Figure 2. Changes in New York Heart Association (NYHA) class, left ventricular ejection fraction (LVEF), and end-diastolic diameter index (iEDD) from initial evaluation to end of follow-up based on enrollment period. Each bar indicates the change from enrollment (horizontal line) to final evaluation (blunt bar extremity). Overall *P* values (ANOVA) for average differences based on enrollment period are shown.

When adjusted for age, sex, NYHA class, LVEF, and left atrial diameter index, event-free rates for the combined end point (all-cause mortality and HTx) at 5 years were 62%, for patients enrolled in period 1; 74% for period 2; 84% for period 3; and 93% for period 4 (Figure 3A). At multivariate analysis, an earlier enrollment period proved the most powerful predictor of the combined end point, independent of age, NYHA functional class, LVEF, left atrial diameter index, and sex (Table 3). Of note, each enrollment period was associated with a 42% reduction in risk compared with the previous one (hazard ratio, 0.58; 95% confidence interval, 0.50–0.67; *P*<0.001).

Likewise, an earlier period of enrollment proved a potent independent predictor of refractory HF death and SD. Risk
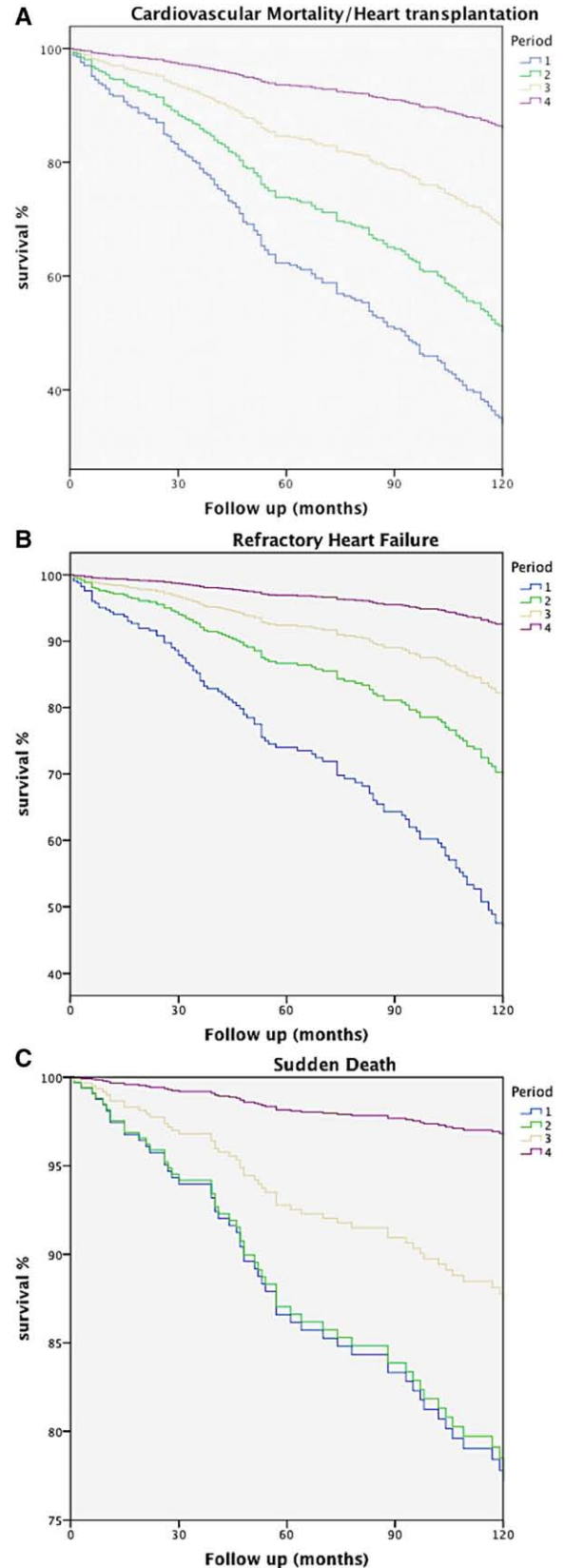

Figure 3. Cox multivariate regression survival plot (adjusted for age, New York Heart Association [NYHA] class, sex, left ventricular ejection fraction and indexed left atrium diameter) indicating differences in outcome based on enrollment period for the 3 study end points: **A**, All-cause mortality/heart transplantation; (**B**) death for refractory heart failure; and (**C**) sudden death.

Table 3.   Independent Predictors of Adverse Outcome and Cardiovascular Mortality, Death for Refractory Heart Failure, and Sudden Death at Multivariate Cox Regression Analysis

Variable*	Cardiovascular Mortality/Heart Transplantation		Refractory Heart Failure		Sudden Death	
	HR (95% CI)	P Value	HR (95% CI)	P Value	HR (95% CI)	P Value
Women	0.69 (0.47–1.02)	0.06	0.58 (0.35–0.97)	0.04	…	NS
Age, per year†	1.02 (1.01–1.04)	<0.001	1.03 (1.01–1.05)	<0.001	…	NS
Period 2 (vs 1)	0.64 (0.42–0.98)	0.04	0.47 (0.28–0.80)	0.005	0.96 (0.44–2.10)	0.96
Period 3 (vs 1)	0.35 (0.24–0.53)	<0.001	0.26 (0.16–0.42)	<0.001	0.52 (0.24–1.12)	0.09
Period 4 (vs 1)	0.14 (0.08–0.23)	<0.001	0.10 (0.06–0.19)	<0.001	0.13 (0.04–0.38)	<0.001
NYHA class†	1.89 (1.53–2.33)	<0.001	2.37 (1.81–3.11)	<0.001	…	NS
Left atrial size, per mm‡	1.07 (1.04–1.1)	<0.001	1.07 (1.03–1.11)	0.001	…	NS
LVEF, per unit†	0.99 (0.97–1.00)	0.06	…	NS	…	NS
Mitral regurgitation (moderate to severe)†	1.2 (1.05–1.42)	0.04	…	…	…	…

CI indicates confidence interval; HR, hazard ratio; LVEF, left ventricular ejection fraction; NS, not significant; and NYHA, New York Heart Association.

*The following baseline variables were also tested but failed to reach statistical significance in the multivariate analysis: family history of idiopathic dilated cardiomyopathy, atrial fibrillation, left bundle-branch block, and indexed left ventricular end-diastolic diameter at enrollment (the latter because of colinearity with LV ejection fraction).

†At enrollment.

‡Left atrium diameter index at enrollment.

of refractory HF mortality decreased (53%, 74%, and 90% in periods 2, 3, and 4, respectively) compared with period 1. Conversely, likelihood of SD declined sharply in patients enrolled after 2000, with a 87% risk reduction in period 4 compared with period 1. Notably there was also a trend of risk reduction from 1991 to 2000 (48%; *P*=0.09; Figure 3B and 3C).

Discussion

The present study demonstrates that the long-term prognosis of patients with IDCM has radically improved during the past 30 years, in terms of overall mortality, refractory HF death, and SD, reflecting continuing progress in pharmacological and device-based management.[19] Among our patients, each of 4 consecutive HF management eras implied a 42% reduction in mortality compared with the previous one, paralleled by greater degrees of reverse LV remodeling.[21] As a consequence, patients enrolled during the past decade, maximally treated with ACEI/ARBs and β-blockers and with unrestricted access to ICD/CRT, showed a 86% relative risk reduction in cardiovascular mortality compared with those enrolled before 1985, who were essentially managed with diuretics and digoxin.[20]

Causes of Death: Changing Patterns Over Time

With regard to specific causes of death, the greatest reduction in refractory HF mortality was observed in the transition from periods 1 to 2 after the introduction of ACE inhibition; thereafter, HF death rates declined at a relatively constant rate. Conversely, marked and progressive impact on SD was evident in the transition to periods 3 and 4. Such advantageous trend of SD mortality reflects both the introduction of the ICD/CRT and the complete penetration of β-blocker treatment for HF in real-world practice. Indeed, because of the relatively small number of patients receiving ICD and ICD/CRT in our cohort, the fall in SD rates suggests a crucial prophylactic role of

β-blockers. Consistent with this concept, Zecchin et al[22] have recently demonstrated the efficacy of optimized pharmacological treatment in reducing SD risk of IDCM. In their cohort, accurate titration of ACEI/ARBs and β-blockers led to a substantial improvement in the clinical and instrumental profile of patients with IDCM initially meeting the Sudden Cardiac Death in Heart Failure Trial (SCD-HeFT)[23] criteria for prophylactic ICD implantation, two thirds of whom did not retain such indication at 6 months. Of note, the long-term outcome in this subgroup was comparable with that of patients with IDCM without SCD-HeFT criteria at enrollment.[22] Of note, similar results in patients with new-onset HF and severely depressed LVEF have been recently reported by Teeter et al.[24]

Impact of Clinical Setting on Pharmacological Therapy Implementation

HF treatment is constantly evolving and has led to remarkable achievements during the past decades. The use of neurohormonal inhibitors has become established during the past 2 decades, following major randomized clinical trials[25–32] and their subsequent impact on international HF guidelines.[19,33,34] Compared with the early observations from the 1980s, when HF treatment was virtually confined to diuretics and digoxin, the 1990s have seen an explosion in the use of neurohormonal blockers: an extensive analysis by Stevenson et al showed that 86% of patients with HF were treated with ACEI after 1990 compared with 46% before 1990.[35] Nonetheless, the use of evidence-based pharmacological therapies in the real world has consistently failed to reach the rates reported in landmark randomized clinical trials. The EuroHeart Failure Survey,[36] investigating the clinical profile and treatment of >11 000 patients hospitalized for or with HF in 115 hospitals from 24 European countries between 2000 and 2001, showed that diuretics were still the most widely used agents in HF, prescribed in 87% of patients at the time, followed by ACEI

(62%), β-blockers (37%), cardiac glycosides (36%), nitrates (32%), calcium channel blockers (21%), and spironolactone (20%). Notably only 17.2% of patients received the combination of diuretic, ACEI, and β-blockers. These rates have significantly improved in more recent HF registries, such as the S-HFR registry[37] or the EURObservational Research Programme: the Heart Failure Pilot.[38] Yet, in the S-HFR registry, as many as 18% of patients with HF did not receive ACEI/ARBs, whereas 11% did not receive a β-blocker in the absence of any reported contraindication or side effect. Similar results originated from other extensive studies of HF cohorts showing that, in spite of the widely available evidence-based international treatment guidelines, recommended therapies are slowly adopted and inconstantly applied in clinical practice, with considerable proportions of eligible patients failing to receive optimal treatment.[39,40]

Conversely, our observations support the view that, in appropriate settings, the treatment rates and benefits of controlled randomized HF trials can be replicated in the real world.[41] The present IDCM cohort is representative of those seen at other community hospitals, in that ≈95% of our patients were from a well-defined geographic region in Central Italy, most of them having lived virtually their entire life in Tuscany. All patients were systematically followed up on a 6-month basis by the same team >3 decades, providing a high level of continuity of care during this extended time period and allowing continuous reassessment of risk profile and management of the primary condition as well as cardiovascular risk factors and comorbidities. This provided the opportunity to gradually optimize pharmacological therapy, including uptitration of ACEI/ARBs and β-blockers to the maximum tolerated dosage.[19] It is noteworthy that 92% and 57% of the overall population received ACEI/ARBs and β-blockers during the whole study period, reflecting stringent implementation of international guidelines even in the earliest cohorts, as soon as new treatments became available. In the past decades, these rates were as high as 97% and 86%, respectively, resembling those of landmark HF randomized control trials, rather than most HF surveys and registries.[36] However, our data are consistent with selected reports from dedicated tertiary hospital settings based on patients with chronic systolic HF because of ischemic heart disease.[42] Overall, these findings considerably expand previous observations showing favorable temporal trends in the outcome of IDCM and HF, based on smaller cohorts and less extended follow-up. Matsumura and Kubo[9,10] have shown significant improvement in the prognosis of a relatively small group of Japanese patients with IDCM, partly explained through an increased use of ACEI/ARBs and β-blockers and a declining use of class Ia/Ib antiarrhythmic drugs after the 1990s, and a similar trend was observed by Aleksova et al[11] in a study focusing mainly on asymptomatic patients with IDCM without prior history of HF.

Changes in Demographics Among Enrollment Periods

It is important to acknowledge that, in our IDCM cohort, patients enrolled later had a less severe profile in terms of symptoms, LV size and function. This trend presumably reflects increasing awareness of the disease and availability of more sensitive diagnostic tools and may have contributed to the reduction in mortality observed during the years, resulting

in an overestimation of benefits related to treatment. However, this trend is counterbalanced by the more advanced age at enrollment of the most recent groups. Although it is virtually impossible to quantify the net result of these trends on outcome, their opposite prognostic weight is likely to limit their combined effect substantially. In our multivariate models, the enrolment period proved to be a very potent predictor of outcome independent of the most relevant baseline clinical and demographic features including well-recognized prognostic factors (ie, age, sex, NYHA functional class, LV and left atrial diameters, LVEF, and degree of functional mitral regurgitation).[43–47] Thus, it is plausible to attribute most of the survival benefit observed to improvements in management rather than to the evolving patient demographics.

Conclusions

The evolution of evidence-based treatment has led to progressive improvement in the prognosis of IDCM, with dramatic reduction in heart failure–related mortality and SD during the past 3 decades. In the appropriate setting, the benefits of controlled randomized trials can be replicated in the real world, emphasizing the importance of tailored and systematic follow-up providing long-term continuity of care.

Disclosures

None.

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CLINICAL PERSPECTIVE

The present study is based on a clinical experience whereby 603 patients with angiographically proven idiopathic dilated cardiomyopathy have been followed by the same team during the past 30 years in a Regional Hospital in Florence, providing substantial continuity of care during an extended time period. Outcome in our cohort was associated with the treatment era in which each patient was enrolled (1977–1984; 1985–1990; 1991–2000; and 2001–2011), reflecting progress in management and changing demographics of patients with idiopathic dilated cardiomyopathy. Of note, each period was associated with a 40% reduction in mortality compared with the previous one, with patients enrolled during the most recent decade showing a 75% relative risk reduction in cardiovascular mortality compared with those enrolled before 1985. With regard to specific causes of death, the greatest reduction in mortality related to refractory heart failure was observed in the transition from periods 1 to 2 after the introduction of angiotensin-converting enzyme inhibition. Conversely, progressive impact on sudden death was evident in the past 2 periods, reflecting the introduction of device-based therapies and full penetration of β-blocker treatment. These results quantify the impact of evolving treatment options for idiopathic dilated cardiomyopathy in the real world, emphasizing the importance of tailored follow-up and long-term continuity of care.

Prognostic Value of N-Terminal Pro-Brain Natriuretic Peptide in Outpatients With Hypertrophic Cardiomyopathy

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In hypertrophic cardiomyopathy, the plasma levels of N-terminal pro-brain natriuretic peptide (NT-proBNP) correlate with functional capacity. However, their prognostic relevance remains unresolved. We followed up 183 stable outpatients with hypertrophic cardiomyopathy (age 50 ± 17 years, 64% men) for 3.9 ± 2.8 years after NT-proBNP measurement. The primary end point included cardiovascular death, heart transplantation, resuscitated cardiac arrest, and appropriate implantable cardioverter-defibrillator intervention. The secondary end point (SE) included heart failure-related death or hospitalization, progression to end-stage disease, and stroke. The median NT-proBNP level was 615 pg/ml (intertertile range 310 to 1,025). The incidence of the primary end point in the lower, middle, and upper tertiles was 0%, 1.3%, and 2.1% annually, respectively (overall $p = 0.01$). On multivariate analysis, the only independent predictors of the primary end point were NT-proBNP (hazard ratio for log-transformed values 5.8, 95% confidence interval 1.07 to 31.6; $p = 0.04$) and a restrictive left ventricular filling pattern (hazard ratio 5.19, 95% confidence interval 1.3 to 21.9; $p = 0.02$). The NT-proBNP cutoff value of 810 pg/ml had the best sensitivity for the primary end point (88%), but the specificity was low (61%). The incidence of the SE in the lower, middle, and upper NT-proBNP tertiles was 4.6%, 12.0%, and 11.2% annually, respectively (overall $p = 0.001$). An NT-proBNP level of <310 pg/ml was associated with a 75% reduction in the rate of SE compared with a level of ≥310 pg/ml (hazard ratio 0.25, 95% confidence interval 0.11 to 0.57; $p = 0.001$), independent of age, left ventricular outflow tract obstruction, or atrial fibrillation. In conclusion, in stable outpatients with hypertrophic cardiomyopathy, plasma NT-proBNP proved a powerful independent predictor of death and heart failure-related events. Although the positive predictive accuracy of an elevated NT-proBNP level was modest, low values reflected true clinical stability, suggesting the possibility of avoiding or postponing aggressive treatment options.   © 2013 Elsevier Inc. All rights reserved. (Am J Cardiol 2013;112:1190−1196)

Natriuretic peptides have been validated as markers of severity and decompensation in various cardiac disorders and represent powerful predictors of outcome in heart failure (HF).[1] They have also been useful as screening tools in large populations at risk of developing cardiovascular events, including diabetic and elderly patients.[2] To date, the prognostic accuracy of natriuretic peptides in patients with hypertrophic cardiomyopathy (HC), the most common genetic heart disease, is unknown.[3−9] In the present study, we assessed the outcomes of a consecutive outpatient cohort of patients with HC, who were largely asymptomatic or mildly symptomatic at enrollment and were followed up for almost 4 years after a comprehensive clinical evaluation that had included serum N-terminal pro-brain natriuretic peptide (NT-proBNP) titration. NT-proBNP, the N-terminal part of the BNP pro-hormone, is released during hemodynamic stress by stretched cardiac myocytes and split by proteolytic enzymes into 2 peptides; the C-terminal fragment (BNP) represents the biologically active hormone.[1,10] Because of a different clearance mechanism (renal for NT-proBNP and receptor mediated for BNP), NT-proBNP represents a more reliable predictor of outcome than BNP[10] and was, therefore, chosen for our analysis.

Methods

The serum NT-proBNP levels were determined in 218 consecutive outpatients with HC who had been referred to the Referral Center for Cardiomyopathies in Florence (Italy) before January 2011 as a part of a comprehensive noninvasive cardiovascular evaluation, including clinical history, physical examination, 12-lead electrocardiography, and echocardiography. The diagnosis of HC was determined by

Table 1
Baseline patient characteristics within each N-terminal pro-brain natriuretic peptide (NT-proBNP) tertile

Variable	Overall Population (n = 183)	Lower Tertile (<310 pg/ml; n = 61)	Middle Tertile (310−1,025 pg/ml; n = 61)	Upper Tertile (>1,025 pg/ml; n = 61)	p Value
Men	118 (64%)	53 (86%)*,†	39 (64%)†	26 (42%)*	<0.01‡
Age (yrs)	50 ± 17	47 ± 17	52 ± 16	50 ± 19	0.256
NYHA class III-IV at first evaluation	33 (18%)	4 (6.5%)*,†	13 (21%)	16 (26%)	0.01‡
Atrial fibrillation	48 (26%)	7 (11%)*,†	19 (31%)	22 (36%)	<0.01‡
LA diameter (mm)	44 ± 7	42 ± 6*,†	45 ± 8	46 ± 7	<0.01‡
Restrictive LV filling pattern	12 (6.5%)	1 (1.6%)	6 (10%)	5 (8%)	0.26
Peak LVOT gradient ≥30 mm Hg	65 (36%)	14 (23%)*,†	25 (41%)	27 (44%)	0.03‡
Positive genotype	98 (53%)	30 (49%)	32 (52%)	36 (59%)	0.14
Maximum LV wall thickness (mm)	22 ± 6	20 ± 3*,†	24 ± 5	23 ± 8	<0.01‡
β-Blocker use	122 (67%)	34 (55%)*	45 (74%)	43 (70%)	0.07
Amiodarone use	23 (12%)	5 (8%)	8 (13%)	10 (16%)	0.38
With ICD	21 (17%)	7 (11%)	11 (18%)	14 (23%)	0.24
Primary end point	8 (4%)	0*,†	3 (4.9%)	5 (8.1%)	0.01‡
Sudden death	4 (2%)	0	2 (3.3%)	2 (3.3%)	0.18
HF-related death	2 (1%)	0	1 (1.6%)	1 (1.6%)	0.44
Cardiac surgery-related death	1 (0.5%)	0	0	1 (1.6%)	0.20
Heart transplantation	1 (0.5%)	0	0	1 (1.6%)	0.20
Appropriate ICD discharge	0	0	0	0	—
Secondary end point	67 (37%)	11 (18%)*,†	29 (47%)	27 (44%)	<0.01‡
Hospitalization for HF	38 (21%)	4 (6.5%)*,†	15 (24.5%)	19 (31%)	<0.01‡
Progression to NYHA III-IV (during follow-up)	20 (11%)	4 (6.5%)	9 (15%)	7 (11.5%)	0.17
Progression to end-stage disease	21 (11.5%)	2 (3%)*,†	9 (15%)	10 (16%)	<0.01‡
New-onset AF	14 (8%)	4 (6.5%)	6 (10%)	4 (6.5%)	0.59
Cerebrovascular event	8 (4%)	0†	2 (3%)	6 (10%)	<0.01‡

ICD = implantable cardioverter-defibrillator; LA = left atrial; LVOT = LV outflow tract; NYHA = New York Heart Association.

* p <0.05 versus middle tertile.

† p <0.05 versus upper tertile.

‡ statistically significant.

2-dimensional echocardiographic identification of left ventricular (LV) hypertrophy associated with nondilated ventricular chambers in the absence of any other cardiac or systemic disease capable of producing the magnitude of hypertrophy evident.[11] Of the 218 patients, 35 with known coronary artery disease (n = 15) or evidence of systolic dysfunction at the first evaluation (defined as a LV ejection fraction <50%; n = 20) were excluded. The remaining 183 patients with HC constituted the study group enrolled in the present project. All had had a stable clinical course, without episodes of acute HF or worsening of symptoms, in the previous 3 months.

The local ethics committee approved the study, and all patients gave informed consent to participate. Peripheral blood samples, collected after 30 minutes of complete rest, were measured using an electrochemiluminescent immunoassay (Elecsys proBNP II assay, Roche Diagnostics, Indianapolis, Indiana) on a Cobas E601 analyzer (Roche Diagnostics).[12]

Transthoracic echocardiography was performed using commercially available equipment. The standard evaluation included M-mode, 2-dimensional, and Doppler study, according to the recommendations of the American Society of Echocardiography.[13] LV systolic dysfunction, characterizing end-stage HC, was defined as an LV ejection fraction <50%. A restrictive LV filling pattern was defined as a ratio of early to late velocity of mitral inflow ≥2, associated with a deceleration time of ≤150 ms (or a deceleration time <120 ms for patients with atrial fibrillation).[14] LV outflow tract obstruction was considered present when a peak instantaneous outflow gradient of ≥30 mm Hg was measured using continuous wave Doppler echocardiography under basal (at rest) conditions or with physiologic provocation (Valsalva maneuver or exercise).

