemoglobin concentration ($P = 0.544$, Kruskal-Wallis test), or platelet count ($P = 0.394$, 1-way ANOVA). Likewise, the total leukocyte count did not differ significantly between the robust, prefrail, and frail patients ($P = 0.113$, 1-way ANOVA) (Fig. 2A). However, when the different leukocyte subpopulations were analyzed (differential count), the lymphocyte counts were significantly lower in both frail (1.6 [±0.1] × $10^3/\mu$l) and prefrail individuals (1.7 [±0.07] × $10^3/\mu$l) than in robust individuals (2.3 [± 0.2] × $10^3/\mu$l) ($P = 0.004$, 1-way ANOVA) (Fig. 2B). No significant differences in lymphocyte counts between prefrail and frail patients were observed ($P = 0.961$, Tukey posthoc test). Unlike in the case of the lymphocyte count, no significant differences were observed in the other leukocyte subtypes among the 3 groups (neutrophil count: $P = 0.187$, 1-way ANOVA [Fig. 2C]; monocyte count: $P = 0.191$, Kruskal-Wallis test [Fig. 2D]; eosinophil count: $P = 0.833$, Kruskal-Wallis test [Fig. 2E]; basophils count: $P = 0.834$, Kruskal-Wallis test [Fig. 2F]). The mean values of the different hematological parameters were within the normal reference ranges (Table 2).

### 3.5. Evaluation of the relationship between frailty syndrome and biochemical parameters

There were no significant differences between the robust, prefrail, and frail patients in terms of most of the biochemical parameters measured in blood (data not shown). The only exception was creatinine, with a significantly higher mean serum concentration in robust individuals (1.02 [±0.1] mg/dl) compared to prefrail (0.8 [±0.02] mg/dl) and frail patients (0.9 [±0.09] mg/dl) ($P = 0.037$, 1-way ANOVA). However, there were no statistically significant differences in the serum creatinine levels between prefrail and frail individuals in the Tukey posthoc test ($P = 0.186$). Likewise, while the mean values of most of these biochemical parameters were also within the normal physiological range, the glucose, and GFR levels were respectively slightly higher and lower than the recommended levels in the 3 groups (data not shown). Biochemical parameters are shown in Table 2.

### 3.6. Evaluation of the relationship between frailty syndrome and blood inflammatory markers

With regard to the different blood inflammatory markers, there were significant differences among the 3 groups in relation to the concentrations of IL-6 ($P = 0.017$, Kruskal-Wallis test), CRP ($P = 0.014$, Kruskal-Wallis test), or

Table 1
Baseline characteristics of patients in this study.

| Category | | | Mean (±SEM) value | Range |
|---|---|---|---|---|
| Age (y) | | | 72.2 (±9.4) | 51–92 |
| Time elapsed since the moment of diagnosis (wk) | | | 106.27 (±13.0) | 16–408 |
| Gleason score | | | 7.2 (±0.1) | 5–9 |
| Testosterone (ng /dl) | | | 1.9 (±0.4) | 0.02–2.08 |
| Total PSA | | | 3.7 (±0.8) | 0.0–29 |
| BMI (Kg/m$^2$) | | | 28 (±0.4) | 20.6–34.4 |
| Abdominal perimeter (cm) | | | 104 (±1.4) | 85–125 |
| Systolic blood pressure (mmHg) | | | 135.8 (±2.1) | 95–170 |
| Diastolic blood pressure (mmHg) | | | 78.7 (±1.2) | 63–98 |
| Ability to perform daily activities (Barthel index) | | | 69.8 (±0.5) | 90–100 |
| Cognitive function (MMSE test) | | | 27.1 (±0.4) | 16–30 |
| Depressive symptoms (Yesavage scale) | | | 2.2 (±0.3) | 0–14 |
| Sleep quality (Athenas scale) | | | 2.8 (±0.5) | 0–17 |
| Charlson Comorbidity Index (age-adjusted) | | | 3.4 (±1.2) | 1–7 |
| | | | *n* | % |
| Civil status | Married | | 40 | 87 |
| | Widower | | 2 | 4.3 |
| | Divorced | | 3 | 65 |
| | Others | | 1 | 2.2 |
| Coliving status | Single | | 5 | 10.9 |
| | With partner | | 33 | 71.7 |
| | With family (with or without partner), children, brothers, etc. | | 8 | 17.4 |
| Educational level | Without studies | | 9 | 19.6 |
| | Primary | | 18 | 39.1 |
| | Secondary | | 12 | 26.1 |
| | University | | 17 | 15.2 |
| Clinical staging | I | | 0 | 0 |
| | II | | 22 | 47.8 |
| | III | | 6 | 13 |
| | IV | | 18 | 39.1 |
| Presence of bone metastases at diagnosis | Yes | | 10 | 21.7 |
| | No | | 36 | 78.3 |
| Previous prostatectomy | Yes | | 30 | 65.2 |
| | No | | 16 | 34.8 |
| ADT treatment | Bicalutamide | | 1 | 2.2 |
| | Leuproreline | | 11 | 23.9 |
| | Triptoreline | | 34 | 73.9 |
| BMI(Kg/m$^2$) | Underweight(<18.5) | | 0 | 0 |
| | Normal (18.5–24.9) | | 10 | 21.7 |
| | Overweight (25–29.9) | | 23 | 50.0 |
| | Obese (>30) | | 13 | 28.3 |
| Abdominal perimeter | <102 cm | | 20 | 43.5 |
| | ≥102 cm | | 26 | 56.5 |
| Dycotomized frailty criteria | Weight loss | Yes | 3 | 6.5 |
| | | No | 43 | 93.5 |
| | Fatigue | Yes | 12 | 26.1 |
| | | No | 34 | 73.9 |
| | Low physical activity | Yes | 27 | 58.7 |
| | | No | 19 | 41.3 |
| | Slow walking speed | Yes | 13 | 28.3 |
| | | No | 33 | 71.7 |
| | Muscle weakness | Yes | 11 | 23.9 |
| | | No | 35 | 76.1 |

ADT = androgen deprivation therapy.

