Predictors of long-term outcome in patients with biopsy proven inflammatory cardiomyopathy

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Abstract

Background The objective of this study was to identify prognostic indicators in patients with inflammatory cardiomyopathy (iCM) on endomyocardial biopsy (EMB). Methods and results Between 2007 and 2011 all consecutive patients with diagnosed with iCM at EMB were retrospectively analyzed. The combined primary endpoint (EP) (1°EP) was cardiac death, aborted sudden cardiac death/appropriate implantable cardioverter defibrillator (ICD) shock, progressive heart failure requiring left ventricular assist device (LVAD) implantation and heart transplantation. 503 patients (mean age 58 ± 12 years, 73% male) were available for analysis. Genomes of cardiotrophic viruses were detected in 396 patients (79%) and immuno-histochemical signs of inflammation were present in 223 individuals (44%). After 3.6 ± 2.4 years of follow-up, cardiac mortality was 3.0% (n = 14) and a total of 8.6% (n = 40) reached the primary endpoint. Independent predictors for the 1°EP were: age ≥ 50 years, presence and duration (≤ 28 days) of symptomatic heart failure. A risk stratification approach based on the results of the multivariate analysis demonstrated that absence of signs and/or symptoms of congestive heart failure in younger (< 50 years) patients with longer (> 28 days) duration of disease appear to have an excellent prognosis with 100% survival and no events during follow-up. The presence of all above mentioned independent risk factors results in an 1°EP occurrence of 35.9%. Conclusions Symptoms of heart failure, short duration of disease, and older age are indicators of poor outcome in patients with iCM.

Keywords: Endomyocardial biopsy; Inflammatory cardiomyopathy; Outcome

1 Introduction

Inflammatory cardiomyopathy (iCM) is a myocardial disease diagnosed by a combination of histological[1] and immuno-histochemical[2] criteria together with the detection of viral genome at endomyocardial biopsy (EMB),[3] and has a large variable clinical manifestation. Although progressive heart failure, chest pain, and ventricular arrhythmias are common presentations of the disease,[4] the natural history of iCM ranges from full recovery to the development of dilated cardiomyopathy or sudden cardiac death.[4] Because prognostic stratification is critical in successful management of this heterogeneous disease, and no reliable parameters exist,[5–7] we thought to retrospectively analyze all consecutive patient diagnosed with iCM at EMB at our hospital, and extrapolate independent risk factors for mid- to long term adverse outcome.

2 Methods

2.1 Study subjects

Between January 2007 and December 2011 all consecutive patients suspected, and subsequently diagnosed, with iCM were enrolled. The patterns of clinical presentation were already presented earlier.[8] Work-up for iCM was started in patients presenting with acute chest pain (pericarditic or pseudo-ischemic), new onset or sub-acute (up to 3 months) dyspnea, palpitations, syncope, aborted sudden cardiac death (SCD) or unexplained cardiogenic shock in the additional presence of: (1) functional or structural abnormalities on cardiac imaging, (2) increase in serum concentrations of myocardial necrosis markers,[9] (3) new 12-lead electrocardiogram (ECG) and/or Holter and/or stress test abnormalities, (4) edema and/or LGE of classic...
myocarditic pattern at cardiac magnetic resonance.[10] Careful history,[11–14] physical examination, thyroid function and measurements of antinuclear antibodies in addition to standard laboratory testing were always collected. A standard echocardiography study was performed in all patients by experienced sonographers, and the left ventricular end-diastolic and end-systolic diameters were measured in M-mode parasternal long axis view.[15] The institutional ethics committee approved the study.

2.2 Cardiac catheterization and endomyocardial biopsy

Relevant coronary stenosis was excluded with coronary angiography. Left ventricular end-diastolic pressure was measured with standard fluid-filled catheters and the ejection fraction was calculated with a left ventricular angiography in the 30° right anterior oblique and 60° left anterior oblique view. If renal failure or excessive end-diastolic pressures did not allow left ventricular angiography, ejection fraction was estimated by echocardiography using the Teichholz method.

