



ORIGINAL ARTICLE

## Body composition and echocardiographic abnormalities associated to anemia and volume overload in heart failure patients

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### KEYWORDS

Anemia;  
Volume overload;  
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### Summary

**Background:** Heart failure (HF) patients with hemodilutional anemia had a worse prognosis than those with true anemia. Volume overload (VO) in anemic patients must be identified in order to select the best treatment.

**Method:** We assessed the prevalence of anemia and VO in systolic and diastolic HF patients, and its effects on clinical status, body composition, and quality of life (QOL). Anemia was defined according to the OMS criteria and VO was assessed by bioelectrical impedance analysis (BIA). Echocardiography variables were also obtained.

**Results:** We studied 132 HF patients, 59.1% with systolic and 40.9% with diastolic HF. Anemia was present in 29.5% patients with systolic and 24.1% with diastolic HF ( $P = 0.7$ ). Anemic patients in the systolic HF group tend to be worse functional class than the non-anemic subjects; moreover, in both groups of cardiac dysfunction, the anemic patients had less grip strength, lower body mass index (BMI) and waist and hip circumferences, and a higher percentage of extracellular water (ECW), a higher proportion of patients with total serum cholesterol concentration below the 25th percentile, and a lower serum albumin. When patients were divided in 4 subgroups according to the presence of anemia and/or VO, we observed that VO affected the body composition not only through lower BMI, and waist and hip circumferences but

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also through a lower phase angle. Systolic HF patients with anemia and VO had the highest dimension of left atrium and pulmonary artery pressure (PAP) levels.

**Conclusion:** Anemia is common in HF and its prevalence is similar in systolic and diastolic HF patients. Anemia was associated to VO, less grip strength and body composition abnormalities; however, VO was related to more severe body composition and echocardiographic abnormalities, as well as to an impairment of QOL. So that, it is important to differentiate between those who have anemia associated to VO and those who have true anemia.

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## Introduction

Anemia in chronic heart failure (HF) is a common finding, with prevalence rates from 4% to 55% depending on the studied population.<sup>1</sup> This range is probably due to differing definitions of what constitutes anemia and to the different types of patients examined. Anemia is more frequent in patients with more severe HF<sup>2–10</sup> and its association to a poor prognosis is well established.<sup>5–10</sup>

Multiple potential mechanisms of interaction exist between anemia and the clinical syndrome of HF, including hemodilution, inflammatory activation, chronic renal failure, and malnutrition.<sup>1</sup> Androne et al.<sup>11</sup> reported a high prevalence (46%) of hemodilution among anemic HF patients. In this study, they also found that patients with hemodilution tended to have worse survival than patients with true anemia and that 56% of patients with volume excess determined by the <sup>131</sup>I-tagged albumin technique were found euvoletic on clinical examination. Witte et al.<sup>12</sup> found a high prevalence (34%) of anemia in patients with symptoms and signs of HF, but few of them had hematinic deficiency (Vitamin B12, iron and folate), without differences between systolic and diastolic left ventricular dysfunction.

Therefore, the evaluation and treatment of anemia in HF must be focused not only to the identification of hemoglobin (Hb) and hematocrit decreased levels, but also on the identification of a volume overload (VO) state in order to select the better treatment for both hemodilutional and true anemic patients.

It is estimated that around 60% of human tissue is made up of water, the majority being intracellular. The extracellular fluid volume is composed of intravascular and extravascular compartments. The intravascular compartment is also known as the plasma volume (PV), whereas the extravascular compartment represents fluid within the interstitial space. Disproportionate fluctuations in PV may result in either hemoconcentration or hemodilu-

tion, thereby influencing the measured packed cell volume. Anand et al. found that untreated congestive HF patients had 34% more PV (determined by the <sup>125</sup>I-HAS method) compared with control subjects, and this excess of water was retained almost entirely within the extracellular space, with both extravascular and PV components increasing in approximately the same proportion (the total extracellular fluid rose by 33% and the PV rose by 34%).<sup>13</sup>

Bioelectrical impedance analysis (BIA) is a non-invasive method for the estimation of total body water (TBW), extracellular water (ECW) and intracellular water (ICW) even in the presence of altered fluid distribution.<sup>14</sup> Using the ratio between two vectors, those of impedance resistance and reactance, we can obtain a phase angle that is an indicator of water distribution between the extra and intracellular space.<sup>15</sup> So BIA, might be a useful tool in the evaluation and monitoring of anemic and non-anemic HF patients with VO (evaluate by an excess of extracellular fluid volume).

The purposes of this study were: (1) to assess anemia and VO prevalence in systolic and diastolic HF patients, and (2) to learn whether anemia or VO contributes to a worse clinical status (symptoms and cardiac function) and quality of life (QOL) in patients with stable systolic and diastolic HF.

## Material and methods

### Patients

This cross-sectional study included 132 male and female patients, attending to the HF Clinic of the Instituto Nacional de Ciencias Médicas y Nutrición "SZ". Patients were recruited if they were  $\geq 18$  years old, with confirmed HF diagnosis as defined by the European Society of Cardiology Guidelines<sup>16</sup> (symptoms of HF as breathlessness or fatigue, either at rest or during exertion, or ankle swelling

and objective evidence of cardiac dysfunction at rest).

Diagnosis of systolic or diastolic dysfunction was done by echocardiography when one or more of the following points were observed in the echocardiogram: Systolic–Ejection fraction  $<45\%$ , end diastolic left ventricular diameter  $\geq 55$  mm, shortening fraction  $\leq 25\%$ ; Diastolic–Preserved systolic function, as echocardiographic evidence of left ventricular hypertrophy (posterior wall+interventricular septum thickness)/2  $\geq 1.3$  cm, posterior wall thickness  $\geq 1.2$  cm, and/or left atrial dilatation  $\geq 4.5$  cm.

We excluded patients with end stage renal disease, non-controlled hypo or hyper thyroid function, hepatic failure, unstable ischemic heart disease (unstable angina and/or acute myocardial infarction), recent myocardial revascularization procedures (percutaneous transluminal coronary artery angioplasty and/or aorto coronary bypass grafting in the last 3 months), and life-threatening arrhythmias.

At the beginning of the study all patients were taking two or more of the following drugs administered at high-dose according to patient tolerance: beta-blockers, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, diuretics, digitalis, oral nitrates, aldosterone receptor antagonists, and calcium channel blockers.

For the purpose of this study, anemia was defined by the World Health Organization criteria; that is, Hb  $<12$  g/dl in women and  $<13$  g/dl in men.<sup>17</sup>

To determinate VO presence, body content of ECW (%) was divided into quartiles as follows: men, 25th: 21.4%; 50th: 22.9% and 75th: 24.6%; females, 25th: 21.0%; 50th: 22.5%; 75th: 24.1%. HF patients were considered to have VO if the percentage of ECW was  $\geq 75$ th for each sex.