Dates are expressed as the mean (±standard error mean [SEM]) and range or frequency (*n*) and percentage (%) for each value. Age was expressed in years, time elapsed since the moment of diagnosis was expressed in months, time elapsed since the last administration of the drug was expressed in days, serum testosterone and free prostate-specific antigen (PSA) levels were expressed in conventional units, body mass index (BMI) was calculated from weight and standing height (Kg/m$^2$), abdominal perimeter was measured in cm, blood pressure numbers were expressed in mmHg, geriatric assessment was evaluated using Barthel index to measure the basic activities of daily living, minimental score test (MMES) to determine cognitive impairment, Yesavage scale for geriatric depression and Athens scale to detect insomnia and frailty was measured according to the 5 criteria proposed by Fried et al (2001).

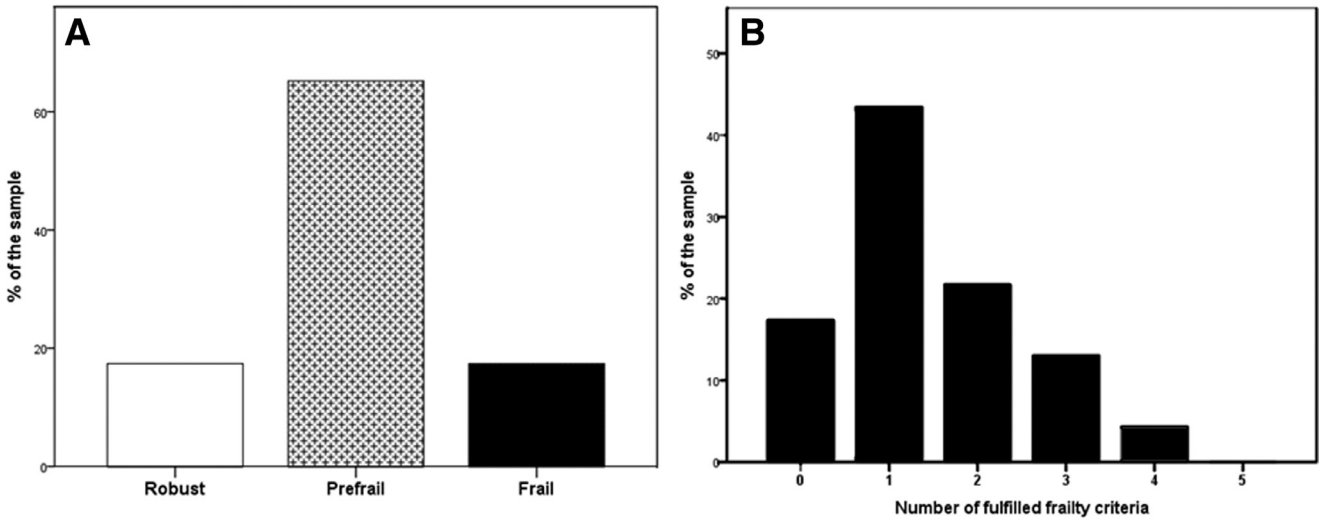


Fig. 1. Evaluation of frailty syndrome in the study sample. Frailty was measured according to the 5 criteria proposed by Fried et al. (2001). (A) Percentage of the categories of frailty expressed as follows: participants who met 3 or more criteria were classified as frail, those who met 1 or 2 were prefrail, and those who did not meet any of the criteria were nonfrail (robust). (B) Number of frailty criteria met in the sample, expressed as percentage of the entire population.

fibrinogen ($P = 0.005$, 1-way ANOVA). Both prefrail and frail patients presented significantly higher mean serum IL-6, CRP, and fibrinogen values compared to robust individuals (Fig. 3A–C). However, the posthoc analysis showed that the frail individuals had significantly higher levels of fibrinogen than the prefrail participants ($P$ =0.005). On the other hand, no significant differences were observed for TNF-$\alpha$ ($P = 0.134$, 1-way ANOVA) (Fig. 3D), for IL-beta ($P = 0.974$, 1-way ANOVA) (Fig. 3E), for IL-8 ($P = 0.181$, 1-way ANOVA) (Fig. 3F). In the multinomial logistic regression analyses, a higher log (IL-6) was associated to a significantly increased OR of being frail (OR = 56.9, 95% CI = 1.23–2.639.8; $P < 0.05$), as was a higher log (fibrinogen) (OR = 1.2, 95% CI = 1.02–1.40; $P < 0.05$). In contrast, no significant effects upon the OR of being frail were observed for either the lymphocyte count (OR = 0.20, 95% CI = 0.01–6.60; $P = 0.39$) or CRP concentration (OR = 2.69, 95% CI = 0.84–8.69; $P = 0.10$).

In order to determine whether the associations between inflammatory markers and frailty syndrome were specific for CaP patients, we compared those biomarkers in a population of older individuals without CaP ($N = 46$) of similar ages (77.3 ± 9.7) and frailty severity (Robust: 13.3% ($N = 10$); Prefrail: 29.3% ($N = 22$); Frail: 18.7% ($N = 14$). The data and comparisons of biomarkers associated with frailty syndrome in CaP and control group are shown in Table 3. There was a significant difference between CRP, IL-6, IL-8, and the severity of frailty syndrome in the control group ($P < 0.05$ in both cases, Kruskal-Wallis test). However, no significant differences were found in the control group for fibrinogen concentration and lymphocytes counts ($P > 0.05$, in all cases, Kruskal-Wallis test). In contrast, IL-8 appeared to be a marker associated with frailty syndrome only in control group ($P < 0.05$, Kruskal-Wallis test).

