Heart failure (HF) has become the dominant cardiovascular disorder in the Western world (1). Cardiac remodeling is implicated in the development and progression of HF (2). Among the 3 decisive components of remodeling, fibrosis, cardiomyocyte (CM) hypertrophy, and damage (3), cell death with deficient regeneration is considered 1 of the key events for the occurrence of failure (4). Although the 3 classical mechanisms of cell death, apoptosis, necrosis, and autophagy (5) have been reported to operate in failing human hearts (6), current data do not allow determination which is more relevant to the progression of HF and death.

To understand the pathogenic mechanisms of HF that may support newer therapeutic modalities designed to act upon remodeling, we investigated in endomyocardial biopsy (EMB) specimens of patients with idiopathic dilated cardiomyopathy (IDCM) and severe HF the relation between CM apoptosis, oncosis, and autophagy and structural alterations of remodeling (fibrosis and CM hypertrophy) with functional ventricular parameters with the aim of determining which is related to prognosis.
ICDM was familiar in 8 patients and sporadic in 92 patients.

The local research ethics committee approved the study protocol, and all patients gave written informed consent for the EMB procedure.

In all patients, 12-lead electrocardiography, digital 24-h Holter monitoring, chest X-ray, M-mode and bidimensional echocardiography, color Doppler, radioisotope ventriculography, right and left catheterization with coronary angiography, EMB, and routine laboratory investigations were performed. LVEF was determined by radionuclide ventriculography.

Clinical status permitted the determination of peak oxygen consumption (\(\text{V}_{\text{O2}}\text{max}\)) in 76 patients and the 6-min walking distance in 63 patients.

**Tissue processing and morphometry.** EMB samples from the right ventricular septum were obtained using a Schultz-Caves bioprome. The EMB tissue samples (mean 8.86 ± 2.76 fragments) were fixed for 4 h in 10% buffered formaldehyde and embedded in paraffin. The tissue blocks were kept at 4°C and processed simultaneously for the immunohistochemical studies. Tissue sections 3 μm thick were stained with hematoxylin and eosin, trichrome, and Picrosirius red.

We present herein evidence that the simultaneous presence of CM autophagic vacuolization (AuV) and oncosis and the magnitude of CM hypertrophy are strong independent predictors of death in patients with ICDM and advanced HF.

**Methods**

**Study group.** We studied retrospectively 100 consecutive patients with chronic HF referred to the Heart Failure Center of the University Hospital Favaloro Foundation between September 1992 and September 2002 as potential candidates for heart transplantation (HT), who met the diagnosis criteria for ICDM, defined as ventricular chamber enlargement, normal wall thickness, and systolic dysfunction with left ventricular ejection fraction (LVEF) <30% in the absence of coronary artery disease as assessed by coronary arteriography (7).

Patients with congenital, ischemic, or valvular heart disease, severe arterial hypertension, histological myocarditis, and Chagas' heart disease were excluded.

Maximal cardiomyocyte diameter (CMD) was determined using a digital analysis system (Image Pro Plus 4.5, Media Cybernetics, Silver Spring, Maryland) in 200 cells transversally sectioned at the nuclear level.

Interstitial fibrosis was expressed as the percent of Picrosirius red–positive areas respective to the whole area of the EMB sample, excluding the endocardium, scanned using the same digital analysis system.