Patients were divided first, as is shown in Fig. 1, into anemic and non-anemic subjects; and then, in four groups: Group A (HF subjects without anemia nor VO), Group B (HF subjects with only VO), Group C (HF subjects with only anemia), and Group D (HF subjects with anemia and VO), in both systolic and diastolic HF groups.

### Anthropometry

Weight and height were measured in accordance to the reference manual of anthropometric standardization,<sup>18</sup> all subjects wore light clothing and were barefoot. Body mass index (BMI), was calculated by dividing total body weight (kg) to the squared height (m<sup>2</sup>). Waist and hip circumferences were also measured, as well as hand-grip strength using the Smedley Hand Dynamometer (Stoelting, Wood Dale, K.).

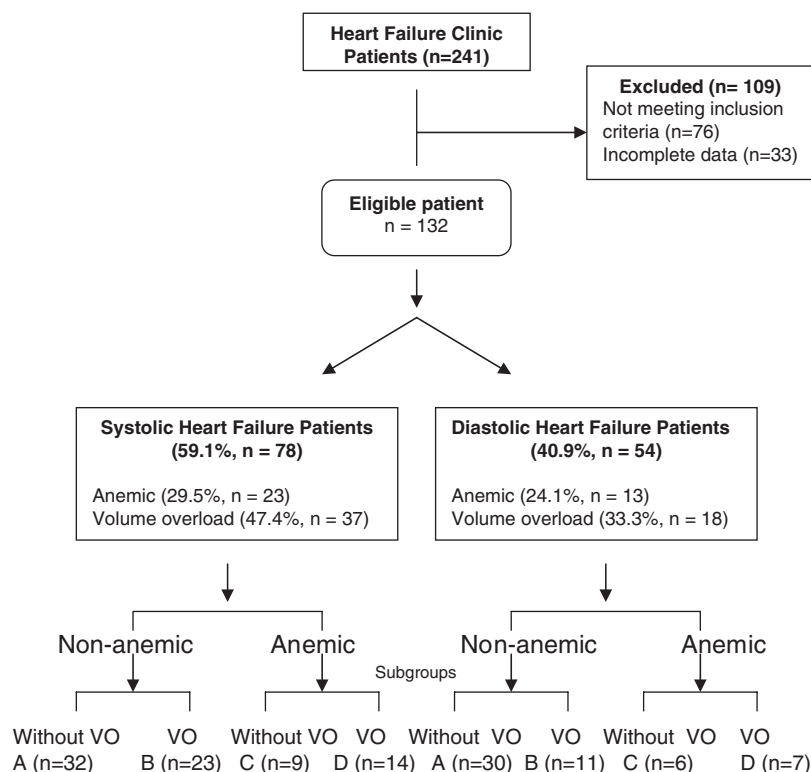


**Figure 1** Flow diagram of participants through each stage of the study analysis. VO = volume overload.

### Bioelectrical impedance analysis

Whole-body bioelectrical impedance was measured by using a tetrapolar and multiple frequencies equipment (BodyStat QuadScan 4000 (BODYSTAT LTD; Isle of Man, British Isles). Volumes of body fluid compartments were estimated by a modeling program supplied by the manufacturer of the analyzer using a 200 kHz signal to assess TBW and a 5 kHz signal to assess ECW. These estimations have been used in patients with varying degree of fluid retention, such as surgical<sup>19</sup> and critically ill<sup>20</sup> patients, in which, the method showed a good reproducibility for fluid measurements and a good correlation with values obtained from radioisotope dilution and deuterium measurement, respectively. An independent panel of BIA experts in 1997 recommended the utilization of multi-frequency BIA in estimating TBW, ECW, and ICW (ICW = TBW - ECW) also in altered fluid distribution.<sup>14</sup>

Phase angle for all patients was also obtained. The phase angle is defined as the relation between the 2 vector components of impedance: resistance and reactance. It may be interpreted as an indicator of water distribution between the extra- and intra-cellular spaces and of cellular health and integrity; a low phase angle is consistent with an inability of cells energy and an indicative of breakdown in the selective permeability of cellular membranes; a high phase angle is consistent with large quantities of intact cell membranes and body cell mass (the range of phase angle in the human body is 1–20°).<sup>21</sup>

### Biochemical markers

Fasting blood samples were obtained for glucose, albumin, Hb, hematocrit, creatinine, triacylglycerol, total cholesterol, HDL-cholesterol, and LDL-cholesterol serum levels. We defined dyslipidemia as: total cholesterol >200 mg/dl, LDL-cholesterol >100, HDL-cholesterol ≤40 and/or triacylglycerol ≥150.

### Clinical data

Current symptoms like fatigue, oedema, congestive state (dyspnoea, oedema, weight increase, decubitus intolerance, and effort-cough) as well as functional class, according to the New York Heart Association (NYHA) classification, were evaluated during the medical interview. These measurements were done by a cardiologist who was blinded to the body composition evaluation.

### Echocardiogram

A wide angle, two-dimensional echocardiogram, was videotaped on a 3/4 in videocassette recorder

with patients in left lateral position, using HP sonos ultrasound 5000 imaging system. Ejection and shortening fractions, and stroke volume were calculated using the telediastolic and telesystolic diameters in the two and four chambers views of the left ventricle. All these parameters were estimated according to the recommendations of the American Society of Echocardiography. The echocardiographer was a cardiologist blinded to the clinical evaluation and laboratory results.

### Quality of life

QOL evaluation was carried out by a questionnaire adapted from the Kansas City Cardiomyopathy Questionnaire<sup>22</sup> and the Minnesota Living With Heart Failure Questionnaire.<sup>23,24</sup> In the test-retest reliability of this instrument we obtained a Kappa value >7.0 ( $P < 0.05$ ) for each item.

### Statistic analysis

All analyses were performed using a commercially available package (SPSS for Windows, Rel. 10.0 1999 Chicago: SPSS Inc.). Continuous variables are given as mean ± standard deviation (SD); categorical variables are presented as absolute and relative frequency. Comparisons between anemic and non-anemic patients were made with Pearson's  $\chi^2$  for categorical variables and unpaired  $t$ -test for continuous variables. Correlation between continuous variables was obtained by Pearson coefficient. The differences among the four subgroups in both, systolic and diastolic HF were assessed by Pearson's  $\chi^2$  for categorical variables and analysis of variance (ANOVA) as well as a post hoc analysis with the LSD method for continuous variables. A  $P$ -value <0.05 was considered statistically significant.