### *3.7. Evaluation of the relationship between each of the 5 frailty syndrome criteria and lymphocyte count and blood inflammatory markers*

The mean serum IL-6 values were significantly higher among the subjects with a slower walking speed (1.7 [±0.06] pg/ml versus 2.1 [±0.1] pg/ml) ($P = 0.022$, Mann-Whitney $U$ test). Furthermore, compared with the patients with greater physical activity, the patients with moderate or light physical activity (<150 minutes a week) had significantly higher serum concentrations of both CRP (5.0 [±0.4] mg/l vs. 3.5 [±0.3] mg/l) and fibrinogen (428.9 [±16.9] mg/dl vs. 369.4 [±14.4] mg/dl) ($P = 0.012$ and $P = 0.016$, Student's $t$ test, respectively). Meanwhile, the mean serum fibrinogen levels were significantly higher among the subjects that reported feeling fatigued more than 3 days a week (441.9 [±23.3] mg/dl vs. 391.1 [±13.9] mg/dl) ($P = 0.047$, Mann-Whitney $U$ test). Significant differences were also observed between self-reported fatigue and lymphocyte counts ($P = 0.039$, Student's $t$ test). However, in this case, and in contrast to the observations for fibrinogen, the subjects who reported low energy were those exhibiting lower lymphocyte counts (1.5 [±0.09] × $10^3/\mu$l vs. 1.8 [±0.08] × $10^3/\mu$l). There were no significant differences between the 2 remaining Fried criteria (involuntary weight loss in the last year and low muscle strength) in relation to blood inflammatory markers ($P > 0.05$ in all cases).

## 4. Discussion

This study, involving a population of older men with CaP subjected to ADT, shows for the first time that certain inflammatory markers, such as serum CRP, IL-6, and fibrinogen concentration, as well as lymphocyte counts, are related to frailty syndrome. These associations were not

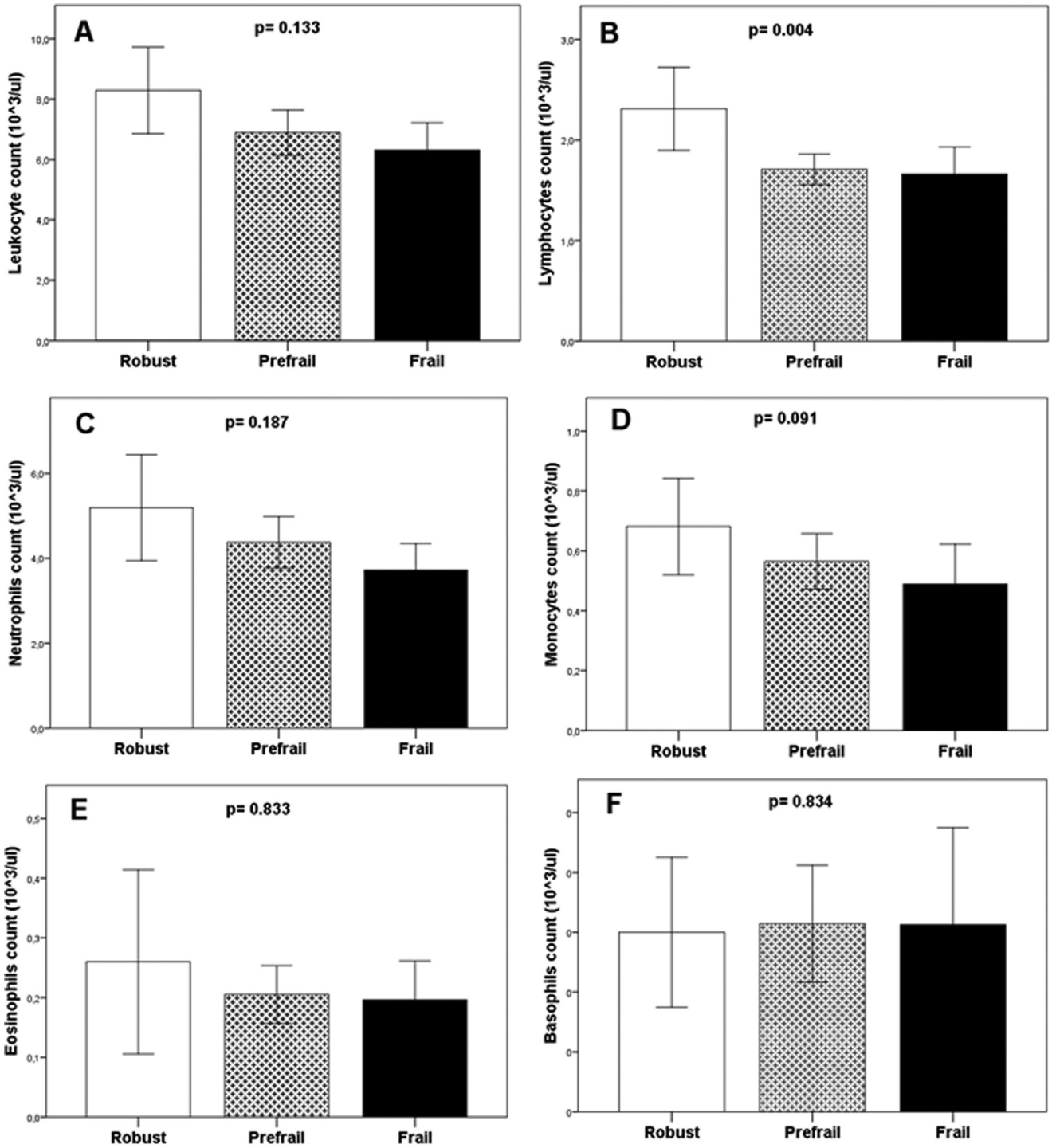


Fig. 2. Evaluation of relationship between frailty syndrome and the total-subtype leukocyte count. Frailty was measured according to the 5 criteria proposed by Fried et al. (2001). (A) Leukocyte ($10^3/\mu$l), (B) lymphocyte ($10^3/\mu$l), (C) neutrophil ($10^3/\mu$l), (D) monocyte ($10^3/\mu$l), (E) eosinophil ($10^3/\mu$l), and (F) basophil ($10^3/\mu$l) counts were measured in the blood and were plotted against the categories of fragility, was expressed as follows: participants who met 3 or more criteria were classified as frail, those who met 1 or 2 were prefrail, and those who did not meet any of the criteria were nonfrail (robust). Dates are expressed as the mean (±standard error mean (SEM)) for each group. The level of significance is indicated in the corresponding panel and indicated with asterisks.