**Investigation of apoptosis, oncosis, and autophagy.** Tissue sections were rehydrated and incubated with monoclonal antibodies against ubiquitin and Beclin-1 for determination of autophagy, and anti-complement factor 9 (C9) for oncosis (Novocastra, New castle-on-Tyne, United Kingdom). The specificity of all antibodies was verified by omission of primary antibodies. The secondary detection system was a commercial kit (Biogenex, San Ramon, California) with biotinylated antimouse immunoglobulin G, peroxidase–linked streptavidin, and AEC (3-amino–9-ethyl carbazole) as chromogen. In addition, in selected EMB specimens, double labeling studies were carried out with antiubiquitin and anti-Beclin-1 antibodies using a commercial kit (ABC, Vector, Burlingame, California) and fluorescein–labeled and Texas red–labeled streptavidin. Observations were made with a 100 ultraviolet lamp and photographed with an AXIOLCAM camera (Carl Zeiss AG, Oberkochen, Germany). Apoptosis was investigated using the terminal deoxynucleotidyl transferase–mediated deoxurydine triphosphate nick end labeling (TUNEL) method with a commercial kit (CardioTACS, R&D Systems, Minneaplis, Minnesota).

The proportion of cells labeled with the markers was related to the number of CM nuclei present in the whole tissue section.

To determine the number of CM nuclei present in each EMB specimen, the area of the tissue sections, after elimination of the endocardial fibrotic area, was determined on images collected at 4× magnification with a commercial digital scanner using a digital analysis system (Image Pro Plus 4.5). The number of CM nuclei (mean 344 ± 112) present in 1 mm² was determined on randomly chosen areas at 400× magnification. From these data and the area of the tissue section, positive cells were expressed as a percent of the total number of CM nuclei. In addition, results were dichotomized as negative or positive when at least 1 CM cytoplasm for the determination of AuV and oncosis, or 1 CM nucleus for apoptosis, was stained with the markers.

The study was carried out under Nomarski optics. CMs were identified by the presence of sarcomeres and by their size and shape.

Studies were carried out on a single-blind basis, and results were included in the patient database after data from 100 biopsies were collected.

**Follow-up.** We observed patients until death or HT or until September 2005, beginning at the time of baseline cardiac catheterization and EMB.
Follow-up information was obtained during routine ambulatory visits and by telephone contact with patients or their physicians.

The end point was all-cause mortality. Deaths were classified as sudden death, HF death, noncardiovascular death, or unknown. HT, either United Network for Organ Sharing status 1 or 2, was coded as a nonfatal end point, and patient follow-up was censored at the time of transplantation. Patients lost to follow-up were also censored at the time of last known alive-and-well date.

**Statistical analysis.** Continuous variables are reported as mean ± SD or median (interquartile range [IQR]), and categorical variables are presented as percents of all patients. Differences between continuous variables were assessed using Student *t* tests or Mann-Whitney *U* tests as applicable. Chi-square or Fisher exact tests were used to compare categorical variables. The relationship between EMB parameters and clinical variables was studied using Pearson’s correlation coefficient. Univariate Cox regression analysis was performed to identify predictors of all-cause mortality. Clinical variables that on univariate analysis had *p* values <0.05 were included in the multivariate models with the EMB parameters that also had *p* values <0.05. When 2 or more selected variables were closely associated (correlation coefficient ≥0.600), the variable chosen was that with the greatest Wald statistic.

Kaplan-Meier survival curves were calculated with the data dichotomized at the mean value for each parameter, and a comparison of groups was made using the log-rank test. Two-sided *p* values <0.05 were considered significant.

Statistical analysis was performed using SPSS version 11.0 (SPSS, Inc., Chicago, Illinois).

**Results**

**Patient characteristics.** One hundred patients (59 men, mean age 42.48 ± 15.38 years) with IDCM and chronic HF were analyzed. Most patients (83%) were in New York Heart Association functional classes III and IV, and 17 were in classes I and II at the moment of evaluation, when baseline cardiac catheterization and EMB were performed. The mean LVEF was 20.8 ± 7.41%. Three patients had histories of sustained ventricular tachycardia (VT). In 24-h Holter recordings, 48 patients presented complex ventricular arrhythmias, 33 patients showed nonsustained VT, and 15 patients showed ventricular premature contractions of Lown grade 4A (couplets). Echo-cardiographic studies showed moderate to intense dilation of the cavities: the mean left ventricular end-diastolic diameter (LVEDD) was 73.6 mm, and the mean left atrial systolic diameter (LASD) was 47.5 mm (Table 1).