## Results

### Patients characteristics

Of the 132 studied patients, 78 (59.1%) had systolic HF and 54 (40.9%) diastolic HF. The anemia prevalence in the total population was 27.3%; for systolic and diastolic HF it was 29.5% and 24.1% ( $P = 0.7$ ), respectively. So anemia prevalence seems to be similar in systolic and diastolic HF patients.

The characteristics of the anemic and non-anemic patients in systolic and diastolic HF groups are given in Tables 1 and 2, respectively. Among systolic HF patients, those with anemia had a lower BMI, weight, waist and hip circumferences, and

**Table 1** Characteristics of patients with systolic dysfunction compared according the presence or absence of anaemia.

| Variables                            | Anemic (n = 23) | Non-anemic (n = 55) | P-value |
|--------------------------------------|-----------------|---------------------|---------|
| Males (%)                            | 39.1            | 69.1                | <0.05   |
| Age (years)                          | 63.44 ± 15.23   | 57.04 ± 16.43       | NS      |
| Grip strength (kg)                   | 17.05 ± 8.29    | 26.68 ± 11.32       | <0.05   |
| Body mass index (kg/m <sup>2</sup> ) | 25.17 ± 4.47    | 27.54 ± 4.57        | <0.05   |
| Weight (kg)                          | 60.09 ± 15.79   | 70.89 ± 14.57       | <0.05   |
| Height (cm)                          | 153.72 ± 12.21  | 159.96 ± 8.60       | <0.05   |
| Waist circumference (cm)             | 85.78 ± 16.28   | 92.75 ± 12.46       | <0.05   |
| Hip circumference (cm)               | 95.74 ± 9.01    | 100.02 ± 9.43       | <0.05   |
| Total body water (%)                 | 52.78 ± 5.52    | 51.52 ± 5.77        | NS      |
| Extra cellular water (%)             | 24.22 ± 2.54    | 22.88 ± 2.28        | <0.05   |
| Phase angle (deg)                    | 4.65 ± 1.23     | 5.48 ± 1.17         | <0.05   |
| Albumin (g/dl)                       | 3.65 ± 0.53     | 3.90 ± 0.42         | 0.09    |
| Hemoglobin (g/dl)                    | 12.33 ± 0.67    | 15.09 ± 1.55        | <0.0001 |
| Hematocrit (%)                       | 37.28 ± 2.49    | 45.01 ± 4.70        | <0.0001 |
| Total cholesterol (mg/dl)            | 172.62 ± 36.59  | 180.21 ± 44.48      | NS      |
| TC < 25th percentile (%)             | 41.7            | 24.1                | <0.05   |
| LDL cholesterol (mg/dl)              | 96.25 ± 33.72   | 110.87 ± 31.97      | NS      |
| HDL cholesterol (mg/dl)              | 43.17 ± 14.99   | 40.02 ± 10.70       | NS      |
| Triacylglycerol (mg/dl)              | 158.54 ± 68.15  | 157.33 ± 93.11      | NS      |
| Glucose (mg/dl)                      | 105.75 ± 29.91  | 114.17 ± 36.27      | NS      |
| Creatinine (mg/dl)                   | 1.12 ± 0.34     | 1.05 ± 0.37         | NS      |
| LVSF (%)                             | 20.38 ± 8.21    | 19.97 ± 8.39        | NS      |
| LVEF (%)                             | 31.33 ± 10.90   | 34.00 ± 10.98       | NS      |
| LVEDd (mm)                           | 54.45 ± 8.35    | 54.32 ± 8.68        | NS      |
| LVESd (mm)                           | 42.85 ± 9.16    | 42.09 ± 10.34       | NS      |
| IVS (mm)                             | 10.53 ± 1.95    | 10.23 ± 2.02        | NS      |
| PW (mm)                              | 10.07 ± 1.62    | 9.57 ± 1.72         | NS      |
| LAD (mm)                             | 47.73 ± 6.82    | 44.52 ± 7.35        | NS      |
| RVDd (mm)                            | 43.83 ± 7.63    | 41.00 ± 9.89        | NS      |
| IVRTI (seg)                          | 96.00 ± 19.41   | 96.90 ± 31.82       | NS      |
| PAP (mmHg)                           | 62.08 ± 15.42   | 54.63 ± 16.93       | NS      |
| NYHA (%)                             |                 |                     |         |
| I                                    | 34.8            | 61.8                | 0.08    |
| II                                   | 47.8            | 30.9                |         |
| III                                  | 17.4            | 7.3                 |         |
| Ischemic etiology (%)                | 60.9            | 59.3                | NS      |
| <i>Symptoms</i>                      |                 |                     |         |
| Congestion (%)                       | 4.3             | 9.4                 | NS      |
| Edema (%)                            | 34.8            | 34.0                | NS      |
| Fatigue (%)                          | 26.1            | 37.2                | NS      |
| <i>Co-morbidities</i>                |                 |                     |         |
| Dyslipidemia (%)                     | 78.9            | 84.4                | NS      |
| Hypertension (%)                     | 70.0            | 50.0                | NS      |
| Diabetes mellitus (%)                | 42.9            | 40.4                | NS      |
| Dysthyroidism (%)                    | 15.0            | 6.5                 | NS      |

TC = total cholesterol, LVSF = left ventricular shorting fraction, LVEF = left ventricular ejection fraction, LVEDd = left ventricular end diastolic diameter, LVESd = left ventricular end systolic diameter, IVS = interventricular septum, PW = posterior wall, LAD = left atrium diameter, RVDd = right ventricular diastolic diameter, IVRTI = isovolumetric relaxation time index, PAP = pulmonary artery pressure. Values are expressed as mean ± standard deviation or percentage when correspond. A  $P > 0.05$  was considered as non-significant (NS).

phase angle, as well as a higher percentage of ECV and less grip strength. Mean serum level of albumin showed a trend to be lower in the anemic patients.

We also found a higher proportion of patients with total serum cholesterol concentration below the 25th percentile (<146 mg/dl). Echocardiography

**Table 2** Characteristics of patients with diastolic dysfunction compared according the presence or absence of anaemia.