identical with those found in the men in the control group with a similar level of frailty and age in which the association between either CRP, IL-6, and frailty syndrome was confirmed, whereas the associations with fibrinogen concentration and lymphocyte counts were only associated with frailty syndrome in CaP patients. In contrast, IL-8 was a marker associated with frailty syndrome only in the control group. The comparison with the control group suggests

Table 2
Blood hematological and biochemical parameters.

| Analytical parameters | Mean (±SEM) value | Range | Normal range |
|---|---|---|---|
| RBCs count ($10^6/\mu$l) | 4.7 (±0.8) | 4–6 | 4.6–6.2 |
| Hgb (g/dl) | 14.0 (±2.3) | 11.7–16.6 | 14–18 |
| Plateletscount ($10^3/\mu$l) | 209.3 (±7.3) | 98–344 | 140–450 |
| WBCs count ($10^3/\mu$l) | 7 (±0.2) | 3.5–12.3 | 4–11 |
| Neutrophils count ($10^3/\mu$l) | 4.4 (±0.2) | 1.9–8.5 | 2.5–7.5 |
| Lymphocytes count ($10^3/\mu$l) | 1.8 (±0.07) | 0.9–3.3 | 1.3–4 |
| Monocytes count ($10^3/\mu$l) | 0.6 (±0.03) | 0.2–1.5 | 0.1–1 |
| Eosinophil count ($10^3/\mu$l) | 0.2 (±0.02) | 0.04–0.7 | 0.05–0.5 |
| Basophils count ($10^3/\mu$l) | 0.03 (±0.004) | 0.0–0.1 | 0–0.2 |
| Glucose (mg/dl) | 115 (±2.9) | 91.8–176.5 | 74–110 |
| Creatinine(mg/dl) | 0.9 (±0.03) | 0.6–1.5 | 0.6–1.4 |
| Urea (mg/dl) | 40.9 (±1.5) | 18.4–80 | 18–45 |
| GFR (ml/min) | 81.6 (±1.8) | 46–90 | 90–120 |
| Sodium (mEq/l) | 141.5(±0.3) | 134–148 | 135–148 |
| Potassium (mEq/l) | 4.4 (±0.9) | 3.3–5.7 | 3.5–5.1 |
| Chloride(mEq/l) | 102.8 (±0.3) | 95.5–107.6 | 98–110 |
| Calcium (mg/dl) | 9.7 (±1.9) | 8.3–11.7 | 8.6–10.4 |
| Phosphate (mg/dl) | 3.2 (±0.8) | 2.2–4.4 | 2.7–4.5 |
| Bilirubin(mg/dl) | 0.5(±0.04) | 0.2–1.6 | 0.3–1.3 |
| GOT (U/l) | 21.4 (±0.9) | 12.7–38.5 | 10–45 |
| GPT(U/l) | 25.5 (±4.3) | 10.9–204 | 10–50 |
| GGT(U/l) | 23.9 (±2.0) | 11–74 | 8–55 |
| Uric acid (mg/dl) | 5.1 (±1.0) | 2.8–8.1 | 3–8 |
| Lactate (mol/l) | 178.5(±4.5) | 123–290 | 90–225 |
| ALP (U/l) | 70.9 (±5.2) | 42–206 | 40–125 |

ALP = alkaline phosphatase; GFR = glomerular filtration rate; GGT = transaminases; GOT = glutamic-oxaloacetic transaminases; GPT = glutamic-pyruvic transaminases; Hgb = hemoglobin; RBCs = red blood cells; WBCs = white blood cells.

Values are reported as the mean (±standard error mean [SEM]), range, and normal range. All of the hematological and biochemical parameter values were expressed in conventional units.

No statistically significant differences were found between groups for any of the analytical parameters studied.

that the pathophysiological bases of frailty syndrome in CaP patients under ADT are not the same, although they share also some common mechanisms. In patients with CaP, the most powerful independent parameters for discriminating between frail vs. nonfrail or robust individuals were the concentration of IL-6 and fibrinogen, as evidenced by the multivariate analysis. Several novel findings of the study are discussed below.

There was a positive association between inflammatory markers and frailty syndrome, since the circulating IL-6 and CRP levels increased significantly in prefrail and frail subjects compared to the robust individuals, but not necessarily at the levels found in acute infections—which suggests the presence of low-grade systemic inflammation. Although the exact mechanisms by which IL-6 contributes to the development of frailty in these patients are unknown, several possible explanations have been proposed. The activation of proinflammatory cytokines promotes anorexia and protein catabolism of the skeletal muscle, which could contribute to a worsening nutritional status and a reduction of muscular mass and consequently to the loss of weight and muscle weakness commonly seen in frailty syndrome [26]. Meanwhile, levels of inflammatory cytokines, including IL-6, are high in men with testosterone deficiency, and are reduced by testosterone therapy [27]. The relationship between IL-6 and frailty may therefore be partially mediated by the low levels of testosterone (near or below castration levels) which these patients present as a result of ADT. However, in our selected population, we observed no mediating effect of this hormone upon the association between IL-6 and frailty, since no statistically significant relationship was observed between the levels of IL-6 and testosterone in the patients under ADT. This suggests that serum IL-6 levels have an independent effect upon frailty, as also demonstrated in frail older individuals without CaP [14,15,18]. The lack of a significant association between IL-8 concentration and frailty in CaP patients may be explained by the higher concentration of this cytokine in nonfrail CaP patients [28] compared to nonfrail men in the control group, leading to a nonsignificant effect where making the comparison. The increase of IL-8 in CaP appeared to be mediated by other inflammatory mechanisms caused by both benign or malign prostate disorder [28–30].