All patients received conventional medical treatment for HF.

**Morphometric studies. CM hypertrophy and fibrosis.** The mean CMD was 17.7 ± 2.55 μm, and all patients showed variable degrees of interstitial fibrosis (mean 14.27 ± 7.34%) (Fig. 1A).

**Apoptosis and oncosis.** Apoptotic CM nuclei stained using the TUNEL method were infrequently observed (in only 4 nuclei in 3 patients) (Fig. 1B).

Staining of the CM cytoplasm with the anti-C9 antibody was usually diffuse (Fig. 1C), with occasional peripheral localization, and was present in 41 patients.

The number of C9-positive CMs varied between 1 and 17, representing 0.109 ± 0.139% of the whole nuclei examined.

**Autophagy.** The criterion for defining AuV was the presence of CMs with large cytoplasm vacuoles containing ubiquitin-positive material (Figs. 1D and 1E). We did not consider CMs with ubiquitin-stained nuclei or small granular cytosolic deposits (Fig. 1F). In addition, we investigated Beclin-1 as a second marker of autophagy. In all ubiquitin-positive CMs, we observed colocalization of Beclin-1 in the same cell, although the pattern of distribution was slightly different (Figs. 1G to 1J).

CMs with evidence of AuV were observed in 28 patients. In the positive EMB samples, the number of CMs with AuV ranged between 1 and 18, representing 0.013 ± 0.012% of the whole nuclei examined.

**Simultaneous presence of oncosis and autophagy.** Nineteen patients showed the simultaneous presence of oncosis and AuV, although in different cells.

**Correlation between clinical and EMB parameters.** CMD was associated with sex (18.1 ± 2.6 μm in men vs. 17.1 ± 2.3 μm in women, *p* = 0.04) and New York Heart Association functional class (16.4 ± 2.1 μm in patients in classes I and II vs. 18.0 ± 2.6 μm in patients in classes III and IV, *p* = 0.02). Statistical analysis showed negative correlations of CMD with VO2max (*r* = −0.353, *p* = 0.002), LVEF (*r* = −0.335, *p* = 0.001), cardiac index (*r* = −0.356, *p* = 0.001), systolic blood pressure (*r* = −0.220, *p* = 0.03), and diastolic blood pressure (*r* = −0.264, *p* = 0.008).

Positive correlations were found between CMD and mean pulmonary artery pressure (*r* = 0.448, *p* < 0.001), PAWP (*r* = 0.387, *p* < 0.001), LVEDD (*r* = 0.283, *p* = 0.004), left ventricular end-systolic diameter (*r* = 0.216, *p* = 0.036), and LASD (*r* = 0.276, *p* = 0.006).

**Interstitial fibrosis.** Fibrosis was associated with NYHA functional class (10.8 ± 3.9% vs. 15.0 ± 7.7% in patients in classes I and II vs. classes III and IV, *p* = 0.002) and with the presence of VT (16.5 ± 8.6% vs. 13.0 ± 6.3% in patients with and without VT, *p* = 0.019). Negative correlations were found between interstitial fibrosis and VO2max (*r* = −0.283, *p* = 0.013) and cardiac index (*r* = −0.249, *p* = 0.017). A positive correlation was found with mean pulmonary artery pressure (*r* = 0.220, *p* = 0.034).

**Apoptosis.** We did not incorporate data obtained with the TUNEL method because only 4 apoptotic CM nuclei were observed in 3 EMB specimens from 3 different patients.
ONCOSIS. The presence of C9 positivity in EMB specimens was associated with VO$_{2\text{max}}$ (15.4 ± 4.5 ml/kg/min vs. 18.3 ± 5.9 ml/kg/min, p = 0.024), LASD (50.6 ± 8.6 mm vs. 45.4 ± 9.5 mm, p = 0.006), and VT (20 of 36 vs. 21 of 64 C9-positive EMB samples in patients with and without VT, p = 0.03).