| Variable                             | Anemic (n = 13) | Non-anemic (n = 41) | P-value |
|--------------------------------------|-----------------|---------------------|---------|
| Males (%)                            | 41.5            | 58.5                | NS      |
| Age (years)                          | 52.39 ± 18.72   | 63.76 ± 15.32       | <0.05   |
| Grip strength (kg)                   | 17.71 ± 8.68    | 24.98 ± 11.52       | <0.05   |
| Body mass index (kg/m <sup>2</sup> ) | 25.57 ± 4.77    | 32.94 ± 8.75        | <0.0001 |
| Weight (kg)                          | 59.26 ± 9.87    | 82.89 ± 23.54       | <0.0001 |
| Height (cm)                          | 152.56 ± 8.45   | 158.38 ± 10.37      | NS      |
| Waist circumference (cm)             | 86.68 ± 11.27   | 104.05 ± 19.93      | <0.05   |
| Hip circumference (cm)               | 97.50 ± 11.73   | 109.23 ± 16.41      | <0.05   |
| Total body water (%)                 | 55.18 ± 6.95    | 48.83 ± 8.28        | <0.05   |
| Extra cellular water (%)             | 24.97 ± 2.92    | 22.01 ± 3.24        | <0.05   |
| Phase angle (deg)                    | 4.67 ± 1.20     | 5.22 ± 1.08         | NS      |
| Albumin (g/dl)                       | 3.23 ± 0.54     | 3.77 ± 0.30         | <0.05   |
| Hemoglobin (g/dl)                    | 11.41 ± 1.88    | 15.54 ± 1.68        | <0.0001 |
| Hematocrit (%)                       | 34.10 ± 5.98    | 46.22 ± 5.77        | <0.0001 |
| Total cholesterol (mg/dl)            | 178.38 ± 48.51  | 187.50 ± 41.84      | NS      |
| TC <25th percentile (%)              | 42.9            | 23.3                | NS      |
| LDL cholesterol (mg/dl)              | 107.63 ± 35.01  | 117.36 ± 32.87      | NS      |
| HDL cholesterol (mg/dl)              | 33.63 ± 8.48    | 43.12 ± 11.19       | <0.05   |
| Triacylglycerol (mg/dl)              | 184.63 ± 137.21 | 154.88 ± 77.77      | NS      |
| Glucose (mg/dl)                      | 132.63 ± 51.85  | 126.32 ± 52.56      | NS      |
| Creatinine (mg/dl)                   | 1.00 ± 0.29     | 0.97 ± 0.37         | NS      |
| LVSF (%)                             | 29.67 ± 7.50    | 33.05 ± 9.77        | NS      |
| LVEF (%)                             | 55.69 ± 12.89   | 57.69 ± 9.64        | NS      |
| LVEDd (mm)                           | 48.08 ± 7.01    | 46.57 ± 7.37        | NS      |
| LVESd (mm)                           | 33.23 ± 8.37    | 29.61 ± 6.71        | NS      |
| IVS (mm)                             | 13.23 ± 2.20    | 14.67 ± 3.34        | NS      |
| PW (mm)                              | 12.08 ± 2.78    | 12.01 ± 1.97        | NS      |
| LAD (mm)                             | 42.18 ± 5.90    | 45.09 ± 7.05        | NS      |
| RVDd (mm)                            | 47.00 ± 9.90    | 41.02 ± 7.27        | NS      |
| IVRTI (seg)                          | 104.75 ± 24.09  | 104.50 ± 18.35      | NS      |
| PAP (mmHg)                           | 59.33 ± 16.70   | 51.83 ± 12.10       | NS      |
| NYHA (%)                             |                 |                     |         |
| I                                    | 38.5            | 48.8                | NS      |
| II                                   | 46.2            | 22.0                |         |
| III                                  | 15.4            | 29.3                |         |
| Ischemic etiology (%)                | 61.5            | 26.8                | <0.05   |
| <i>Symptoms</i>                      |                 |                     |         |
| Congestion (%)                       | 7.7             | 5.0                 | NS      |
| Edema (%)                            | 30.8            | 52.5                | NS      |
| Fatigue (%)                          | 61.5            | 52.5                | NS      |
| <i>Co-morbidities</i>                |                 |                     |         |
| Dyslipidemia (%)                     | 85.7            | 84.4                | NS      |
| Hypertension (%)                     | 55.6            | 80.0                | NS      |
| Diabetes (%)                         | 55.6            | 45.7                | NS      |
| Dysthyroidism (%)                    | 33.3            | 22.9                | NS      |

TC = total cholesterol, LVSF = left ventricular shorting fraction, LVEF = left ventricular ejection fraction, LVEDd = left ventricular end diastolic diameter, LVESd = left ventricular end systolic diameter, IVS = interventricular septum, PW = posterior wall, LAD = left atrium diameter, RVDd = right ventricular diastolic diameter, IVRTI = isovolumetric relaxation time index, PAP = pulmonary artery pressure. Values are expressed as mean ± standard deviation or percentage when correspond. A  $P > 0.05$  was considered as non-significant (NS).

characteristics, functional class, ischemic etiology, symptoms, and co-morbidities did not differ between anemic and non-anemic patients; however,

there was a tendency to have a higher proportion of patients in NYHA II and III among the anemic than in the non-anemic group.

Regarding diastolic HF patients, those with anemia were younger and had less grip strength than non-anemic patients. As was observed in systolic HF group, anemic patients showed more body composition abnormalities than non-anemic patients as lower BMI, weight, waist and hip circumferences; as well as higher percentages of TBW and ECW. Mean serum level of albumin was lower in the anemic patients. Echocardiography measures, functional class, symptoms, and comorbidities did not differ between anemic and non-anemic patients. The ischemic etiology was more frequent among anemic patients.

In Table 3 the pharmacological treatment of patients that were anaemic in comparison with the ones who are not, in both systolic and diastolic HF groups is shown. The major difference was the use of loop diuretics, which was higher among anemic subjects, this due to that anemic patients, who have the higher amount of ECW as was previously mentioned, require a more potent diuretic treatment such as loop diuretics, to reduce edema and congestive status.

### VO evaluation

VO prevalence in total population was 41.7% (55); and of 47.4% (37) and 33.3% (18) for systolic and diastolic HF, respectively. Clinical presence of

oedema and BIA (ECW) fluid assessment were not concordant in all cases, it means that some patients with high ECW according BIA have not clinical evidence of edema; thus, a higher proportion of patients with VO appeared euvolemic. (Fig. 2)

ECW (%) in total population showed a negative correlation with phase angle  $r = -0.367$ ,  $P \leq 0.0001$ , Hb  $r = -0.326$ ,  $P < 0.0001$ , and grip strength  $r = -0.302$ ,  $P \leq 0.0001$  (Figs. 3–5). This suggest that anemia is associated with VO, probably due to a higher activation of rennin–angiotensin–aldosterone system, which joint to less grip strength and lower phase angle could be reflecting a greater severity of disease.

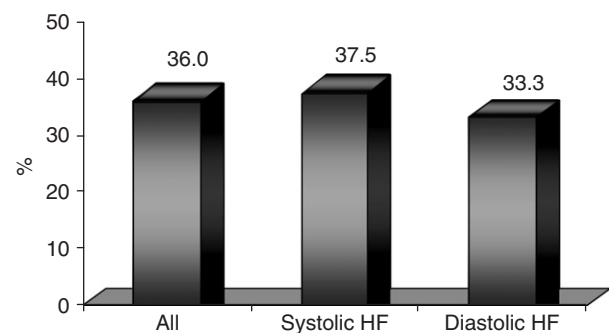


Figure 2 Volume overload patients without edema.