After enrollment, the patients were evaluated annually or more often if dictated by the clinical status, using 12-lead electrocardiography, echocardiography, and 24-hour electrocardiographic Holter monitoring. Pharmacologic therapy was prescribed as appropriate, according to the current HC guidelines.[3] Patients with refractory symptoms related to LV outflow tract obstruction despite optimal medical therapy were referred for surgical myectomy; alcohol septal ablation was reserved for patients at high operative risk owing to advanced age and co-morbidities.[3] An implantable cardioverter-defibrillator was offered to survivors of cardiac arrest or sustained ventricular arrhythmias, for primary prevention in high-risk patients evaluated using established risk markers for sudden cardiac death, and in patients with end-stage disease.[3]

Genetic counseling and mutational analysis were offered to all patients as a part of the standard policy at our center. After providing informed consent, 133 of the study patients (73%) were screened for mutations in the protein-coding exons and splice sites of 8 myofilament genes, including myosin binding protein C, β-myosin heavy chain, the regulatory and essential light chains (MYL2 and MYL3), troponin-T, troponin-I, α-tropomyosin, and α-actin. Direct
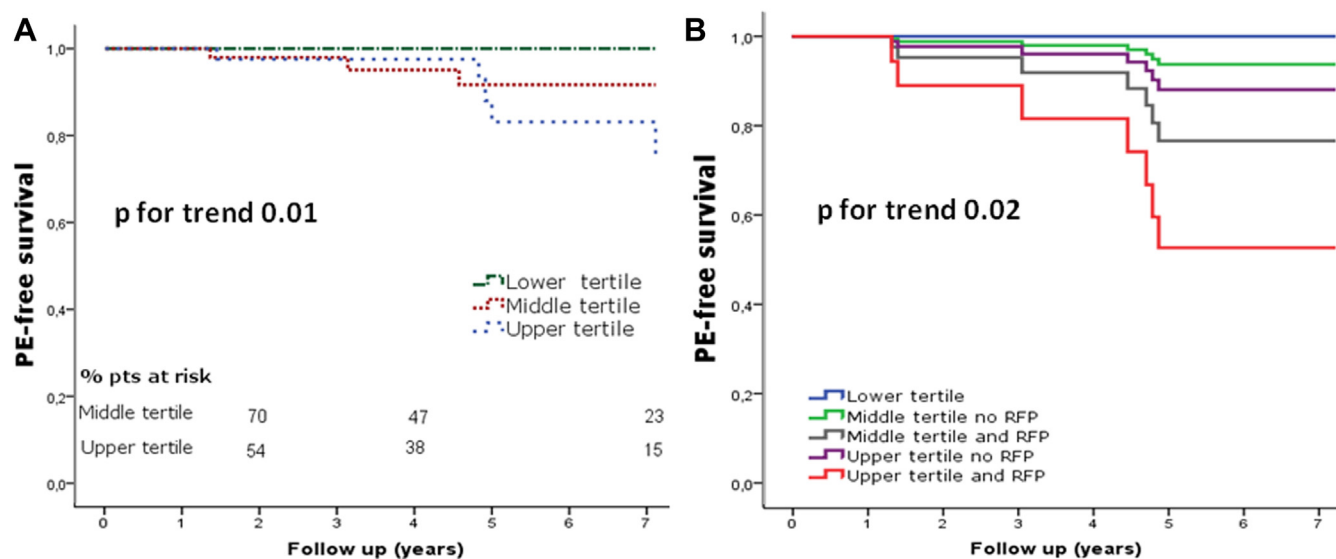
Figure 1. *(A)* Survival free from the primary end point (PE; a composite of cardiovascular death, heart transplantation, and resuscitated cardiac arrest) stratified by NT-proBNP tertiles (overall log-rank p = 0.01). After pairwise comparison with Bonferroni correction, only the lower to upper tertile comparison reached statistical significance (p = 0.01; HR 9.26, 95% CI 1.58 to 53.9). *(B)* Freedom from PE stratified by NT-proBNP tertiles and presence or absence of mitral pulsed wave restrictive filling pattern (RFP).

deoxyribonucleic acid sequencing was performed using ABI-Prism 3730 (Applied Biosystems, Foster City, California), as previously described.[15]

The primary end point was defined as a composite of cardiovascular death, heart transplantation, and resuscitated cardiac arrest. Death was considered to be of cardiovascular origin if due to worsening HF, sudden death, or related to cardiac surgery. Sudden cardiac death was defined as unexpected sudden collapse or death occurring during sleep in patients who had previously experienced a stable clinical course.[16] The secondary end point (SE) was a composite of HF-related events, including HF death, pulmonary edema requiring hospitalization, progression to advanced symptomatic status (New York Heart Association class III or IV), end-stage disease,[4,17] onset of atrial fibrillation, and stroke.

The differences between groups were analyzed using Pearson's chi-square test for discrete variables and the unpaired Student *t* test or 1-way analysis of variance, as appropriate, for continuous variables. Because the NT-proBNP values were not normally distributed in our population, logarithmic transformation was applied. For the same reason, the patients were divided into tertiles according to their NT-proBNP levels. Nonparametric tests were used for the comparisons among the groups (Mann-Whitney *U* test) and for correlation analysis (Spearman's test). Survival curves were estimated using the Kaplan-Meier method, and the log-rank test was used to compare the survival distributions of the groups. The performance of NT-proBNP in predicting the primary end point and SE was assessed using receiver operating characteristic analysis. Calculations were performed using the Statistical Package for Social Sciences, version 19.0.0, software (SPSS, Chicago, Illinois).

Results

The mean age of the 183 study outpatients was 50 ± 17 years. Most were men (64%) and had no or only mild

Table 2
Univariate predictors of the primary end point*

Variable	Univariate		
	p Value	HR	95% CI
Log NT-proBNP	0.02	5.56	1.21–25.5
Restrictive LV filling pattern	0.01	6.32	1.49–26.8
Age (per yr increase)	0.38	1.02	0.97–1.06
Male gender	0.62	1.42	0.34–5.96
Atrial fibrillation	0.28	2.14	0.53–8.59
NYHA class III-IV	0.40	1.96	0.39–9.79
LA diameter	0.53	1.03	0.93–1.13
Maximum LV wall thickness	0.28	0.94	0.84–1.05
Peak LVOT gradient >30 mm Hg	0.97	0.97	0.23–4.10
Positive genotype	0.88	1.13	0.22–5.85

Abbreviations as in Table 1.

* Multivariate predictors are reported in the text.

symptoms at their initial evaluation (New York Heart Association class I or II for 82%); 26% had a history of atrial fibrillation (Table 1). On echocardiographic examination, the maximum LV wall thickness was 22 ± 6 mm, 36% had at rest or inducible LV outflow tract obstruction, and 12 patients (6.5%) had a restrictive LV filling pattern. Of the study population, 2/3 were receiving long-term therapy with β blockers, and 12% were taking amiodarone. Also, 32 patients (17%) received an implantable cardioverter-defibrillator for primary prophylaxis of sudden cardiac death because of their risk profile (Table 1).

The median NT-ProBNP value for the study group was 615 pg/ml (range 22 to 16,013). Because the NT-ProBNP distribution was not normally distributed in our population, the patients were grouped according to the tertiles, with the 33rd and 67th percentile values used as cutoffs (i.e., 310 and 1,025 pg/ml, respectively). Patients in the upper tertile had a larger left atrial diameter and maximum LV wall thickness
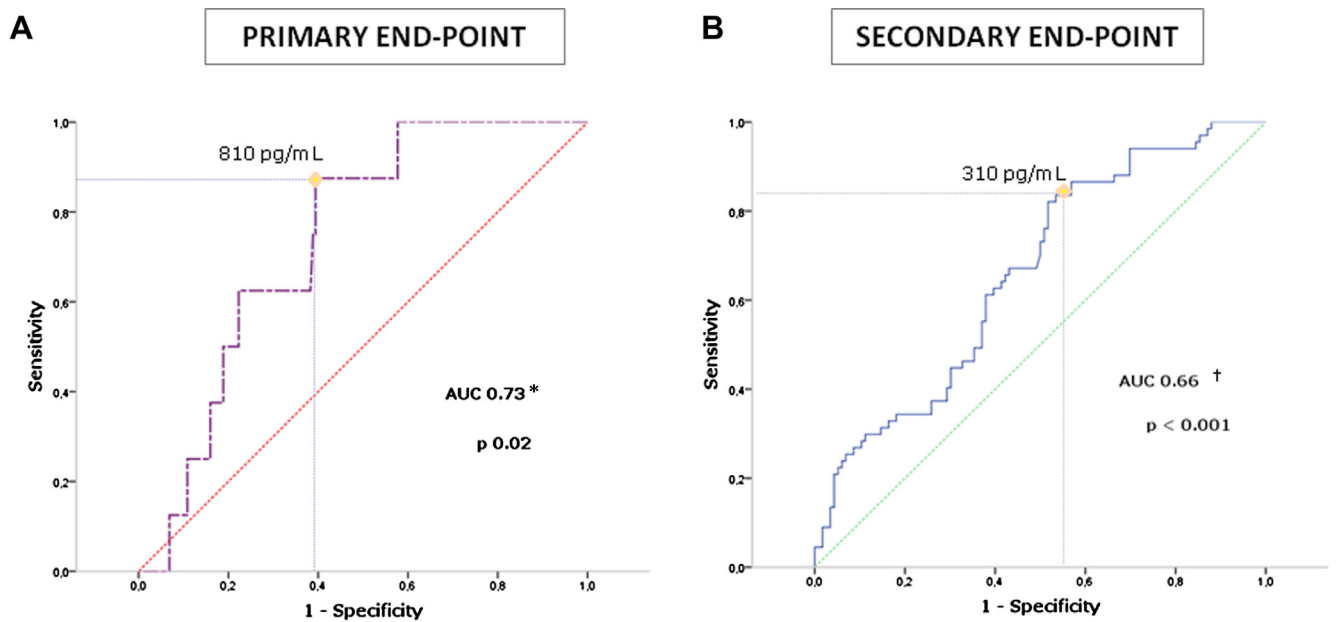
Figure 2. Receiver operating characteristic curves evaluating accuracy of NT-proBNP levels for prediction of *(A)* primary end point and *(B)* SE. *95% CI 0.61 to 0.86; †95% CI 0.58 to 0.74.

Table 3
Univariate and multivariate predictors of the secondary end point (SE)

Variable	Univariate			Multivariate		
	p Value	HR	95% CI	p Value	HR	95% CI
Log NT-proBNP	0.00	2.73	1.67–4.4	<0.01	2.6	1.5–4.3
Restrictive LV filling pattern	0.06	1.96	0.9–4.0			
Age (per yr increase)	0.001	1.02	1.01–1.04	<0.01	1.02	1.0–1.04
Male gender	0.06	1.58	0.96–2.5			
Atrial fibrillation	0.03	1.72	1.0–2.8	0.07	0.51	0.25–1.05
NYHA class III-IV	0.001	2.61	1.5–4.5	0.02	2.07	1.1–3.8
LA diameter	0.03	1.03	1.0–1.06	0.28	1.02	0.98–1.05
Maximum LV wall thickness	0.30	0.97	0.9–1.02			
Peak LVOT gradient >30 mm Hg	0.41	1.22	0.7–2.5			
Positive genotype	0.29	1.37	0.7–2.5			

Abbreviations as in Table 1.

and were more likely to have advanced symptoms (New York Heart Association class III), atrial fibrillation, and dynamic LV outflow tract obstruction (Table 1). The women had greater NT-proBNP values than the men (median 1,074 vs 401 pg/ml, respectively; p = 0.001) and represented most patients in the upper tertile (Table 1). The NT-proBNP values were comparable in patients taking and not taking β blockers (1,097 ± 1,314 vs 1,178 ± 2,362 pg/ml, respectively; p = 0.76), verapamil (1,021 ± 1,328 vs 1,147 ± 1,844 pg/ml, p = 0.69), and amiodarone (1,107 ± 1,828 vs 1,231 ± 1,250 pg/ml, p = 0.27). Of the 119 patients who underwent cardiac magnetic resonance imaging, we found no differences in the NT-proBNP values between the 73 patients with late gadolinium enhancement and the 46 without (1,098 ± 1,485 vs 1,143 ± 1,903 pg/ml, respectively; p = 0.86).

Of the 134 patients who had underwent genetic screening, 98 (73%) were genotype positive and 36 (27%) had negative test results. The relative prevalence of genes involved was

45% for myosin binding protein C, 35% for β-myosin heavy chain, 5% for both regulatory light chain and troponin-T, and 1% for troponin-I. Of genotype-positive patients, 8% had multiple mutations (including 7% with double and 1% with triple mutations). The NT-proBNP values were similar among the genotype-positive and genotype-negative patients (median value 1,297 vs 1,041 pg/ml; p = 0.44). Furthermore, no differences were found among the genotype-positive patients regarding the specific gene affected (overall p [analysis of variance] = 0.97).

During 47 ± 34 months of follow-up, 8 patients (4%) reached the primary end point. Of these, 4 died suddenly—2 patients died from HF-related causes, 1 patient died peri-operatively after surgical myectomy, and 1 patient underwent transplantation. None of the patients experienced appropriate implantable cardioverter-defibrillator intervention. The SE occurred in 67 patients (37%) during the follow-up period, at a rate of 9.4% annually; 33 patients
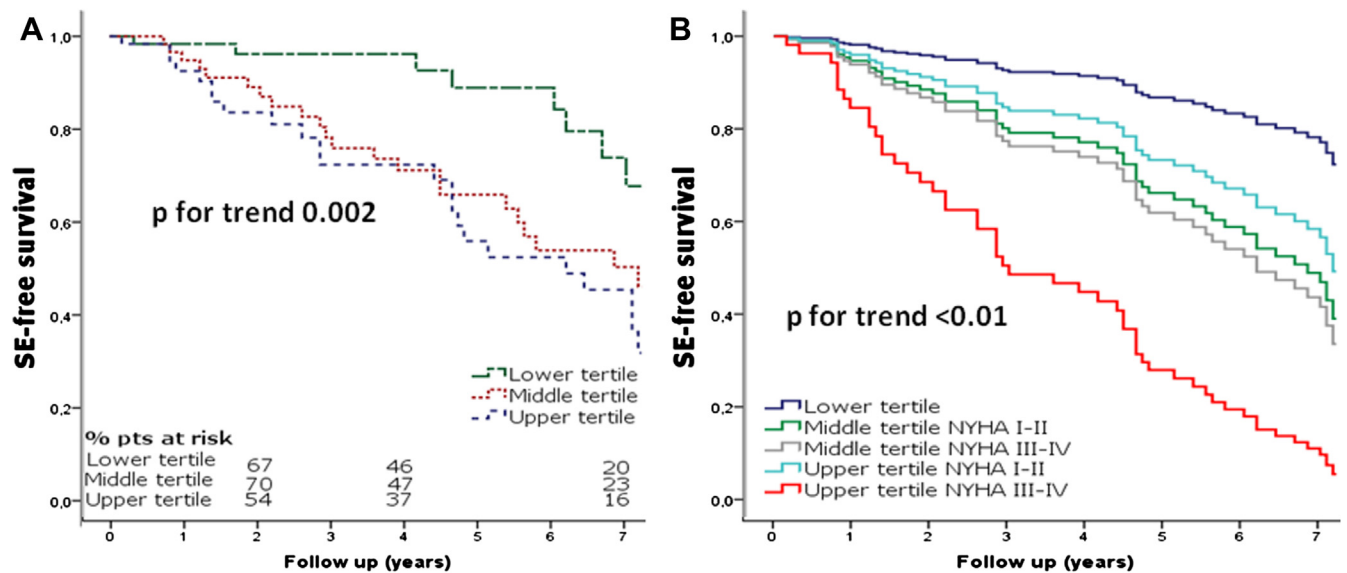
Figure 3. *(A)* Survival free from the SE (a composite of HF death, acute decompensation, progression to end-stage disease, and stroke) stratified by NT-proBNP tertiles (overall log-rank p = 0.002). After Bonferroni correction, the lower to middle and lower to upper tertile comparisons reached statistical significance (p = 0.001 and p = 0.002, respectively). *(B)* In a Cox regression model including other independent predictors of SE, only the initial New York Heart Association (NYHA) functional class helped to further stratify high-risk patients.

(18%) experienced >1 event. The cumulative rates of HF-related events were 21% (5.3%/yr) for acute HF requiring hospitalization, 11% (2.8%/yr) for worsening of symptomatic status to New York Heart Association class III or IV; and 8% (1.9%/yr) for new-onset atrial fibrillation. Eight patients (4%; 1.1%/yr) experienced cerebrovascular events judged to be cardioembolic. The global rate of progression to end-stage disease was 11.5% (n = 21, 2.8%/yr).

When the events were analyzed with regard to the NT-proBNP distribution, the cumulative rate of the primary end point at the end of the follow-up period was 5% (1.3%/yr) and 8% (2.1%/yr) in the middle and upper tertile, respectively. No event occurred in the lower tertile. As shown in Figure 1, the patients in the lower tertile (<310 pg/ml) had a favorable outcome compared with those in the other tertiles (overall p = 0.01). The patients in the middle and upper tertiles, however, had comparable survival rates. On multivariate Cox regression analysis, only NT-proBNP and a restrictive LV filling pattern were independently associated with the primary end point (hazard ratio [HR] for log-transformed NT-proBNP 5.8, 95% confidence interval [CI] 1.07 to 31.6, p = 0.04; HR for a restrictive LV filling pattern 5.19, 95% CI 1.23 to 21.9, p = 0.02; Table 2). Of the patients in the middle NT-proBNP tertile, the presence of a restrictive LV filling pattern conferred additional prognostic stratification, although it had no additional value for patients in the upper tertile (Figure 1). Receiver operating characteristic curve analysis demonstrated moderate power for NT-proBNP in the prediction of the primary end point (area under the curve 0.73, p = 0.02). The NT-proBNP value of 810 pg/ml proved the best threshold for the identification of patients at risk of cardiovascular death, with high sensitivity (88%) but low specificity (61%; Figure 2).

On multivariate analysis, NT-proBNP proved a powerful predictor of the SE, independent of other relevant clinical variables such as age, atrial fibrillation, and left atrial dimensions (HR for log-transformed NT-ProBNP 2.6, 95% CI 1.5 to 4.3, p <0.01; Table 3). Specifically, an NT-proBNP level <310 pg/ml was associated with a 75% reduction in risk of HF-related events compared with values of ≥310 pg/ml (HR for HF-related events 0.25, 95% CI 0.11 to 0.57; p = 0.001). On receiver operating characteristic analysis, the sensitivity for the cutoff value of NT-proBNP of 310 pg/ml was 85%; the specificity, however, was low (45%; Figure 2). Overall, the negative predictive accuracy for the SE associated with an NT-proBNP level <310 pg/ml was 85%. Similarly, the positive predictive accuracy for end-stage progression was low (25%), despite an excellent negative predictive value (88%). The combination of an NT-proBNP level in the upper tertile with left atrial dilation of ≥50 mm was associated with a 2.8 HR for the SE (95% CI 1.39 to 5.69; p = 0.004).

The cumulative rates of SE were 18% (4.6%/yr) in the lower, 47% (12.0%/yr) in the middle and 44% (11.2%/yr) in the upper tertile. The rate of the SE among the patients in the lower tertile was similar for the patients with and without LV outflow tract obstruction (p = 0.73). Survival free from the SE was favorable in the lower NT-proBNP tertile compared with the middle and upper tertiles (overall p <0.01; Figure 3). As observed for the primary end point, however, the middle and upper tertiles had comparable event-free SE survival rates. The presence of congestive symptoms at enrollment (expressed as New York Heart Association functional class) significantly contributed to the risk stratification of patients within the middle and upper NT-proBNP tertiles (Figure 3).

Although serial NT-proBNP testing was not systematically performed, repeat values were available at variable intervals from the original titration (2.6 ± 1.2 years) for 48 of the 183 study patients (26%). Of these 48 patients, 24 showed an increase in NT-proBNP during the follow-up period (range 30 to 5,958 pg/ml; average 1,207 ± 1,412) and 24 a decrease (range −5 to −3,558 pg/ml; average −663 ± 740). The

prevalence of the SE was almost 3 times more common in the group with an NT-BNP increase (n = 8; 33%) than in the patients with an NT-BNP decrease (n = 3; 12%; p = 0.03, univariate survival analysis).

Discussion

In patients with HC, the natriuretic peptides are often elevated and have been shown to correlate with the degree of symptomatic impairment and functional capacity,[18] LV mass index, left atrial volume, and LV outflow tract obstruction.[19,20] To date, however, the predictive accuracy of natriuretic peptides with regard to outcomes in this complex disease has received limited attention.[21,22] In our HC cohort, followed up for an average of almost 4 years, NT-proBNP proved to be an independent predictor of cardiovascular mortality (our primary end point), with a sixfold increase in the likelihood for each increment in the log NT-proBNP. The relatively high NT-proBNP value of 810 pg/ml proved the best cutoff for the identification of patients at risk of cardiovascular death, although with limited specificity (61%) owing to the expected paucity of events during the follow-up period. In the present analysis, however, sudden death represented a potential confounder with regard to the predictive values of NT-proBNP, because life-threatening ventricular arrhythmias can occur in stable and often asymptomatic or only mildly symptomatic patients, lacking LV overload and hemodynamic decompensation at death and, thus, might not be predictable using the biomarkers of HF.

