Cardiomyocyte desmin abnormalities – an accurate predictor of long-term survival in patients with chronic heart failure

Agnieszka Pawlak[1], Robert J. Gil[1, 2], Ewa Walczak[3], Andrzej Słysz[1]

[1] Department of Invasive Cardiology, Central Hospital of Internal Affairs and Administration Ministry, Warsaw, Poland
[2] Mossakowski Institute of Experimental and Clinical Medicine, Polish Academy of Science, Warsaw, Poland
[3] Department of Pathology, Institute of Rheumatology, Warsaw, Poland

Abstract

Background: The proteins of cardiomyocytes are interesting targets for investigations as they may directly reflect intracellular changes within the heart. Desmin is one of the fundamental cytoskeleton proteins of cardiomyocytes, and has a mechanical, structural and regulatory function. In comparison with healthy individuals, patients with heart failure (HF) present different expression of desmin content, that can be associated with its abnormal structure, different distribution, localisation and disturbed function. Abnormal expression of desmin in cardiomyocytes plays a key role in progression of HF.

Aim: To evaluate desmin expression in cardiomyocytes of patients with HF and to asses it's prognostic value during long-term follow-up.

Methods: Diagnostic endomyocardial biopsy (DMB) was performed in 135 patients (86.7% males, mean age 49.4 ± 14.1 years) with clinical symptoms of HF (left ventricular ejection fraction < 45%). In each case four specimens were taken from the right ventricle. Desmin was detected with immunohistochemical staining of cardiomyocytes. The study population was divided into three groups: group I – 48 patients with normal expression of desmin; group II – 54 patients with abnormal accumulation of desmin; group III – 33 patients with low expression of desmin in cardiomyocytes. The ROC curves and Kaplan-Meier survival curves were constructed to analyse predictive value of examined parameters.

Results: The mean duration of follow-up was 33.2 ± 14.6 (6-72) months. Cardiac cause of death was confirmed in 2.08% of cases in group I, 7.4% in group II and 22.86% in group III. Group I vs. group II: Cox's F test – p = 0.07647; log-rank test – p = 0.15047, group I vs. group III: Cox's F test – p = 0.007, log-rank test – p = 0.005, group II vs. group III: Cox's F test – p = 0.033, log-rank test – p = 0.079.

Conclusions: Our results suggest that desmin content in cardiomyocytes directly affects the long-term prognosis in HF patients. The low expression of desmin in cardiomyocytes with immunohistochemical assay is associated with unfavourable clinical course.

Key words: heart failure, desmin, prognostic factor

Kardiol Pol 2009; 67: 724-733

Introduction

Chronic heart failure (HF) is a frequent cause of death and disability. Despite appropriate treatment annual mortality of HF patients exceeds 20%. About 50% of patients die within the first 5 years after diagnosis of HF is established [1]. In the United States alone, approximately one million patients are hospitalised annually due to HF. The latest data suggest that HF may become the leading cause of disability by the year 2020 [2].

Established prognostic factors in HF include clinical parameters, such as New York Heart Association (NYHA) functional class, echocardiographic variables, such as left ventricular (LV) diameters and ejection fraction (EF), and also biochemical ones, such as NT-proBNP and CRP levels. However, the relationship between LVEF and mortality has not been definitely documented [3].

Proper identification of factors predicting mortality and morbidity is fundamental in the treatment of HF patients. It allows identification of patients requiring more intensive monitoring, modification or intensification of therapy. Thus, searching for novel risk factors of mortality in HF is warranted.

The proteins of cardiomyocytes are interesting targets of such investigations as they may directly reflect intracellular changes within the heart. Desmin is one of the fundamental cytoskeleton proteins of cardiomyocytes, and has a mechanical, structural and regulatory function [4]. In comparison with healthy individuals, patients with

Address for correspondence:
Agnieszka Pawlak MD, PhD, Klinika Kardiologii Inwazyjnej, Centralny Szpital Kliniczny MSWiA, ul. Wołoska 137, 02-507 Warszawa,
tel.: +48 22 508 11 00, fax: +48 22 845 41 30, e-mail: a.pawlak1@wp.pl
Received: 25 November 2008. **Accepted:** 25 March 2009.

HF present different expression of desmin content, that can be associated with its abnormal structure, different distribution, localisation and disturbed function [5-7].

So far, the cellular role of desmin has not been elucidated in detail. Also, the impact of abnormal expression of this protein on long-term prognosis has not been yet established.

The aim of the present study was to assess the prognostic value of desmin expression abnormalities during long-term follow-up in patients with HF, in comparison with common clinical parameters.

Methods

Patient characteristics

The study population consisted of 135 patients (mean age 49.4 ± 14.1 years; 86.7% males) presenting with HF of unknown origin. The inclusion criterion was a clinical diagnosis of HF with LVEF below 45%, assessed by echocardiography. Echocardiography was carried out using Sonos 75 V ultrasound apparatus.

After enrolment, endomyocardial biopsy (EMB) was performed by femoral approach. In each patient 3-4 tissue samples were collected using a 7 F bioptome [Cordis; from the following areas of the right ventricle (RV): free RV wall, apex and interventricular septum]. The procedure was performed under continuous ECG monitoring. The mean duration of follow-up was 33.2 ± 14.6 (6-72) months.

All subjects from the study population underwent coronary angiography to exclude significant coronary artery disease (defined as the presence of any coronary artery stenotic lesion producing a greater than 50% reduction in lumen diameter).

All patients underwent two-dimensional echocardiography including measurements of LV end-diastolic diameter (LVEDD) and LVEF using the Simpson equation (from apical four and two chamber view). It was performed before the patient's inclusion in the study. Mean LVEF was 31.4 ± 11.2% and LVEDD 65.1 ± 10.8 mm.

The patients were assigned to NYHA classes I (31%), II (36%), III (27%) or IV (6%). All patients were treated with pharmacotherapy. Most of them received ACE-inhibitors, beta-blockers, diuretics and aldosterone antagonists (Table I).