To reduce the sampling error and maximize the sensitivity and specificity, sites of EMB were chosen according to echocardiography or heart magnetic resonance imaging (1.5-T Magnetom Sonata, Siemens Medical Solutions, Erlangen, Germany) information (hypo- or a-kinetic areas).[16] Biopsy specimens were taken with a dedicated biopome (B-18110-S; 4.5 mm², Mohrheim, Germany) advanced through a 7 or 8 French coronary guiding catheters (JR4/AL1/JL4, Medtronic, Danvers, Mass) in order to increase stability and precision. At least 4 biopsy specimens with a diameter of 1 to 3 mm were harvested under sterile conditions. Two to 3 biopsy specimens were fixed in 4% buffered paraformaldehyde for hematoxylin and eosin, Masson’s trichrome, and Giemsa staining and performance of immunohistology. In order to avoid loss of sensitivity, 2 to 3 cardiac tissue samples were quick-frozen or fixed in RNA later (Ambion Inc, Foster City, Calif) for PCR detection of viral genomes.[10,17] All biopsy specimens were investigated within 24 hours.

2.3 Analysis of endomyocardial biopsies

Endomyocardial biopsy findings were classified by immunohistochemistry, and presence or absence of viral genomes.

2.4 Immunohistochemistry

For immunohistological staining, paraffin-embedded tissue sections were treated with an avidin-biotin-immunoperoxidase method according to the manufacturer’s protocol (Vectorstain Elite ABC Kit, Vector, Burlingame, Calif). The following monoclonal antibodies were applied for identification, localization, and characterization of mononuclear cell infiltrates: CD3 for T cells (DAKO, Hamburg, Germany), CD45RO clone UCHL1 for activated T-cells (DAKO, Hamburg, Germany), MAC 387 for macrophages and natural killer cells (Linares, Dossenheim, Germany), HLA-ABC clone w6/32, and HLA-DP clone CR3/43 (DAKO, Hamburg, Germany) to assess HLA class I or II expression in professional antigen-presenting immune cells, respectively. HLA class II expression has mainly been used in to define pathological forms of inflammation. Clone 1304 (Biologo, Kronshagen, Germany) was used to assess CD54/ICAM.

2.5 Inflammatory severity index

The inflammatory severity index is routinely used in the department of pathology to quantify the extent of myocardial inflammation. This index is the arithmetic mean of the analysis results for mononuclear cell infiltrates (CD3, CD45 and CD68), interstitial and endothelial HLA activation (graded from 1 to 4). “No inflammation”, “inflammation”, and “severe inflammation” is diagnosed with values < 1.2, 1.2 to < 1.7, and ≥ 1.7, respectively.

2.6 Molecular biological detection of viral genomes

Enterovirus species (comprising coxsackieviruses and echoviruses), parvovirus B19, adenoviruses, and human herpesvirus type 6 were evaluated by nested PCR/RT-PCR from deep-frozen or RNA later-fixed endomyocardial biopsy specimens as previously described.[10] For RT-PCR analyses, RNA was transcribed into cDNA by reverse transcriptase according to the protocol of the manufacturer (AGS, Heidelberg, Germany). The enzymatic amplification of cDNA was performed as nested PCR on a Perkin-Elmer GeneAmp PCR System 9600 (Applied Biosystems, Weiterstadt, Germany) in two 30-cycle programs. As an internal control for successful isolation of nucleic acids, the housekeeping gene GAPDH was detected by PCR. A biopsy was considered positive for viral infection if viral genome was detected by PCR, and confirmed by automatic DNA sequencing of viral amplification products.

2.7 Endpoints and definitions

The primary combined endpoint included cardiac death, aborted SCD, appropriate implantable cardioverter defibrillator (ICD) therapy, heart transplantation, and implantation of a left ventricular assist device. Aborted SCD was defined by successful cardiopulmonary resuscitation in a patient with sudden (1 hour after onset of any symptoms) most likely cardiac in origin arrest, who survived the following 28 days.
In patients with ICDs, appropriate ICD therapy (shock and/or anti-tachycardia-pacing) was considered as aborted SCD.

All clinical variables were collected directly from patients and/or medical records. Follow-up included outpatient hospital visits in larger intervals, or standardized telephone contact by trained nurses. Patients were considered lost at follow-up when the last medical contact was longer than 12 months and one of the above mentioned end-point did not occur.