Our SE was more specifically focused on HF-related events and, as expected, was more accurately predicted by the NT-proBNP levels. However, NT-proBNP appeared most useful for its high negative predictive value (i.e., as a screening test to identify low-risk patients, rather than to single out those with a greater risk of events). The threshold of 310 pg/ml, representing the cutoff value for the lower tertile, had very high negative predictive accuracy (85%) and retained important prognostic value even after adjustment for other powerful adverse clinical features, such as age, New York Heart Association functional class, and LV outflow tract obstruction. However, neither this nor other cutoffs were able to further stratify the risk in the 2 upper tertiles, despite a wide spectrum of values ranging to >16,000 pg/ml. These data have confirmed and expanded those of previous studies[21−23] showing a significant association between the NT-proBNP level and HF-related prognosis in patients with HC. Very recently, Coats et al[23] reported a sevenfold increased risk of death and transplantation in patients with HC and abnormal NT-proBNP values. That study enrolled patients with overt systolic dysfunction at baseline, a subgroup with particularly ominous short-term outcomes[9]; however, that subgroup was excluded from our cohort to avoid a potential confounder.[23]

The most important clinical implication of our findings was that low NT-proBNP values can identify truly stable disease and allow cautious reassurance of patients with HC regarding the risk of disease progression and HF, even in the presence of potentially adverse clinical features. For patients with HC and dynamic LV outflow tract obstruction, for example, low NT-proBNP values might represent a useful arbitrator, supporting the decision to avoid or postpone invasive septal reduction therapies, particularly when the

symptoms are sufficiently well-controlled by pharmacologic treatment.[24] This hypothesis deserves to be tested in specifically designed, longitudinal studies. In contrast, greater NT-proBNP values represent a rather nonspecific "red flag," lacking sufficient specificity for the identification of the truly high-risk subset, and needs to be complemented by additional clinical and instrumental workup.[4] For example, the presence of a restrictive LV filling pattern significantly increased the risk of the primary end point in our patients with HC belonging to the intermediate NT-proBNP tertile.[14] Furthermore, in a subgroup of 48 of the 183 study patients for whom NT-BNP repeat values were available during follow-up, the prevalence of the SE was almost 3 times more common in the group with an NT-BNP increase than in those with decreasing titers. Thus, it is plausible that longitudinal, serial testing of NT-proBNP during follow-up could provide incremental clinical benefit compared with a single baseline evaluation, by revealing the trend of over time and the response to treatment.[25]

Our results have suggested that the prognostic value of NT-proBNP is only partly explained by other covariables traditionally regarded as determinants of pressure overload, such as diastolic dysfunction and LV outflow tract obstruction, indicating that some additional mechanism, other than myocyte stretch, must be involved. In nonobstructive HC, the ventricular expression of BNP has been suggested to partly reflect myocardial disarray, hypertrophy, and fibrosis, rather than solely a response to the hemodynamic load.[26] For example, recent evidence that a quota of BNP can be produced by cardiac fibroblasts, involved in the regulation of extracellular matrix turnover,[27] might explain the correlation observed among the NT-proBNP levels, the extent of late gadolinium enhancement at cardiac magnetic resonance imaging,[19] and plasma biochemical markers of collagen turnover.[28] Finally, microvascular dysfunction, a key pathophysiologic feature of HC, might represent an additional stimulus to NT-proBNP production in asymptomatic patients.[29] This concept is consistent with the work by Nakamura et al,[30] demonstrating that myocardial ischemia might stimulate peptide release in patients with HC and angiographically normal coronary arteries.

In conclusion, in stable outpatients with HC, plasma NT-proBNP proved a powerful independent predictor of death and HF-related events. Although the positive predictive accuracy of the elevated NT-proBNP values was modest, low values reflected true clinical stability, suggesting the possibility of avoiding or postponing aggressive treatment options.

Disclosures

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Anderson-Fabry, the *histrionic disease*: from genetics to clinical management

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Abstract

Anderson-Fabry disease (AFD) is an X-linked lysosomal storage disorder of glycosphingolipid catabolism, due to deficiency or absence of a galactosidase A (α-gal A) enzyme. The disease may affect males and females, the latter with an average 10 years delay. Metabolites storage (mostly Gb3 and lyso-Gb3) leads to progressive cellular and multiorgan dysfunction, with either early and late onset variable clinical manifestations that usually reduce quality of life and life expectancy. Heart and kidney failure, stroke and sudden death are the most devastating complications. AFD is always been considered a very rare disease, although new epidemiologic data, based on newborn screening, showed that AFD prevalence is probably underestimated and much higher than previously reported, especially for late-onset atypical phenotypes. Currently, the diagnosis may be easier and simpler by evaluating α-gal A enzyme activity and genetic analysis for *GLA* gene mutations on dried blood spot. While a marked α-gal A deficiency leads to diagnosis of AFD in hemizygous males, the molecular analysis is mandatory in heterozygous females. However, referral to a center with an expert multidisciplinary team is highly advisable, in order to ensure careful management and treatment of patients, based also on accurate molecular and biochemical data interpretation. While long-term efficacy of enzyme replacement therapy (ERT) in advanced stage is still debated, increasing evidence shows greater efficacy of early treatment initiation. Concomitant, organ-specific therapy is also needed. New treatment approaches, such as chemical chaperone therapy, alone or in combination with ERT, are currently under investigation. The present review illustrates the major features of the disease,
focusing also on biochemical and genetic aspects.

Introduction

Anderson-Fabry disease (AFD) (OMIM #301500) is named after two dermatologists, W. Anderson and J. Fabry, who separately described two cases of patients with *angiocheratoma corporis diffusum* in 1898.[1,2] Almost a century later AFD was showed to be a rare X linked multisystemic lysosomal storage disorder of glycosphingolipid catabolism, due to the deficiency of the lysosomal enzyme α galactosidase A (α-Gal A; EC 3.2.1.22).[3,5] This deficiency leads to systemic accumulation of globotriaosylceramide (Gb3) and its deacylated Gb3, the globotriaosylsphingosine (lysoCTH or lyso-Gb3), throughout the body within the lysosomes and in the plasma. In symptomatic AFD male patients Gb3 and lyso-Gb3 are both increased, in plasma as well as in urine.[6] However, symptomatic female patients often have Gb3 values within the normal range. Contrary to Gb3, lyso-Gb3 is absent in healthy controls and it is also markedly increased in symptomatic female patients. Furthermore, the absence of manifestations in male AFD children with no residual enzyme activity, and Gb3 accumulation within the cells, suggests that Gb3 may not be directly responsible for disease signs and symptoms, while lyso-Gb3 is likely to play a direct role in the pathogenesis of AFD.[7]

A specific therapy for AFD, enzyme replacement therapy (ERT), is available since 2001. The clinical and scientific interest in this rare disease is growing all over the world, with more than 2600 papers published up to date (Figure 1).

Lysosomes and α-galactosidase A

Glycosphingolipids are an essential part of the lipid bilayer in the intra and extracellular human membranes. The lysosomal enzyme α-Gal A is involved in the catabolism of glycosphingolipids within the lysosomes whereas it catalyzes the hydrolysis of α-galactosidic linkages of glycosphingolipids, glycoproteins and polysaccharides.[3] This complex catabolic pathway allows a reutilization of the membrane components. Mutations in the *GLA* gene encoding the α-Gal A may affect the synthesis, processing, and stability of the enzyme, leading to the impairment of glycosphingolipids catabolism and to their progressive accumulation within the lysosomes. The storage of Gb3 and other toxic metabolites involves every cell of the human body and starts during the fetal life.[8]

However, cellular dysfunction and organ damage usually become evident with early signs and symptoms in infancy (classic phenotype), or decades later, in the late-onset variant.[5]

Genetic bases

The lysosomal enzyme α-GAL A is encoded by the *GLA* gene (MIM 300644) mapping on the X chromosome at locus Xq22.1 and organized in seven exons encompassing over 12 Kb.[9] The *GLA* cDNA of 1290 bp encodes for a precursor protein of ~50kDa (429 amino acids), which is proteolytically cleaved into the lysosomal mature protein.[10,11]

The mature α-GAL A enzyme is a homodimeric glycoprotein of about 46 kDa (398 amino acids). Each monomer is composed of two domains. The domain 1 contains the active site (encompassing amino acids 32-330), at the center of the β strands in the $(\beta/\alpha)_8$ barrel, while the domain 2 contains antiparallel β strands.[12]

At present 665 *GLA* gene mutations, most of

them private and spread throughout all exons, have been reported (HGMD professional 2012.2), confirming a high genetic heterogeneity. Over 65% of the AFD causing genetic variants are missense or nonsense mutations, while 5% are splicing mutations, 22% small deletions/small insertions or regulatory mutations, and only 6% are gross deletion/insertion. While missense mutations not affecting the active site may lead to partial reduction of α-GAL A activity, no residual enzyme activity is expected for the remaining mutations.

AFD is transmitted as an X-linked disorder. Sons of affected males are always disease free, while their daughters are obligate heterozygotes. The heterozygous females can transmit their mutated allele to their male and female offspring. Thus in each pregnancy 50% of their sons can be hemizygotes and 50% of their daughters can be heterozygotes.

Although X-linked, penetrance and expressivity rate are high in both genders (100% in males and 70% in females).[13] Heterozygous females are usually affected with a milder and late-onset disease (about 10 years later than males), but in a minority, symptoms and organ damage can be as severe and early as in males. Therefore it would be preferable to discontinue *female carrier* and *X-linked recessive* terms, as misleading.[13] This huge variability of female phenotype can be partially explained by the random X inactivation process.

A genotype-phenotype correlation is often difficult, as most of the AFD mutations are private and unique within single families. However some common missense mutations, leading to residual α-GAL activity, have been linked mainly to specific organ involvement in AFD patients (*e.g.* N215S [c.644A>G➔p.Asn215Ser] to hypertrophic cardiomyopathy).[14,15]

Epidemiology

AFD is rare disease, with a reported prevalence in the general population ranging from 1:117,000 to 1:467,000.[16,17] However, recent biochemical and genetic screening data in newborns report an unexpectedly much higher incidence (Table 1).[18-21] While the classic phenotype of AFD is actually rare, these data show that milder variant with late-onset phenotype may be much more frequent than expected. The late-onset subgroup comprises patients with disease onset much later in life than patients with classic AFD, usually in the fourth to six decades, and manifestations confined mainly to one organ system. Patients with atypical late-onset variants have residual enzyme activity, which ranges from 2 to 20% of normal values and results in a milder phenotype. However the correlation between residual enzyme activity and disease phenotype is not strong.[22] The real strength of these newborn-screening initiatives goes well beyond the simple data itself. They shifted a historic paradigm of a rare disease, with multisystemic involvement in young males, to a more common disease, with a wide phenotype, sometimes with disease manifestation confined to one organ system.

Furthermore, screening for AFD among selected groups of patients, *i.e.* patients with juvenile cryptogenic stroke, renal failure or left ventricular hypertrophy, suggests that many patients are misdiagnosed.[23] Indeed, the prevalence of AFD among patients with hypertrophic cardiomyopathy (HCM) ranges from 0.5% to 6%, depending on different selection criteria for age and gender, and diagnostic methods.[24,25] It is about 1% if only males older than 35 years at diagnosis of HCM are selected.

AFD is a pan-ethnic disease, with few areas of high prevalence (*i.e.* Canada and West Virginia, USA), probably due to a founder effect.[4]

Clinical manifestations, clinical course and prognosis

AFD is a progressive disease, characterized by a wide variability of signs, symptoms and patterns, which, for the classical phenotype, are clearly different in three periods of life (Figure 2):
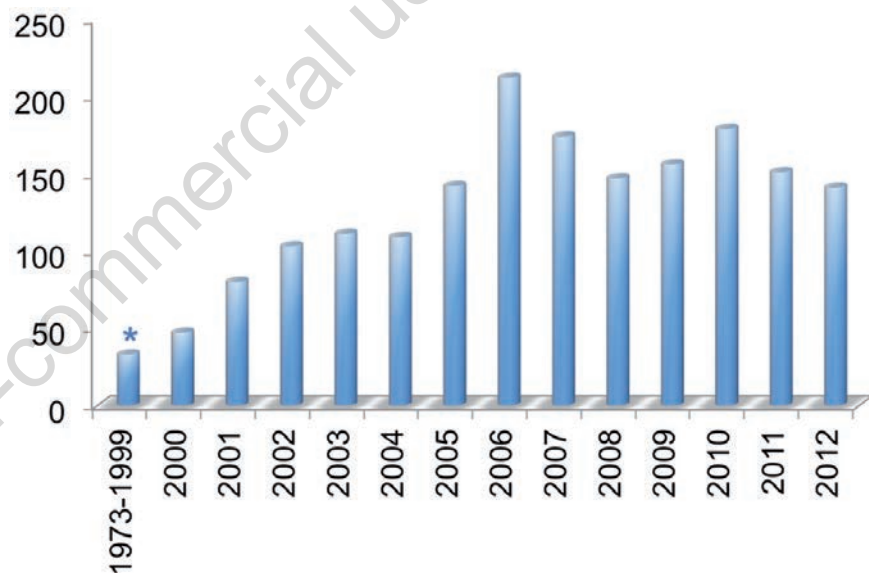

Figure 1. The number of papers on Anderson-Fabry disease is significantly increased since the advent of enzyme replacement therapy, in 2001.

Table 1. Epidemiology of Anderson-Fabry disease. Data from newborn screening based on α-Gal A activity and *GLA* gene analysis on dried blood spot.

Gender	Country[Ref]	Total incidence	Male incidence	Classic phenotype	Late-onset phenotype
M	Italy[18]	-	~1:3100	~1:37,000	~1:3400
M+F	Taiwan[19]	1:2300*	~1:1250*	~1:22,600 (M)*	~1:1400 (M)*
M+F	Taiwan[20]	1:2500*	~1:1400*	~1:57,000 (M)*	~1:1500 (M)*
M+F	Austria[21]	~1:3900	-	~1:17,000	~1:4100

M, male; F, female. *Newborn screening in Taiwan reveal a high incidence of the IVS4+919G>A cryptic splicing mutation, probably due to a founder effect. This mutation has been described in late-onset cardiac phenotype.[19,20]

- Childhood: although the pathologic accumulation of sphingolipids in various tissues throughout the body usually starts in the fetal life,[8] early symptoms, mostly neuropathic pain, usually emerge in infancy.[26]
- Age 20 to 30: progressive cellular dysfunction, related to the slow but continuous intracellular storage, leads to organ damage and failure. Symptoms tend to progress, and the first, subclinical signs of organ involvement may be detected [e.g. proteinuria, electrocardiogram (ECG) changes and silent cerebral abnormalities].
- Over age 30: major organ involvement, such as renal, cerebrovascular and cardiovascular, may lead to disease progression and eventually to an end-stage phase.[27] Average reduction of life expectancy is about 20 years in males and 10 in females.[28]

Anderson-Fabry disease in the childhood: first signs and symptoms

The first clinical symptom is usually neuropathic pain, which occurs in 70% of patients and usually emerges between age 4 and 12, earlier in males than in females.[29] The pain is located at the extremities and can either be chronic or characterized by crisis. It may be triggered by exercise, fever, sun exposure, changes in temperatures or stress.[30] The burning pain may be so intense to interfere with every-day life and substantially reduce quality of life.[31] Neurologic examination is normal, as well as electromyography (EMG) and electroneurography.[32] Both examinations only assess large nerve fibers function, while in AFD small nerve fibers are involved, and their reduced density on skin biopsy is sometimes required to diagnose AFD neuropathy.[33] The neuropathic pain is difficult to manage with standard analgesic and may respond to narcotic analgesic (such as codeine or morphine) or anticonvulsant drugs (such as carbamazepine).[34] Anhidrosis and hypohidrosis, a reduced or absent ability to sweat, can cause heat and exercise intolerance, often reported as *recurrent fever after exercise* by the patients and their parents. They may also trigger AFD pain crisis.[35]

Gastrointestinal involvement, characterized by abdominal pain, diarrhea, nausea, can also be reported during childhood as well as in the adult life.[36]

Retinal vessel tortuosity and cornea verticillata, a corneal lesion caused by Gb3 deposition, can also be detected in childhood.[37] Vertigo, tinnitus and sudden hearing loss have been reported at an early stage and are thought to be related to small vessel involvement.[38]

Angiokeratoma is another early feature of AFD.[39] Small purple skin lesions may be present

in children and tend to increase in size and number with age. They can be found typically on the lower back, thighs, genital areas, umbilicus and sometimes on mucosae (Figure 3). Histologically they are characterized by vessel dilatation (angiomas) and hyperkeratosis.

Major organ involvement, such as renal, cerebrovascular or cardiovascular is uncom-

mon in children.[40] Microalbuminuria and proteinuria, signs of early renal damage, may be detected in the second decade of life.[41] Small white matter lesion on magnetic resonance imaging (MRI) may reveal an initial central nervous system involvement, as well as ECG changes of the heart.

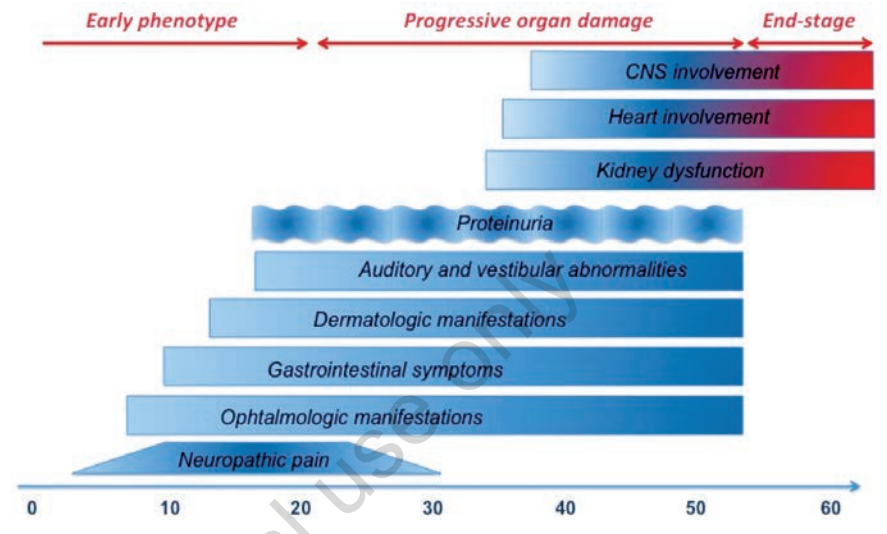Although the early clinical picture is sugges-

Figure 2. Symptoms onset and disease progression in classic Anderson-Fabry disease (AFD). Signs and symptoms of AFD progressively increase with age. In infancy and adolescence neuropathic pain, gastrointestinal manifestations and angiokeratomas are the most frequent manifestations. Proteinuria, the first sign of kidney involvement, is also present in young affected males (Modified from Zarate and Hopkin[4]).
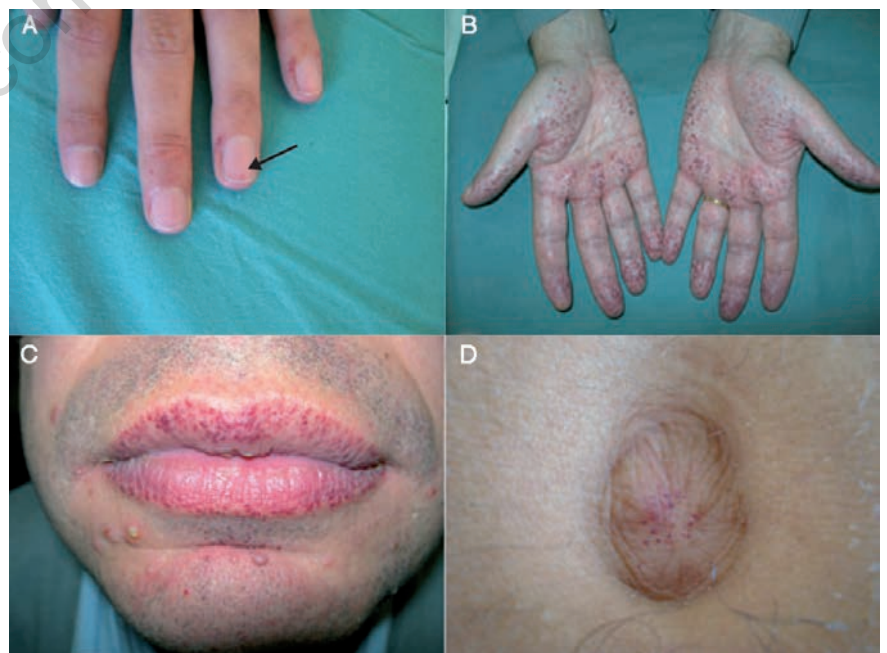
Figure 3. Various aspects of angiokeratoma in Anderson-Fabry disease patients: angiokeratoma of the periungual and subungual area (panel A), of the palms, lips and umbilicus (panel B-D respectively) (Reprinted with permission of the publisher from Zampetti et al.[39]).

tive of AFD, the diagnosis is rarely made in childhood and adolescence, with an average diagnostic delay of 10 years for both genders. Recognition of early and advanced clinical manifestations of AFD represents a real challenge for clinicians, due to variable clinical manifestations and symptoms that may resemble other common diseases.[42]

Anderson-Fabry disease in the adult life

The full spectrum of AFD phenotype slowly develops with age.

Cardiac involvement

Gb3 and related sphingolipids storage may occur in cardiomyocytes, cardiac valves, endothelial cells and conduction system.[43] While ECG changes may be found in young patients, an overt cardiomyopathy with left ventricular hypertrophy (LVH), with diastolic dysfunction, preserved systolic function, often with mild valvular regurgitation emerges in the third or fourth decade of life in affected males, more rarely in a few females.[44]

Conduction abnormalities and arrhythmias

ECG changes are often present in AFD (Figure 4). A short PQ interval (<120 ms), described as a feature of AFD, is a non-specific and rather uncommon finding with a prevalence of 14%.[45] Signs of LVH on ECG, along with repolarization changes (including deep T waves inversion) are frequent. They may be detected even in patients without a marked increase of left ventricular wall thickness at echocardiography.[45,46]

Bradycardia, sinus node disease, prolonged PQ intervals with variable degree of AV-block, and QRS broadening (particularly with a right bundle branch block pattern) are ECG alterations often seen later in life.[47] PQ interval and QRS duration are directly proportional to age, suggesting a relationship to progressive storage of sphingolipids and development of fibrosis. Apoptosis and autonomic nervous system involvement might also play a role. Recently AV and sinus node disease were showed to be common in AFD patients.[47] The prevalence of atrial fibrillation and non-sustained ventricular-tachycardia (VT) are higher in AFD patients when compared to the general population.[48] Furthermore the risk of arrhythmias increases with age, while there are no gender-related differences. Sudden cardiac death may occur, usually in the end stage phase, although its prevalence is unknown.[48] It may be triggered by episodes of sustained VT.

Anderson-Fabry disease cardiomyopathy

AFD cardiomyopathy is characterized by diastolic dysfunction and LVH, usually concentric and non-obstructive. Basal LV outflow tract obstruction (LVOTO) is rare, while exercise provokable LVOTO has been detected in 43% of patients,[49] most of them with LV small cavity size.