Histological and immunohistological evaluation of specimens

Myocardial tissue samples were immediately fixed in 4% formaldehyde, buffered by PBS and embedded in paraffin. Serial sections (4-µm thick) were mounted on poly-L-lysine coated slides. Non-consecutive sections were stained with haematoxylin-eosin, azan (azocarmine G) [8], Mason's method [9] and Mallory's method [8]. Mastocyte content of the tissue was identified with monoclonal mouse antihuman tryptase antibody (DAKO inc. M-7052).

Myocarditis was diagnosed based on morphological assessment of specimens according to Dallas criteria [10] and using immunohistochemical methods for identification of inflammatory cells – T lymphocytes, granulocytes, vascular endothelium and MHC II antigens [11]. Antibodies against CD3 (UCHL-1) (Rabbit antibody anti-human T cell CD3, DAKO, catalogue number A-0452; dilution 1 : 10) and CD45RO (Mouse monoclonal antibody anti-human T cell-Clone OPD4, DAKO, catalogue number M-0834; dilution 1 : 10) were applied to detect T lymphocytes. Antibodies against antigen DPQR (Mouse monoclonal antibody anti-human HLA – DQ, DP, DR Antigen, DAKO, catalogue number M-0775; dilution 1 : 25) identified the expression of MHC class II antigens. Antibodies against CD15 (Mouse monoclonal antibody anti-human granulocyte-associated antigen CD15, DAKO, catalogue number M-0733; dilution 1 : 10) identified granulocytes. Inflammatory process on endothelium was identified with antibodies against CD34 (Mouse monoclonal antibody anti-human endothelium CD34, Novocastra, catalogue number NCL-END; dilution 1 : 25).

Dilated cardiomyopathy (DCM) was diagnosed when histological examination revealed the presence of myocytes of abnormal morphology or signs of increased local hypertrophy, local degenerative changes (mainly myocytolysis), abnormal myocyte distribution, including myocyte branching and stroma fibrosis or the presence of lymphocyte infiltration as well as endocardial fibrosis [12].

Vascular myocardial injury was diagnosed when there were thrombi in coronary arteries, fibrosis of interstitium, presence of numerous mastocytes (cell promoting fibrosis) or thickening or lumen closure of small vessels. The clinical profile of these patients did not reveal coronary artery disease as a cause of heart failure.

Identification and evaluation of desmin expression

The expression of desmin in myocardial biopsy samples was determined by immunohistochemical assay as previously described [13]. Three types of desmin expression were identified (Figure 1), i.e. normal, high and low. Normal expression of desmin was defined as even distribution of

Table I. Medical treatment used in the study patients

Agents	Study population (n = 135) [%]
ACE inhibitors	91.9
Angiotensin receptors blockers	3.0
Beta-blockers	91.1
Diuretics	77.7
Aldosterone antagonists	59.3
Digoxin	43.0
Statins	39.3
Acetylsalicylic acid	39.3
Nitrates	18.5
Amiodarone	25.1

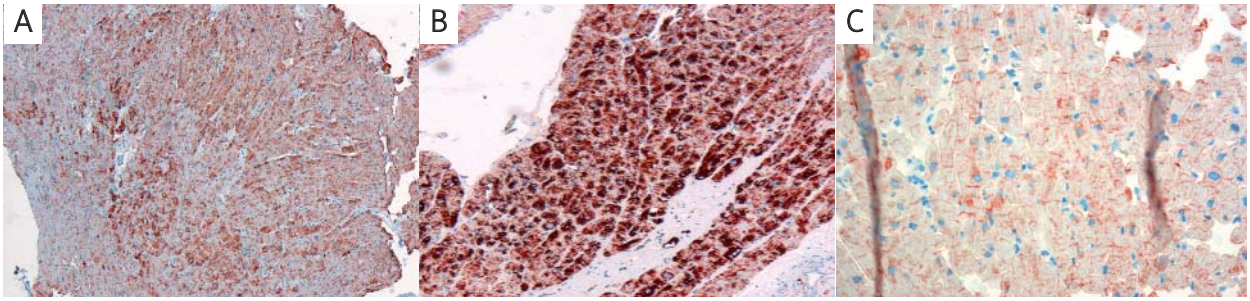

Figure 1. Immunohistochemical desmin staining. Myocardial sample obtained from a patient with heart failure. **A** – normal expression of desmin in cardiomyocytes, magnification 200 ×. **B** – accumulation of forming aggregates of desmin in the cardiomyocyte, magnification 200 ×. **C** – low expression of desmin in the cardiomyocytes, magnification 300 ×

Table II. Cut-off values for desmin, EF, LV, NYHA and follow-up

Test result variables	Positive if greater than or equal to	Sensitivity	1-specificity
Desmin	low expression	0.769	0.410
LVEF [%]	29	0.154	0.500
LVEDD [mm]	64	0.846	0.500
NYHA class	2	0.961	0.459
Follow-up duration [months]	26	0.154	0.500