AUTOPHAGY. AuV was associated only with VT (17 of 36 patients with VT vs. 4 of 64 patients without VT, p < 0.01) in patients with and without both markers, respectively.

CMD, fibrosis, oncosis, and AuV were not correlated with the remaining clinical parameters selected for the study. CMD and fibrosis did not show correlations among them and with oncosis and AuV.

OUTCOMES. A total of 27 deaths were recorded, 13 attributable to progressive HF, 8 to sudden cardiac death, and 3 to noncardiac causes. In 3 patients, the cause of death could not be determined. Twelve patients underwent urgent HT and 16 underwent elective HT. Four patients were lost to follow-up.

The median time of follow-up was 3.1 years (IQR: 0.6 to 5.3 years). The median follow-up of United Network

Table 1  Baseline Characteristics and Outcomes of 100 Patients With IDCM

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total Population (n = 100)</th>
<th>All-Cause Death (n = 27)</th>
<th>Survivors (n = 73)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic parameters</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Age (yrs)</td>
<td>42.5 ± 15.4</td>
<td>43.4 ± 16.0</td>
<td>42.1 ± 15.3</td>
<td>0.727</td>
</tr>
<tr>
<td>Men</td>
<td>59 (59%)</td>
<td>20 (74.1%)</td>
<td>39 (53.4%)</td>
<td>0.062</td>
</tr>
<tr>
<td>Functional parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NYHA functional class III and IV</td>
<td>83 (83%)</td>
<td>24 (88.9%)</td>
<td>59 (80.8%)</td>
<td>0.549</td>
</tr>
<tr>
<td>6-min walking distance (m) (n = 63)</td>
<td>341 ± 69.1</td>
<td>315 ± 75.2</td>
<td>349 ± 66.2</td>
<td>0.107</td>
</tr>
<tr>
<td>VO$_{2\text{max}}$ (ml/kg/min) (n = 76)</td>
<td>17.0 ± 5.49</td>
<td>15.9 ± 4.21</td>
<td>17.4 ± 5.84</td>
<td>0.306</td>
</tr>
<tr>
<td>Ventricular function</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>20.8 ± 7.41</td>
<td>20.8 ± 8.44</td>
<td>20.8 ± 7.07</td>
<td>0.978</td>
</tr>
<tr>
<td>MR (moderate to severe)</td>
<td>53 (53%)</td>
<td>18 (66.7%)</td>
<td>35 (47.9%)</td>
<td>0.096</td>
</tr>
<tr>
<td>Hemodynamic parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac index (l/min/m$^2$)</td>
<td>2.46 ± 0.80</td>
<td>2.29 ± 0.83</td>
<td>2.51 ± 0.79</td>
<td>0.263</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>102 ± 18.4</td>
<td>98.1 ± 14.2</td>
<td>104 ± 19.5</td>
<td>0.157</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>62.4 ± 11.6</td>
<td>59.7 ± 10.4</td>
<td>63.3 ± 11.9</td>
<td>0.177</td>
</tr>
<tr>
<td>Mean PAP (mm Hg)</td>
<td>23.5 ± 10.5</td>
<td>30.0 ± 11.9</td>
<td>21.3 ± 9.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PAWP (mm Hg)</td>
<td>15.9 ± 8.61</td>
<td>21.6 ± 8.97</td>
<td>14.0 ± 7.59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Right atrial pressure (mm Hg)</td>
<td>5.89 ± 4.61</td>
<td>7.00 ± 4.97</td>
<td>5.51 ± 4.46</td>
<td>0.182</td>
</tr>
<tr>
<td>Electrophysiological parameters</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>87.2 ± 16.6</td>
<td>89.4 ± 18.2</td>
<td>86.4 ± 16.0</td>
<td>0.456</td>
</tr>
<tr>
<td>Left bundle branch block</td>
<td>57 (57%)</td>
<td>15 (55.6%)</td>
<td>42 (57.5%)</td>
<td>0.859</td>
</tr>
<tr>
<td>Atrial Fibrillation</td>