LVH is usually mild, although male patients may also show severe and asymmetric septal or apical hypertrophy, both in classical and late-onset disease (Figure 5). Systolic function is usually preserved in most patients until advanced stage, but a few patients may gradually develop a systolic dysfunction, often leading to progressive refractory heart failure.[50] The use of some echocardiographic technique, such as tissue doppler imaging or strain rate imaging, may detect early cardiac involvement before LVH develops.[51,52] Replacement fibrosis, detected by cardiac MRI usually in the inferior or posterolateral wall, is considered a sign of disease progression (Figure 6).[53] Myocardial fibrosis may also be present in female patients
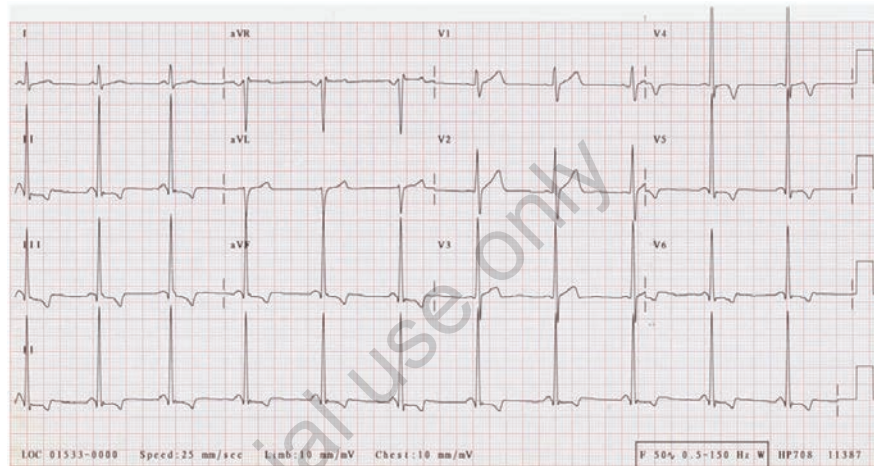

Figure 4. Electrocardiogram in a male patient with Anderson-Fabry disease showing left ventricular hypertrophy with T-waves inversion and a relatively short PR (133 ms) (*Reprinted with permission of the publisher from O'Mahony and Elliott*[46]).
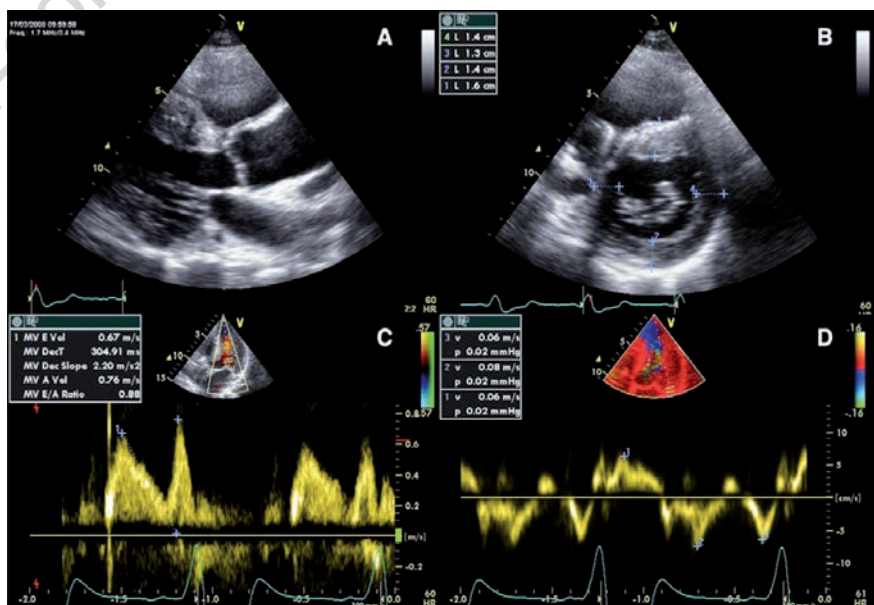

Figure 5. Typical echocardiographic aspect of Anderson-Fabry disease-related cardiomyopathy. The 2-D images in panel A and B show mild, concentric left ventricular hypertrophy. There is also mild diastolic dysfunction (panel C and D) (*Reprinted with permission of the publisher from O'Mahony and Elliott*[46]).

without LVH, suggesting that hypertrophy and fibrosis are not necessarily associated.[54] The endocardial binary appearance (*binary sign*) was suggested as an echocardiographic hallmark of AFD cardiomyopathy;[55] however, recent studies showed that these echo features can be detected also in patients with sarcomeric HCM.[56] The *binary sign* is of limited utility in distinguishing the cause of LVH. Of note, right ventricular hypertrophy, is detected by MRI in about 2/3 of patients with LVH.[57]

Angina and coronary artery disease

Chest discomfort and angina may be present, despite the absence of atherosclerotic coronary artery disease. Both are likely to be related to microvascular dysfunction (Figures 7 and 8).[58-60] Coronary microvascular blood flow impairment can be detected in both genders, even without LVH. It can lead to chronic ischemia and myocardial replacement fibrosis.

Other cardiac abnormalities

The prevalence of mild aortic root enlargement is higher in male AFD patients,[61] while exercise capacity is usually reduced, when measured by a cardiorespiratory test.[62]

Kidney involvement

Microalbuminuria and proteinuria, due to kidney impairment related to Gb3 accumulation and glomerular fibrosis, are usually present by the third decade of life, sometimes earlier when α-GAL enzyme activity is <1% (Figure 9).[63,64] Proteinuria levels may vary widely, but tend to increase in severity with age and are inversely correlated to kidney function, based on glomerular filtration rate, which is often normal or even higher in young patients.[65] After renal dysfunction has progressed to end-stage, dialysis and renal transplant may be necessary.[66-67]

Cerebrovascular involvement

Transient ischemic attack and strokes are the most relevant and disabling complication of AFD and may significantly contribute to disease-related morbidity and mortality.[68] Multifocal leukoencephalopathy is the typical lesions of AFD and results from small vessels involvement and microvascular dysfunction (Figure 10).[69] The prevalence of strokes in AFD is about 7% in males and 5% in females, usually in the third and the fourth decade of life. However, the prevalence of cerebrovascular involvement rises up to 60-70% if minor signs or symptoms and abnormal brain MR image are considered.[68] Psychiatric disorders, depression and dementia may be present and they can only be partially attributed to the cerebral microvascular disease.[70] The severity of cerebrovascular disease may vary widely.[71] Combining neurologic symptoms and instru-
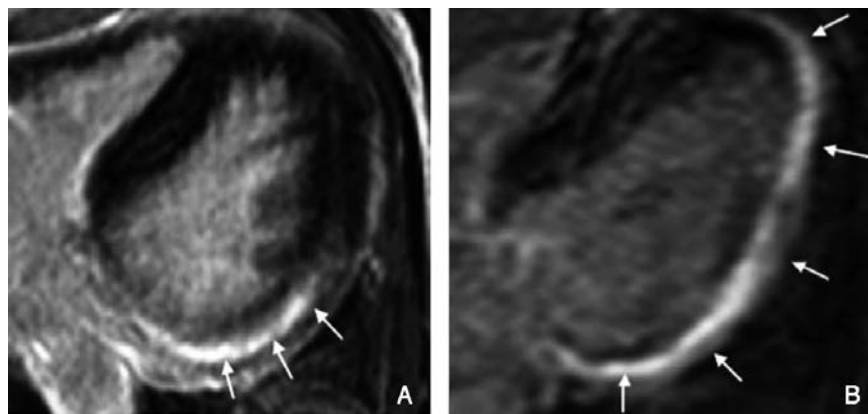

Figure 6. Myocardial fibrosis as a sign of disease progression. Panel A) cardiovascular magnetic resonance image showing an area of myocardial fibrosis involving the posterolateral wall (arrows in four-chamber horizontal long-axis view). Panel B) the extension of fibrosis is significantly increased, despite 6 years of enzyme replacement therapy (*Reprinted with permission of the publisher from Imbriaco et al.*[101]).
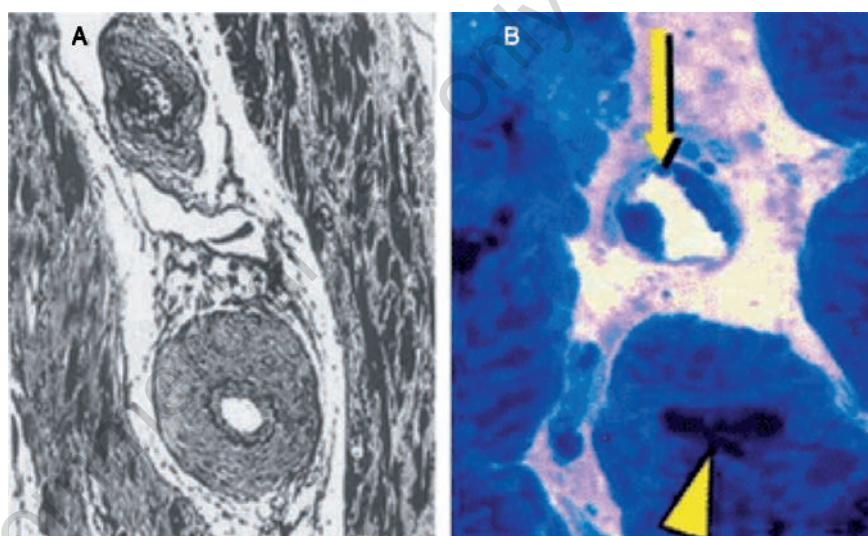

Figure 7. Gb3 inclusions in the endothelium and cardiomyocytes. Panel A shows an intramural coronary artery in a patient with hypertrophic cardiomyopathy. Wall thickening is due primarily to severe thickening of medial wall, while the intima is mildly thickened. Panel B shows interstitial capillaries in the myocardium of a patient with Anderson-Fabry disease. Glycosphingolipid inclusions are present in the endothelium (arrow) and cardiomyocytes (arrowhead) (*Reprinted with permission of the publisher from Camici e Crea*[58]).
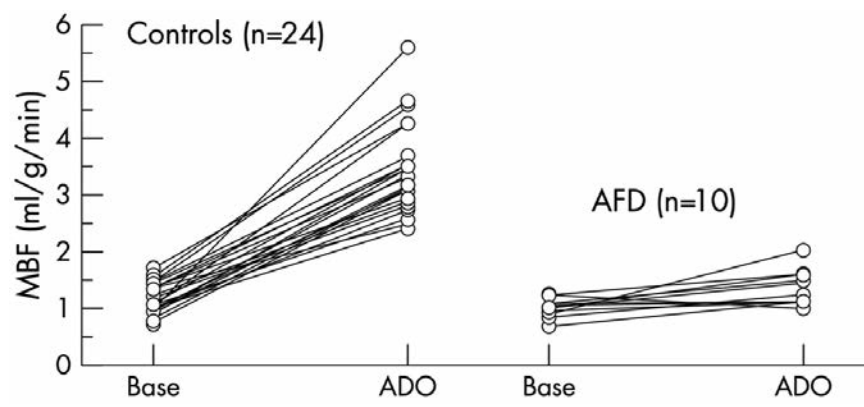

Figure 8. Microvascular dysfunction detected by myocardial positron emission tomography. Male patients with Anderson-Fabry disease (AFD) show a marked reduction in myocardial blood flow (MBF) during adenosine-induced hyperaemia (ADO), compared to controls (*Reprinted with permission of the publisher from Elliott et al.*[59]).

mental findings, CNS disease can be briefly classified into 3 categories: i) symptomatic patients with repeated cerebrovascular events, ii) patients with minor neurological manifestation such as vertigo, dizziness, hearing loss, headache and migraine; iii) asymptomatic patients with abnormal brain MRI findings.[68]

Other findings

Symptoms compatible with autonomic dysfunction, such as arterial hypotension, sinus bradycardia or gastrointestinal symptoms, have always been attributed to autonomic nervous system neuropathy,[72] which is often neglected in the single patient.

Obstructive airway disease and osteopenia/osteoporosis in AFD patients have also been reported.[73,74]

Diagnosis

Once the clinical pictures rises a suspicion of AFD, biochemical and genetic tests are required to confirm the diagnosis.

Biochemical diagnosis

The detection of markedly reduced or absent α-gal A activity in leukocytes is sufficient to confirm AFD diagnosis,[3] although it should be carefully evaluated with clinical and genetic molecular findings. While patients with a milder AFD phenotype usually have residual α-gal A activity, in classically affected young males enzyme activity it is very low or undetectable.[75,76]

α-gal A activity can be assessed in plasma. However, when reduced, its value needs to be confirmed in leukocytes or fibroblasts, in order to avoid diagnostic pitfalls, due to enzyme pseudodeficiency in plasma.[77] The *GLA* gene sequence analysis should always be performed and correctly interpreted, in order to reveal polymorphisms, that potentially lead to an enzymatic pseudodeficiency.[78] Biochemical diagnosis may be unreliable in heterozygous females, as they can have a normal or only mildly reduced α-gal A activity, due to random X-chromosome inactivation. Therefore, it is crucial to combine biochemical assays with appropriate molecular analyses of the *GLA* gene in order to attribute or exclude the AFD heterozygous status in females.[79]

More recently, screening tests on dried blood spot (DBS), using fluorescent methods, were introduced,[80] together with multiplex assays of lysosomal enzymes by tandem mass spectrometry.[81,82] The availability of multiplex technology using DBS on filter paper made possible newborn screening programs of treatable lysosomal storage diseases.[83] DBS is simply transportable and represent an easier,

faster and less expensive method for enzyme activity evaluation in selected at risk population. As false positive or negative results may occur, it is necessary to confirm a reduced residual enzyme activity by standard laboratory diagnostic procedures.[84,85]

Genetic analysis

Molecular analysis is usually performed in probands by direct sequencing analysis of the *GLA* gene coding regions, of its exons/introns boundaries and of the intronic region encompassing the known deep intronic mutations.[86,87] Rarely, in males with low or no enzyme activity, and females with AFD clinical manifestations and normal or low enzyme activity, exon-based sequence analysis may fail to identify the disease-causing mutation. Such in these cases, further molecular and cellular laboratory investigations are needed to evaluate the *GLA* gene expression profile at mRNA and protein level. Both Ishii and Filoni identified, by mRNA analysis, deep intronic pathogenic mutations, which alter the GLA splicing regulation.[86,87] In females, due to the presence of the wild-type allele, gross *GLA* gene rearrangements, such as deletion or insertion of entire exons, may be missed, unless specific laboratory technique, such as multiplex ligation-dependent probe amplification analysis and/or quantitative polymerase chain reaction amplification, are used.[88,89]

Additional investigations are also necessary in order to assess the pathogenicity of new variants. *In silico* and mRNA analysis, computational modeling, and functional studies are needed to prove or exclude the effect of the new identified variant as a disease-causing mutation.[78] Co-segregation in male family members, tissue biopsies and determination of specific metabolites (*i.e.* Gb3 or lyso-Gb3) may also be necessary. Recently, several authors suggest to consider *GLA* gene mutations on the bases of their pathogenic significance, with variants predict-

ed to have high (Class 1) or low probability to be disease-causing (Class 2, polymorphisms).[90] Single nucleotide polymorphisms (SNPs) are exonic or intronic nucleotide variants detected with incidence higher than 1% in a healthy control population. They are usually interpreted as not disease-causing mutation. Several *GLA* gene polymorphisms, leading to molecular heterogeneity have been described (SNP, http://www.ncbi.nlm.nih.gov/SNP).[79] Sometime exonic polymorphisms, including rare variants leading to aminoacid change, may be found in males with normal or decreased enzyme activity, but still well above the pathologic range of values. They are almost certainly non-pathogenic variants or *Class 2 mutations* and they include polymorphisms or sequence changes described as pseudodeficiency alleles, *e.g.* c.937 G>T (p.D313Y).[89,90] Pseudodeficiency is a condition in which individuals show a reduced α-Gal A activity *in vitro*, but remain clinically healthy.

However, in a few patients, when the combination of clinical, histological, biochemical and functional data are still not clear-cut, the AFD diagnosis may remain uncertain[91] (Figure 11).

Role and significance of Gb3 and lyso-Gb3

Biomarkers are chemical molecules that can be clinically useful to assess disease progression, therapeutic efficacy, as well as diagnostic confirmation. Although many attempts have been made in order to identify a biomarker for AFD, a valid and reliable molecule is still missing. Gb3 is the primary storage molecule and can be find inside cells and in biologic fluids, such as urine and plasma. For many years Gb3 has been considered a reliable marker for AFD, so that its reduction in tissue biopsies has served has a major criteria to prove the efficacy of the enzyme replacement therapy. With
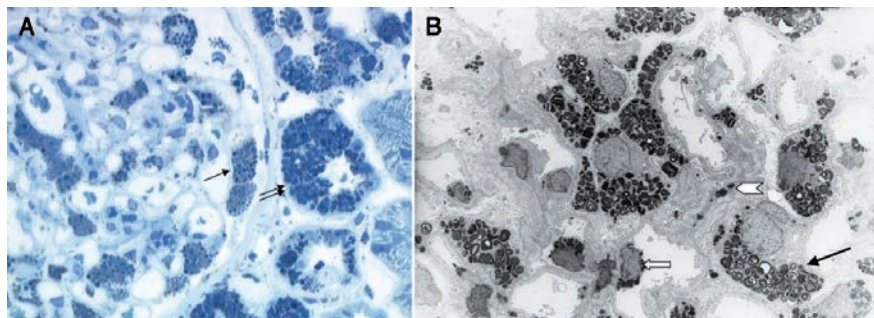

Figure 9. Evidence of Gb3 inclusions in the kidney. Panel A) sphingolipid inclusions in podocytes (arrow) and distal tubule epithelial cells (double arrow). Panel B) lysosomal inclusions in podocytes (arrow), endothelial (open arrow) and mesangial cells (open arrowhead) (*Reprinted with permission of the publisher from Tøndel et al.*[64]).

time, new research investigations proved that Gb3 do not correlate with disease manifestations. Plasma Gb3 levels are abnormally high at a very young age (sometimes detectable even *in utero*), while clinical manifestations develop much later in life.[92] Furthermore, Gb3 might be within the normal range in symptomatic female. The diagnostic value of this parameter has been questioned as well, since it can be detected in normal healthy subjects.[7]

Globotriaosylsphingosine, also known as lyso-Gb3, is the deacylated form of Gb3. In contrast to Gb3, this relatively new biomarker is abnormally high in male and female patients with AFD while this is absent in control healthy subjects.[7,93] lyso-Gb3 may be a useful additional element for confirmation of AFD diagnosis in patients with *GLA* variant of unknown significance (Figure 11). Furthermore, lifetime exposure to lyso-Gb3 correlates with disease severity, even after adjustments for age, gender and cardiovascular risk factors, and suggests its pathophysiological role. However, the usefulness of lyso-Gb3 as a biomarker for assessing the response to ERT is still under investigation.[94,95]

Genetic counseling and multi-disciplinary approach

Once biochemical and molecular genetic data are ascertained and/or available, their interpretation should be performed by a multi-disciplinary team with expertise in AFD, usually available in referral centers, in order evaluate the appropriate treatment strategy. Genetic counseling should then be offered to the proband, and his family members, who should undergo clinical screening.
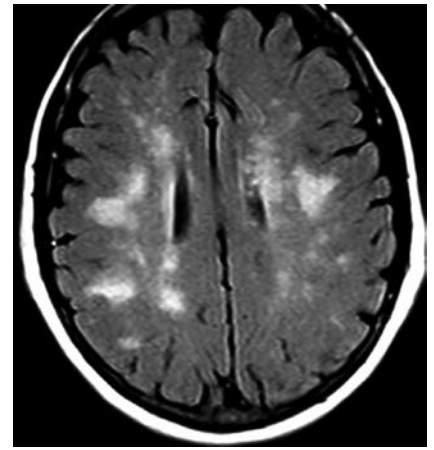

Figure 10. Brain magnetic resonance imaging of an asymptomatic 54-year old female showing multifocal leukoencephalopathy (*Reprinted with permission of the publisher from Buechner et al.*[68]).
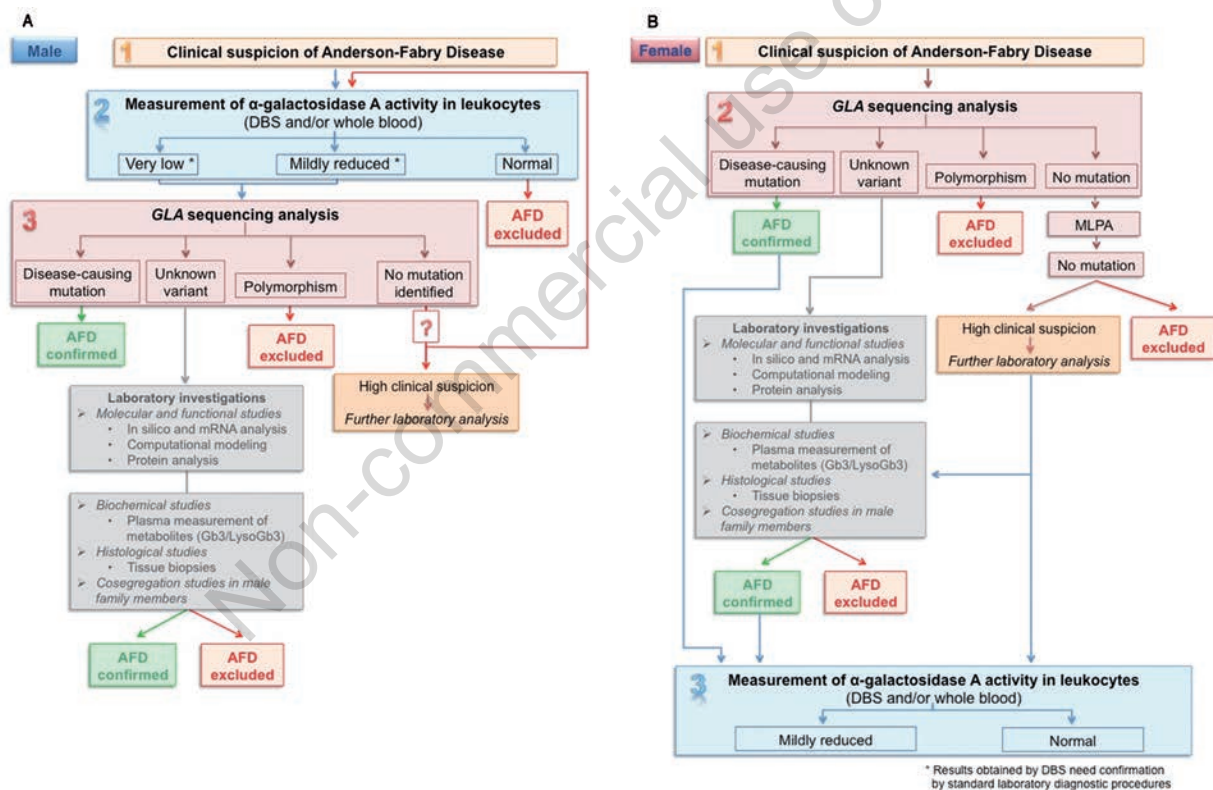

Figure 11. Diagnostic flow chart of Anderson-Fabry disease (AFD) in males and females: step-by-step approach. Panel A) males. 1) Clinical picture indicative of AFD, family history compatible with an X-linked transmission. 2) In males, normal values of α-gal A activity exclude the diagnosis of AFD. Reduced (<20%) or absent enzyme activity values confirm the suspicion of AFD and require genetic testing. Biochemical analysis on dried blood spot (DBS) must be confirmed by standard laboratory procedures (*i.e.* whole blood analysis). 3) If no mutations are identified by genetic analysis, and the α-gal A activity is low or undetectable, further laboratory analyses are required to confirm the diagnosis (*e.g.* mRNA and protein analysis, tissue biopsies). Comparably, the identification of new unknown variant requires laboratory analysis, along with co-segregation studies within the family. Panel B) females. 1) Clinical picture indicative of AFD, family history compatible with an X-linked transmission. 2) In female the α-gal A activity can be within the normal range. Therefore genetic analysis is mandatory to confirm the diagnosis; if no mutations are identified by genetic analysis, and the clinical suspicion is high, further laboratory analyses are needed (*e.g.* multiplex ligation-dependent probe amplification to detect deletions or insertions, mRNA and protein analysis, tissue biopsies). Comparably, the identification of new unknown variant requires laboratory analysis, along with co-segregation studies within the family. 3) Enzyme activity may be helpful in diagnosis and management of female AFD patients and should be measured after the genetic diagnosis is made. Biochemical analysis on DBS must be confirmed by standard laboratory procedures (*i.e.* whole blood analysis).