2.8 Statistical analysis

Continuous variables were reported as mean value ± standard deviation or median and interquartile ranges (25th–75th percentiles) if appropriate. Normality of distribution was proved with the D’Agostino-Pearson test. Categorical variables were presented as absolute (n) and relative (%) frequencies. The Student T or the Kruskal-Wallis tests were used depending on presence or absence of normal distribution. The Chi-Square test was used for categorical variables. To determine independent risk factors for the occurrence of the combined primary endpoint a backward stepwise Cox regression analysis was performed. All parameters with a probability value of \( P \leq 0.05 \) were included into the model, metric and non-metric variables were transferred into dichotomized variables. The hazard ratio (HR) with 95% confidence interval (CI) was calculated to determine the relative risk of each suspected risk factor, but only if the number of events was \( \geq 5 \) per group. Survival curves of patients were calculated by the Kaplan-Meier method and compared with the log-rank test (Mantel-Cox). A probability value of \( < 0.05 \) was considered to be statistically significant. Statistical analysis was performed using the GraphPad Prism version 6.02 for windows (GraphPad Software, La Jolla, California, USA).

3 Results

Between January 2007 and December 2011, 24,275 patients were treated at our institution. EMB due to suspicion of iCM was performed in 695 (2.8%) patients and the diagnosis was confirmed at EMB in 503 patients who represent the population of this study. Thus, the incidence of iCM at our institution is 2.1% (503/24,275).

Clinical information and the EMB result are given in Table 1. Patients were relatively young, mostly male, and 40% presented with moderately severe or severe heart failure [New York Heart Association (NYHA) class III or IV].

3.1 Endomyocardial biopsy

At EMB, at least 1 viral genome was detected in 78.7% (396/503) of patients. In 12.7% (64/503) more than 1 viral genome was present. The following viruses were detected: parvovirus B19 (n = 286, 56.9%), human herpesvirus type 6 (n = 48, 9.5%), enterovirus species (n = 61, 12.1%), and adenovirus (n = 1, 0.2%). Among patients with \( \geq 1 \) viral genome, parvovirus B19 was always present. The most frequent combination of myocardial co-infection was parvovirus B19 and enterovirus (n = 32, 6.4%) - Figure 1. A significant inflammatory infiltrate was detected at immuno-histochemical staining in 44.3% (n = 223) of patients.

3.2 Follow-up

Thirty-seven (7.4%) patients were lost at follow-up, and
therefore excluded from the analysis. After a mean follow-up of 43.4 ± 29 months, 40/466 (8.6%) patients experienced the combined primary endpoint resulting in an event-rate per year of 2.4% (95% CI: 1.7–3.2).

Twenty-one (4.5%) patients died, and 27 (5.8%) experienced aborted SCD. The most common cause for death was cardiac and accounted for 14 (3%) losses (four sudden cardiac death, nine terminal cardiac pump failure, one acute myocardial infarction). Non-cardiac causes of death were malignant tumors (n = 5), gastro-intestinal bleeding (n = 1), and cirrhosis related terminal liver failure (n = 1). Four patients (0.9%) underwent heart transplantation. Three (0.6%) patients (2 subsequently underwent cardiac transplantation) received a left ventricular assist (Figure 2).

Figure 2. Flow chart of the patients. AMI: acute myocardial infarction; CHF: congestive heart failure; EMB: endomyocardial biopsy; EP: end-point; FU: follow-up; HTx: heart transplantation; ICD: implantable cardioverter defibrillator; iCM: inflammatory cardiomyopathy; LVAD: left ventricular assist device; SCD: sudden cardiac death; w/o: without.

3.3 Predictors of outcome

At bi-variate analyses, resuscitation prior to admission, signs (pulmonary edema and/or rales) or symptoms of heart failure, syncope, low systolic and mean blood pressure, reduced left ventricular (LV) ejection fraction, and echocardiographic evidence of pericardial effusion were all associated with an increased risk of adverse events during follow-up (Table 2 and 3).

Cardiac catheterization and magnetic resonance imaging were not useful for risk stratification of subsequent events (Table 4). At unadjusted Kaplan-Meier, low ejection fraction (≤ 30%) compared to preserved ejection fraction (≥ 50%) was associated with worse outcome. Immuno-histology and presence of a virus were no predictors of outcome.

At multivariate analysis, age ≥ 50 years, signs (rales) and symptoms of congestive heart failure, and short (≤ 28 days) duration of symptoms at presentation clearly identified patients at elevated risk for subsequent events (Table 5).