**Table 3** Drug medication in patients with systolic and diastolic dysfunction compared according to the presence or absence of anaemia.

| Variables                            | Anemic (n = 23) | Non-anemic (n = 55) | P-value |
|--------------------------------------|-----------------|---------------------|---------|
| <i>Systolic HF patients</i>          |                 |                     |         |
| Beta-blockers (%)                    | 100             | 87.3                | NS      |
| ACE inhibitor (%)                    | 72.7            | 70.9                | NS      |
| ARB (%)                              | 45.5            | 34.5                | NS      |
| Thiazide diuretics (%)               | 81.8            | 61.8                | NS      |
| Loop diuretics (%)                   | 36.4            | 9.1                 | <0.05   |
| Digitalis (%)                        | 63.6            | 67.3                | NS      |
| Oral nitrate (%)                     | 36.4            | 41.8                | NS      |
| Aldosterone receptor antagonists (%) | 63.6            | 53.7                | NS      |
| Calcium channel blocker (%)          | 4.5             | 0                   | NS      |
| <i>Diastolic HF patients</i>         |                 |                     |         |
| Beta-blockers (%)                    | 84.6            | 66.7                | NS      |
| ACE inhibitor (%)                    | 30.8            | 30.8                | NS      |
| ARB (%)                              | 76.9            | 71.8                | NS      |
| Thiazide diuretic (%)                | 61.5            | 61.5                | NS      |
| Loop diuretic (%)                    | 23.1            | 2.6                 | <0.05   |
| Digitalis (%)                        | 30.8            | 25.6                | NS      |
| Oral nitrate (%)                     | 41.7            | 23.1                | NS      |
| Aldosterone receptor antagonists (%) | 23.1            | 41.0                | NS      |
| Calcium channel blocker (%)          | 7.7             | 10.3                | NS      |

ACE inhibitor = angiotensin-converting enzyme inhibitors, ARB = angiotensin II receptor blockers.

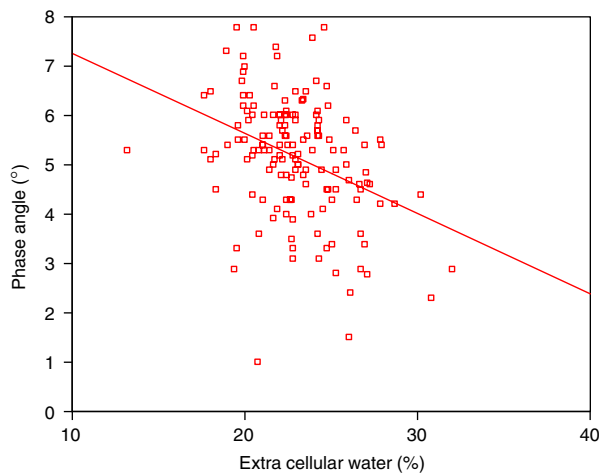


**Figure 3** Correlation between ECW (%) and phase angle (deg) in all HF patients;  $r = -0.367$ ,  $P \leq 0.0001$ .

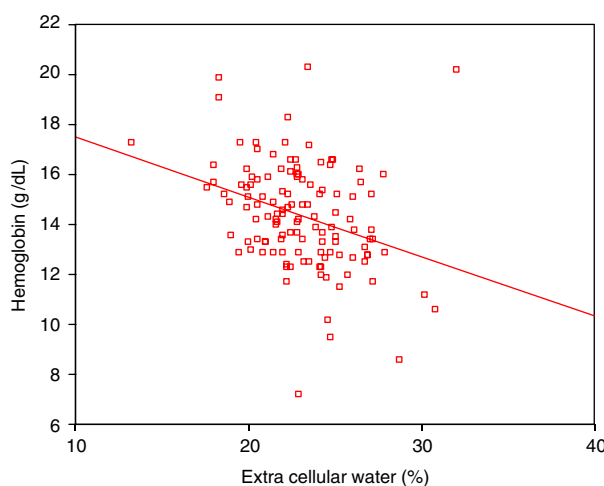


**Figure 4** Correlation between ECW (%) and hemoglobin (mg/dl) in all HF patients;  $r = -0.326$ ,  $P < 0.0001$ .

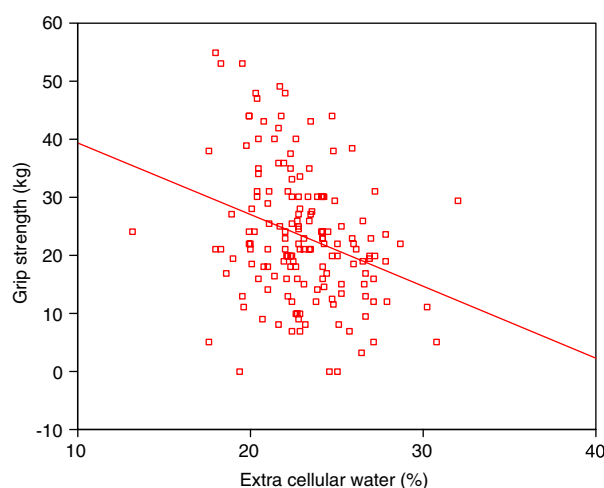


**Figure 5** Correlation between ECW (%) and grip strength (kg) in all HF patients;  $r = -0.302$ ,  $P \leq 0.0001$ .

## VO and anemia effects

When the four subgroups (A–D, performed according to the presence or absence of anemia and VO) were compared, we found that in systolic HF patients, the four subgroups were significantly different with regard to grip strength, body composition, frequency of patient with total cholesterol concentrations below 25th percentile, Hb and hematocrit serum levels, left ventricular shortening and ejection fractions, and pulmonary artery pressure (Table 4).

After post hoc analysis, we observed that the patients with VO (Group B), anemia (Group C), and with both characteristics (Group D) had less grip strength and a lower phase angle versus patients without anemia neither VO (Group A). Subjects in Groups B and D had lower weight and waist and hip circumferences than those in Groups A and C. These groups (B and D) also showed lower left ventricular ejection fraction (LVEF) than Group A. Moreover, in comparison with Group A, patients in Groups B had lower left ventricular shortening fraction (LVSF) and a larger left ventricular end systolic diameter (LVESd), meanwhile subjects in Group D had a larger left atrium diameter (LAD). The highest pulmonary artery pressure (PAP) was observed in this group of patients with anemia and VO.

Subgroups in diastolic HF patients (Table 5) were significantly different in age, body composition parameters, albumin, Hb, and hematocrit serum levels, as well as in the interventricular septum diameter.