The role of CRP in the development of frailty is biologically plausible through the amplification of inflammation [9,13]. However, in this study, the serum concentrations of IL-6 and CRP were not significantly correlated to each other, suggesting that these 2 different markers of the

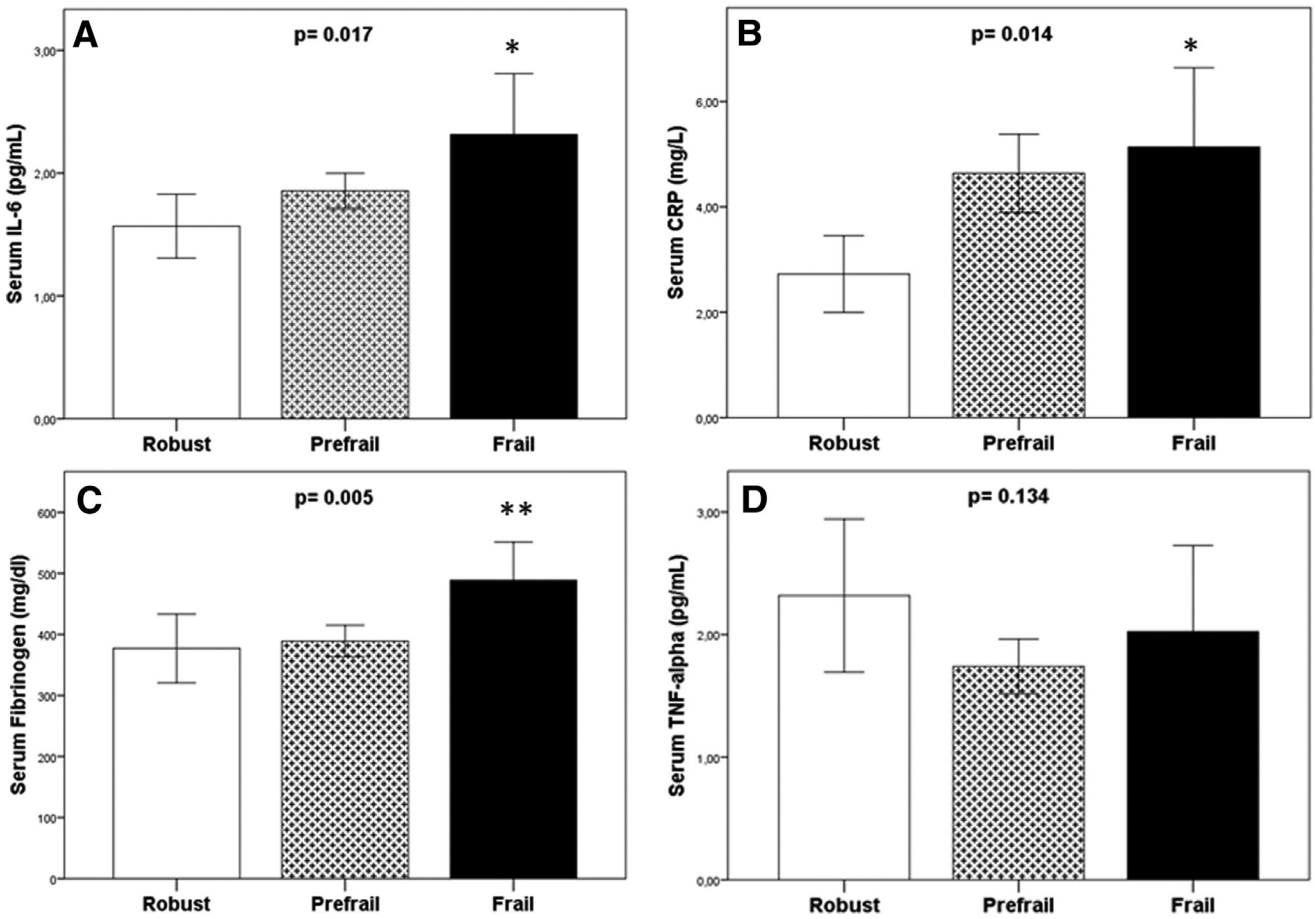


Fig. 3. Evaluation of relationship between frailty syndrome and serum levels of IL-6, CRP, Fibrinogen, and TNF-alpha. Frailty was measured according to the 5 criteria proposed by Fried et al. (2001). (A) IL-6 (pg/ml), (B) CRP (mg/l), (C) Fibrinogen (mg/dl), and (D) TNF-alpha (pg/ml) were measured in the blood and were plotted against the categories of fragility, which was expressed as follows: participants who met 3 or more criteria were classified as frail, those who met 1 or 2 were prefrail, and those who did not meet any of the criteria were nonfrail (robust). Dates are expressed as the mean (±standard error mean (SEM)) for each group. The level of significance is indicated in the corresponding panel and indicated with asterisks.

inflammatory system may contribute to frailty through different mechanisms. On the other hand, excess body fat has been associated with frailty by significantly increasing inflammatory parameters [31]. In fact, although it is mainly produced by the cells of the immune system, IL-6 can also be produced by adipocytes, and since IL-6 is a potent stimulus for the expression of CRP in the liver, this is believed to be a reason why obese or overweight individuals have higher endogenous levels of CRP [32]. Accordingly, we observed that prefrail and frail individuals have more overweight than robust individuals; suggesting ADT in these patients could have incremented body weight through a loss of lean mass and a parallel increase in fat mass [1]. Likewise, this adverse effect of ADT upon body composition could justify the fact that "involuntary weight loss in the last year" was found to be the least representative criterion in the overall sample.