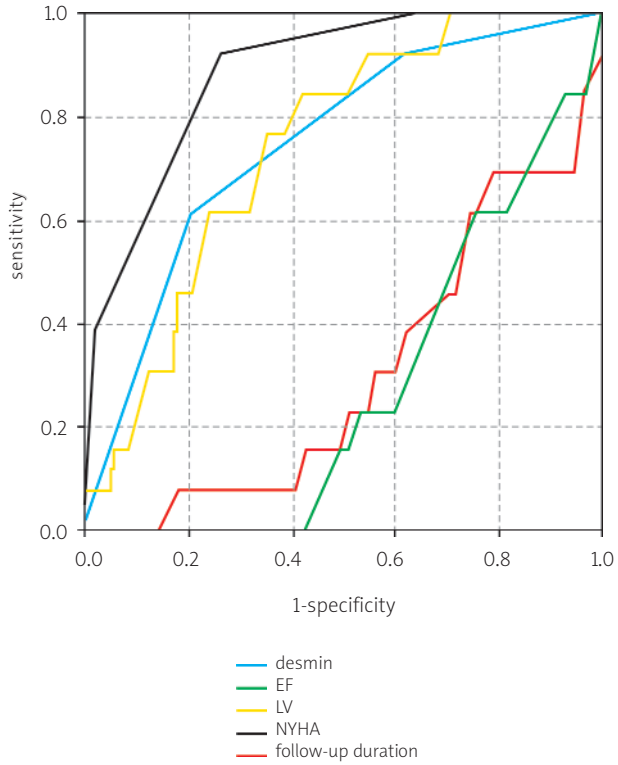

Figure 2. ROC curves for desmin, EF, LV, NYHA class, and follow-up

desmin in slides (not intensive reaction) – Figure 1. A. High expression of desmin – irregular, more intensive accumulation of desmin in stained slides with formation of aggregates – Figure 1. B. Low expression of desmin was assigned when desmin was barely visible or invisible in cells – Figure 1. C. All samples were evaluated by two independent histopathologists.

Statistical analysis

Variables are presented as mean ± standard deviation, or as numbers and percentages. Continuous variables of normal distribution were compared using Student's t-test. Discrete variables were compared using χ^2 test (with Yates' correction when needed) or exact Fisher's test. Multivariable Cox proportional hazards analysis was used to adjust for baseline differences between groups. Kaplan-Meier estimates of survival with respect to desmin group, NYHA class, LVEF and diameter of LV were calculated and compared using the log-rank test. Spearman's correlation was used to examine the relationship between ranks and when evaluating the variable subsidiary. The cut-off values for desmin, diameter of LV, LVEF and NYHA class were analysed using ROC curves. A p value < 0.05 was considered significant. The Statistica 6.0 package was used.

Results

Sensitivity and specificity of desmin assessment in relation to the clinical parameters of HF

When analysing the variables using ROC curves, the highest sensitivity in predicting the outcome was observed for the following parameters: NYHA class (area under the curve 0.883, sensitivity – 96.1%, specificity – 45.9%), desmin (area under the curve 0.749, sensitivity – 76.9%, specificity – 41%) and also LV diameter (area under the curve 0.742, sensitivity – 84.6%, specificity – 50%). Significantly lower sensitivity was observed for LVEF (area under the curve 0.278, sensitivity – 15.4%, specificity 50%). The results are given in Table II and Figure 2.

The analysis of the study group with respect to the prediction of survival based on parameters such as LV

diameter, LVEF, NYHA class and cellular desmin expression showed LV diameter > 64 mm, LVEF less than 29%, NYHA class higher than II (i.e. III or IV) and reduced desmin expression to be predictors of poor prognosis (Table II).

Mean survival of patients exceeding these borderline values were as follows: 3 months for LV more than 64 mm, 9 months for NYHA class III or IV and LVEF less than 29%, and 16 months for desmin deficiency in cardiomyocytes.

Desmin expression and survival

Deaths from cardiac causes were noted in 2.08% of the patients in group I, 7.4% in group II and 22.86% in group III. In the whole study population cardiac mortality was 9.63%.

A comparison of the three desmin groups with respect to the survival rate demonstrated the best prognosis in patients with normal desmin content. Less favourable survival was found in patients with high desmin expression (norm vs. high – F Cox test, p = 0.076; log-rank test, p = 0.150), whereas the worst outcome was found in patients with deficiency of this protein. The most significant difference of survival was seen between patients with normal desmin level and subjects with desmin deficiency (F Cox test, p = 0.007, log-rank test, p = 0.005, Figure 3). Also, significant differences with respect to survival were observed between HF patients with excessive cellular desmin content and subjects with deficiency of this protein (F Cox test, p = 0.033, log-rank test, p = 0.079).

Univariate analysis revealed that deficiency of desmin, apart from NYHA class, remained a strong and independent predictor of unfavourable prognosis, stronger than LV diameter and LVEF, also reaching statistical significance (desmin, p = 0.001 vs. LV, p = 0.005 and EF, p = 0.005).

The independent predictors (i.e. prognostic factors) of death in multivariate analysis of HF patients were NYHA class and desmin deficiency (Table III).

LV diameter and long-term prognosis

The analysis of survival curves in relation to LV diameter revealed that mortality rate was significantly higher in the group of patients with LV diameter more than 64 mm than in patients with LVEDD ≤ 64 mm (F Cox test, p = 0.001, log-rank test, p = 0.011, Figure 4).

NYHA and long-term prognosis

Likewise, significantly worse survival rate was observed in patients with NYHA class III or IV, in comparison with those with NYHA I (NYHA I vs. III log-rank test, p = 0.003, NYHA I vs. IV log-rank test, p = 0,000) and NYHA II (NYHA II vs. III F Cox test, p = 0.002 log-rank test, p = 0.007; NYHA II vs. IV F Cox test, p = 0.00001, log-rank test p = 0.000). There were also statistically significant differences between patients with NYHA class III or IV (F Cox test, p = 0.01796, log-rank test, p = 0.06900, Figure 5).

EF and long-term prognosis

The analysis revealed that LVEF value less than 29% is associated with adverse prognosis in comparison with patients in whom LVEF was higher than 29% (F Cox test, p = 0.006, log-rank test, p = 0.03, Figure 6).