After post hoc analysis, subjects in Group C were younger. Patients in Groups B–D had lower BMI and waist and hip circumferences with respect to those in Group A. Phase angle was lower in patients with VO without or with anemia (Groups B and D), and the lowest muscular strength was observed in Group D.

The main echocardiography differences were found in Groups B and C, which showed the larger ISV and LVESd, respectively.

The QOL among diastolic HF patients was lower in Group B ( $P = 0.01$ ). The same tendency was observed in those patients with systolic HF ( $P = 0.09$ ). The rest of the groups did not show significant differences regarding QOL (data not shown).

When the four subgroups were compared according to systolic or diastolic HF, we did not find any significant differences.

## Discussion

In our population of HF patients without renal disease, the prevalence of anemia ( $Hb < 12$  g/dl in

**Table 4** Comparisons of body composition and echocardiographic characteristics between the groups in patients with systolic dysfunction.

| Variable                             | Group A (nor anemia neither volume overload) (n = 32) | Group B (volume overload) (n = 23) | Group C (anemia) (n = 9)    | Group D (anemia and volume overload) (n = 14) | P-value between groups |
|--------------------------------------|-------------------------------------------------------|------------------------------------|-----------------------------|-----------------------------------------------|------------------------|
| Age (years)                          | 57.72 ± 14.86                                         | 57.86 ± 21.83                      | 62.88 ± 18.38               | 62.64 ± 13.83                                 | NS                     |
| Grip strength                        | 29.44 ± 11.10                                         | 20.19 ± 10.49*                     | 16.38 ± 9.16*               | 17.43 ± 8.08*                                 | <0.0001                |
| <i>Body composition</i>              |                                                       |                                    |                             |                                               |                        |
| Body mass index (kg/m <sup>2</sup> ) | 29.24 ± 3.78                                          | 22.85 ± 3.71*                      | 28.97 ± 4.04 <sup>†</sup>   | 23.11 ± 3.38*, <sup>†</sup>                   | <0.0001                |
| Weight (kg)                          | 75.16 ± 11.99                                         | 57.91 ± 13.84*                     | 72.69 ± 16.30 <sup>†</sup>  | 53.90 ± 11.17*, <sup>§</sup>                  | <0.0001                |
| Height (cm)                          | 160.21 ± 8.28                                         | 158.11 ± 9.23                      | 157.81 ± 12.75              | 152.37 ± 11.73*                               | NS                     |
| Waist circumference (cm)             | 97.06 ± 9.97                                          | 80.98 ± 11.01*                     | 95.80 ± 19.22 <sup>†</sup>  | 79.09 ± 10.04*, <sup>§</sup>                  | <0.0001                |
| Hip circumference (cm)               | 103.19 ± 7.97                                         | 90.81 ± 7.83*                      | 101.93 ± 9.28 <sup>†</sup>  | 91.93 ± 6.82*, <sup>§</sup>                   | <0.0001                |
| Total body water (%)                 | 48.91 ± 4.16                                          | 58.24 ± 3.40*                      | 47.25 ± 4.46 <sup>†</sup>   | 55.94 ± 3.00*, <sup>§</sup>                   | <0.0001                |
| Extra cellular water (%)             | 21.73 ± 1.36                                          | 25.86 ± 1.15*                      | 21.70 ± 1.46 <sup>†</sup>   | 25.66 ± 1.77*, <sup>§</sup>                   | <0.0001                |
| Phase angle (deg)                    | 5.69 ± 0.95                                           | 4.75 ± 1.55*                       | 4.76 ± 1.39*                | 4.39 ± 0.92*                                  | <0.05                  |
| <i>Metabolism</i>                    |                                                       |                                    |                             |                                               |                        |
| Albumin (g/dl)                       | 3.94 ± 0.37                                           | 3.85 ± 0.59                        | 3.60 ± 0.50                 | 3.67 ± 0.57                                   | NS                     |
| TC < 25th percentile (%)             | 19.6                                                  | 33.3                               | 55.5                        | 100                                           | <0.05                  |
| Hemoglobin (g/dl)                    | 15.21 ± 1.55                                          | 14.74 ± 1.18                       | 12.66 ± 0.29*, <sup>†</sup> | 12.15 ± 0.78*, <sup>†</sup>                   | <0.0001                |
| Hematocrit (%)                       | 45.23 ± 4.52                                          | 44.33 ± 3.87                       | 37.93 ± 1.09*, <sup>†</sup> | 36.68 ± 2.99*, <sup>†</sup>                   | <0.0001                |
| <i>Cardiologic</i>                   |                                                       |                                    |                             |                                               |                        |
| LVSF (%)                             | 23.27 ± 7.55                                          | 14.10 ± 6.54*                      | 18.40 ± 9.34                | 21.40 ± 8.38 <sup>†</sup>                     | <0.05                  |
| LVEF (%)                             | 37.03 ± 9.87                                          | 26.42 ± 10.76*                     | 34.43 ± 13.24               | 29.45 ± 10.02*                                | <0.05                  |
| LVEDd (mm)                           | 52.87 ± 7.27                                          | 57.64 ± 11.07                      | 55.00 ± 10.55               | 54.96 ± 7.15                                  | NS                     |
| LVESd (mm)                           | 39.36 ± 9.32                                          | 48.09 ± 10.31*                     | 42.43 ± 9.78                | 43.75 ± 9.19                                  | NS                     |
| IVS (mm)                             | 10.48 ± 2.06                                          | 9.70 ± 2.26                        | 10.57 ± 1.40                | 10.81 ± 2.33                                  | NS                     |
| PW (mm)                              | 9.69 ± 1.83                                           | 8.90 ± 1.60                        | 10.33 ± 0.82                | 9.88 ± 2.17                                   | NS                     |
| LAD (mm)                             | 44.97 ± 6.56                                          | 44.70 ± 7.39                       | 45.40 ± 4.04                | 49.09 ± 7.70*                                 | NS                     |
| PAP (mmHg)                           | 51.15 ± 17.12                                         | 62.17 ± 15.13                      | 50.80 ± 15.58               | 72.00 ± 9.06*, <sup>§</sup>                   | <0.05                  |

TC = total cholesterol, LVSF = left ventricular shorting fraction, LVEF = left ventricular ejection fraction, LVEDd = left ventricular end diastolic diameter, LVESd = left ventricular end systolic diameter, IVS = interventricular septum, PW = posterior wall, LAD = left atrium diameter, PAP = pulmonary artery pressure.

\*Statistically significant differences vs. Group A.

<sup>†</sup>Statistically significant differences vs. Group B.