Management and follow-up

AFD is a multi-systemic disorder and a thorough evaluation of organ involvement is highly recommended.

Neurologic assessment comprises diagnosis and staging of neuropathic pain, through EMG and skin biopsy, if needed.[33] Since leukoencephalopathy may be present even in young patients without symptoms and with a normal neurologic exam, brain MRI is required at initial assessment.[29] It should be repeated at a 2-3 years interval, depending on clinical picture.

Nephrologic assessment is based on microalbuminuria, 24 h proteinuria, renal function (creatinine level and estimated glomerular filtration rate), which should be routinely and repeatedly assessed.[5] Ultrasound abdominal examination is also required for kidney morphology, as well as, in selected cases, renal biopsy.

Cardiologic evaluation

Patients should undergo ECG, colordoppler echocardiography, 24 h ambulatory ECG monitoring and exercise (possibly cardiorespiratory) test, in order to define cardiac involvement and future risk.[96] Since arterial hypertension, although rare in these patients, may increase the risk of cerebrovascular and cardiovascular disease, blood pressure should be carefully controlled even with 24 h blood pressure monitoring. Pulmonary function tests are sometimes required in patients whose main symptom is dyspnea.

A rigorous control of conventional atherosclerotic risk factors is mandatory. Indeed, measurement of blood cholesterol, homocysteine level, and fasting glucose, should be performed once or twice a year, although it was suggested that statin treatment might be deleterious in AFD patients. Lifestyle change, especially smoking cessation, dietary modifications and mild physical activity, are also recommended.

Therapy

Since 2001 ERT is available and it has been validated as standard treatment for AFD patients in more than 45 countries. In Europe ERT is available in two commercial formulations: agalsidase α (Replagal®; Shire, Cambridge, MA, USA) and agalsidase β (Fabrazyme®; Genzyme Corp., Cambridge, MA, USA), but the former is not yet approved by Food and Drugs Administration in USA. Randomized, placebo-controlled clinical trials assessed safety and efficacy of both enzyme formulations. However, these trials enrolled small cohort of patients and, despite over 10 years of clinical practice, many questions about ERT long-term efficacy remain unanswered. Both preparations are infused intravenously once every other week, at different dose regimens (0.2 mg/kg for Replagal®; 1 mg/kg for Fabrazyme®). Patients should be treated with licensed recommended doses of agalsidase α and agalsidase β. Currently there is no level 1 scientific evidence showing superiority of one enzyme preparation over the other.[97,98] Treatment failures may occur with both drugs and are probably related to age and advanced disease stage, because severe organ damage is often irreversible (Figure 6).[99,100] Moreover patients can develop complications despite receiving optimal treatment, with concomitant therapy and ERT at licensed recommended dose.[97]

There is increasing evidence that ERT is more effective when started in the early stage of disease, and early diagnosis is highly preferable.[100,101] However, timing of ERT, especially in children and females, is still a matter of debate and may vary in different countries. Current expert opinions about timing of ERT are presented in Table 2,[98] but real guidelines have never been written.

Concomitant treatment: appropriate organ-specific and adjuvant therapy must always be considered for all patients, along with ERT infusion, for symptom control and disease stabilization (Table 3).

Conclusions and future perspectives

AFD is a lysosomal storage disease with multiorgan involvement and reduced life

Table 2. Priority stages for treatment in naïve male and female patients with Anderson-Fabry disease.

High priority	
Children	**Adults**
Neuropathic pain unresponsive to optimal medical therapy	Neuropathic pain unresponsive to optimal medical therapy
Persistent microalbuminuria	Normal or mild to moderate reduction of renal function (GFR 30-90 mL/min)
Proteinuria >250 mg/day	Proteinuria >300 mg/day
Left ventricular mass index >90th percentile (age adjusted)	LVH without extensive fibrosis (on MRI)
TIA/stroke or white matter lesion on MRI	Cerebrovascular disease
-	Disease onset <50 years
Intermediate priority	
Age onset >50 years	→ Less severe disease
LVH with fibrosis	
Severe renal dysfunction (GFR<30 mL/min)	→ Less reversible disease
Low priority	
Severe cardiac or CNS disease (end stage)	
Multiple organ failure	
Other comorbidities with reduction of life expectancy <1 year	
Very mild multiorgan disease (*e.g.* normal renal function, no LVH, neuropathic pain well controlled by conventional medical therapy)	

GFR, glomerular filtration rate; LVH, left ventricular hypertrophy; MRI, magnetic resonance imaging; TIA, transient ischemic attack; CNS, central nervous system.
A consensus of expert wrote this document, on October 2010, in order to support clinicians' decisions during the period of enzyme replacement therapy (ERT) shortage. Although the present recommendations are not supposed to represent real treatment guidelines, they underline some important issues about the efficacy of ERT in advanced stage of Anderson-Fabry disease. Priority to receive ERT was based on disease severity, potential reversibility and rate of disease progression, while age and gender did not represent valid criteria (*Modified from G.E. Linthorst et al. 2012*[93]).

Table 3. Conventional medical treatment in Anderson-Fabry disease.

Symptomatic management of Anderson-Fabry disease
Analgesics to control neuropathic pain
Avoidance of triggers of acute pain crisis
Anti-arrhythmic drugs
Rigorous control of any coronary risk factor (smoke, dyslipidemia, hypertension, hyperhomocysteinemia)
Long-term therapy to prevent organ damage
ACEi/ARB for proteinuria and kidney dysfunction
Anti-aggregant (aspirin, clopidogrel) or anticoagulant (warfarin) to prevent TIA and strokes
End-stage disease management
Dialysis or kidney transplantation for renal failure
Heart transplantation for patient with refractory heart failure

ACEi, angiotensin-converting-enzyme inhibitors; ARB, angiotensin receptor blockers; TIA, transient ischemic attack.

expectancy in many affected patients. AFD has always been considered a very rare disease. However, new screening data reported a much higher incidence. Currently, AFD diagnosis may be easier and simpler by evaluating α-gal A enzyme activity and genetic analysis for *GLA* gene mutations on dried blood spot. A marked α-gal A deficiency leads to diagnosis of AFD in hemizygous males, while molecular analysis is mandatory in heterozygous females. However, referral to a center with an expert multidisciplinary team is highly advisable, in order to ensure careful management and treatment of patients, based also on accurate molecular and biochemical data interpretation.

Enzyme replacement therapy in patients with early symptoms and initial organ disease, such as microalbuminuria and neuropathic pain, seem to be effective. Furthermore, new treatment approaches are currently under investigation, such as chemical chaperone therapy alone (phase III clinical studies; http://www.clinicaltrials.com) or in combination with ERT (preclinical studies).[102-104]

While many aspects of this disease have been elucidated, further clinical and laboratories studies are still needed to clarify residual areas of doubt.

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Male

1 Clinical suspicion of Anderson-Fabry Disease

2 Measurement of α-galactosidase A activity in leukocytes
(DBS and/or whole blood)

Very low * | Mildly reduced * | Normal

AFD excluded

3 GLA sequencing analysis

Disease-causing mutation | Unknown variant | Polymorphism | No mutation identified

AFD confirmed

AFD excluded

?

High clinical suspicion

Further laboratory analysis

Laboratory investigations
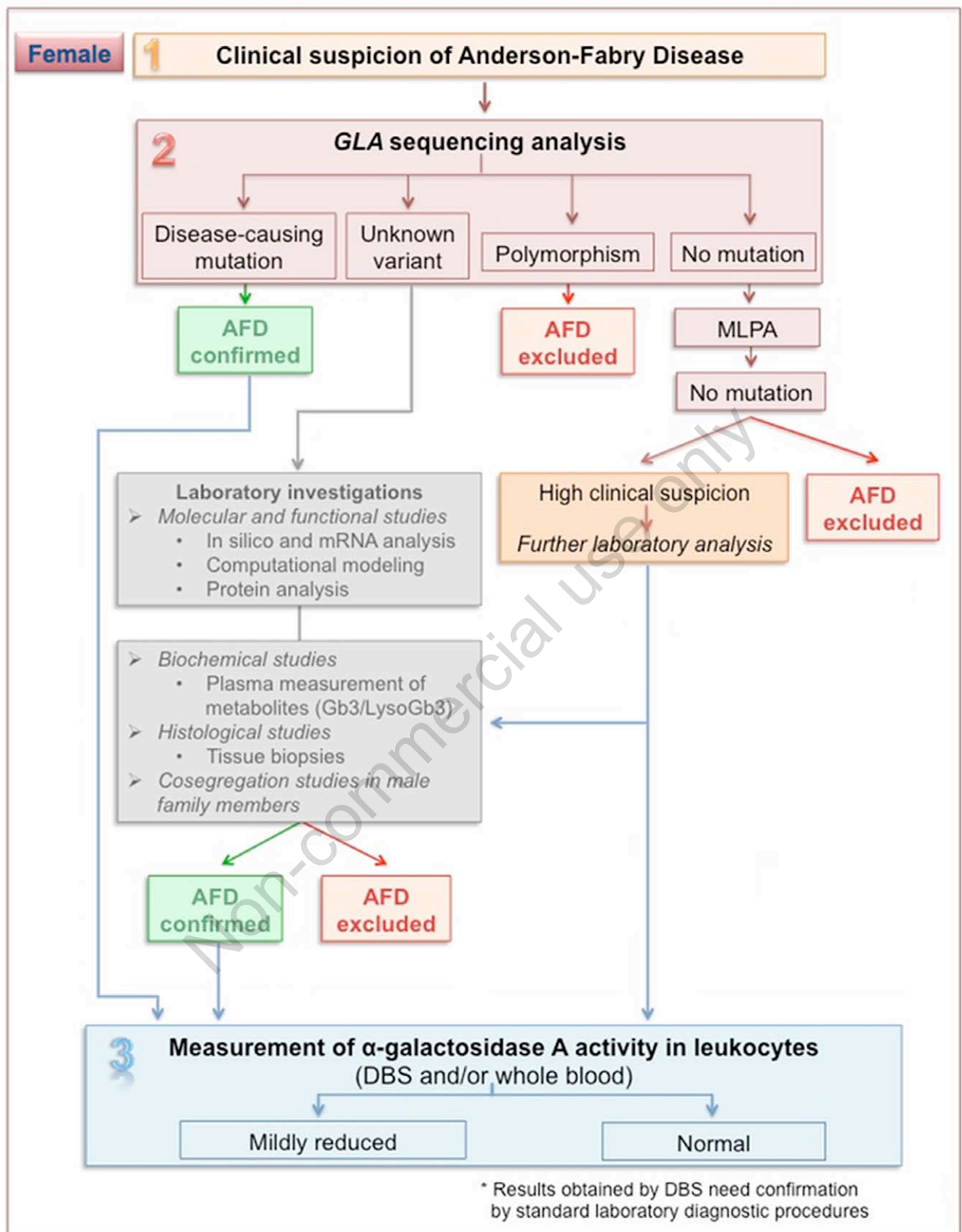> *Molecular and functional studies*
  • In silico and mRNA analysis
  • Computational modeling
  • Protein analysis

> *Biochemical studies*
  • Plasma measurement of metabolites (Gb3/LysoGb3)
> *Histological studies*
  • Tissue biopsies
> *Cosegregation studies in male family members*

AFD confirmed | AFD excluded

* Results obtained by DBS need confirmation by standard laboratory diagnostic procedures

Female **1** **Clinical suspicion of Anderson-Fabry Disease**

2 *GLA* sequencing analysis

| Disease-causing mutation | Unknown variant | Polymorphism | No mutation |

AFD confirmed

AFD excluded

MLPA

No mutation

Laboratory investigations
➢ *Molecular and functional studies*
  • In silico and mRNA analysis
  • Computational modeling
  • Protein analysis

High clinical suspicion
Further laboratory analysis

AFD excluded

➢ *Biochemical studies*
  • Plasma measurement of metabolites (Gb3/LysoGb3)
➢ *Histological studies*
  • Tissue biopsies
➢ *Cosegregation studies in male family members*

AFD confirmed

AFD excluded

3 **Measurement of α-galactosidase A activity in leukocytes**
(DBS and/or whole blood)

Mildly reduced

Normal

* Results obtained by DBS need confirmation by standard laboratory diagnostic procedures

Review

Hypertrophic cardiomyopathy: The need for randomized trials

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ABSTRACT

Hypertrophic cardiomyopathy (HCM) is a complex cardiac condition characterized by variable degrees of asymmetric left ventricular (LV) hypertrophy, generally associated with mutations in sarcomere protein genes. While generally perceived as rare, HCM is the most common genetic heart disease with over one million affected individuals in Europe alone and represents a prevalent cause of sudden cardiac death in the young. To date, HCM remains an orphan disease, as recommended treatment strategies are based on the empirical use of old drugs with little evidence supporting their clinical benefit in this context. In the six decades since the original description of the disease, less than fifty pharmacological studies have been performed in HCM patients, enrolling little over 2,000 HCM patients, mostly comprising small non-randomized cohorts. No specific agent has been convincingly shown to affect outcome, and critical issues such as prevention of myocardial energy depletion, microvascular ischemia, progressive myocardial fibrosis and the peculiar mechanisms of arrhythmogenesis in HCM still need to be addressed in a systematic fashion. However, there is increasing evidence that a variety of drugs may counter the effects of sarcomere protein mutations and the resulting pathophysiological abnormalities at the molecular, cellular and organ level. Following major advances in our understanding of HCM and increasing opportunities for networking among large international referral centres, the opportunity now exists to identify potentially effective treatments and implement adequately designed pharmacological trials, with the ultimate aim to impact the natural course of the disease, alleviate symptoms and improve quality of life in our patients.

Keywords: hypertrophic cardiomyopathy, translational research, pharmacological therapy, clinical trials, outcome

Figure 1. Levels of therapeutic intervention: towards the core.

Imagining a disease as a fruit or a planet, there are several levels at which one can intervene with any therapeutic approach (Figure 1). The first and most obvious is to simply scratch the surface and control symptoms. This objective can be achieved in most cardiac patients: however, it is the very least that we can do. The second step is to interfere and possibly halt disease progression, thus preventing its consequences on outcome: this can be done in several cardiac conditions, but is definitely harder to achieve.[1,2] Third, we can try to prevent the development of full-blown disease in patients who are predisposed due to acquired risk factors and/or genetic substrate.[3] And fourth, we can address the core of the problem by acting directly on the etiology, removing the actual cause and ultimately cure the patient.[1,4] Despite extraordinary progress over the last decades, these last two steps have hardly if ever been achieved in cardiovascular medicine.

As a consequence, it is important to realize that our practice is based on highly sophisticated palliation. What this approach usually does is change a disease into a milder one. For example, septal myectomy turns obstructive into non-obstructive hypertrophic cardiomyopathy (HCM), a highly significant change for the patient.[5,6] Therefore, this kind of very effective palliation is something we should definitely keep on doing and improving until a cure becomes available.[2] However, all efforts should be directed at improving the state of things by accumulating new evidence and knowledge.[4,7]

HYPERTROPHIC CARDIOMYOPATHY AS AN ORPHAN DISEASE

HCM is a complex cardiac condition characterized by variable degrees of left ventricular (LV) hypertrophy occurring in the absence of secondary triggers, generally associated with mutations in sarcomeric protein genes.[2,5] Although perceived as rare, HCM is the most common genetic heart disease, as well as a prevalent cause of sudden cardiac death in the young. Based on the reported prevalence of 1:500 in the general population, a total estimate exceeding one million individuals with HCM are expected in Europe alone. HCM is characterized by a very complex pathophysiological background that accounts for the heterogeneity of its clinical manifestations and natural history.[5] Established features of the disease, besides left ventricular (LV) hypertrophy, include a constellation of deranged cardiomyocyte energetics, diastolic dysfunction, microvascular ischemia, enhanced myocardial fibrosis, autonomic dysfunction and enhanced arrhythmogenesis.[8,9] In addition, most patients exhibit dynamic LV outflow tract obstruction either at rest or with physiological provocation.[10] Outflow obstruction is a major determinant of symptoms, such as dyspnea, chest pain or presyncope and, together with sudden death prevention, has represented the most visible and consistent target of therapeutic efforts in HCM.[2,5 – 7]

Despite decades of increasing attention and research efforts by the scientific community, treatment strategies for HCM remain largely based on a small number of clinical studies, or empirically based on personal experience or extrapolation from other cardiac conditions. As stated in the recent Report of the Working Group of the National Heart, Lung, and Blood Institute on Research Priorities in Hypertrophic Cardiomyopathy,[7] "nearly 50 years after the identification of HCM as an autosomal dominant disease, and 20 years after its linkage to sarcomeric protein mutations, we still do not understand the most proximal mechanism(s) that initiates the disorder"; and "treatment recommendations in HCM are based on observational series without prospective randomized controls. While clinical usage provides support that various pharmacologic agents reduce HCM symptoms, no evidence has demonstrated that they alter disease progression or outcomes."

In a recent review of original articles, reviews and editorials addressing any pharmacological agent ever used in HCM cohorts, only forty-five studies were identifiedover the last sixty years (i.e. less than 1 per year), enrolling a total of 2,121 HCM patients.[4] Of these, only 5 were randomized double blind

placebo-controlled trials. Remarkably, a comparison of the period 1991–2011 vs. 1971–1990 demonstrated no increase in the number of studies, and only a modest increase in the number of patients enrolled (627 vs. 1,473, patients respectively). With regard to sample size, only 7 studies (15%) enrolled more than 50 patients, whereas 22 (49%) had less than 20 patients.[4] The maximum number of patients in a single prospective study was 118, in a multicenter registry evaluating the efficacy and safety of disopyramide.[11] Overall, these data eloquently demonstrate how poorly pharmacological research in HCM compares with that performed in other, more prevalent conditions such as coronary artery disease and heart failure.[1]

Notably, when randomized trials have been performed in HCM, results have been far less intuitive than expected. The story of dual-chamber pacing for control of LV outflow obstruction is certainly the best case in point. Based on anecdotal observations showing an effect of LV pacing on obstruction, several and often intriguing pathophysiological explanations were provided, leading to three randomized trials. All three showed that pacing had in fact little if any effect on the gradient or exercise capacity in most patients, and that any improvement in symptoms was largely related to a placebo effect of the device.[5,12] Thus, we should be aware that several widely accepted recommendations for management of HCM based on expert opinion or case series still need to stand the test of properly designed trials.

INHERENT CHALLENGES OF CLINICAL TRIALS IN HCM

Several reasons – some obvious, other less so – stand behind this state of things. The first lies with the practical challenges inherent in designing trials with HCM patients (Table 1). The epidemiology of HCM is complex and as yet only partially resolved, due to issues such as incomplete penetrance and prevalence of subclinical disease.[3,13] Despite not being rare, HCM is uncommonly encountered and possibly neglected at many community-based cardiac centers and outpatient clinics. Furthermore, even when overt and correctly diagnosed, its clinical spectrum is highly heterogeneous, encompassing different stages that may not be directly comparable.[9] A preventive trial in genotype-positive/phenotype-negative individuals will necessarily enroll subsets that are different from HCM patients with classic phenotype and dynamic outflow obstruction, or in the end-stage phase. Each research question should be addressed by targeting the appropriate patient subgroups, with imaginable problems in achieving the desired yield in any given cohort.[7,9]

Another very complex issue concerns the assessment of outcome in HCM, and the effects of treatment on survival. Even at tertiary referral centers, cardiovascular mortality rates in contemporary HCM cohorts are very low, i.e. 1–2%/year, although the selected subset with systolic dysfunction and end-stage progression may exceed 10%/year.[2,5,9,14] Rates of sudden cardiac death, formerly believed to be very common among HCM patients, are even lower, i.e. <1% in most cohorts and only 3%/year even in patients carrying implantable defibrillators because judged to have a "high-risk" status.[15] Based on these estimates, large patient cohorts and extended follow-up duration are required to detect differences in survival, even when a highly effective drug or procedure is tested. Thus, the relatively favorable long-term prognosis of most HCM patients may represent a formidable obstacle to clinical trials targeting outcome, including a hypothetical study comparing surgical myectomy and percutaneous alcohol septal ablation.[16]

In order to overcome the limitations associated with the low hard event rates, surrogate end-points have often been employed in HCM trials, including indexes of LV performance, functional capacity and oxygen consumption, progression of symptoms, prevalence of arrhythmias and appropriate

Table 1. Challenges for clinical trials in HCM.