Inflammatory cytokines stimulate the release of procoagulant factors [33], confirming the findings of increased fibrinogen concentrations in prefrail and frail subjects vs. nonfrail individuals. Previous studies have also shown that frail older adults have increased fibrinogen levels [34]. Likewise, when evaluating the serum concentrations of fibrinogen among prefrail and frail patients, we observed a significant difference in these values, indicating that the level of fibrinogen could be a suitable biomarker for determining the severity of frailty syndrome. Larger studies are required to confirm whether the determination of this biomarker could help identify prefrail older adults before the onset of clinically recognizable frailty. An increase in procoagulant markers such as fibrinogen has been associated with the pathogenesis of atherosclerosis [35], and several authors have in turn considered arteriosclerosis to be an important component in the development of frailty syndrome [36]. In this context, the alteration in perfusion leads to a decrease in nerve and muscle irrigation, which aggravates the loss of muscle mass and strength—a key component in the development of frailty. The increase in fibrinogen levels in frail individuals with CaP may suggest a pathophysiological alteration linked to several cardiovascular diseases, and this study provides the basis for identifying frail men treated with ADT who might benefit from

Table 3
Inflammation markers associated with frailty syndrome in CaP patients and control group.

| Biomarker | CaP patients | *P* value | Control group | *P* value |
|---|---|---|---|---|
| CRP | Robust: 2.7 (±0.3)<br>Prefrail: 4.6 (±0.3)<br>Frail: 5.1 (±0.7) | $P = 0.01$ | Robust: 2.6 (±0.5)<br>Prefrail: 3.7 (±0.3)<br>Frail: 4.3 (±0.3) | $P = 0.04$ |
| IL-6 | Robust: 1.5 (±0.1)<br>Prefrail: 1.8 (±0.07)<br>Frail: 2.3 (±0.2) | $P = 0.02$ | Robust: 1.7 (±0.1)<br>Prefrail: 2.1 (±0.2)<br>Frail: 2.4 (±0.2) | $P = 0.01$ |
| Fibrinogen | Robust:377.1 (±28.1)<br>Prefrail: 389.2 (±12.9)<br>Frail: 488.6 (±31.2) | $P = 0.005$ | Robust: 384.3 (±28.1)<br>Prefrail: 375.6 (±26.7)<br>Frail: 404.5 (±14.1) | $P = 0.56$ |
| Lymphocytes count | Robust: 2.3 (±0.2)<br>Prefrail: 1.7 (±0.07)<br>Frail: 1.6 (±0.1) | $P = 0.004$ | Robust: 2.1 (±0.08)<br>Prefrail: 2.0 (±0.09)<br>Frail: 2.0 (±0.09) | $P = 0.90$ |
| IL-8 | Robust: 34.7(±2.7)<br>Prefrail: 42.6 (±2.2)<br>Frail: 43.9 (±3.6) | $P = 0.18$ | Robust: 27.8(±3.5)<br>Prefrail: 44.0(±2.8)<br>Frail: 42.1(±2.7) | $P = 0.002$ |

regular cardiac monitoring and lifestyle modification recommendations [37].

As regards cellular markers of inflammation, low lymphocyte counts are associated with frailty syndrome only in CaP patients, but not in the control group. Expression of the androgen receptor in lymphoid and nonlymphoid cells of the thymus and bone marrow suggests that these hormones play an important physiological role in developing lymphoid precursors [38]. Accordingly, cellular immunity can be modulated by testosterone and/or its active metabolites. In this regard, the low serum testosterone levels (near or below castration levels) present in our patients as a consequence of ADT may result in a deficient number or function of mature peripheral lymphocytes. As a confirmation for this explanation, the interplay between reduced testosterone levels and reduced lymphocyte counts in frailty syndrome has been demonstrated in older women [20], but not in men or in samples including both genders [39,40]. However, the fact that the lymphocyte counts were similar in the prefrail and frail subjects indicates it is not a suitable biomarker of the severity of frailty syndrome, and suggests that there is a threshold below which the lymphocyte counts do not influence the progression of frailty after onset has occurred. However, reduced lymphocyte counts, even within the normal range, have also been associated with an increased mortality risk in healthy older people, including men and women [41] and people with various diseases [42], including cancer [43], and this may be relevant for ADT treatment, which usually spans a long period of time [44]. CaP patients with diabetes show higher levels of frailty. It is widely accepted that diabetes accelerates the ageing process and could provide an early pathophysiologic environment for frailty, and the close relationship between diabetes and sarcopenia may be a common factor [45]. Diabetes can contribute to frailty by increasing the incidence of the core components of frailty or through some of the comorbidities and complications associated with this condition, and our results suggest a strict glucemic control is necessary in CaP patients.

The present study has some limitations. First, the sample size was relatively small. Nevertheless, in our opinion, the sample was large enough because it suggests future lines of research in this field, and tailors clinical decisions in this population. Second, although frailty has been conceptualized as a multidimensional geriatric syndrome, we evaluated it using the criteria proposed by Fried et al [9], which are closely linked to physical condition. However, from our point of view these criteria have several advantages that make them suitable for the clinical setting, since they are objective, brief, and easy to use [46]. Finally, the cross-sectional design of the study prevented us from evaluating the causality of the identified associations between frailty and inflammatory markers, and did not allow us to rule out the possibility that these associations may be due to noncausal relationships.