<sup>§</sup>Statistically significant differences vs. Group C.

women and <13 mg/dl in men) was 27.3%, which is lower than the one reported by other authors. Wexler et al.<sup>2</sup> informed a 59.8% prevalence (with the same anemia definition) in out-patients. In that study all the patients were NYHA class III or IV, whereas in our study we did not have patients in functional class IV. Considering the type of ventricular dysfunction, we found that anemia prevalence was similar between systolic (29.5%) and diastolic (24.1%) HF patients. Witte et al.<sup>12</sup> found similar results. They reported a slightly higher prevalence of anemia ( $\leq 12.5$  g/dl in women and men) in patients with systolic and diastolic ventricular dysfunction (35% and 33%, respectively). So that, this results encourage the findings of Witte et al. regarding the fact that patients with HF have

a high prevalence of anemia no matter if they have left ventricular systolic or diastolic dysfunction.

There are multiple potential mechanisms by which the HF syndrome could contribute to the development of anemia. These include hemodilution, renal dysfunction, proinflammatory cytokines, malnutrition due to right-sided HF, decreased perfusion to the bone marrow, and drug therapy (such a ACE inhibitors), and it is believed that in reality, anemia in HF is a result of a complex interaction between cardiac performance, neurohormonal and inflammatory activation, renal function, and bone marrow responsiveness. Thus, the presence of anemia may be a marker for greater severity of HF or greater burden of comorbidity,<sup>1</sup> similar to the anemia of chronic disease states,

**Table 5** Comparisons of body composition and echocardiographic characteristics between the groups in patients with diastolic dysfunction.

| Variable                             | Group A (nor anemia neither volume overload) (n = 30) | Group B (volume overload) (n = 11) | Group C (anemia) (n = 6)     | Group D (anemia and volume overload) (n = 7) | P-value between groups |
|--------------------------------------|-------------------------------------------------------|------------------------------------|------------------------------|----------------------------------------------|------------------------|
| Age (years)                          | 63.77 ± 12.43                                         | 63.73 ± 22.16                      | 41.20 ± 18.63 <sup>*,†</sup> | 58.14 ± 16.90                                | <0.05                  |
| Grip strength                        | 25.52 ± 11.94                                         | 23.55 ± 10.75                      | 20.50 ± 10.54                | 17.21 ± 8.20 <sup>*</sup>                    | NS                     |
| <i>Body composition</i>              |                                                       |                                    |                              |                                              |                        |
| Body mass index (kg/m <sup>2</sup> ) | 35.81 ± 8.32                                          | 25.12 ± 3.65 <sup>*</sup>          | 26.89 ± 3.79 <sup>*</sup>    | 22.98 ± 2.11 <sup>*</sup>                    | <0.001                 |
| Weight (kg)                          | 90.86 ± 20.71                                         | 61.16 ± 16.19                      | 61.12 ± 3.29                 | 54.63 ± 8.39 <sup>*</sup>                    | <0.001                 |
| Height (cm)                          | 159.55 ± 9.08                                         | 155.18 ± 13.24                     | 151.46 ± 9.37                | 153.86 ± 8.90                                | NS                     |
| Waist circumference (cm)             | 110.25 ± 18.75                                        | 87.14 ± 11.81 <sup>*</sup>         | 90.90 ± 9.10 <sup>*</sup>    | 80.81 ± 8.82 <sup>*</sup>                    | <0.001                 |
| Hip circumference (cm)               | 114.91 ± 15.26                                        | 94.25 ± 7.59 <sup>*</sup>          | 98.60 ± 5.19 <sup>*</sup>    | 92.43 ± 8.74 <sup>*</sup>                    | <0.001                 |
| Total body water (%)                 | 45.73 ± 6.54                                          | 57.27 ± 6.57 <sup>*</sup>          | 49.04 ± 3.46 <sup>†</sup>    | 59.56 ± 5.16 <sup>*,§</sup>                  | <0.001                 |
| Extra cellular water (%)             | 20.60 ± 2.25                                          | 25.85 ± 2.29 <sup>*</sup>          | 22.32 ± 0.62 <sup>†</sup>    | 26.86 ± 2.32 <sup>*,§</sup>                  | <0.001                 |
| Phase angle (deg)                    | 5.43 ± 0.95                                           | 4.70 ± 1.25 <sup>*</sup>           | 5.73 ± 0.31                  | 4.17 ± 1.17 <sup>*,§</sup>                   | <0.05                  |
| <i>Metabolics</i>                    |                                                       |                                    |                              |                                              |                        |
| Albumin (g/dl)                       | 3.73 ± 0.31                                           | 3.87 ± 0.24                        | 3.35 ± 0.21 <sup>†</sup>     | 3.26 ± 0.68 <sup>*,†</sup>                   | <0.05                  |
| TC < 25th percentile (%)             | 22.2                                                  | 28.6                               | 33.3                         | 57.1                                         | NS                     |
| Hemoglobin (mg/dl)                   | 15.64 ± 1.52                                          | 15.25 ± 2.08                       | 11.42 ± 2.41 <sup>*,†</sup>  | 11.24 ± 1.71 <sup>*,†</sup>                  | <0.001                 |
| Hematocrit (%)                       | 46.51 ± 5.19                                          | 45.43 ± 7.36                       | 33.14 ± 7.20 <sup>*,†</sup>  | 34.13 ± 5.71 <sup>*,†</sup>                  | <0.001                 |
| <i>Cardiologic</i>                   |                                                       |                                    |                              |                                              |                        |
| LVSF (%)                             | 34.67 ± 6.13                                          | 29.57 ± 15.03                      | 27.00 ± 10.00                | 31.83 ± 5.56                                 | NS                     |
| LVEF (%)                             | 57.00 ± 10.18                                         | 59.50 ± 8.26                       | 51.20 ± 18.42                | 59.00 ± 8.76                                 | NS                     |
| LVEDd (mm)                           | 47.19 ± 5.98                                          | 44.90 ± 10.47                      | 49.00 ± 8.00                 | 48.57 ± 6.60                                 | NS                     |
| LVESd (mm)                           | 30.07 ± 6.01                                          | 28.22 ± 8.76                       | 36.60 ± 11.06 <sup>*,†</sup> | 31.57 ± 6.43                                 | NS                     |
| IVS (mm)                             | 13.82 ± 2.13                                          | 17.00 ± 4.90 <sup>*</sup>          | 14.00 ± 2.55                 | 12.71 ± 2.14 <sup>†</sup>                    | <0.05                  |
| PW (mm)                              | 11.97 ± 2.24                                          | 12.11 ± 1.05                       | 12.00 ± 3.81                 | 12.00 ± 2.28                                 | NS                     |
| LAD (mm)                             | 44.61 ± 7.27                                          | 46.33 ± 6.69                       | 40.75 ± 9.22                 | 42.50 ± 3.73                                 | NS                     |
| PAP (mmHg)                           | 50.45 ± 12.46                                         | 54.00 ± 12.12                      | 57.33 ± 16.20                | 56.40 ± 17.50                                | NS                     |

TC = total cholesterol, LVSF = left ventricular shorting fraction, LVEF = left ventricular ejection fraction, LVEDd = left ventricular end diastolic diameter, LVESd = left ventricular end systolic diameter, IVS = interventricular septum, PW = posterior wall, LAD = left atrium diameter, PAP = pulmonary artery pressure.