Relatively low prevalence (1:500)	Need for adequate networking, hard to perform single-center trials
Heterogeneous disease spectrum	Need to tailor trials to specific subsets, hard to include all patients in a single study
Low event rates	Difficult to power studies to assess differences in survival/hard outcome end-points
Surrogate end-points	End-points such as oxygen consumption, variation in symptoms, regression of LV hypertrophy or arrhythmias may be difficult to define and/or have un uncertain relation to outcome
Complex, unresolved pathophysiology	Need to identify appropriate targets for treatment
Perceived as economically irrelevant to the pharmaceutical industry	However, this is a wrong perception based on absolute number of patients and need for very extended treatment

defibrillator shocks, circulating markers of collagen synthesis and natriuretic peptides.[4,13,17–19] Advanced imaging techniques such as positron emission tomography (PET) and cardiac magnetic resonance (CMR) can be exploited today to quantify the effect of novel treatments on relevant features including coronary microvascular dysfunction and tissue fibrosis, respectively.[13,20–23] Each of these may represent reasonable end-points in certain contexts, particularly in proof-of-concept and pilot studies. Furthermore, pilot studies based on these surrogate end-points might provide the rationale and justify the effort of large, multicenter, prospective studies enrolling the number of patients necessary to determine the benefit of novel treatments on more robust measures of outcome.[4,7] At the same time, the favourable long-term prognosis associated with HCM identifies control of symptoms and improvement in quality of life as major objectives for pharmacological treatment, to be pursued and investigated independent of outcome.[4]

Last but not least, HCM has never been the focus of strong economic interest by pharmaceutical companies, because of the perceived rarity of the disease as well as the lack of novel and expensive therapeutic agents such as have been approved, for example, in rare conditions such as Fabry disease or pulmonary arterial hypertension.[24] This aspect, however, may be expected to change soon. In recent years, the widespread use of imaging and genetic testing for familial screening, as well as an increasing awareness in the medical community, have led to the identification of large HCM populations, regularly followed at international referral centers.[2,4] For the first time in the history of this disease, it has become feasible to address HCM management issues in an evidence-based fashion, by means of adequately designed clinical trials. The full exploitation of this potential may lead to a paradigm shift in research methodology involving the whole field of genetic heart diseases. Such favorable conjunction is beginning to attract the interest of private companies, promoting investments that are likely to reach a critical mass for effective clinical research in a very near future.[4,25] When this time comes, the powerful combination of basic science, clinical knowledge and adequate trial methodology may finally come to fruition.

POTENTIAL TARGETS OF PHARMACOLOGIC TREATMENT

Progress in HCM treatment is hindered by the incomplete knowledge of its pathophysiologic mechanisms and lack of specific agents capable of interfering with disease-causing pathways.[7–9] However, several targets for treatment have been identified (Figure 2), some of which overlap with other cardiac diseases and with heart failure at large.[8,26] For example, interventions aimed at normalizing energy homeostasis represent a viable approach, as shown by a recent study on perhexiline, a metabolic modulator which inhibits the metabolism of free fatty acids and enhances carbohydrate utilization by the cardiomyocyte. In a recent randomized double-blind placebo-controlled trial, perhexilinehas have shown the capacity to improve the energetic profile of the LV, resulting in improved diastolic function and exercise capacity in HCM patients.[18]

Furthermore, HCM cardiomyocytes exhibit with well-established abnormalities in intracellular calcium handling, contributing to excessive energy expenditure and enhanced arrhythmogenesis that are largely due to enhanced membrane late sodium current.[26] Such defect may be selectively and dramatically reversed *in vitro* by ranolazine.[26] Following the demonstration of its beneficial effects on HCM cardiomyocytes, a multi-center, double blind, placebo-controlled pilot study is currently underway in Europe to test the efficacy of ranolazine on exercise tolerance and diastolic function in symptomatic HCM patients (RESTYLE-HCM; EUDRA-CT 2011-004507-20). Besides the specific merits of ranolazine, similar examples of translational approach to HCM identifies a fundamental pre-requisite for the identification of novel, potentially effective agents, based on thorough investigation of the molecular basis of the disease.[1] In the future, a more specific approach may be tailored to specific mutations or groups of mutations, by screening large panels of candidate molecules in assays based on induced pluripotent stem cells isolated from human fibroblasts.[27] As shown recently, the possibility of modulating the activity of sarcomere contractile proteins, such as beta-myosin, is beginning to surface, with huge potential implications for HCM treatment.[28]

Finally, an option that is less seductive but should not be underestimated, is to investigate potential role in HCM patients of drugs that represent the standard of care for other cardiac patients. Agents such as modulators of the renin-angiotensin system, statins and calcium antagonists have all shown promise in preventing development of LV hypertrophy in genotype positive individuals,[3] and may potentially normalize cellular homeostasis, prevent microvascular remodeling and ischemia, reduce fibrosis and prevent end-stage progression.[4,8,9,17,21,22] However, the indications, timing and doses of
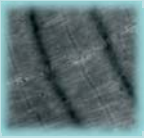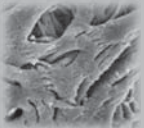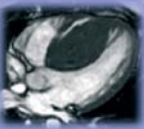
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LEVEL OF INTERVENTION	TARGET	GOAL
MOLECULAR	Abnormal Sarcomere Function Enhanced Ca^{++} Sensitivity Unfavourable EP Remodeling	Improve Contractile Efficiency Inhibit Triggers for LVH Reduce Arrhythmogesis
CELLULAR	Deranged Energy Homeostasis Disturbed Biomechanical Stress Sensing Abnormal Ca^{++} Handling	Prevent Energy Depletion Prevent Hypertrophy/Apoptosis Control Collagen Synthesis
ORGAN	Microvascular remodelling Myocardial Fibrosis Diastolic Dysfunction Reentry Arrhythmias	Reduce Myocardial Ischemia Preserve Systolic Function Improve Diastole Prevent Arrhythmias

Figure 2. Therapeutic targets and goals in HCM.

these treatments remain unresolved in HCM, and their efficacy in impacting its natural history remains untested. Likewise, the effects of betablockers and antiarrhythmics, such as amiodarone, in preventing appropriate defibrillator shocks and sudden cardiac death has only been addressed retrospectively and inconclusively.[29] As noted above, many recommendations present in current HCM guidelines still require adequate evidence to their support, including several that few would dare question. And while it is unrealistic to expect that each of these indications will become evidence-based in the near future, it is the change of attitude that really matters, from passive acceptance of the empirical to uncompromising quest for the methodologically appropriate and convincing.[2,4,7]

CONCLUSIONS

HCM is not uncommon, but remains an orphan disease with regard to pharmacological treatment. In the era of translational research, physicians and researchers have been very good at asking the right questions, but need to direct more effort into finding the best clinical answers for HCM patients. In a broader perspective, cardiomyopathies should be seen as paradigms providing invaluable insights on disease mechanisms that may be of general relevance to patients with more prevalent cardiac conditions. What has long been considered impossible to achieve is now closer at hand: the time is now ripe to promote robust clinical research in this complex condition in order to advance and standardize management of HCM patients.

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Trattamento dei sintomi refrattari nella cardiomiopatia ipertrofica con fisiopatologia restrittiva: nuove prospettive per la ranolazina

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The management of patients with hypertrophic cardiomyopathy (HCM) and refractory symptoms due to massive hypertrophy and severe diastolic dysfunction represents a real challenge for the clinical cardiologist. Such patients often require novel therapeutic approaches, both invasive and pharmacological, involving multidisciplinary teamwork; however, the implementation of potentially viable treatment options is hindered by lack of disease-specific evidence.

We report the case of a young woman with severe HCM and restrictive physiology, who underwent extensive myectomy via the transaortic and transapical approach, followed by biventricular pacing for cardiac resynchronization, with significant but incomplete symptomatic improvement. The subsequent introduction of ranolazine, based on promising preclinical data, has led to an excellent final result. An ongoing randomized clinical trial is currently testing the efficacy of ranolazine in symptomatic HCM.

Key words. Angina; Hypertrophic cardiomyopathy; Massive hypertrophy; Ranolazine; Restrictive cardiomyopathy.

La cardiomiopatia ipertrofica (CMI) è la più frequente malattia genetica del muscolo cardiaco, con una prevalenza di 1:500 nella popolazione generale[1] ed è caratterizzata da espressione morfologica e decorso clinico estremamente eterogenei[2]. In un sottogruppo di pazienti con CMI, ad espressione fenotipica estrema, si riscontrano gradi molto marcati di ipertrofia associata a ridotte dimensioni cavitarie del ventricolo sinistro (VS) e fisiopatologia restrittiva. Tali pazienti presentano quasi sempre sintomi limitanti, quali dispnea o angina per sforzi anche lievi, e sono di difficile gestione clinica[3,4]. Le strategie terapeutiche vengono rese ancor più complesse dalla mancanza di evidenze scientifiche specifiche per la malattia[5].

Viene qui presentato un caso clinico, la cui gestione è risultata particolarmente impegnativa ed ha richiesto nel tempo il ricorso ad approcci innovativi, sia da un punto di vista chirurgico che farmacologico. Il caso rappresenta un buon esempio di come, per una corretta gestione del paziente con cardiomiopatia, sia necessario un team davvero multidisciplinare.

CASO CLINICO

La paziente è una giovane donna giunta alla nostra osservazione all'età di 30 anni, in seguito alla diagnosi di CMI, sulla base di accertamenti per limitazione funzionale. Alla prima valutazione presso il nostro centro riferiva palpitazioni, astenia, dolore toracico e dispnea da sforzo inquadrabile in una classe NYHA III, di intensità molto variabile a seconda dei giorni, ma abbastanza marcati da impedire lo svolgimento delle normali attività lavorative nel suo negozio (Figura 1). L'esame obiettivo si caratterizzava per bassi valori pressori a riposo (100/55 mmHg) ed un terzo tono, in assenza di soffi patologici o segni di scompenso. L'ECG a 12 derivazioni mostrava ritmo sinusale con ipertrofia e sovraccarico del VS (Figura 2, pannello 1C). All'ecocardiografia era presente un'ipertrofia diffusa del VS di grado marcato, a massima espressione medio-apicale, con spessore massimo 30 mm a livello del setto basale anteriore (Figura 3, pannelli A-C). Il ventricolo sinistro appariva ipercinetico, con una cavità ridotta, a "piede di ballerina" e obliterazione completa dell'apice in sistole. Era presente una grave disfunzione diastolica del VS, con *pattern* del flusso transmitralico restrittivo (Figura 2, pannello 1B; Figura 3, pannelli D). I lembi mitralici erano ridondanti, con accenno a movimento sistolico anteriore, ma non erano misurabili gradienti sistolici al tratto di efflusso né in sede medio-ventricolare. Si riscontravano inoltre una insufficienza mitralica centrale, di grado lieve, ed un'ipertensione polmonare moderata (Figura 2, pannello A1; Figura 3, pannelli D1-D2). In tale occasione venne iniziata una terapia farmacologica con na-
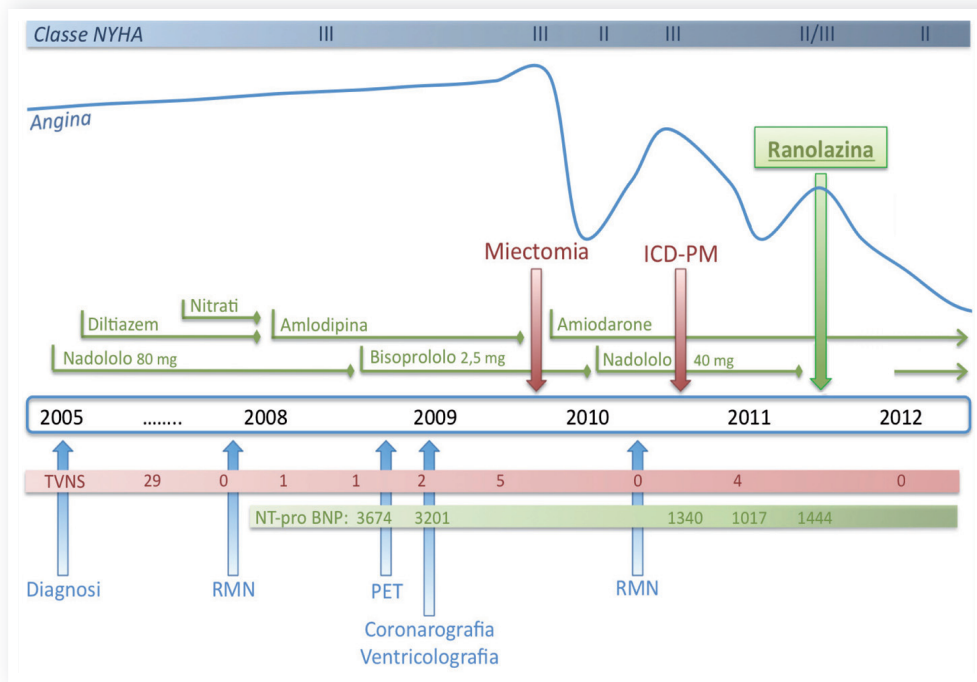
Figura 1. Decorso clinico ed interventi terapeutici. *Dall'alto in basso:* classe funzionale NYHA per la dispnea, angina, terapie (farmacologica e chirurgica), episodi di TVNS (registrati all'ECG dinamico o al controllo ICD), livelli di NT-proBNP ed esami strumentali.

ICD, defibrillatore impiantabile; NT-proBNP, porzione N-terminale del propeptide natriuretico di tipo B; PET, tomografia ad emissione di positroni; PM, pacemaker; RMN, risonanza magnetica nucleare; TVNS, tachicardia ventricolare non sostenuta.
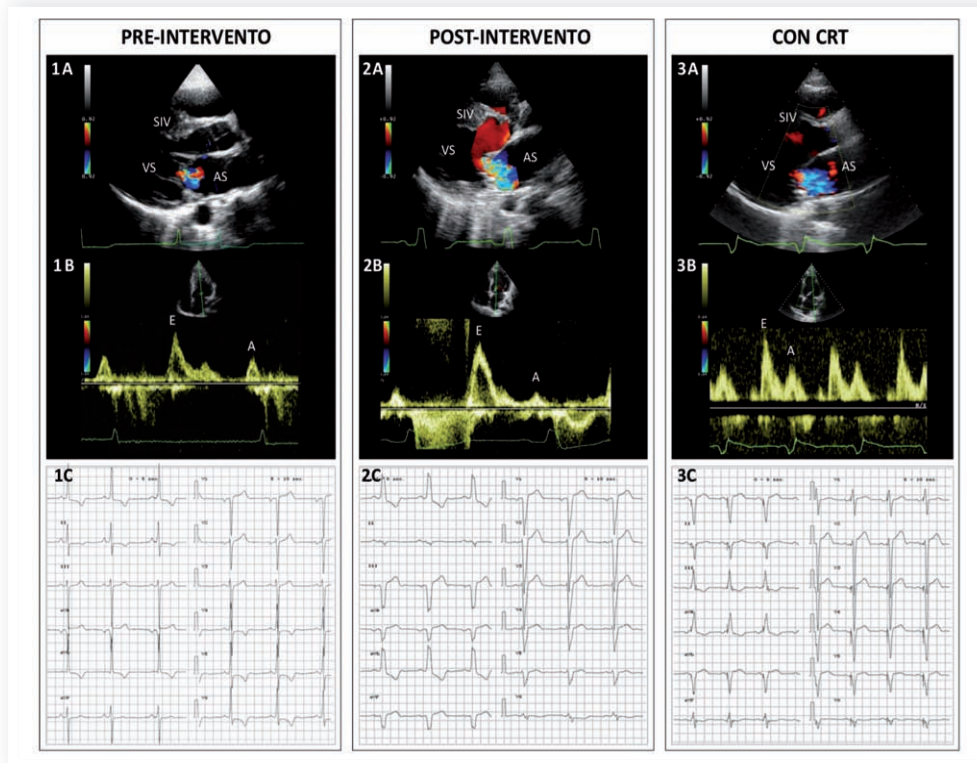

Figura 2. Quadro elettrocardiografico ed ecocardiografico alla valutazione preoperatoria, postoperatoria e dopo terapia di resincronizzazione cardiaca (CRT). *Pannello 1:* alla prima valutazione è presente un'insufficienza mitralica di grado lieve, una disfunzione diastolica severa ed un quadro elettrocardiografico di ipertrofia e sovraccarico del ventricolo sinistro. *Pannello 2:* a 6 mesi dall'intervento di miectomia si riscontra un'insufficienza mitralica di grado marcato con dilatazione della cavità ventricolare sinistra, *pattern* diastolico restrittivo sostanzialmente invariato. L'ECG presenta ritmo sinusale con blocco di branca sinistra. *Pannello 3:* 3 mesi dopo impianto di pacemaker biventricolare si osserva una netta riduzione del grado di insufficienza mitralica, con miglioramento del *pattern* diastolico. L'ECG conferma il buon grado di resincronizzazione.

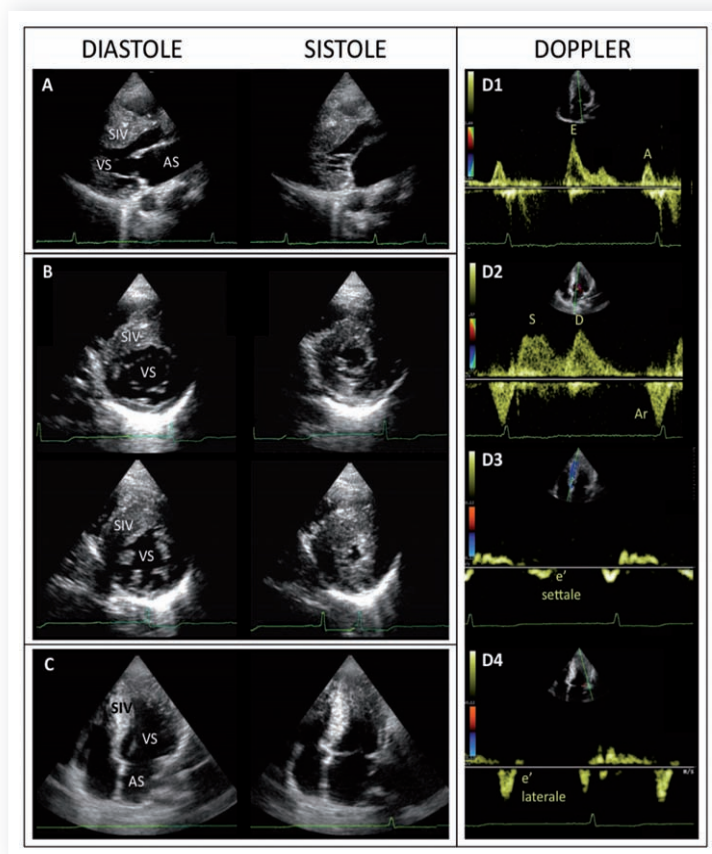AS, atrio sinistro; SIV, setto interventricolare; VS, ventricolo sinistro.

Figura 3. Ecocardiogramma preoperatorio. Proiezione parasternale asse lungo (*A*), asse corto (*B*) e apicale 4 camere (*C*). È presente ipertrofia severa e diffusa, con spessore massimo a livello del setto medio. Si noti la completa obliterazione della cavità in sistole nelle varie proiezioni e l'iper-riflettenza irregolare del setto interventricolare. L'analisi Doppler del flusso mitralico mostra una disfunzione diastolica severa, con *pattern* restrittivo ad andamento trifasico (*D1*) e notevole riduzione della velocità dell'anulus mitralico al Doppler tissutale (*D3-D4*). Il flusso delle vene polmonari è alterato, con una *A reverse* prominente (*D2*).
AS, atrio sinistro; SIV, setto interventricolare; VS, ventricolo sinistro.

dololo, titolato fino alla massima dose tollerata di 80 mg/die, ottenendo una parziale remissione dei sintomi.

Nel corso di controlli successivi, una tomografia ad emissione di positroni mostrava una disfunzione microvascolare diffusa, complessivamente di grado lieve (flusso medio per l'intero VS dopo dipiridamolo 2.6 ml/min/g), ma con aree settali marcatamente ipoperfuse; alla risonanza magnetica cardiaca veniva confermata l'ipertrofia massiva con obliterazione medio-apicale (Figura 4); erano inoltre presenti estese aree di impregnazione tardiva con gadolinio, suggestive per fibrosi miocardica. Un eco-color-Doppler cardiaco da sforzo, in terapia, non evidenziava gradienti significativi in sede subaortica o medio-ventricolare; venivano evidenziati una ridotta capacità funzionale (test interrotto per dispnea al termine 100 W), anche se con adeguato incremento pressorio (90/60 mmHg al basale, 160/80 mmHg al picco). La paziente presentava inoltre frequenti salve di tachicardia ventricolare non sostenuta all'ECG dinamico e valori della porzione N-terminale del propeptide natriuretico di tipo B (NT-proBNP) ripetutamente elevati (Figura 1). Infine, all'esame genetico veniva documentata una doppia mutazione in eterozigosi sul gene della proteina C legante la miosina (Figura 5).

In seguito a peggioramento della dispnea e soprattutto dell'angina, la terapia farmacologica è stata progressivamente potenziata; nel corso di un follow-up di circa 5 anni sono stati ag-

giunti al nadololo sia calcioantagonisti che nitrati transdermici (Figura 1), con modesti risultati. All'età di 35 anni, in seguito ad aggravamento dei sintomi, la paziente veniva ricoverata per ulteriori accertamenti. Una coronarografia mostrava vasi epicardici indenni da lesioni, con modesto *bridging* miocardico al tratto medio dell'arteria interventricolare anteriore, di dubbio significato clinico; alla ventricolografia veniva confermata la grave riduzione di cavità del VS, con obliterazione sistolica dell'apice *in toto* (Figura 6).