<td>15 (15%)</td>
<td>5 (18.5%)</td>
<td>10 (13.7%)</td>
<td>0.549</td>
</tr>
<tr>
<td>VT</td>
<td>36 (36%)</td>
<td>17 (63.0%)</td>
<td>19 (26.0%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Echocardiographic parameters (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEDD</td>
<td>73.6 ± 10.0</td>
<td>79.1 ± 12.3</td>
<td>71.5 ± 8.29</td>
<td>0.005</td>
</tr>
<tr>
<td>LVESD</td>
<td>62.1 ± 11.2</td>
<td>68.1 ± 12.8</td>
<td>59.8 ± 9.79</td>
<td>0.001</td>
</tr>
<tr>
<td>LASD</td>
<td>47.5 ± 9.45</td>
<td>52.3 ± 7.42</td>
<td>45.7 ± 6.55</td>
<td>0.002</td>
</tr>
<tr>
<td>EMB morphometry</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMD (µm)</td>
<td>17.7 ± 2.55</td>
<td>19.6 ± 2.58</td>
<td>17.0 ± 2.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Interstitial fibrosis (%)</td>
<td>14.3 ± 7.34</td>
<td>15.3 ± 5.64</td>
<td>13.9 ± 7.88</td>
<td>0.390</td>
</tr>
<tr>
<td>EMB cardiomyocyte damage or death</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AuV</td>
<td>28 (28%)</td>
<td>14 (51.9%)</td>
<td>14 (19.2%)</td>
<td>0.001</td>
</tr>
<tr>
<td>AuV, quantitative</td>
<td>0 (0–5)</td>
<td>1 (0–5)</td>
<td>0 (0–0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Oncosis (C9 positive)</td>
<td>41 (41%)</td>
<td>14 (51.9%)</td>
<td>27 (37%)</td>
<td>0.180</td>
</tr>
<tr>
<td>C9 positive, quantitative</td>
<td>0 (0–2)</td>
<td>1 (0–3)</td>
<td>0 (0–2)</td>
<td>0.102</td>
</tr>
<tr>
<td>AuV plus oncosis</td>
<td>19 (19%)</td>
<td>11 (40.7%)</td>
<td>8 (11%)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD, n (%), or median (interquartile range). AuV = autophagic vacuolization; CMD = cardiomyocyte diameter; C9 = complement factor 9; DBP = diastolic blood pressure; EMB = endomyocardial biopsy; IDCM = idiopathic dilated cardiomyopathy; LASD = left atrial systolic diameter; LVEDD = left ventricular end-diastolic diameter; LVEF = left ventricular ejection fraction; LVESD = left ventricular end-systolic diameter; MR = mitral regurgitation; NYHA = New York Heart Association; PAP = pulmonary artery pressure; PAWP = pulmonary artery wedge pressure; SBP = systolic blood pressure; VO$_{2\text{max}}$ = peak oxygen consumption; VT = ventricular tachycardia.
for Organ Sharing status 1 HT patients was 0.24 years (IQR: 0.1 to 0.3 years), of status 2 HT patients was 1.14 years (IQR: 0.3 to 2.6 years), and of surviving nontransplanted patients was 5.3 years (IQR: 4.0 to 7.6 years).

As can be seen in Table 1, many of the clinical parameters, the magnitude of CMD, the presence of AuV, and the simultaneous presence of AuV with oncosis showed correlations with outcomes. **Univariate analysis.** On Cox regression analysis of the baseline variables, the univariate clinical predictors of survival were mitral regurgitation, systolic blood pressure, mean pulmonary artery pressure, PAWP, right atrial pressure, VT, LVEDD, left ventricular end-systolic diameter, and LASD. The structural predictors were CMD and the presence of AuV, either alone or associated with oncosis in the same EMB specimen. In contrast, the magnitude of fibrosis and the presence of oncosis as the only modality of CM damage or death were not predictors of outcomes (Table 2).