A risk stratification approach based on the results of the multivariate analysis is depicted in Figure 3. Absence of signs (rales) or symptoms congestive heart failure (CHF), younger age (< 50 years old), and a prolonged duration of the disease before presentation, identified a subset of patients at very low risk of subsequent events. In contrast, presence of signs and/or symptoms of heart failure (CHF +) in older (≥ 50 years) patients with shorter duration (≤ 28 days) of the disease, results in a primary end-point occurrence of 35.9% during 3.6 years of follow-up. Patients who present with only one or two risk factors have an intermediate prognosis, with an event-rate of 27.3% during follow up.

4 Discussion

The main finding of this study is that a combination of “simple” clinical information (signs and symptoms of heart failure, age and disease duration) can predict mid- to long-term outcome in patients with iCM. A subgroup of patients at very low risk of adverse events at mid- to long-term follow-up (cardiac death, aborted SCD, implantation of a left ventricular assist device, and heart transplantation) is identified by young age (< 50 years), absence of heart failure, and long (> 28 days) duration of symptoms.

4.1 Follow-up and predictors of events

In our population, moderate to severe (NYHA III/IV) heart failure was present in 40% of patients and the mean ejection fraction was 40%. Nevertheless, cardiac mortality, at a follow-up > 3 years, was only 3%, and increased to about 9% only if aborted SCD was added. This is in contrast with previous older studies, reporting cardiac death rates of 14%/3/ and 24.9%.[6,18] This might be due to the improvements in medical treatment, since up to 10 years separate ours from previous studies.[6] and in this series most patients were on guideline recommended medical therapy for heart failure. Nevertheless, it cannot be excluded that a relatively healthy series of patients was captured from this analysis, as other more recent studies still showed higher mortality rates.[19]

Whenever iCM is suspected, cardiovascular magnetic resonance can localize and quantify tissue injury, including edema, hyperaemia, and fibrosis.[20,21] In the past, the sensi-
activity of this diagnostic test proved to be very high in patients with biopsy-proven iCM disease,[22,23] but newer studies reported that cardiac magnetic resonance abnormalities do not closely correlate with EMB evidence of iCM.[24] The relative cumbersome and long scanning program, together with the necessity of using more than one single parameter,[20,25] the variability of the results depending from the presentation (acute vs. chronic),[26] and the high expertise needed for the execution and interpretation of this test,[8,25,26] are all possible explanation for our results, which failed to detect any association between abnormalities detected at magnetic resonance scan and events at follow-up. Some studies were able to show a role for EMB analysis in risk stratification for patients with iCM,[3,10,27,28] lymphocytic infiltration has been linked to cardiovascular death and need for heart transplantation,[3,10,27] and eosinophilic dominant iCM, more frequent in East Asian countries, is associated with an excellent long-term prognosis.[28] Nevertheless, information derived from histopathological and immunohistochemical analyses in our cohort showed no additional prognostic value, this is in line with previous reports,[6,29] and underlines the conflicting evidence regarding the long-