Despite these limitations, the results of our study provide new evidence linking frailty to a state of low-grade systemic inflammation in older adults with CaP under ADT, presenting both similarities and differences with men without CaP and offering possibilities for limiting the progression of frailty, one of the factors that influence clinical, therapeutic, and palliative decisions. In clinical practice, the identification of biomarkers could be helpful in the early identification of elderly people with CaP exposed to ADT who are at risk of becoming frail. These individuals would benefit from a comprehensive integral geriatric assessment, and treatment of the individual patient should be based on their health status as regards frailty syndrome [47,48] according to the recommendations of the International Society of Geriatric Oncology task force [49]. Routine monitoring of frailty syndrome during ADT is recommended in order to limit its adverse consequences, and to guide clinical decisions regarding CaP patients.

## References


[1] Connolly RM, Carducci MA, Antonarakis ES. Use of androgen deprivation therapy in prostate cancer: indications and prevalence. Asian J Androl 2012;14(2):177–86.

[2] Widmark A, Klepp O, Solberg A, Damber JE, Angelsen A, Fransson P, et al. Endocrine treatment, with or without radiotherapy, in locally advanced prostate cancer (SPCG-7/SFUO-3): an open randomised phase III trial. Lancet 2009;373(9660):301–8.

[3] Galvão DA, Spry NA, Taaffe DR, Newton RU, Stanley J, Shannon T, et al. Changes in muscle, fat and bone mass after 36 weeks of maximal androgen blockade for prostate cancer. BJU Int 2008;102(1):44–7.

[4] Smith MR, Finkelstein JS, McGovern FJ, Zietman AL, Fallon MA, Schoenfeld DA, et al. Changes in body composition during androgen deprivation therapy for prostate cancer. J Clin Endocrinol Metab 2002;87(2):599–603.

[5] Galvão DA, Taaffe DR, Spry N, Joseph D, Turner D, Newton RU. Reduced muscle strength and functional performance in men with prostate cancer undergoing androgen suppression: a comprehensive cross-sectional investigation. Prostate Cancer Prostatic Dis 2009;12(2):198–203.

[6] Bylow K, Hemmerich J, Mohile SG, Stadler WM, Sajid S, Dale W. Obese frailty, physical performance deficits, and falls in older men with biochemical recurrence of prostate cancer on androgen deprivation therapy: a case-control study. Urology 2011;77(4):934–40.

[7] Ensrud KE, Ewing SK, Cawthon PM, Fink HA, Taylor BC, Cauley JA, et al. A comparison of frailty indexes for the prediction of falls, disability, fractures, and mortality in older men. J Am Geriatr Soc 2009;57(3):492–8.

[8] Fernández-Garrido J, Ruiz-Ros V, Navarro-Martínez R, Buigues C, Martínez-Martínez M, Verdejo Y, et al. Frailty and leucocyte count are predictors of all-cause mortality and hospitalization length in non-demented institutionalized older women. Exp Gerontol 2018;103:80–6.

[9] Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, et al. Cardiovascular Health Study Collaborative Research Group. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci 2001;56(3):M146–56.

[10] Fahey JL, Schnelle JF, Boscardin J, Thomas JK, Gorre ME, Aziz N. Distinct categories of immunologic changes in frail elderly. Mech Ageing Dev 2000;15:1–20.

[11] Franceschi C, Bonafe M, Valensin S, Olivieri F, De Luca M, Ottaviani E, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci 2000;908:244–54.

[12] Monti D, Ostan R, Borelli V, Castellani G, Franceschi C. Inflammaging and human longevity in the omics era. Mech Ageing Dev 2017;165:129–38.

[13] Yao X, Li H, Leng SX. Inflammation and immune system alterations in frailty. Clin Geriatr Med 2011;27(1):79–87.

[14] Cardoso AL, Fernandes A, Aguilar-Pimentel JA, de Angelis MH, Guedes JR, Brito MA, et al. Towards frailty biomarkers: candidates from genes and pathways regulated in aging andage-related diseases. Ageing Res Rev 2018;47:214–77.

[15] Fernández-Garrido J, Ruiz-Ros V, Buigues C, Navarro-Martinez R, Cauli O. Clinical features of prefrail older individuals and emerging peripheralbiomarkers: a systematic review. Arch Gerontol Geriatr 2014;59(1):7–17.

[16] Kane AE, Sinclair DA. Frailty biomarkers in humans and rodents: current approaches and future advances. Mech Ageing Dev 2019;180:117–28.

[17] Velissaris D, Pantzaris N, Koniari I, Koutsogiannis N, Karamouzos V, Kotroni I, et al. C-reactive protein and frailty in the elderly: a literature review. J Clin Med Res 2017;9(6):461–5.

[18] Wang J, Maxwell CA, Yu F. Biological processes and biomarkers related to frailty in older adults: a state-of-the-science literature review. Biol Res Nurs 2019;21(1):80–106.

[19] Collerton J, Martín-Ruiz C, Davies K, Hilkens CM, Isaacs J, Kolenda C, et al. Frailty and the role of inflammation, immunosenescence and cellular ageing in the very old: cross-sectional findings from the Newcastle 85+ Study. Mech Ageing Dev 2012;133(6):456–66.

[20] Fernández-Garrido J, Navarro-Martínez R, Buigues-González C, Martínez-Martínez M, Ruiz-Ros V, Cauli O. The value of neutrophil and lymphocyte count in frail older women. Exp Gerontol 2014;54:35–41.

[21] Handforth C, Clegg A, Young C, Simpkins S, Seymour MT, Selby PJ, et al. The prevalence and outcomes of frailty in older cancer patients: a systematic review. Ann Oncol 2015;26(6):1091–101.

[22] Mandelblatt JS, Cai L, Luta G, Kimmick G, Clapp J, Isaacs C, et al. Frailty and long-term mortality of older breast cancer patients: CALGB 369901 (Alliance). Breast Cancer Res Treat 2017;164(1):107–17.

[23] Youngerman BE, Neugut AI, Yang J, Hershman DL, Wright JD, Bruce JN, et al. The modified frailty index and 30-day adverse events in oncologic neurosurgery. J Neurooncol 2018;136(1):197–206.