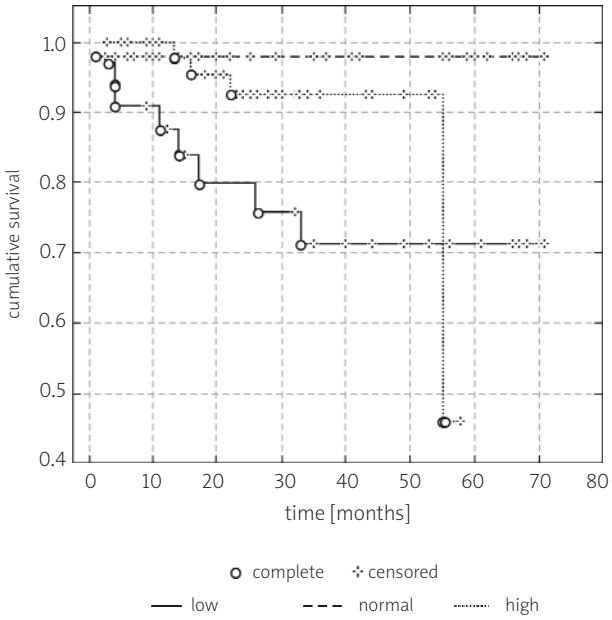

Figure 3. Kaplan-Meier survival curves of patients with normal, high and low expression of desmin

Table III. Uni- and multivariate analysis of prognostic value of desmin content, LVEF, LV diameter and NYHA class in predicting survival [odds ratios (OR) and 95% CI are given]

Parameter	P-value – univariate analysis	P-value – multivariate analysis	OR	Lower limit	Upper limit
Desmin (low)	0.0002	0.0053	16.65	1.91	91.57
EF	0.0058	0.5626	0.96	0.82	1.11
LV	0.0054	0.6515	0.97	0.86	1.09
NYHA	0.0000	0.0003	46.30	3.39	631.11

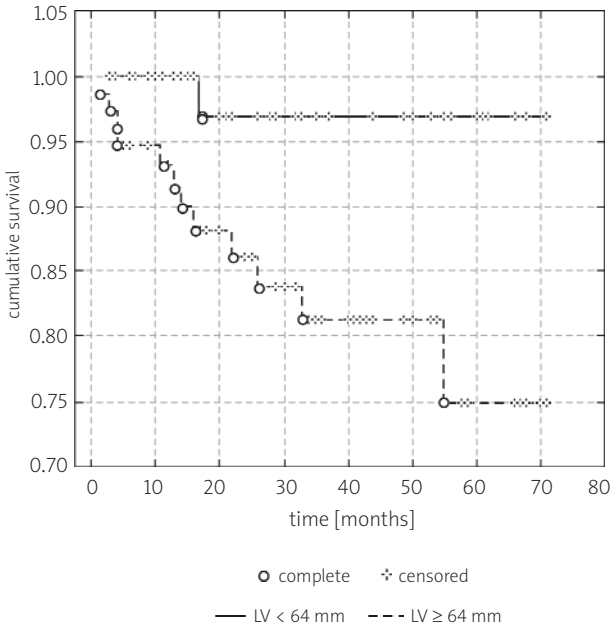

Figure 4. Kaplan-Meier survival curves of patients with LV diameter greater or lower than 64 mm

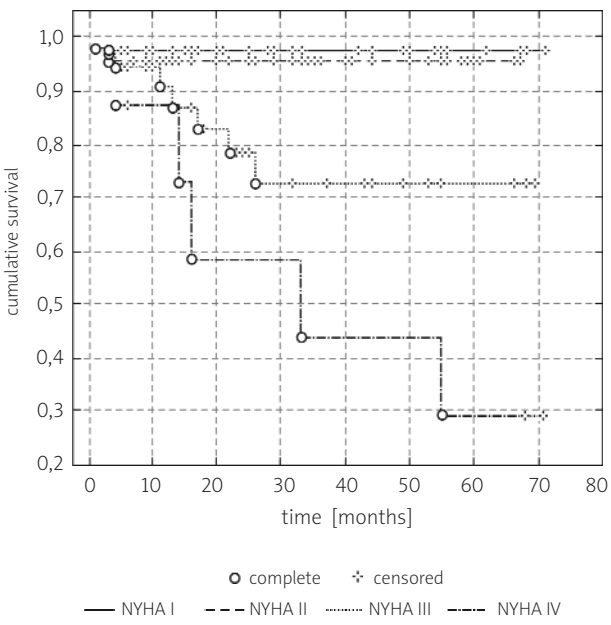

Figure 5. Kaplan-Meier survival curves of patients with NYHA class I-IV

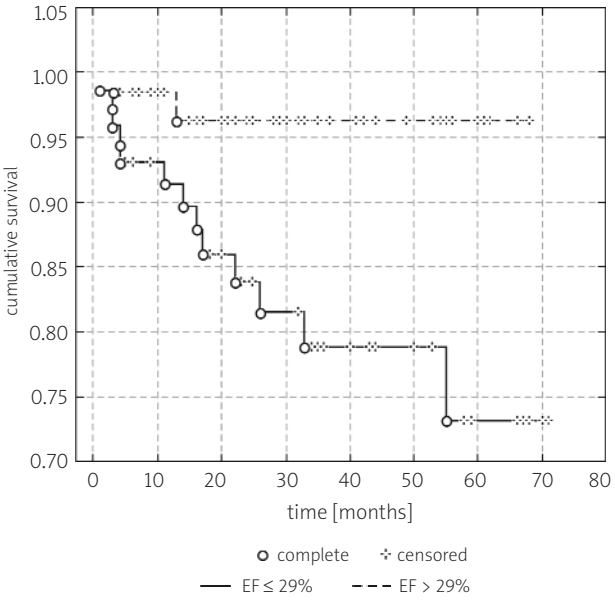

Figure 6. Kaplan-Meier survival curves of patients with LVEF greater or lower than 29%

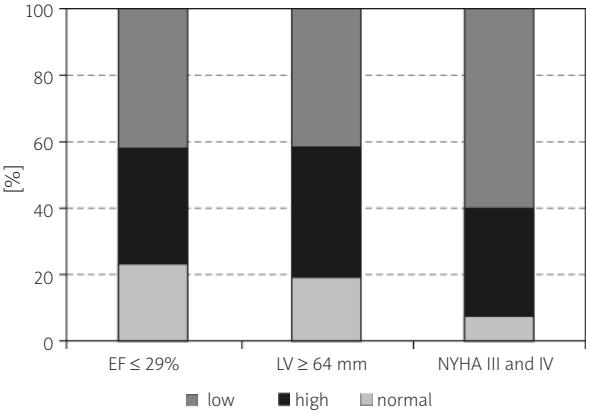

Figure 7. Desmin distribution in population with poor prognosis

Desmin distribution in groups with poor prognosis

Patients with LVEF < than 29% constituted 54% (73 subjects); with LV diameter more than 64 mm – 59.3% (80 subjects); and with NYHA class III or IV – 32.6% (44 subjects) of the whole study population.