## Table 2. Bi-variate analyses of the baseline characteristics.

| Variable | Dichotomization | Patients n (%) | Events n (%) | Event-rate per year (95% CI) | Hazard Ratio (95% CI) | P-Value |
|----------|-----------------|----------------|--------------|-----------------------------|-----------------------|---------|
| Total patients in follow-up | 466 (100%) | 40 (8.6%) | 2.4 (1.7–3.2) | - | - |
| Age, yrs | < 40 | 58 (46.4%) | 1 (1.7%) | 0.5 (0.1–3.9) | Ref | - |
| | ≥ 40 | 67 (53.6%) | 4 (6.0%) | 1.5 (0.5–3.9) | 2.31 (0.30–17.74) | 0.420 |
| | ≥ 50 | 130 (69.1%) | 15 (11.5%) | 3.1 (1.9–5.1) | 5.49 (0.72–42.05) | 0.101 |
| | ≥ 60 | 117 (66.9%) | 9 (7.7%) | 2.0 (1.0–3.9) | 3.94 (0.48–32.50) | 0.203 |
| | ≥ 70 | 94 (61.8%) | 11 (11.7%) | 3.7 (2.1–6.8) | 8.57 (1.00–73.79) | 0.050 |
| Sex | female | 128 (27.5%) | 14 (10.9%) | 3.0 (1.7–5.0) | Ref | - |
| | male | 338 (72.5%) | 26 (7.7%) | 2.1 (1.5–3.2) | 0.75 (0.40–1.42) | 0.383 |
| Diabetes | no | 338 (72.8%) | 26 (7.7%) | 2.1 (1.5–3.2) | 1.74 (0.91–3.36) | 0.096 |
| | yes | 126 (27.2%) | 14 (11.1%) | 3.2 (1.9–5.4) | Ref | - |
| Resuscitation prior admission | no | 449 (96.6%) | 33 (7.3%) | 2.0 (1.4–2.9) | Ref | - |
| | yes | 16 (3.4%) | 7 (43.8%) | 12.2 (5.8–25.5) | - | 0.009 |
| Pulmonary edema | no | 408 (87.7%) | 30 (7.4%) | 2.0 (1.4–2.9) | Ref | - |
| | yes | 57 (12.3%) | 10 (17.5%) | 5.1 (2.7–9.5) | 2.63 (1.34–5.17) | 0.005 |
| Duration of symptoms, days | ≤ 28 | 229 (49.2%) | 22 (9.6%) | 2.0 (1.3–3.2) | 0.73 (0.39–1.34) | 0.310 |
| | > 28 | 236 (50.8%) | 18 (7.6%) | 2.8 (1.8–4.2) | 0.009 |
| Acute coronary syndrome | no | 349 (74.9%) | 27 (7.7%) | 2.2 (1.6–4.8) | 1.19 (0.62–2.27) | 0.597 |
| | yes | 117 (25.1%) | 13 (11.1%) | 3.5 (2.4–5.1) | Ref | - |
| CCS functional class | 0 | 224 (48.3%) | 28 (12.5%) | 3.5 (2.4–5.1) | Ref | - |
| | I/II | 133 (28.7%) | 6 (4.5%) | 1.3 (0.6–2.8) | 0.37 (0.15–0.90) | 0.029 |
| | III/IV | 107 (23.1%) | 5 (4.7%) | 1.2 (0.5–2.9) | 0.30 (0.12–0.78) | 0.014 |
| NYHA functional class | 0 | 123 (26.5%) | 5 (4.1%) | 1.1 (0.4–2.6) | Ref | - |
| | I/II | 142 (30.6%) | 14 (9.9%) | 2.6 (1.5–4.3) | 2.65 (0.93–7.55) | 0.067 |
| | III/IV | 199 (42.9%) | 20 (10.1%) | 3.0 (2.0–4.7) | 3.35 (1.20–9.34) | 0.021 |
| Fatigue | no | 304 (65.5%) | 8 (26.7%) | 2.2 (1.5–3.2) | 0.75 (0.38–1.50) | 0.498 |
| | yes | 160 (34.5%) | 15 (9.4%) | 2.5 (1.5–4.2) | 1.25 (0.66–2.36) | 0.000 |
| Syncope | no | 435 (93.5%) | 29 (7.2%) | 2.0 (1.4–2.8) | Ref | - |
| | yes | 30 (6.5%) | 1 (3.3%) | 7.5 (3.8–15.0) | 4.45 (2.19–9.02) | 0.000 |
| Blood pressure, mmHg | systolic | ≥ 100 | 438 (96.5%) | 34 (7.8%) | 2.1 (1.5–3.0) | Ref | - |
| | < 100 | 16 (3.5%) | 3 (18.8%) | 5.8 (1.9–17.9) | - | 0.002 |
| mean | ≥ 60 | 451 (99.6%) | 36 (8.0%) | 2.2 (1.6–3.1) | Ref | - |
| | < 60 | 2 (0.4%) | 1 (50.0%) | 20.0 (2.8–141.9) | - | 0.017 |
| Rales | no | 404 (86.9%) | 29 (7.2%) | 1.9 (1.3–2.8) | Ref | - |
| | yes | 61 (13.1%) | 10 (16.4%) | 5.2 (2.8–9.7) | 3.09 (1.53–6.25) | 0.002 |

CCS: Canadian Cardiovascular Society; CI: confidence interval; NYHA: New York Heart Association.
Table 3. Bi-variate analyses of electrocardiographic, and echocardiographic data.