**Multivariate analysis.** Both CMD and the simultaneous presence of AuV and oncosis were independent predictors of mortality when they were incorporated in 2 base models adjusted for the 2 strongest clinical predictor variables: LVEDD and PAWP. In addition, we included progres-
in our study, as it is known that in HF, most parameters that
are associated with the 2 main causes of death, HF and
VT. We used both univariate and multivariate models for
patients with advanced HF. Those parameters were found
to be associated with the 2 main causes of death, HF and
VT. We used both univariate and multivariate models for

In this study, we correlated the magnitude of fibrosis, CM
hypertrophy, oncosis, and extensive AuV with mortality in
patients with advanced HF. Those parameters were found
to be associated with the 2 main causes of death, HF and
VT. We used both univariate and multivariate models for
our study, as it is known that in HF, most parameters that
have significant predictive value in univariate models do not
retain that value in multivariate regression analysis (8). The
main results were that the magnitude of CMD, the presence
of AuV, and the simultaneous occurrence of AuV and
oncosis in the same EMB specimen were predictors of
mortality.

Fibrosis and CMD as predictors of outcomes. Coinci-
dent with previous reports (9–11), the fractional area of
collagen was not associated with death. Recently, fibrosis,
determined by cardiovascular magnetic resonance, has been
linked to prognosis in dilated cardiomyopathy (12). This
discrepancy could be because in EMB, the study is limited
to the subendocardial area, and cardiovascular magnetic
resonance shows mesocardial fibrosis.

As opposed to fibrosis, CMD was an important indepen-
dent predictor of death. More than 50% of patients with
CMD over 17.7 μm died during follow-up, compared with
about 10% of patients with CMD below 17.7 μm. The few
studies published in the past correlating CMD with out-
comes in patients with advanced HF have shown dissimilar
results (9–11,13). More recent reports also claim that CMD
does not correlate with outcomes in HF (14,15). This
discrepancy with our results could be ascribed to the
different origin of the EMB samples (left ventricle vs. right
ventricle), to the small number of EMB fragments evaluated
(1 vs. 8.6), to the smaller number of cells analyzed (50 vs.
200), and to the different end points considered (death or
HT and major arrhythmic events during the follow-up vs.
death) in 1 study (14) and to the smaller number of patients
included in another study (35 vs. 100) (15). Moreover, in
those studies, multivariate analysis was not performed.

Oncosis and AuV as predictors of outcomes. Previous
reports have stressed the importance of apoptosis as a
component of HF (16) and have even proposed that its
magnitude is associated with outcomes (17). In our study,
the percent of TUNEL-positive nuclei was very small, in
agreement with recent data from other studies (4,18–20).
This low frequency of apoptosis could be ascribed to the fact
that our patients were in an advanced stage of HF, and it is
known that in this condition, the proportion of apoptotic
CMs is very low or nil (21). For this reason, apoptotic cell
death was not considered for the statistical analysis.
To our knowledge, few studies exist on the presence of oncosis in IDCM (6,18,20). The introduction of C9, a part of the membrane attacking complex C5b-9, is currently accepted as a tool for the detection of early oncosis (22). Our observation that CMs with sarcoplasmic C9 were present in 41% of EMB specimens indicates that oncosis is frequent and may play an important role in HF. In aortic stenosis, the proportion of oncotic myocytes increases significantly during progression to HF (4).

The presence of oncosis as the only modality of cell death did not show a correlation with prognosis, but its association with AuV was a strong independent predictor of mortality. Autophagy has been considered an adaptive mechanism for degradation and recycling of cytoplasmic components, such as long-lived proteins and organelles (23). In situ demonstration of autophagy in the human heart has been based on electron microscopy and the presence of large deposits of ubiquitin in the myocytes (4,6,20).