In all groups with poor prognosis, i.e. in the groups of patients with EF < 29%, LV diameter > 64 mm and in NYHA class III or IV, patients with normal, excessive and decreased content of desmin were seen. However, patients with desmin deficiency were in the majority within each of these groups.

There were significantly lower numbers of patients with normal cellular desmin presentation than of subjects with excess and deficiency of this protein in each of the analysed subgroups (EF < 29%, p = 0.005, p = 0.0004, LV > 64 mm, p = 0.01, p = 0.001, NYHA > class II, p = 0.006, p = 0.000) (Figure 7).

The LVEDD > 64 mm was significantly more frequently seen in the groups of patients with excess and with deficiency of desmin in the biopsy specimen (excess

p = 0.0003, deficiency p = 0.0000), whereas LV dilatation was infrequent in patients with normal desmin expression (p = 0.006, Figure 8).

Similar correlations were seen for the LVEF. Patients with desmin deficiency significantly more frequently belonged to the subgroup of subjects with reduced LVEF (p = 0.0009), (Figure 9).

In the case of NYHA classification, the most striking difference was observed in patients with normal desmin expression, where advanced HF was observed only occasionally (NYHA III and IV; p = 0.0000). A similar, but of lower magnitude, difference was observed in the group with excess of desmin (p = 0.001). Severe HF (NYHA III or IV) was more frequently seen in patients with desmin deficiency (p = 0.01), (Figure 10).

With increasing NYHA class a reduction of the percentage of patients with normal cellular desmin expression was observed. In NYHA classes I to III a comparable number of patients with excessive desmin expression in cardiomyocytes was demonstrated, while the contribution of this group was reduced in NYHA class IV (Figure 11).

The presence of all four parameters of poor prognosis, i.e. LVEF less than 29%, LV diameter more than 64 mm and NYHA higher than class II, was found in 11.8% of patients. The majority of patients were free from these factors or presented no more than one of them (Figure 12).

The analysis of the entire study population demonstrated a statistically significant correlation between desmin expression and LV diameter (p = 0.0002), LVEF (p = 0.012) and NYHA class (p = 0.00007).

The analysis of the population with inappropriate desmin expression (excess and deficiency) and with LV diameter > 64 mm, LVEF < 29%, and NYHA class III or IV, demonstrated a significant association only between desmin and NYHA class (Table IV).

Discussion

It is beyond question that progress in medicine contributes to the increase in numbers of patients with HF. They constitute a very heterogeneous group, with respect to the aetiology and prognosis. Thus, stratification of mortality risk in this group of patients is of utmost importance. It impels and promotes the development of new, more specific measures relating to the prognosis in this group of patients.

Analysis of validated echocardiographic (LV, LVEF) and clinical parameters allows to detect HF and to stratify the risks in this population with poor prognosis. However, such an approach, based on assessment of a single parameter, does not adequately differentiate the HF population into groups with good or poor prognosis. In one of the recent studies on prognostic models in patients with HF and performed on a large study population of 7500 subjects, LVEF < 45% was found to be a predictor of poor prognosis

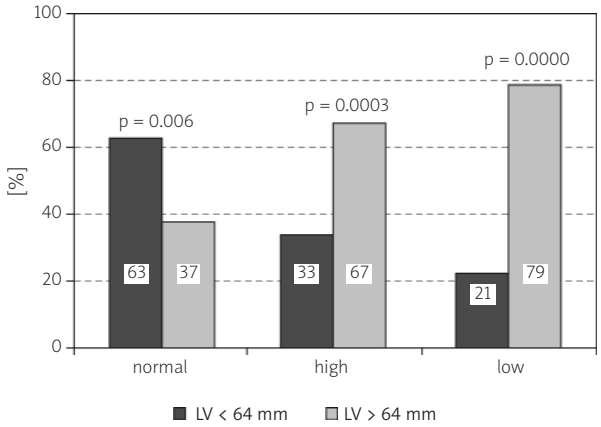

Figure 8. Distribution of patients in each desmin subgroup with respect to LV diameter

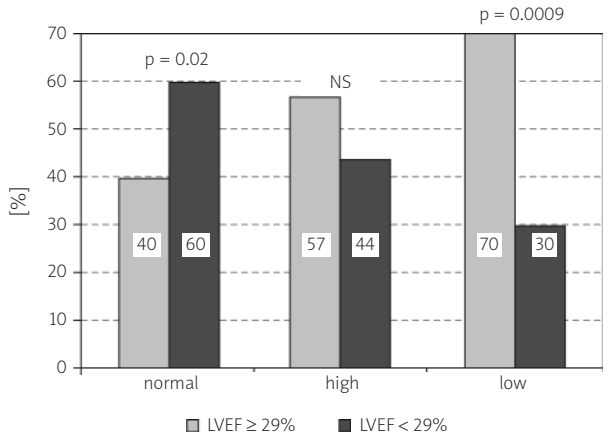

Figure 9. Distribution of patients in each desmin subgroup with respect to LVEF

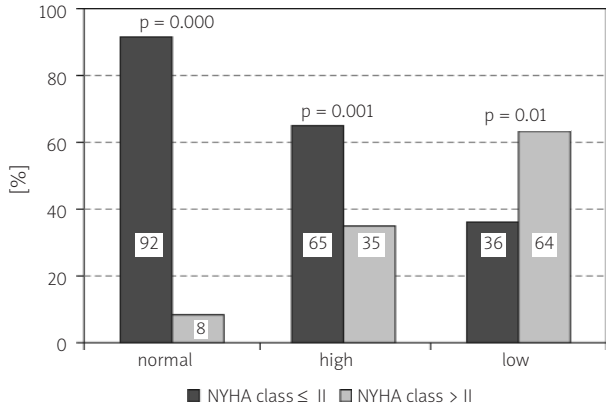

Figure 10. Distribution of patients in each desmin subgroup with respect to NYHA functional classification