<sup>\*</sup>Statistically significant differences vs. Group A.

<sup>†</sup>Statistically significant differences vs. Group B.

<sup>§</sup>Statistically significant differences vs. Group C.

which is partly mediated by cytokines, such as tumor necrosis factor and interleukin-1 that are also elevated in advanced HF and are associated to increased morbidity and mortality.

Anemic patients in the systolic HF group tend to be in a worse functional class than the non-anemic subjects; moreover, in both groups of cardiac dysfunction, the anemic patients had less grip strength, more body composition alteration (lower BMI and waist and hip circumferences, as well as a higher percentage of ECW), a higher proportion of patients with total serum cholesterol concentration below the 25th percentile (<146 mg/dl), and a lower mean serum level of albumin. These findings probably reflect a grater severity of the disease among anemic patients and agree with what was

found by Horwich et al.<sup>6</sup> who observed that patients in the lower Hb quartile (Hb < 12.3) were characterized by makers of malnutrition, such as lower levels of albumin and lower BMI. They suggest that the poor prognosis of anemia in HF stems from malnutrition due to cardiac cachexia, a catabolic state seen in advanced HF that is related to the neurohormone and immune disorders and associated with increases HF mortality. In this study, due the nature of the cross-sectional design it was not possible to determine the presence of cardiac cachexia.

As was mentioned before, expansion of PV is a characteristic of the HF syndrome, and, therefore, some anemia may be dilutional rather than due to a true decrease in red blood cell mass.<sup>1</sup>

The measurement of PV in this study was made by assuming the increased extracellular fluid, the increased PV.<sup>13</sup> In this matter, we observed that the anemic subjects had more ECW than the non-anemic ones, in both systolic and diastolic HF groups. A significantly higher percentage of TBW was also observed among the anemic patients of diastolic group (Tables 1 and 2). Indeed, the amount of ECW correlated negatively with levels of Hb in all HF patients (Fig. 4). This suggest that anemia in this study was strongly associated with VO, probably due to a higher activation of rennin–angiotensin–aldosterone system.

Regarding the relation between VO and anemia, Androne et al.<sup>11</sup> in a study with advanced HF patients distinguished between dilutional and true anemia through a technique such as  $I^{131}$ -tagged albumin, and they found that almost half (46%) of patients categorized as anemic had hemodilution (normal red blood cells with excess PV). Moreover, they concluded that patients with hemodilution tend to have a worse prognosis than patients with true anemia. This is important because the therapeutic approach could be different in anemic patients. Probably, in patients with dilutional anemia the first step in their treatment may be to adjust diuretic doses; however, Witte et al.<sup>12</sup> reported that in their study population, which included renal failure patients, those with anemia were not more likely to be hematinic deficient than patients without anemia, but that the hematocrit levels were not influenced by diuretic dose. Probably a prospective study is needed to try to explain that situation, in which some aspects such as renal function and inflammatory activation must be controlled.

Several methods are available for determining PV, all of which involve the principles of dilutional analysis employed after the administration of tracer molecules,<sup>13</sup> however these methods are invasive and expensive. Even though BIA, the method used in our study to assess fluid status, does not differentiate between true and dilutional anemia, it could be a useful method to make an easy and non-invasive evaluation of ECW in HF patients, even before the appearance of edema.

Using a percentage of ECW  $\geq 24.6\%$  in men and  $\geq 24.1\%$  in women as cut point of VO, we found a VO prevalence in total population of 41.7%; and for systolic and diastolic HF patients it was 47.4% and 33.3%, respectively. However, an important finding in this study was that many of those patients considered as hypervolemic by BIA did not show clinical manifestations of edema (global 36%, systolic 37.5%, and diastolic 33.3%). This finding is in agreement with that reported by Androne

et al.,<sup>11</sup> in which those with excessive PV appeared euvolemic in 56%. This is an important clinical finding because it reflects the necessity of monitoring fluid balance by BIA, as a useful tool for early detection of VO even in the absence of clinical evidence of edema.

When patients with systolic and diastolic HF were divided in subgroups, as seen in Tables 4 and 5, we observed that VO affected the body composition not only through lower BMI, and waist and hip circumferences but also through a lower phase angle. These effects were more apparent in the presence of anemia. It is note worthy that Group D of the systolic HF patients had the highest dimension of left atrium and PAP levels, meaning a more severe hemodynamic alteration and over stimulation of rennin–angiotensin–aldosterone system. Perhaps in this group of patient with fluid overload echocardiographic parameters such as inferior caval vein and mitral valve flow could be abnormal because they give information about congestion status, unfortunately in this study was not included because was not available in almost all patient. We also observed a poor QOL among these patients.

According to the results of Rauchhaus et al.<sup>25</sup> who demonstrated a relationship between higher levels of cholesterol and increased survival in patients with HF, we could suggest a worse prognosis in patients whose anemia are associated to VO (Group C), in which the frequency of patients with total cholesterol serum levels  $< 146$  mg/dl is higher.

Phase angle, as a BIA parameter that indicates the distribution of water between the intra and extracellular spaces, corresponds to a low ECW–ICW ratio. The prognostic value of the phase angle has been proved in renal failure and HIV-infected patients. Maggiore et al.<sup>26</sup> showed that phase angle is a good predictor of death in patients on hemodialysis (with a significantly relative risk of 2.6). In HIV patients<sup>27</sup> a low phase angle (defined as the lowest quartile in the study population:  $< 5.3$ ) was found to be an independent adverse prognostic marker of clinical progression and survival. In patients with liver cirrhosis,<sup>28</sup> a lower phase angle (males:  $4.0^\circ$ , females:  $4.2^\circ$ ) was present in patients with a higher disease progression (patients with cirrhosis, edema, and ascitis). The role of phase angle in HF and anemia has not yet been studied, but according to our findings it could be a good prognostic marker, that guarantees further research.

## Conclusion

Prevalence of anemia is similar in systolic and diastolic HF patients. Anemia was associated with

evidence of increased VO, less grip strength and body composition abnormalities in both preserved and low ejection fraction patients; however, VO was related to more severe body composition and echocardiographic abnormalities, as well as to an impairment of QOL, which means worse clinical status.

So that, in HF patients with anemia it is important to differentiate between those who have anemia associated to VO and those who have true anemia. A higher activation of rennin-angiotensin-aldosterone system, which is present in worse clinical conditions, could be related to hemodilutional anemia due to a higher fluid retention.

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