Data la gravità del quadro clinico e la refrattarietà dei sintomi alla terapia medica, venne proposta alla paziente la possibilità di un intervento di miectomia estesa, allo scopo di aumentare le dimensioni del VS e la gittata sistolica. In assenza di indicazioni "classiche" all'intervento, tale proposta era basata su una positiva esperienza, maturata presso questo ed altri centri, nel trattamento delle forme di CMI con ostruzione medio-ventricolare, che presentano una fisiopatologia per molti aspetti simili al caso in questione[6]. La paziente è stata quindi operata nel 2009 dal prof. Magdi Yacoub e dall'equipe del dr. Pierluigi Stefano, mediante un approccio combinato per via transaortica e transapicale. Quest'ultimo, mediante incisione a "bocca di pesce" dell'apice ventricolare, ha permesso di rimuovere porzioni di miocardio a livello del VS medio-apicale, con significativo ampliamento della cavità (Figura 4). Il decorso postoperatorio è stato complicato da fibrillazione atriale parossistica, per cui è stata
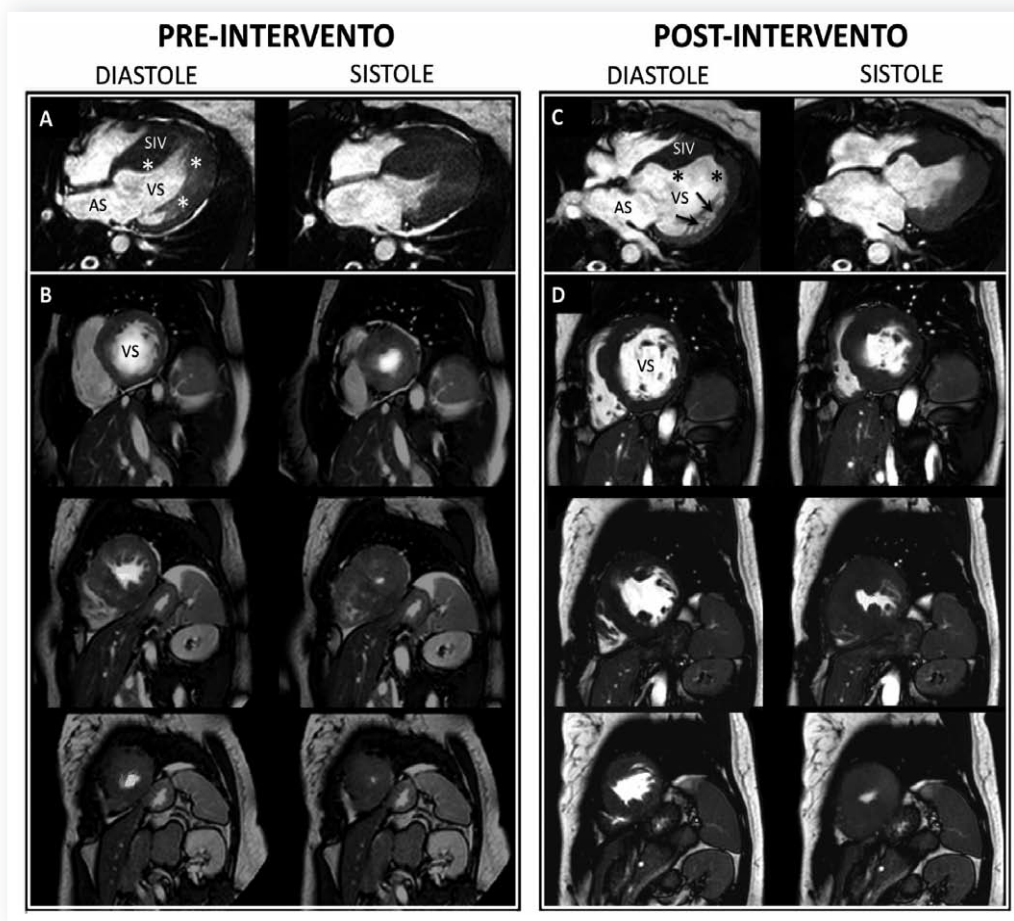
Figura 4. Risonanza magnetica nucleare pre- e postoperatoria. Immagini di risonanza magnetica nucleare ottenute con sequenze cine in telediastole e telesistole, 2 mesi prima (*A, B*) e 8 mesi dopo la miectomia (*C, D*). *A* e *C:* le immagini in 4 camere mostrano l'importante ispessimento del setto interventricolare e della parete laterale medioapicale. La rimozione di miocardio a livello del setto e della parete postero-laterale (*) mediante miectomia ha permesso la creazione di una cavità ventricolare. Evidente in *C* il rimodellamento post-chirurgico della parete laterale (*frecce*). *B* e *D:* sezione coronale asse corto a livello della valvola mitrale e dei muscoli papillari: è evidente l'obliterazione sistolica a livello medio-apicale prima dell'intervento (*B*).
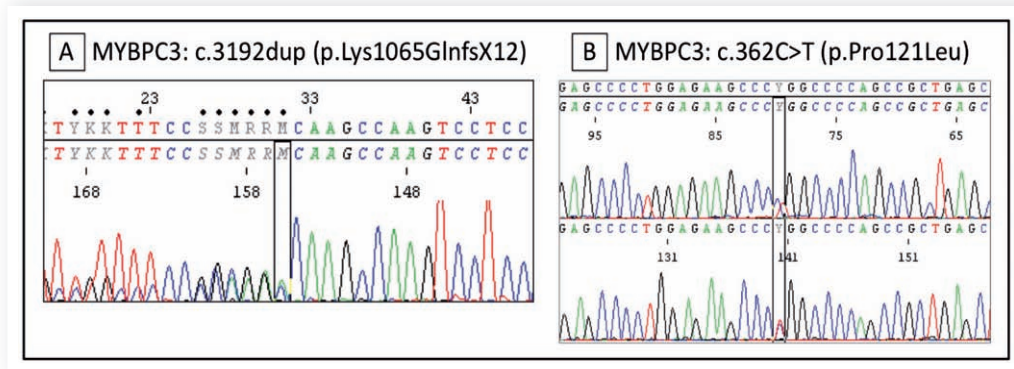AS, atrio sinistro; SIV, setto interventricolare; VS, ventricolo sinistro.

Figura 5. Elettroferogramma della sequenza del DNA che mostra la presenza di un genotipo complesso, caratterizzato da due mutazioni nello stesso gene (*MYBPC3*, proteina C legante la miosina). I *rettangoli* indicano la posizione della mutazione nella sequenza. *A:* mutazione di *frameshift* caratterizzata dalla duplicazione di una singola base che ha come conseguenza la creazione di un codone di stop a valle. La sintesi della corrispondente proteina si arresta prematuramente. *B:* mutazione *missense* caratterizzata dalla sostituzione di una singola base (citosina) con un'altra (timina) che comporta a livello della proteina il cambiamento dell'aminoacido prolina con leucina.

iniziata terapia con amiodarone. L'ecocardiogramma alla dimissione confermava l'ottimo esito dell'intervento, con un volume ventricolare residuo ora ai limiti della norma per dimensioni corporee, una funzione sistolica globale conservata ed una insufficienza mitralica lieve. A poche settimane dall'intervento, la paziente riferiva netto miglioramento della dispnea (classe funzionale NYHA II stabile), associato a riduzione dei valori di NT-proBNP (Figura 1).
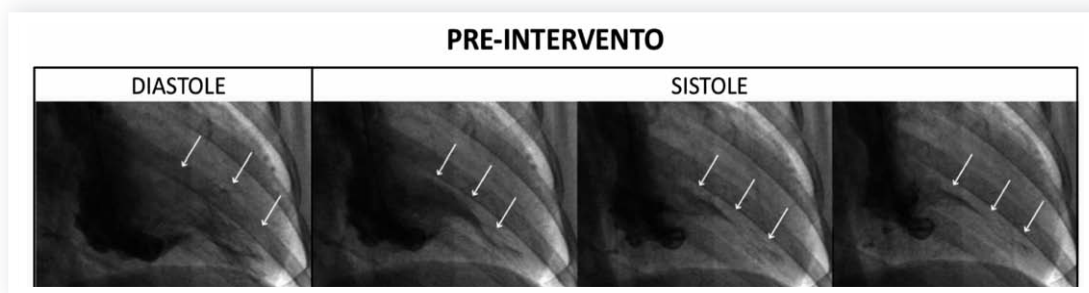
Figura 6. Ventricolografia preoperatoria. La ventricolografia mostra la riduzione della cavità ventricolare sinistra durante la sistole, che appare molto ridotta (*frecce*).

Dopo circa 6 mesi veniva effettuato un nuovo controllo clinico per significativa riaccentuazione della dispnea nelle ultime settimane. Veniva evidenziato un aumento spiccato del grado di insufficienza mitralica, attribuibile a rimodellamento della parete laterale, che si associava a dilatazione dell'anulus, ipomobilità e *tethering* del lembo mitralico posteriore (Figura 2, pannelli 2A-C). Veniva inoltre ipotizzato che la presenza di un'asincronia marcata del VS, secondaria al blocco di branca sinistra postoperatorio, costituisse un fattore aggravante della valvulopatia. Negli anni era stata più volte discussa con la paziente l'indicazione ad un defibrillatore impiantabile in prevenzione primaria, dati i numerosi fattori di rischio presenti. Venne pertanto proposto di procedere all'impianto di un dispositivo biventricolare, con il razionale che la terapia di resincronizzazione potesse migliorare il grado di insufficienza mitralica[7], come confermato dal successivo decorso. A 3 mesi dall'impianto del dispositivo, l'insufficienza mitralica era solo di poco superiore a quella pre-miectomia, stimabile di grado moderato (Figura 2, pannelli 3A-C), e si rifletteva in un notevole miglioramento della dispnea. Restava invece invariata la severità dell'angina, sia come intensità che come frequenza degli episodi.

Nel tentativo di potenziare in modo massimale la terapia medica, data la scarsa tolleranza ai betabloccanti ed un'intolleranza assoluta agli inibitori dell'enzima di conversione dell'angiotensina, veniva aggiunto al trattamento in atto (nadololo a basse dosi ed amiodarone), una dose scalare di ranolazina. Tale raccomandazione si basava sulla letteratura disponibile per la cardiopatia ischemica[8,9] e sugli ottimi risultati preliminari ottenuti *in vitro* su campioni di miectomia di pazienti con CMI, compreso quello della paziente (Figura 7). Il farmaco è stato titolato fino ad un dosaggio di 1000 mg 2 volte/die, ben tollerato eccetto che per disturbi gastrointestinali lievi, che si sono attenutati dopo le prime somministrazioni. A pochi giorni dall'assunzione della dose piena di ranolazina la paziente riferiva un netto miglioramento dei sintomi, con scomparsa degli episodi anginosi durante le normali attività quotidiane e gli sforzi moderati. Non si sono osservate variazioni significative del QTc. Nei 6 mesi successivi la paziente è rimasta stabilmente in classe funzionale NYHA II, con buona qualità di vita, mantenendo invariata una terapia a base di nadololo e ranolazina.

COMMENTI E IMPLICAZIONI CLINICHE

Il caso descritto, fortunatamente raro nella sua complessità, presenta numerosi spunti di interesse. Anzitutto, un grado massivo di ipertrofia nel giovane con CMI si associa molto spesso a vari altri marcatori di gravità della malattia, quali un genotipo complesso[10], disfunzione microvascolare e fibrosi del VS, aritmie
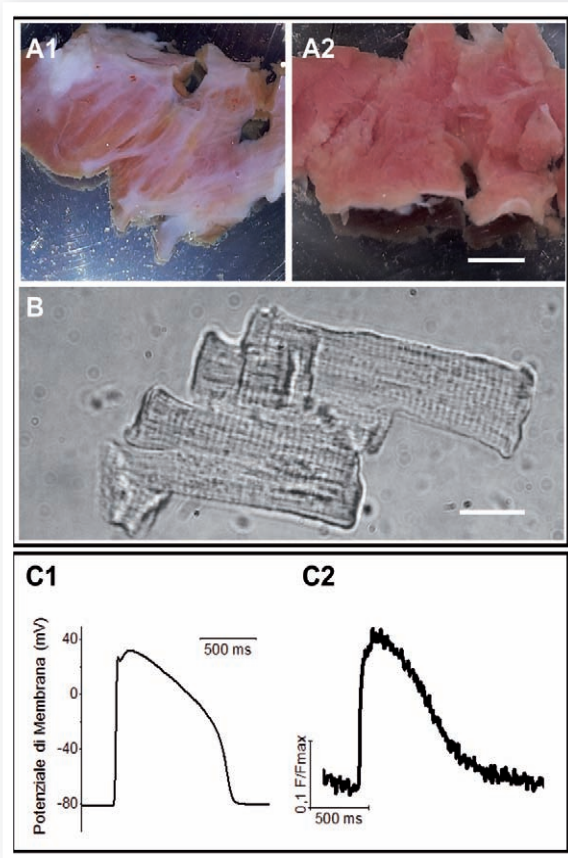

Figura 7. Misure del potenziale d'azione e del transiente di calcio intracellulare sui cardiomiociti isolati al momento della miectomia. *A:* pezzo chirurgico di miectomia settale della paziente. Il pezzo è mostrato sul lato endocardico (*A1*), dove è possibile apprezzare ampie zone di fibrosi, e nella sua porzione profonda (*A2*). La barra corrisponde a 3 mm. *B:* tramite digestione enzimatica del pezzo chirurgico sono stati ottenuti cardiomiociti ventricolari isolati, mostrati nell'immagine fotomicroscopica in figura. La barra corrisponde a 20 µm. *C:* le cellule sono state utilizzate per simultanee misure di *patch-clamp* e calcio intracellulare (previo caricamento con il colorante fluo-4). Il *pannello C1* mostra un potenziale d'azione ottenuto da una cellula della paziente, nettamente prolungato rispetto a un normale potenziale d'azione ventricolare; il *pannello C2* mostra un transiente di calcio ottenuto dalla stessa cellula, anch'esso di durata aumentata. L'esposizione al farmaco accorcia la durata del potenziale d'azione e del transiente di calcio, con potenziali benefici sulla funzione miocardica.

ventricolari all'ECG dinamico, elevati livelli di biomarcatori e disfunzione diastolica di grado severo con *pattern* restrittivo, tutti indicatori di prognosi avversa. Spesso si osserva anche un ridotto incremento pressorio allo sforzo, non presente in questo

caso, che esprime il precario equilibrio emodinamico di questi pazienti durante l'esercizio fisico. Sul piano clinico, l'esasperata espressione fenotipica e fisiopatologica della malattia si traduce nella comparsa di una sintomatologia severa, in particolare angina e dispnea da sforzo, causati dal notevole aumento delle pressioni di riempimento, dall'assenza di cavità con riduzione della gittata sistolica e dalla disfunzione microvascolare. I sintomi, limitanti e difficili da trattare, si collocano nel contesto di un progressivo rimodellamento del VS, caratterizzato da gradi crescenti di fibrosi alla risonanza magnetica cardiaca[3]. Alcuni studi italiani hanno dimostrato che un *pattern* di riempimento restrittivo del VS nella CMI è un fattore predittivo forte e indipendente per l'evoluzione verso la fase terminale e lo scompenso refrattario[4,11].

In casi di questa gravità una terapia farmacologica standard, basata su betabloccanti e calcioantagonisti, è raramente in grado di raggiungere un adeguato controllo dei sintomi. Inoltre, i pazienti con fisiopatologia restrittiva tendono a non tollerare dosi massimali di questi farmaci e sono notoriamente intolleranti ai diuretici. D'altra parte il trattamento chirurgico di queste forme è un territorio ancora inesplorato. Appaiono invece promettenti i risultati ottenuti nella risoluzione chirurgica dell'ostruzione medio-ventricolare nella CMI[12,13], un intervento tecnicamente complesso, che, se eseguito in centri altamente specializzati, può portare ad ottimi risultati a distanza. Nel nostro caso era di fatto presente la fisiopatologia dell'ostruzione medio-ventricolare (e cioè il contatto sistolico di papillari anomali con il setto interventricolare), anche se la totale obliterazione dell'apice del VS non consentiva lo sviluppo di gradienti a questo livello. Il razionale per l'impiego di una miectomia nella paziente presentata appariva quindi promettente, purché associato ad un gesto specificamente rivolto alla "creazione" di volume intraventricolare. La difficoltà di ottenere tale risultato senza interferire con la meccanica del VS è ben esemplificata dalla complicanza osservata nel breve termine: un eccessivo rimodellamento della parete laterale, secondario alla miectomia, ha infatti prodotto una perdita di coaptazione dei lembi mitralici, dalle conseguenze potenzialmente gravi. Fortunatamente, il ricorso ad una terapia di resincronizzazione, anch'essa suggerita dalla letteratura[7], ha permesso di correggere in buona parte il problema. Il decorso postoperatorio della nostra paziente pone tuttavia l'accento sulla necessità di approfondire le nostre conoscenze sulla fisiopatologia di questa condizione, per standardizzare un intervento chirurgico risolutivo e privo di danni collaterali. Le nuove possibilità offerte dall'imaging contemporaneo[14] possono consentirci oggi un tale risultato.

Razionale dell'utilizzo della ranolazina in pazienti con cardiomiopatia ipertrofica

Le terapie farmacologiche impiegate nella CMI si basano su farmaci antichi, utilizzati in modo empirico, e privi di azioni specifiche sui meccanismi fisiopatologici peculiari della malattia[2].

Terapie sperimentali, testate su modelli animali, hanno fornito risultati promettenti soprattutto per quanto riguarda la prevenzione dello sviluppo di ipertrofia nei soggetti con genotipo positivo, ma non esistono dati riguardo ad interventi potenzialmente efficaci sui meccanismi molecolari della disfunzione ventricolare sinistra e della progressione di malattia. Un recente studio del gruppo di Oxford ha mostrato dei risultati incoraggianti per quanto riguarda l'efficacia della perexilina sui sintomi e sulla limitazione funzionale nei pazienti con CMI. La perexilina è un farmaco noto da molti anni che svolge un'azione

favorevole dal punto di vista metabolico, spostando il metabolismo cardiomiocitario dalla catena ossidativa alla glicolisi[15-17]. Tale farmaco risulta però poco maneggevole per una tossicità epatica ed una neuropatia dose-dipendente, che rendono necessaria la misurazione della concentrazione ematica del farmaco. Ne appare pertanto improbabile l'uso clinico su vasta scala nel prossimo futuro. Lo studio rappresenta tuttavia un utile *proof of concept*, in quanto ha evidenziato il potenziale effetto favorevole di farmaci metabolici nella CMI.

La ranolazina è un inibitore selettivo della corrente lenta del sodio, che ha dimostrato una notevole efficacia antischemica nei pazienti con malattia coronarica, senza interferire in maniera significativa sui parametri emodinamici, in quanto legata ad una normalizzazione delle elevate concentrazioni di calcio intracitoplasmatico tipica del cardiomiocita ischemico. Oltre all'effetto antischemico la ranolazina ha mostrato un promettente effetto antiaritmico e lusitropo, entrambi oggetto di studi clinici in corso[8,9]. Questi dati fanno ipotizzare un effetto favorevole della ranolazina in una patologia come la CMI, caratterizzata da elevata calcio-sensibilità miocardica ed elevati valori di calcio intracellulare. A conferma di ciò, dati preliminari ottenuti presso il laboratorio di farmacologia dell'Università di Firenze hanno dimostrato che la corrente lenta del sodio è iperattivata nei cardiomiociti umani di pazienti con CMI, provocando un marcato allungamento del potenziale d'azione e la comparsa di post-potenziali tardivi[18]. In tali cellule la ranolazina, a concentrazioni terapeutiche, è in grado di accorciare significativamente il potenziale d'azione e di produrre un netto miglioramento del profilo elettrofisiologico, con scomparsa dei post-potenziali e riduzione del calcio intracitoplasmatico. A livello clinico tali effetti hanno prodotto nella nostra paziente un buon controllo dei sintomi legati ad ischemia microvascolare. Tale riscontro, sebbene aneddotico, rappresenta un risultato promettente per l'impiego del farmaco su più ampia scala in pazienti con CMI. Oltre all'azione antischemica, un possibile effetto sulla disfunzione diastolica e sul rischio aritmico, suggerito dai dati *in vitro*, completano il profilo di un razionale estremamente forte per un impiego clinico della ranolazina in questo contesto.

Seguendo un approccio traslazionale fortemente invocato dalla comunità scientifica internazionale[5], i dati preclinici ottenuti a Firenze sono sfociati in uno studio multicentrico randomizzato europeo, volto a valutare l'effetto della ranolazina su capacità funzionale, biomarcatori e parametri diastolici in pazienti con CMI sintomatica, attualmente in corso. Questo studio rappresenta una delle primissime iniziative metodologicamente solide in questo ambito. Dopo cinque decadi di empirismo appassionato la speranza è che stia iniziando anche per i pazienti con cardiomiopatie genetiche l'era di una medicina basata sull'evidenza.

RIASSUNTO

La gestione dei sintomi nei pazienti con cardiomiopatia ipertrofica (CMI) associata a gradi marcati di ipertrofia, ridotte dimensioni cavitarie del ventricolo sinistro e fisiopatologia restrittiva, rappresenta una vera sfida per il cardiologo clinico. Le strategie terapeutiche vengono rese ancor più complesse dalla mancanza di dati relativi alle opzioni mediche e chirurgiche potenzialmente più idonee per i pazienti con CMI; soprattutto sul piano farmacologico, la malattia è a tutt'oggi da considerarsi orfana di opzioni specifiche e basate sulle evidenze.

Presentiamo il caso di una giovane paziente affetta da CMI con fisiopatologia restrittiva, sottoposta ad un intervento chirurgico di miectomia estesa, con approccio transaortico e transapicale, ed a terapia di resincronizzazione cardiaca, con miglioramento significativo ma incompleto dei sintomi. Il successivo potenziamento della terapia con ranolazina, introdotta sulla base di da-ti preclinici promettenti, ha portato ad un netto miglioramento. È in corso uno studio clinico multicentrico randomizzato per valutare l'efficacia della ranolazina in pazienti sintomatici con CMI.

Parole chiave. Angina; Cardiomiopatia ipertrofica restrittiva; Ipertrofia massiva; Ranolazina.

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Review article

Clinical and molecular classification of cardiomyopathies

Franco Cecchi*, Benedetta Tomberli, Iacopo Olivotto

ABSTRACT

The term "cardiomyopathies" was used for the first time 55 years ago, in 1957. Since then awareness and knowledge of this important and complex group of heart muscle diseases have improved substantially. Over these past five decades a large number of definitions, nomenclature and schemes, have been advanced by experts and consensus panel, which reflect the fast and continued advance of the scientific understanding in the field.

Cardiomyopathies are a heterogeneous group of inherited myocardial diseases, which represent an important cause of disability and adverse outcome. Although considered rare diseases, the overall estimated prevalence of all cardiomyopathies is at least 3% in the general population worldwide. Furthermore, their recognition is increasing due to advances in imaging techniques and greater awareness in both the public and medical community.

Cardiomyopathies represent an ideal translational model of integration between basic and clinical sciences. A multidisciplinary approach is therefore essential in order to ensure their correct diagnosis and management.

In the present work, we aim to provide a concise overview of the historical background, genetic and phenotypic spectrum and evolving concepts leading to the various attempts of cardiomyopathy classifications produced over the decades.

Keywords: classification, cardiomyopathies, myocardial disease

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INTRODUCTION

Cardiomyopathies (CM) are a fascinating group of myocardial diseases, which constitute an important cause of disability and adverse outcome due to heart failure or sudden and unexpected death. Their recognition is increasing due to advances in imaging techniques and greater awareness in the medical community, although the majority of patients are still likely to be undiagnosed or misdiagnosed with more prevalent cardiac conditions. Population cross-sectional studies show that the overall estimated prevalence of all cardiomyopathies is at least 3% in the general population worldwide. They are often inherited heart muscle diseases, generally with an autosomic dominant, more rarely recessive or X-linked transmission. As a variety of gene abnormalities are identified as the cause of cardiomyopathies, the need for a close cooperation among clinicians, geneticists and molecular biologists, in addition to imaging experts, pathologists, neurologists, nephrologists and paediatricians is well recognized: a multidisciplinary approach is essential in order to ensure their correct diagnosis and management. Furthermore, cardiomyopathies represent an ideal translational model of integration between basic and clinical sciences. In the present work, we aim to provide a concise overview of the historical background, genetic and phenotypic spectrum and evolving concepts leading to the various attempts of cardiomyopathies classifications produced by experts over the decades.