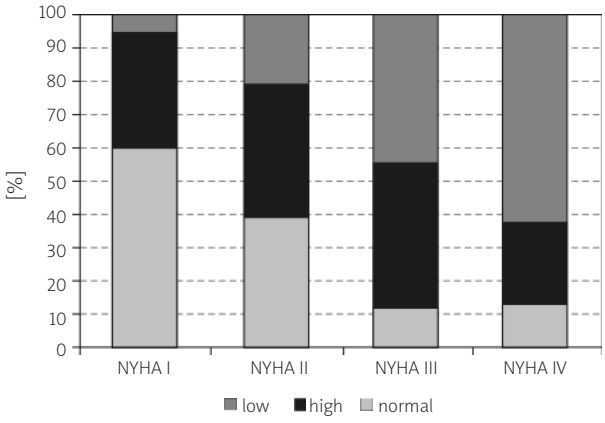

Figure 11. Desmin expression vs. NYHA class

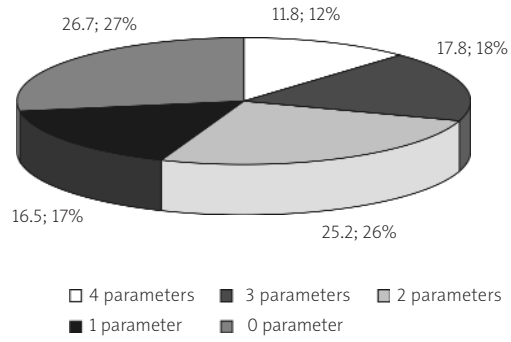

Figure 12. Co-existence of predictors of poor prognosis in entire study population (n = 135)

Table IV. Correlations between LVEF, LV, NYHA class and desmin in entire study population, and in the population of patients with the results below borderline values

	Correlations	R Spearman	p
In entire population (high and low)	Desmin & LVEF	–0.215987	0.011870
	Desmin & LV	0,316876	0.000181
	Desmin & NYHA	0.336491	0.000066
In population with values below borderline values	Desmin & LVEF	0.122896	0.256786
	Desmin & LV	–0.096363	0.374605
	Desmin & NYHA	–0.265756	0.012851

[14]. Neither ESC nor AHA guidelines define LVEF threshold for HF diagnosis. Some accept the value of 45%, while others of 30%. Much more difficult is to quantify LVEF with respect to the prognosis in HF. An additional difficulty concerns patients with diastolic HF, who present HF symptoms despite preserved LVEF, but their prognosis is as poor as in the remaining HF patients. Thus, the role of LVEF as an ideal parameter predicting the outcome in HF patients has been questioned. The present study enrolled patients with LVEF < 45%. Our analyses revealed that patients with LVEF < 29% belonged to the subgroup of definitely poorer prognosis (log-rank test, p = 0.03).

Also, LV diameter is a variable for which cut-off values to assess the prognosis have not been established. It appears that in the case of LVEF and LV diameter in patients with systolic HF a general rule can be accepted that as EF decreases and LV diameter increases, the prognosis worsens. The present study enrolled patients regardless of LV diameter. The performed analysis revealed that LV diameter > 64 mm was a significant predictor of poor prognosis, in comparison with the group of patients with LV diameter less than 64 mm (log-rank test p = 0.01).

Our analyses show that NYHA scale remains the most valuable predictor of the outcome. Patients with advanced HF – presenting with NYHA class III or IV symptoms – have worse prognosis than patients in NYHA class I or II (p = 0.000) [15, 16]. The problem is that in patients with severe HF, haemodynamic changes and multi-organ damage are so advanced that the effects of therapy are modest. Heart transplantation appears to be the only effective method of treatment in this group.

Our results not only confirm that quantification of HF by means of haemodynamic or clinical parameters is not precise, but also that these parameters do not allow an assessment of alterations at the cellular level, as well as evaluation of HF severity. Advances in technology enable better and safer assessment of myocardial changes at the cellular level, making it possible to evaluate single cardiomyocytes. It seems to be highly useful for understanding of pathological processes and for more precise evaluation of prognosis in HF patients.

As far as we are aware, our study is the first one investigating the expression of the cardiomyocyte structural protein desmin with respect to the prognosis in patients with HF. This fundamental protein of the cardiomyocyte skeleton plays a role in numerous cellular processes [5, 17, 18]. Desmin abnormalities significantly affect cardiomyocyte function analysed both in *in vitro* and *in vivo* studies, as well as in an animal model [19].

The analysis of desmin expression in biopsy specimens in combination with parameters such as LVEF and LV diameter suggests that there is a specific sequence of desmin expression in the course of HF. In early stages cellular desmin content is normal, and increases together with worsening of echocardiographic and clinical

parameters (stage II). At the end-stage of this process, a deficiency of desmin occurs (stage III). We demonstrated significant differences of distribution of parameters such as LVEF, LV diameter and NYHA class in consecutive stages.

The most unfavourable values were observed in patients with desmin deficiency in the cardiomyocytes. Increased desmin expression seems to be an effect of activation of compensatory mechanisms. This results in increased production, and perhaps impaired degradation of desmin [20, 21]. However, in the end-stage of the disease, the failing cells are incapable of producing an appropriate amount of this protein. Such conclusions have been confirmed by an Italian study, which demonstrated cellular desmin deficiency in patients with NYHA class IV, undergoing heart transplantation [22]. It should be emphasised that it is difficult to definitely establish whether the changes in desmin expression are the results of haemodynamic abnormalities of injured myocardium, or whether a damaging factor modifies desmin expression and causes haemodynamic compromise.

The analysis shows that desmin expression in cardiomyocytes is a parameter of high sensitivity (76.9%) and slightly lower specificity (41%) in evaluation of HF patients, reaching values comparable with well-established predictors such as NYHA class or LV diameter and being more precise than LVEF.