| Variable                                | Dichotomization | Patients n (%) | Events n (%) | Event-rate per year (95% CI) | Hazard Ratio (95% CI) | P-Value |
|-----------------------------------------|-----------------|----------------|--------------|-----------------------------|-----------------------|---------|
| **Electrocardiogram**                   |                 |                |              |                             |                       |         |
| Sinusrhythm                             | no              | 87 (18.8%)     | 9 (10.3%)    | 3.1 (1.6–5.9)               | Ref                   | -       |
|                                         | yes             | 375 (81.2%)    | 28 (7.5%)    | 2.0 (1.4–2.9)               | 0.62 (0.30–1.28)      | 0.197   |
| Heart rate at admission, min            | < 60            | 58 (12.7%)     | 3 (5.2%)     | 1.5 (0.5–4.5)               | 0.68 (0.21–2.20)      | 0.519   |
|                                         | 60–100          | 351 (76.6%)    | 30 (8.5%)    | 2.3 (1.6–3.3)               | Ref                   | -       |
|                                         | > 100           | 49 (10.7%)     | 4 (8.2%)     | 2.6 (1.0–7.0)               | 1.27 (0.44–3.65)      | 0.654   |
| Bundle branch block                     | no              | 296 (66.1%)    | 18 (6.1%)    | 1.6 (1.0–2.6)               | Ref                   | -       |
|                                         | yes             | 152 (33.9%)    | 16 (10.5%)   | 2.9 (1.8–4.8)               | 1.85 (0.95–3.59)      | 0.068   |
| PQ-interval, s                          | ≤ 0.2           | 334 (89.1%)    | 22 (6.6%)    | 1.8 (1.2–2.7)               | Ref                   | -       |
|                                         | > 0.2           | 41 (10.9%)     | 4 (9.8%)     | 2.8 (1.1–7.5)               | 1.56 (0.54–4.53)      | 0.415   |
| QRS-width, s                            | ≤ 0.12          | 330 (75.9%)    | 25 (7.6%)    | 2.1 (1.4–3.1)               | Ref                   | -       |
|                                         | > 0.12          | 48 (11.0%)     | 3 (6.3%)     | 1.6 (0.5–4.8)               | 0.73 (0.23–2.34)      | 0.596   |
| QT-interval, s                          | ≤ 0.39          | 205 (47.9%)    | 13 (6.3%)    | 1.7 (1.0–3.0)               | Ref                   | -       |
|                                         | > 0.39          | 223 (52.1%)    | 18 (8.1%)    | 2.2 (1.4–3.6)               | 1.36 (0.65–2.85)      | 0.409   |
| ST-segment alterations                   | no              | 284 (63.4%)    | 21 (7.4%)    | 2.1 (1.4–3.2)               | Ref                   | -       |
|                                         | yes             | 164 (36.6%)    | 12 (7.3%)    | 1.9 (1.1–3.4)               | 0.90 (0.44–1.82)      | 0.766   |
| **Echocardiography**                    |                 |                |              |                             |                       |         |
| Ejection fraction, %                    | < 30            | 113 (25.6%)    | 15 (13.3%)   | 3.7 (2.3–6.2)               | -                     | <0.001  |
|                                         | ≤ 40            | 105 (23.8%)    | 8 (7.6%)     | 2.3 (1.1–4.6)               | -                     | 0.012   |
|                                         | < 60            | 161 (36.5%)    | 17 (10.6%)   | 3.0 (1.9–4.9)               | -                     | 0.001   |
|                                         | ≥ 60            | 62 (14.1%)     | 0 (0%)       | 0.0 (–)                     | Ref                   | -       |
| Pericardial effusion/tamponade          | no              | 371 (89.6%)    | 29 (7.8%)    | 2.1 (1.5–3.1)               | Ref                   | -       |
|                                         | yes             | 43 (10.4%)     | 8 (18.6%)    | 5.2 (2.6–10.4)              | 2.57 (1.18–5.60)      | 0.018   |
| Intracardiac thrombus                   | no              | 381 (96.1%)    | 31 (8.1%)    | 2.2 (1.5–3.1)               | Ref                   | -       |
|                                         | yes             | 13 (3.3%)      | 3 (23.1%)    | 9.0 (2.9–27.8)              | -                     | -       |
| RV-pressure, mmHg                       | ≤ 30            | 190 (64.2%)    | 17 (6.9%)    | 2.6 (1.6–4.1)               | Ref                   | -       |
|                                         | > 30            | 106 (35.8%)    | 12 (11.3%)   | 3.1 (1.7–5.4)               | 1.16 (0.56–2.40)      | 0.697   |
| LVEDD, mm                               | ≤ 54            | 153 (41.2%)    | 23 (8.5%)    | 2.3 (1.3–4.0)               | Ref                   | -       |
|                                         | > 54            | 218 (58.8%)    | 21 (9.6%)    | 2.8 (1.8–4.2)               | 1.34 (0.67–2.68)      | 0.401   |
| LVESD, mm                               | ≤ 36            | 43 (25.7%)     | 5 (11.6%)    | 2.3 (1.0–5.6)               | Ref                   | -       |
|                                         | > 36            | 124 (74.3%)    | 14 (11.3%)   | 3.1 (1.8–5.3)               | 1.71 (0.68–4.26)      | 0.253   |

CI: confidence interval; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; RV: right ventricle.

term prognostic value of histopathological and immuno-histochemical information derived from EMB.