[24] Guralnik JMJ, Simonsick EME, Ferrucci LL, Glynn RJ, Berkman LF, Blazer DG, et al. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. J Gerontol 1994;49:M85–94.

[25] Ottenbacher KJ, Branch LG, Ray L, Gonzales VA, Peek MK, Hinman MR. The reliability of upper- and lower-extremity strength testing in a community survey of older adults. Arch Phys Med Rehabil 2002;83:1423–7.

[26] Ershler WB, Keller ET. Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. Annu Rev Med 2000;51:245–70.

[27] Traish A, Bolanos J, Nair S, Saad F, Morgentaler A. Do androgens modulate the pathophysiological pathways of inflammation? Appraising the contemporary evidence. J Clin Med 2018;7(12):pii: E549.

[28] Veltri RW, Miller MC, Zhao G, Ng A, Marley GM, Wright GL Jr., et al. Interleukin-8 serum levels in patients with benign prostatic hyperplasia and prostate cancer. Urology 1999;53(1):139–47.

[29] Lehrer S, Diamond EJ, Mamkine B, Stone NN, Stock RG. Serum interleukin-8 is elevated in men with prostate cancer and bone metastases. Technol Cancer ResTreat 2004;3(5):411.

[30] Roumeguère T, Legrand F, Rassy EE, Kaitouni MI, Albisinni S, Rousseau A, et al. A prospective clinical study of the implications of IL-8 in the diagnosis, aggressiveness and prognosis of prostate cancer. Future Sci OA 2017;4(2):FSO266.

[31] Buch A, Carmeli E, Boker LK, Marcus Y, Shefer G, Kis O, et al. Muscle function and fat content in relation to sarcopenia, obesity and frailty of old age—an overview. Exp Gerontol 2016;76:25–32.

[32] Blaum CS, Xue QL, Michelon E, Semba RD, Fried LP. The association between obesity and the frailty syndrome in older women: the women's health and aging studies. J Am Geriatr Soc 2005;53:927–34.

[33] Hubbard RE, O'Mahony MS, Savva GM, Calver BL, Woodhouse KW. Inflammation and frailty measures in older people. J Cell Mol Med 2009;13(9B):3103–9.

[34] Pérez-Tasigchana RF, León-Muñoz LM, López-García E, Gutierrez-Fisac JL, Laclaustra M, Rodríguez-Artalejo F, et al. Metabolic syndrome and insulin resistance are associated with frailty in older adults: a prospective cohort study. Age Ageing 2017;46(5):807–12.

[35] Wu IC, Shiesh SC, Kuo PH, Lin XZ. High oxidative stress is correlated with frailty in elderly Chinese. J Am Geriatr Soc 2009;57(9):1666–71.

[36] Chang CC, Hsu CY, Huang PH, Liu LK, Chen LK, Chen JW, et al. Association between frailty and carotid intima media thickness and inflammatory marker in an elderly population. Geriatr Gerontol Int 2017;17(12):2449–54.

[37] Haque R, UlcickasYood M, Xu X, Cassidy-Bushrow AE, Tsai HT, Keating NL, et al. Cardiovascular disease risk and androgen deprivation therapy in patients with localized prostate cancer: a prospective cohort study. Br J Cancer 2017;117(8):1233–40.

[38] Olsen NJ, Kovacs WJ. Effects of androgens on T and B lymphocyte development. Immunol Res 2001;23(2–3):281–8.
[39] De Fanis U, Wang GC, Fedarko NS, Walston JD, Casolaro V, Leng SX. T-lymphocytes expressing CC chemokine receptor-5 are increased in frail older adults. J Am Geriatr Soc 2008;56(5):904–8.
[40] Marcos-Pérez D, Sánchez-Flores M, Maseda A, Lorenzo-López L, Millán-Calenti JC, Gostner JM, et al. Frailty in older adults is associated with plasma concentrations of inflammatory mediators but not with lymphocyte subpopulations. Front Immunol 2018;9:1056.
[41] Izaks GJ, Remarque EJ, Becker SV, Westendorp RG. Lymphocyte count and mortality risk in older persons. The Leiden 85-Plus Study. J Am Geriatr Soc 2003;51(10):1461–5.
[42] Semba RD, Margolick JB, Leng S, Walston J, Ricks MO, Fried LP. T cell subsets and mortality in older community-dwelling women. Exp Gerontol 2005;40(1–2):81–7.
[43] Zhao W, Wang P, Jia H, Chen M, Gu X, Liu M, et al. Lymphocyte count or percentage: which can better predict the prognosis of advanced cancer patients following palliative care? BMC Cancer 2017;17(1):514.
[44] Connolly RM, Carducci MA, Antonarakis ES. Use of androgen deprivation therapy in prostate cancer: indications and prevalence. Asian J Androl 2012;14(2):177–86.
[45] Sinclair AJ, Rodriguez-Mañas L. Diabetes and frailty: two converging conditions? Can J Diabetes 2016;40(1):77–83.
[46] Morley JE, Vellas B, van Kan GA, Anker SD, Bauer JM, Bernabei R, et al. Frailty consensus: a call to action. J Am Med Dir Assoc 2013;14(6):392–7.
[47] Winters-Stone KM, Moe E, Graff JN, Dieckmann NF, Stoyles S, Borsch C, et al. Falls and frailty in prostate cancer survivors: current, past, and never users of androgen deprivation therapy. J Am Geriatr Soc 2017;65(7):1414–9.
[48] Molina-Garrido MJ, Guillén-Ponce C. Use of geriatric assessment and screening tools of frailty in elderly patients with prostate cancer. Rev Aging Male 2017;20(2):102–9.
[49] Droz JP, Albrand G, Gillessen S, Hughes S, Mottet N, Oudard S, et al. Management of prostate cancer in elderly patients: recommendations of a task force of the international society of geriatric oncology. Eur Urol 2017;72(4):521–31.