Our study shows that desmin is a predictor of poor prognosis in HF patients. Decreased desmin expression in cardiomyocytes is associated with increased mortality rate of about 30%. Combined with other risk factors, such as LV diameter, LVEF and NYHA class, desmin expression remains as an independent predictor of increased mortality. It is also interesting that the excess of desmin is an abnormality *per se*, not associated yet however with increased mortality (log-rank test, $p = 0.15$). It seems that at this stage therapeutic interventions such as resynchronisation therapy or heart transplantation may occur effective.

In the group of patients with normal desmin expression in cardiomyocytes, there was a significantly lower number of subjects with adverse prognosis, predicted by LV diameter > 64 mm, LVEF < 29%; patient with advanced HF with NYHA class III or IV symptoms were practically absent in this group. The situation is different in patients with abnormal desmin expression. Echocardiographic parameters unfavourable in terms of prognosis are observed more frequently and become more pronounced in the case of deficiency of the analysed protein. Only in the case of NYHA classification is an increased percentage of patients with advanced HF observed in the group with deficiency of desmin. This may reflect the fact that NYHA classes III and IV include patients with very advanced HF.

The analysis of individual subgroups associated with increased risk of mortality demonstrated the presence of all three types of desmin expression, although deficiency and excess were more frequent than a normal desmin content. Moreover, the data do not indicate a specific value of LV or NYHA class above which no normal desmin expression in cardiomyocytes can be seen. As the statistical analyses reveal, although there are strong correlations between specific clinical and echocardiographic parameters in the entire study population, the correlations between groups of poor prognosis are definitely weaker. This may suggest the absence of simple translation of structural changes into clinical parameters and suggests the contribution of other factors. Such a factor with a significant impact on the function of cardiomyocytes and other myocardial cells is the energetic potential of the cell. The results also show that changes in cardiomyocytes do not always translate into changes in parameters such as NYHA class, LV diameter or LVEF.

Additionally, desmin changes are associated with a long mean survival time with a borderline value of 16 months, increasing the time window for treatment modification, and, perhaps, for reversibility of desmin alteration at the cellular level. Our previous experience (n = 12) showed that with HF progression worsening desmin abnormalities can be expected in follow-up biopsy despite administration of optimal treatment [23]. From the experiments on animal models there seems to be a chance to stop or reverse this abnormal process with administration of growth factors, such as G-CSF, or exercise [24, 25].

Study limitation

The main limitation of our study is desmin assessment only in immunohistochemical assay in all of the examined population. However, immunohistochemical assay was confirmed by immunofluorescence assay in part of our study group and our results were published in a previous paper [10]. We know that in the group of patients with high expression of desmin, aggregates can consist of proteins other than desmin (selenoprotein or mothylin), too. But we chose this method for desmin evaluation because we were looking for a simple, easy to perform and relatively low cost method for long-term prognosis.

Conclusions

Abnormal cardiomyocyte desmin expression, i.e. its deficiency, is a strong and independent predictor of long-term prognosis in patients with HF. The study also reveals associations between normal, increased and decreased desmin expression and clinical parameters such as NYHA class, LV diameter and LVEF. Based on these observations desmin expression analysis can be expected to form a new, useful prognostic tool in patients with HF.

References

1. Alpert NR, Mulieri LA, Warshaw D. The failing human heart. *Cardiovasc Res* 2002; 54: 1-10.

2. Corrí U, Mezzani A, Bosimini E, et al. Prognostic value of time-related changes of cardiopulmonary exercise testing indices in stable chronic heart failure: a pragmatic and operative scheme. *Eur J Cardiovasc Prev Rehabil* 2006; 13: 186-92.
3. Wu AH, Omland T, Wold Knudsen C, et al. Relationship of B-type natriuretic peptide and anemia in patients with and without heart failure: a substudy from the Breathing Not Properly (BNP) Multinational Study. *Am J Hematol* 2005; 80: 174-80.
4. Costa ML, Escaleira R, Cataldo A, et al. Desmin: molecular interactions and putative functions of the muscle intermediate filament protein. *Braz J Med Biol Res* 2004; 37: 1819-30.
5. Kumarapeli AR, Wang X. Genetic modyfication of the heart: chaperones and the cytosceleton. *J Mol Cell Cardiol* 2004; 37: 1097-109.
6. Thornell L, Carlsson L, Li Z, et al. Null mutation in the desmin gene gives rise to cardiomyopathy. *J Mol Cell Cardiol* 1997; 29: 2107-24.
7. Goldfarb LG, Vicart P, Geoble HH, et al. Desmin myopathy. *Brain* 2004; 127: 723-34.
8. Zawistowski S. Technika histologiczna, histologia oraz podstawy histopatologii. *PZWL* 1975; 132-3.
9. Carleton HM. Histological technique for normal and pathological tissues and the identification of parasites. *Oxford University Press,* London-New York-Toronto, 1957; 106.
10. Aretz HT. Myocarditis. The Dallas criteria. *Hum Pathol* 1987; 18: 619-24.
11. Noutsiasis M, Pauschinger M, Schulthesis HP, et al. Advances in the immunohistochemical diagnosis of inflammatory cardiomyopathy. *Eur Heart J* 2002; 4: 154-62.
12. Nożyński J, Konecka-Mrówka D, Zembala-Nożyńska E, et al. Biopsja mięśnia sercowego w kardiologii i kardiochrurgii. *Kardiochir Torakochir Pol* 2004; 1: 110-17.
13. Pawlak A, Gil RJ, Walczak E, et al. Evaluation of desmin activity using immunohistochemical and immunofluorescent staining of myocardial biopsies in patients with chronic heart failure. Comparison of the two methods. *Kardiol Pol* 2007; 65: 229-35.
14. Pocock SJ, Wang D, Pfeffer MA, et al. Predictors of mortality and morbidity in patients with chronic heart failure. *Eur Heart J* 2006; 27: 65-75.
15. Bhatia RS, Tu JV, Lee DS, et al. Outcome of heart failure with preserved ejection fraction in a population-based study. *N Engl J Med* 2006; 355: 260-9.
16. Metra M, Ponikowski P, Dickstein K, et al. Heart Failure Association of the European Society of Cardiology. Advanced chronic heart failure: A position statement from the Study Group on Advanced Heart Failure of the Heart Failure Association of the European Society of Cardiology. *Eur J Heart Fail* 2007; 9: 684-94.
17. Li Z, Mericskay M, Agbulut O, et al. Desmin is essential for the tensile strength and integrity of myofibrils but not for myogenic commitment, differentation, and fusion of sceletal muscle. *J Cell Biol* 1997; 139: 1-16.
18. Cartaud A, Jaśmin BJ, Changeux JP, et al. Direct involvement of a lamin-B-related (54 kDa) protein in the association of intermediate filaments with the postsynaptic membrane of the Torpedo Marmorata electrocyte. *J Cell Sci* 1995; 108: 153-60.
19. Paulin D, Li Z. A major intermediate filament protein essential for the structural integrity and function of muscle. *Expl Cell Research* 2004; 301: 1-7.
20. Kitamura S, Ando S, Shibata M, et al. Protein kinase C phosphorylation of desmin at four serine residues within the non-alpha-helical head domain. *J Biol Chem* 1989; 264: 5674-8.
21. Goebel HH. Congenital myopaties at their molecular dawning. *Muscle Nerve* 2003; 27: 527-48.
22. Di Somma S, Di Benedetto MP, Salvatore G, et al. Desmin-free cardiomyocytes and myocardial dysfunction in end stage heart failure. *Eur J Heart Fail* 2004; 6: 389-98.
23. Gil RJ, Pawlak A, Walczak E, et al. Presence of desmin in cardiomyocytes and long-term prognosis in patients with heart failure. *Eur Heart J* 2005; 26 (Abstract Suppl.): 380.
24. Li Y, Takemura G, Okada H, et al. Treatment with granulocyte colony-stimulating factor ameliorates chronic heart failure. *Lab Invest* 2006; 86: 32-44.
25. Maloyan A, Gulick J, Glabe CG, et al. Exercise reverses preamyloid oligomer ond prolongs survival in alphaB-crystalin-based desmin-related cardiomyopathy. *Proc Natl Acad Sci USA* 2007; 104: 5995-6000.