No correlation was found between invasive hemodynamic assessment and prognosis. This is indeed unexpected as one series found that higher pulmonary artery pressures were associated with worse outcome in biopsy proven iCM. As the patients in this study were investigated more than 20 years ago, this might also relate to improvements in guideline suggested medical therapy.

Of interest, was the combination of 3 readily available clinical parameters that could offer the best approach for long-term risk stratification for patients with iCM. Age, presence vs. absence of CHF symptoms, and duration of symptoms could identify both a very low risk subgroup, without events at follow-up, and a high-risk group with an annual event-rate of 10%.

4.2 Limitations

The patient population was collected over a long-time period, and diagnostic pathways did not change during this time. Nevertheless, limitations inherent to a retrospective observational single academic medical center study cannot be excluded. Second, absence of any threshold for positive parvovirus B19 copy numbers might have influenced the results of our study and might explain, at least in parts, the low death and transplantation rate. However, this approach was not established as a clinical routine during the analyzed period.

4.3 Conclusions

In the present population, biopsy-proven iCM is associated with an 8.6% long-term incidence (mean follow-up 3.6
Table 4. Bi-variate analyses of magnet resonance imaging and cardiac catheterization data.

| Variable                                      | Dichotomization | Patients n (%) | Events n (%) | Event-rate per year (95% CI) | Hazard Ratio (95% CI) | P-Value |
|------------------------------------------------|-----------------|----------------|--------------|-----------------------------|-----------------------|---------|
| Magnetic Resonance Imaging                     |                 |                |              |                             |                       |         |
| Edema                                         | no              | 164 (82.0%)    | 8 (4.9%)     | 1.3 (0.7–2.7)               | Ref                   | -       |
|                                               | yes             | 36 (18.0%)     | 3 (8.3%)     | 2.2 (0.7–6.7)               | 1.59 (0.44–5.69)      | 0.477   |
| Late gadolinium enhancement                    | no              | 126 (58.6%)    | 7 (5.6%)     | 1.5 (0.7–3.2)               | Ref                   | -       |
|                                               | yes             | 89 (41.4%)     | 5 (6.6%)     | 1.5 (0.6–3.6)               | 1.11 (0.35–3.51)      | 0.857   |
| LVESD, mm                                      | ≤ 36            | 34 (26.4%)     | 1 (2.2%)     | 1.0 (0.1–7.2)               | Ref                   | -       |
|                                               | > 36            | 95 (73.6%)     | 5 (5.3%)     | 1.5 (0.6–3.6)               | 0.97 (0.11–8.31)      | 0.976   |
| LVEDD, mm                                      | ≤ 54            | 46 (34.6%)     | 1 (2.2%)     | 0.7 (0.1–5.2)               | Ref                   | -       |
|                                               | > 54            | 87 (65.4%)     | 5 (5.7%)     | 1.7 (0.7–4.1)               | 1.54 (0.18–13.14)     | 0.693   |
| Pericardial effusion                           | no              | 121 (74.7%)    | 9 (7.4%)     | 1.9 (1.0–3.6)               | Ref                   | -       |
|                                               | yes             | 41 (25.3%)     | 2 (4.9%)     | 1.5 (0.4–6.1)               | 1.01 (0.21–4.85)      | 0.986   |
| Heart catheterization                          |                 |                |              |                             |                       |         |
| LVEDP, mmHg                                    | ≤ 15            | 138 (36.0%)    | 8 (5.8%)     | 1.5 (0.8–3.1)               | Ref                   | -       |
|                                               | > 15            | 245 (64.0%)    | 24 (9.8%)    | 2.8 (1.9–4.2)               | 1.98 (0.91–4.31)      | 0.084   |
| PA systolic pressure, mmHg                     | ≤ 30            | 121 (68.0%)    | 12 (9.9%)    | 2.7 (1.5–4.7)               | Ref                   | -       |
|                                               | > 30            | 57 (32.0%)     | 9 (15.8%)    | 4.7 (2.5–9.1)               | 1.78 (0.76–4.18)      | 0.186   |
| RV systolic pressure, mmHg                     | ≤ 30            | 90 (32.3%)     | 8 (8.9%)     | 2.4 (1.2–4.8)               | Ref                   | -       |
|                                               | > 30            | 189 (67.7%)    | 21 (11.1%)   | 3.3 (2.1–5.0)               | 1.45 (0.65–3.24)      | 0.369   |
| Cardiac index, (l/min)/m²                      | ≤ 1.8           | 56 (33.5%)     | 8 (14.3%)    | 3.5 (1.8–7.1)               | 2.74 (0.38–19.97)     | 0.321   |
|                                               | ≥ 1.8           | 83 (49.7%)     | 11 (13.3%)   | 3.9 (2.1–7.0)               | 3.08 (0.43–21.93)     | 0.262   |
|                                               | ≥ 2.5           | 28 (16.8%)     | 1 (3.6%)     | 1.2 (0.2–8.4)               | Ref                   | -       |
| Cardiac power index [w/m²]                    | < 0.5           | 129 (77.2%)    | 19 (14.7%)   | 3.9 (2.5–6.1)               | 0.080                 |         |
|                                               | ≥ 0.5           | 38 (22.8%)     | 11 (14.3%)   | 4.7 (0.7–33.1)              |                       |         |
| Pulmonary vascular resistance [dyn x s/m³]    | ≤ 240           | 123 (74.5%)    | 11 (8.9%)    | 2.5 (1.4–4.6)               | Ref                   | -       |
|                                               | > 240           | 42 (25.5%)     | 8 (19.0%)    | 5.4 (2.7–10.8)              | 2.24 (0.93–5.41)      | 0.073   |