In addition, we decided to use Beclin-1, a bcl-2–interacting protein that promotes autophagy, as a second in situ marker of autophagy. Increased levels of Beclin-1 were elevated during cardiac injury induced by ischemia-reperfusion (24) and down-regulated in failing human hearts after mechanical unloading (18).

Figure 2 Kaplan-Meier Curves Against All-Cause Mortality for Clinical and EMB Parameters

Kaplan-Meier survival curves for left ventricular end-diastolic diameter (LVEDD) (A), pulmonary artery wedge pressure (PAWP) (B), cardiomyocyte diameter (CMD) (C), and autophagic vacuolization plus oncosis (D), stratified at greater than (dashed line) and less than (solid line) the mean value against all-cause mortality.
The role of autophagy in cell death is controversial, and most evidence suggests that it is an adaptive response (25,26). With the present evidence, it is not possible to state if the presence of large deposits of ubiquitin or Beclin-1 contributes directly to cell death or represents a mechanism for its prevention, as has been previously stated (27), but it appears reasonable to suppose that CMs with extensive loss of organelles and sarcomeres, although they may not be dead, are unable to produce a significant amount of contractile power, to contributing to a worsening of cardiac function and HF (28). In the literature, large deposits or extensive vacuolization has been termed autophagic cell death (4,6,20). However, we prefer to use the term “autophagic vacuolization,” as has recently been recommended (29), because it is not definitely known if those cells are really dead. The role of autophagy in HF probably depends on the severity of HF. It has been reported that in cardiac hypertrophy and in the transition to HF, autophagy may be beneficial, but above a certain threshold, autophagic activity might lead to HF (26,27). This is supported by the presence of increased ubiquitinated proteins in failing human hearts (6,30).

Evidence of AuV was present in 28% of EMB specimens. Autophagy has been observed in dilated cardiomyopathy, and in terminal HF, 0.08% to 0.34% of CMs of left ventricular sections in explanted hearts showed sarcoplasmic ubiquitin inclusions (6,20).

Although in our study, AuV was a significant predictor of death in the univariate analysis, in the multivariate analysis, its statistical value was not retained. However, AuV was a strong predictor of outcome when associated with oncosis in the same EMB specimen. This observation suggests that both modalities of cell damage may be relevant for the progressive functional deterioration of the heart that ends in death.

**Study limitations.** The main limitations of our study are that it was an observational and retrospective analysis of data that were generated for clinical purposes and not prospectively, the relatively small number of patients, and the small size of tissue samples on EMB. Although it is known that right ventricular EMB specimens are representative of the state of the myocardium when a sufficient number (more than 5 samples) have been obtained (31), and quantitative representation of cell death in EMB specimens mirrors that in the whole heart (32), and thus, it can be assumed that oncosis and AuV are diffuse processes, the possibility of an underestimation of the proportion of CMs with evidence of oncosis and AuV should be considered. As a consequence of the small number of events, most of the multivariate models presented were overfitted. Such models may capitalize on the particular characteristics of the sample, and thus our findings may not be consistent on replication.

**Conclusions**

Our results, stressing that CM hypertrophy and cell damage by AuV, especially when associated with oncosis, are independent predictors of death in patients with IDCM and advanced HF, not only give support to the established opinion that remodeling is an important component of HF but indicate that in a population of patients with IDCM and advanced HF, the magnitude of those phenomena—the greater the extent of CM hypertrophy and cell damage, the poorer the prognosis—is related with outcome and suggests that to modify the ominous prognosis of patients with advanced HF, prevention or reversal of CM hypertrophy (33,34) and cell damage by AuV and oncosis (35) should become major targets for therapeutic intervention in heart pathophysiology.

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Key Words: autophagy • heart failure • myocyte hypertrophy • oncosis • prognosis • remodeling.