Nieprawidłowa ekspresja desminy w kardiomiocycie jako czynnik odległego rokowania co do przeżycia u chorych z niewydolnością serca

Agnieszka Pawlak[1], Robert J. Gil[1, 2], Ewa Walczak[3], Andrzej Słysz[1]

[1] Klinika Kardiologii Inwazyjnej, Centralny Szpital Kliniczny MSWiA, Warszawa

[2] Instytut Medycyny Doświadczalnej i Klinicznej im. M. Mossakowskiego, Polska Akademia Nauk, Warszawa

[3] Zakład Anatomii Patologicznej, Instytut Reumatologii, Warszawa

Streszczenie

Wstęp: Białka kardiomiocytu są przedmiotem wielu badań zarówno eksperymentalnych, jak i klinicznych, jako że mogą bezpośrednio odnosić się do wewnątrzkomórkowych zmian w sercu. Desmina jest jednym z podstawowych białek cytoszkieletu kardiomiocytu i pełni w jego obrębie funkcję mechaniczną, strukturalną i regulatorową. W porównaniu ze zdrowymi osobnikami, chorzy z niewydolnością serca wykazują odmienną ekspresją desminy, która może być efektem zmian w budowie białka, nieprawidłowego rozmieszczenia i zaburzonej funkcji. Nieprawidłowa ekspresja desminy w kardiomiocycie odgrywa główną rolę w progresji niewydolności serca.

Cel: Ocena wpływu poziomu ekspresji desminy w kardiomiocycie chorych z niewydolnością serca na rokowanie odległe.

Metody: Diagnostyczną biopsję mięśnia serca (DMB) wykonano u 135 chorych (86,7% mężczyźni, średni wiek 49,4 ± 14,1 roku) z klinicznymi cechami niewydolności serca, z frakcją wyrzutową lewej komory (LVEF) poniżej 45%. W każdym przypadku pobierano 3 lub 4 wycinki z prawej komory serca. Desminę w kardiomiocytach oznaczano metodami immunohistochemicznymi. Badana populacja została podzielona na trzy grupy: I grupa – 48 chorych z prawidłową ekspresją desminy, II grupa – 54 chorych z nadmierną ekspresją desminy, III grupa – 33 chorych z obniżoną ekspresją desminy w kardiomiocycie.

Wyniki: Średni czas obserwacji wynosił 33,2 ± 14,6 miesiąca (6–72). Zgon z przyczyn sercowych wystąpił w 2,08% przypadków w grupie I, 7,4% w grupie II i 22,86% w grupie III. Grupa I *vs* grupa II: test F Coksa – p = 0,07647, test log-rank – p = 0,15047; grupa I *vs* grupa III: test F Coksa – p = 0,007, test log-rank – p = 0,005; grupa II *vs* grupa III: test F Coksa – p = 0,033, test log-rank – p = 0,079.

Wnioski: Nasze wyniki sugerują, że poziom ekspresji desminy w kardiomiocytach bezpośrednio wpływa na rokowanie długoterminowe u chorych z niewydolnością serca. Obniżona ekspresja lub jej brak w kardiomiocytach w oznaczeniu immunohistochemicznym są związane z niekorzystnym przebiegiem klinicznym.

Słowa kluczowe: niewydolność serca, desmina, czynnik rokowniczy

Kardiol Pol 2009; 67: 724-733

Adres do korespondencji:

dr n. med. Agnieszka Pawlak, Klinika Kardiologii Inwazyjnej, Centralny Szpital Kliniczny MSWiA, ul. Wołoska 137, 02-507 Warszawa,

tel.: +48 22 508 11 00, faks: +48 22 845 41 30, e-mail: a.pawlak1@wp.pl

Praca wpłynęła: 25.11.2008. **Zaakceptowana do druku:** 25.03.2009.