CI: confidence interval; LVEDD: left ventricular end-diastolic diameter; LVEDP: left ventricular end-diastolic pressure; LVESD: left ventricular end-systolic diameter; PA: pulmonary artery; RV: right ventricle.

Table 5. Multivariate analysis: independent risk factors for occurrence of the combined endpoint during follow-up.

| Variable                  | Dichotomization | Hazard Ratio 95% CI | P-Value |
|---------------------------|-----------------|---------------------|---------|
| Age, yrs                  | ≥ 50 vs. < 50    | 4.13 (1.44–12.05)   | 0.008   |
|                           | ≥ 60 vs. < 50    | 1.89 (0.58–6.15)    | 0.291   |
|                           | ≥ 70 vs. < 50    | 3.14 (1.09–9.06)    | 0.034   |
| NYHA functional class     | III/IV vs. NYHA 0 | 3.47 (1.16–10.40)  | 0.026   |
|                           | III/IV vs. NYHA 0 | 1.81 (0.62–5.28)    | 0.277   |
| CCS functional class     | III/IV vs. CCS 0 | 0.37 (0.14–0.95)    | 0.039   |
|                           | III/IV vs. CCS 0 | 0.38 (0.13–1.07)    | 0.067   |
| Syncope                   | yes vs. no       | 7.40 (3.23–16.96)   | 0.000   |
|                           | yes vs. no       | 2.93 (1.26–6.82)    | 0.013   |
| Duration of symptoms, days| > 28 vs. ≤ 28   | 0.42 (0.20–0.86)    | 0.017   |

CCS: Canadian Cardiovascular Society; CI: confidence interval; NYHA: New York Heart Association.

Figure 3. Unadjusted event-free survival from primary endpoint according to the findings of endomyocardial biopsy, left ventricular function at the time of endomyocardial biopsy, and according to a triple-parameter risk stratification model using presence of signs and/or symptoms (NYHA class I – IV) of congestive heart failure (CHF +), age, and disease duration. CHF: congestive heart failure; CI: confidence interval; NYHA: New York Heart Association.

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years old), prolonged duration of symptoms (> 28 days), and presentation with congestive heart failure symptoms identify a subgroup of patients at higher risk (10%/year rate of the cumulative end-point). Absence of any of these characteristics classifies patients in a very low risk subgroup without events at follow-up. Histopathological and immunohistochemical analyses, invasive hemodynamic study as well as cardiac magnetic resonance did not provide additional prognostic information.

Acknowledgments

The authors would like to acknowledge the valuable work of Hiltrud Niggemann (statistical analyses). None of the authors has any conflict of interest to disclose.

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