HISTORY

The term '*cardiomyopathy*' was first used in 1957 by Brigden, who described a group of uncommon, non-coronary myocardial diseases [1]. In 1961 Goodwin defined cardiomyopathies as "*myocardial diseases of unknown cause*" [2]. He described three different entities, namely "*dilated, hypertrophic and restrictive*", terms which are still in use today. In the 70s, the expanding clinical use of non-invasive imaging, such as m-mode and 2D echocardiography, allowed cardiologists and internists to easily measure left ventricular (LV) wall thickness, cavity dimension and systolic function. Cardiomyopathies began to be recognized with increasing frequency in different populations. In an attempt to provide a useful intellectual framework for clinicians involved in the care of these patients, the first classification of cardiomyopathies was published in 1980, by the World Health Organization (WHO) and International Society and Federation of Cardiology (ISFC), and included the three subgroups proposed by Goodwin [3]. The definition of "*myocardial diseases of unknown cause*" was maintained to define *cardiomyopathies*, which were distinguished from "*specific heart muscle diseases*", the latter comprising heart diseases with similar phenotypes, but due to an identifiable cause.

In the last 30 years, intensive genetic investigation carried out with linkage analysis on large affected families lead to major breakthroughs in the identification of genes associated with familial cardiomyopathies [4,5]. Meanwhile, new nosologic entities were described and the new revision of the classification was carried out in 1996 by the WHO and ISFC [6].

Representing a major advancement, both "*arrhythmogenic right ventricular dysplasia*" (with the inappropriate term "dysplasia" later changed to "cardiomyopathy") and a group of "*unclassified cardiomyopathies*", defined as "*those that do not fit in any group*", were added to the three original subgroups. The definition of cardiomyopathy was changed to "*diseases of the myocardium associated with myocardial dysfunction*". Moreover, three additional subgroups termed "hypertensive", "valvular" and "ischemic" cardiomyopathies were – somewhat confusingly – added to the group of "specific heart muscle diseases" in order to resolve a terminology controversy between US and European experts [6]. These were defined as cardiac conditions characterized by the presence of hypertension, coronary or valvular disease, in a degree that would not explain the magnitude of LV dysfunction observed. Nevertheless, a substantial difference in terminology persisted on the two sides of the Atlantic, reflecting the refusal of these fine distinctions by US experts [7].

In 2006, an American Historical Association (AHA) panel of experts published a scientific statement on the "*Contemporary classification and definitions of Cardiomyopathies*" [7]. They proposed a novel approach, by which "*Primary*" cardiomyopathies were defined as those "*involving only the heart*", as opposed to the "*secondary*", characterized by a "*generalized multiorgan involvement*". Primary cardiomyopathies for the first time also included "*ion channel diseases*" and were differentiated in three subgroups based on their etiology as "*genetic, mixed and acquired*" [Fig. 1]. The radical shift from a phenotypic to an etiological classification, as well as the inclusion of ion channel diseases among cardiomyopathies, although proposed to guide future research rather
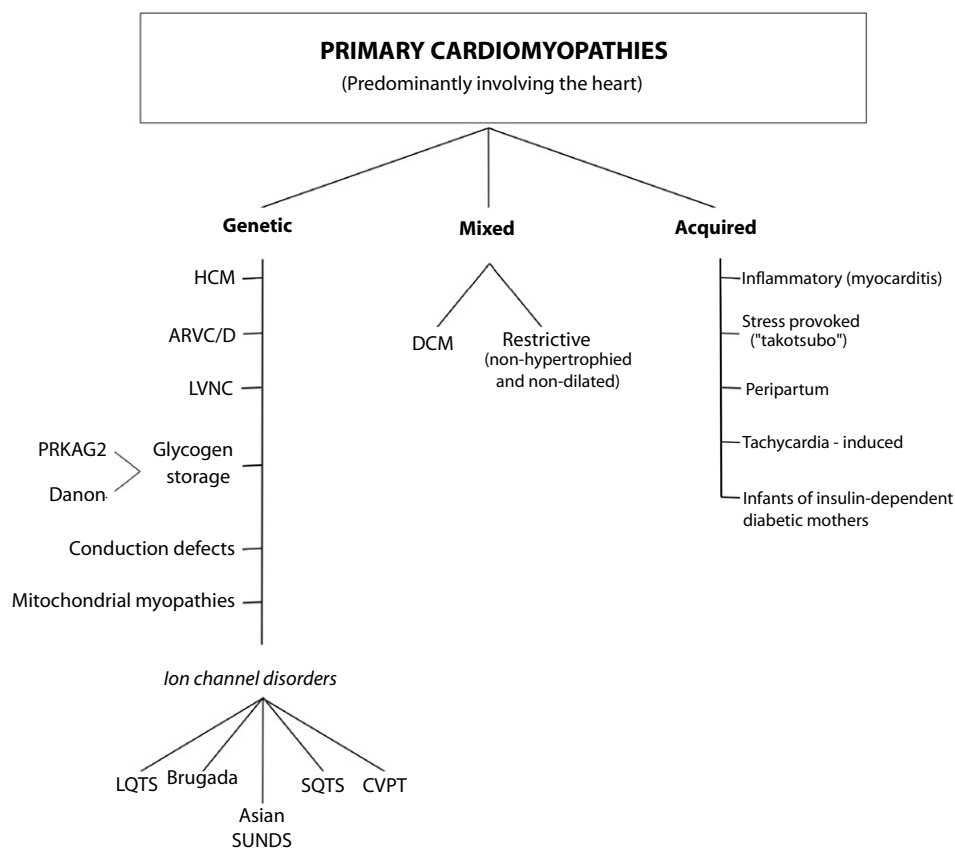
Figure 1. Classification of the cardiomyopathies – American Heart Association. Modified from [7].

than to be employed in the clinical arena, sparked a passionate transatlantic debate, culminating in a thorough reworking of the original 1995 classification by the European Society of Cardiology (ESC) Working Group on Myocardial and Pericardial diseases, in 2008 [8]. Intrinsically faithful to the concept of classifying cardiomyopathies based on phenotype, the 2008 classification aimed to provide a simple operational framework for the medical community, which might have a direct impact in diagnosing and managing these complex diseases. Each of the time-honoured categories *dilated, hypertrophic, restrictive and arrhythmogenic right ventricular* were maintained, divided into familial and non-familial to replace the pre-genetic era concept of "unknown etiology". Furthermore, only two new entities were included into the unclassified group, while the confusing "hypertensive", "valvular" and "ischemic" categories were removed.

CURRENT CLASSIFICATION OF CARDIOMYOPATHIES (ESC WORKING GROUP ON MYOCARDIAL AND PERICARDIAL DISEASES)

The panel felt the proposed classification should be useful for everyday clinical practice. The very definition of *cardiomyopathies* was changed, from "*myocardial diseases of unknown cause*", to "*myocardial disorders in which the heart muscle is structurally and functionally abnormal in the absence of coronary artery disease, hypertension, valvular or congenital heart disease sufficient to cause the observed myocardial abnormality*" [8]. Ion channel diseases were excluded, despite their genetic nature, in view of their lack of a structural cardiac phenotype affecting the heart muscle.

Because cardiomyopathies are diagnosed based on clinical examination and imaging, the four classical morphological subgroups of "*hypertrophic, dilated, restrictive, arrhytmogenic*" were maintained, with a fifth subgroup of "*unclassified*", comprising the recently described "*LV non-compaction*" and "*Takotsubo cardiomyopathy*" [Fig. 2].

Each category was subdivided in a familial and non-familial subset, with the latter including all known causes that might be responsible for that phenotype. A list of potential genetic and non-genetic causes is provided for each subgroup of cardiomyopathies [Tables 1−5]. The precise
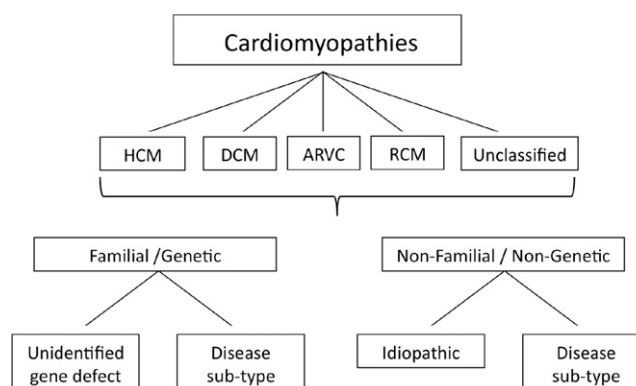
Figure 2. Classification of the cardiomyopathies – European Society of Cardiology. Modified from [8].

Table 1. Hypertrophic cardiomyopathy.

FAMILIAL   Unknown gene

Sarcomeric protein disease ß myosin heavy chain, Cardiac myosin binding protein C Cardiac troponin I, T and C, a-tropomyosin, Essential myosin light chain, Regulatory myosin light chain, Cardiac actin, a- myosin heavy chain, Titin

Glycogen storage diseases (e.g. GSD II (Pompe's disease); GSD III (Forbes' disease), AMP kinase (WPW, HCM, conduction disease)

Lysosomal storage diseases (e.g. Anderson-Fabry disease, Hurler's syndrome)

Disorders of Fatty Acid Metabolism Carnitine, Phosphorylase B kinase deficiency

Mitochondrial cytopathies (e.g. MELAS, MERFF, LHON)

Syndromic HCM Noonan's syndrome, LEOPARD syndrome, Friedreich's ataxia, Beckwith-Wiedermann syndrome; Swyer's syndrome (pure gonadal dysgenesis)

Other: Muscle LIM protein Phospholamban promoter Familial Amyloid

NON-FAMILIAL

Obesity; Infants of diabetic mothers; Athletic training; Amyloid (AL / prealbumin)

Table 2. Dilated cardiomyopathy.

FAMILIAL, unknown gene

Sarcomeric protein mutations (see HCM)

Z band : Cypher/Zasp, Muscle LIM protein, TCAP

Cytoskeletal genes: Dystrophin, Desmin, Metavinculin, Sarcoglycan complex, CRYAB, Epicardin

Nuclear membrane: Lamin A/C, Emerin

Mildly dilated CM

Intercalated disc protein mutations (see ARVC)

Mitochondrial cytopathy

NON FAMILIAL

Myocarditis (infective/toxic/immune)

Kawasaki disease

Eosinophilic (Churg Strauss syndrome)

Viral persistence

Drugs, Pregnancy, Endocrine

Nutritional: thiamine, carnitine, selenium, hypophosphataemia, hypocalcemia.

Alcohol

Tachycardiomyopathy

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identification of the disease etiology has obvious clinical implications, by virtue of its direct impact to totally different management. For example, amyloidosis, Anderson Fabry diseases and glycogen storage diseases may be diagnosed as hypertrophic cardiomyopathy (HCM); yet their treatment varies widely. Of note, the inclusion of amyloidosis in this classification was widely debated [9]. Substantial doubts also regarded takotsubo, a disease that is generally transient, has no proven inherited cause, and appear related to regional myocardial hypoperfusion rather than to heart muscle abnormalities. Ultimately, both were included as this was felt to be conceptually useful in clinical practice.

Finally, a stepwise approach was proposed for diagnostic work-up. Step one is the identification of cardiomyopathies on the basis of the presenting morphologic features. Following diagnosis in the proband, a comprehensive family screening by ECG and echocardiography should be offered to first-degree relatives, in order to assess whether there is a familiar transmission. The third step

Table 3. Restrictive cardiomyopathy.

FAMILIAL, unknown gene
    Sarcomeric protein mutations: Troponin I (RCM +/− HCM), Essential myosin light chain
    Familial Amyloidosis Transthyretin (RCM + neuropathy)
                            Apolipoprotein (RCM + nephropathy)
    Desminopathy
    Pseuxanthoma elasticum
    Haemochromatosis
    Anderson-Fabry disease
    Glycogen storage disease
    Endomyocardial fibrosis (Familial) (Fusion FIP1-like-1 / PDGFRA genes)
NON FAMILIAL
    Amyloid (AL/prealbumin)
    Scleroderma
    Endomyocardial fibrosis
        Hypereosinophilic syndrome, Idiopathic chromosomal cause
    Drugs: serotonin, methysergide, ergotamine, mercurial agents, busulfan, anthracyclines
    Carcinoid heart disease, Metastatic cancers, Radiation

Table 4. Arrhythmogenic right ventricular cardiomyopathy.

FAMILIAL, unknown gene
    Intercalated disc protein mutations: Plakoglobin,
                                  Desmoplakin
                                  Plakophilin 2
                                  Desmoglein 2
                                  Desmocollin 2
    Cardiac ryanodine receptor (RyR2)
    Transforming growth factor-β3 (TGFβ3)
NON FAMILIAL
    Inflammation?

Table 5. Unclassified cardiomyopathies.

FAMILIAL   unknown gene
    Left ventricular non-compaction:
        Barth Syndrome
        Lamin A/C
        ZASP
        a-dystrobrevin
NON FAMILIAL
    Takotsubo cardiomyopathy

consists in the search for the specific cause of the disease, with the help of genetic analysis, metabolic and biochemical laboratory tests, additional imaging and, in selected instances, myocardial biopsy.

ROLE OF GENETIC TESTING
Many cardiomyopathies are believed to derive from the interaction between one or more genetic mutations, often unidentified modifier genes and environmental factors [10]. When genetic analysis is performed in candidate genes, the probability of identifying the pathogenic gene mutation is in the range of 40–60%, for patients with HCM, with approximately 5% of complex mutations [11,12]. Results for dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM) and isolated LV non-compaction are considerably less rewarding [10], although the advent of next generation, genome-wide techniques may increase the yield substantially, as recent data on titin in DCM suggests [13].

   In the meantime, however, cross-talk between geneticists and clinicians has developed slowly, with modalities of interaction and degree of mutual comprehension that vary wildly in various settings. In many institutions, particularly in the US, a geneticist is not available on-site, and genetic testing is often performed remotely via private companies [14]. In addition, clinicians often question the clinical utility of genetic testing in cardiomyopathy patients and their families. The apparent lack of practical benefit, in the face of considerable costs, has long hindered large-scale diffusion of genetic testing, and still accounts for understandable (but not always justifiable) resistance by the clinician. An indisputable benefit of systematic genetic testing lies in the cross-fertilization between

cardiologists and geneticists. The former, generally show limited expertise and propensity at investigating the hereditary nature of cardiac diseases, and at identifying complex, syndromic phenotypes associated with cardiomyopathies (e.g. Noonan's, Leopard's, mitochondrial disease and Anderson Fabry) [15–18]. Standard protocols for genetic testing routinely include pre-test counselling by a multidisciplinary team involving clinical geneticists [15]. This is a valuable moment for reciprocal education among professionals, ultimately benefitting a wide spectrum of patients with rare conditions.

HYPERTROPHIC CARDIOMYOPATHY

HCM is a genetic disease characterized by unexplained LV hypertrophy, associated with non-dilated ventricular chambers, in the absence of another cardiac or systemic disease capable of producing that degree of hypertrophy [Fig. 3]. HCM is diagnosed by a maximal LV wall thickness greater than 15 mm, based on echocardiography (ECHO) or cardiac magnetic resonance (CMR) [19]. This value is lowered to 13–14 mm, when family members are screened. In children, a wall thickness greater than 2 standard deviations (SD) for age, sex or body size is considered diagnostic.
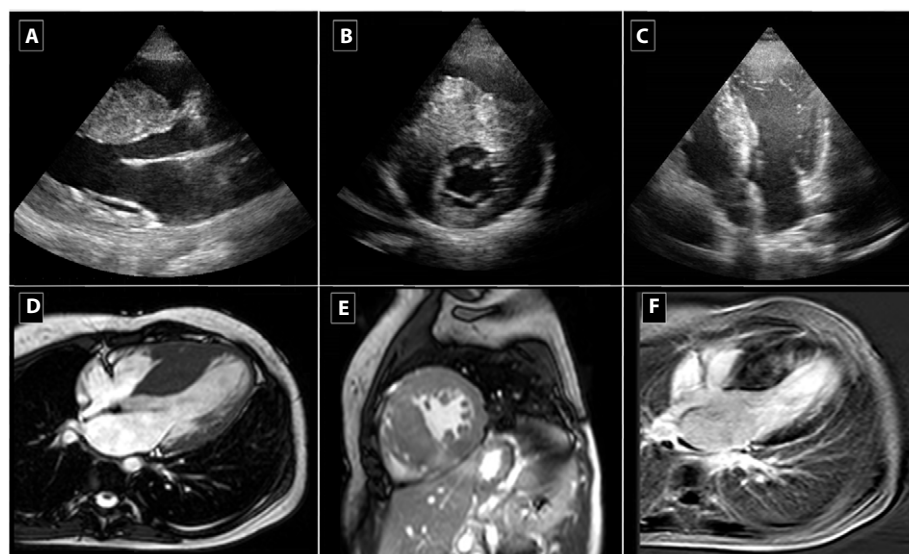

Figure 3. Hypertrophic cardiomyopathy. Echocardiographic and cardiac magnetic resonance images from a 17-year old female patient with HCM. Parasternal long and short axis views show severe LV thickness values (max LV wall thickness 31 mm), with redundant mitral leaflets (panels A, B and D) and small cavity size. Apical 4 chambers view shows massive hypertrophy of the septum and the antero-lateral wall (panels C and E). Image of late gadolinium enhancement showing limited and nontransmural area of fibrosis of the IVS (panel F: black arrow). Abbreviations: LV = left ventricle, RV = right ventricle IVS = inter-ventricular septum.

The distribution of hypertrophy is usually asymmetric and sometimes confined to one or two LV segments. As a consequence, LV mass (measured by CMR) can be within the normal range. LV outflow tract obstruction is an important feature of HCM, and may be demonstrated in up to 70% patients [20]. Overall, the clinical course of patients with HCM is relatively benign, with an annual mortality rate of about 1%. Contrary to prior perceptions, the risk of sudden cardiac death is relatively low [21], although still a major concern in young individuals and athletes. Furthermore, about half of patients show some degree of disease progression and functional limitation, with a small subset of about 5% developing the so-called end-stage HCM. Family screenings, following the introduction of genetic testing has led to the identification of genotype-positive phenotype-negative individuals, a novel category within the HCM spectrum, characterized by absence of LV hypertrophy, assessed by ECG and ECHO [19].

Sarcomeric gene mutations, often private, are the most frequent cause of HCM, accounting for approximately 30–65% of probands in different cohorts [22]. In the remaining subset the genetic substrate is unknown. Furthermore, a small proportion of patients with the HCM phenotype are affected by cardiofacial syndromes (e.g. Noonan, LEOPARD, Costello), neuromuscular diseases (e.g. Frederich's ataxia), mitochondrial diseases [23], metabolic disorders of lysosomal storage

diseases (i.e. Fabry, Pompe, Danon) [24]. These rare conditions sometimes exhibit an X-linked rather than the autosomal pattern of inheritance, usually observed in HCM [Table 1].

DILATED CARDIOMYOPATHY

DCM is characterized by LV dilatation and global systolic dysfunction (EF < 50%), in the absence of coronary artery disease or other identifiable causes (such as systemic hypertension, valve disease, drugs, inflammatory heart diseases) capable of causing that magnitude of impairment [Table 2]. In familial DCM, screening of first-degree relatives will identify the disease in up to 50% [10]. As for many other cardiomyopathies, the prevalence of DCM is underestimated, because many patients may have a subclinical form of the disease which may be difficult to diagnose for the lack of symptoms. Familial and sporadic forms of DCM have similar morphological manifestation and clinical course [Fig. 4]. They are progressive diseases, with a prognosis that, although improved in the last decades, is usually poor due to heart failure, atrial and ventricular arrhythmias, stroke and sudden death [25]. In patients with refractory heart failure, heart transplant represent the final option.
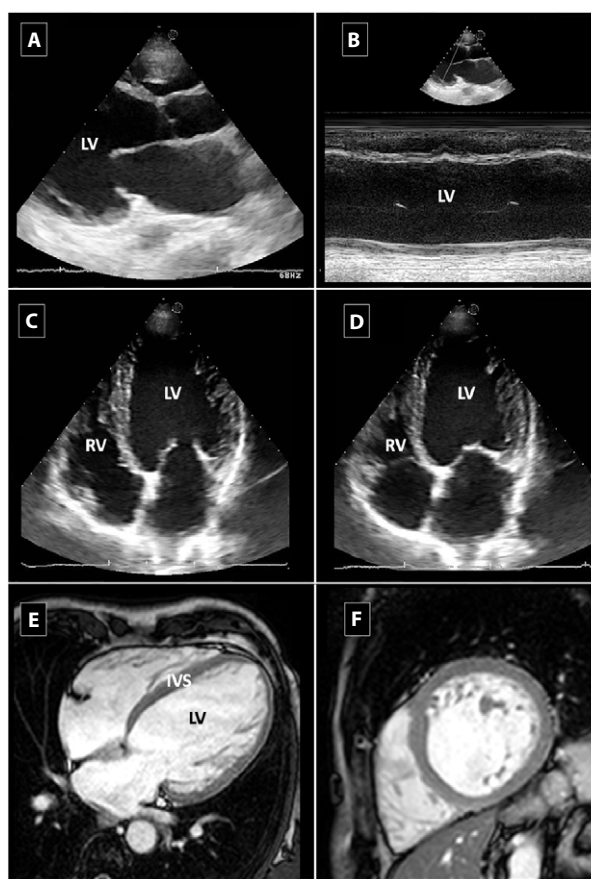

Figure 4. Dilated cardiomyopathy. Echocardiographic and cardiac magnetic resonance images from a 57-year old female patient with DCM and normal coronary angiogram. Parasternal long axis view and CMR images show dilated LV (panels A–B and E–F), with severe systolic dysfunction - EF = 21%; (panel C = diastole, panel D = systole). Abbreviations: LV = left ventricle, RV = right ventricle IVS = inter-ventricular septum.

The low yield of genetic testing for DCM (i.e. 30%) limits its clinical use. This is related to the large number of potentially disease-causing genes. Furthermore, genetic mutations are usually private and the interpretation of the analysis results may be difficult [10]. As noted above, the advent of next generation sequencing may radically change this scenario [13]. However some gene mutations, such as Lamin A/C seem to carry a more adverse outcome, in particular for sudden death [26–28].

RESTRICTIVE CARDIOMYOPATHY

RCM is defined by the presence of a restrictive LV physiology, with normal or more often reduced diastolic/systolic volumes, normal wall thickness and systolic function, marked diastolic flow

impairment and biatrial dilatation. RCM are rather uncommon, although their prevalence is still unknown. Either Amyloid Light-chain (AL) amyloidosis or amyloidosis due to transthyretin gene mutations with heart involvement, often cause RCM [Table 3] [9]. A striking subtype of disease with restrictive physiology, endomyocardial fibrosis, endemic in areas of the African continent, has an unknown etiology and very poor prognosis [29]. Moreover a "restrictive phenotype" may be part of the clinical spectrum of end-stage HCM [30], and may occasionally originate as a primary, non HCM-related phenotype from sarcomere gene mutations (generally in the thin filament protein coding genes). RCM is usually associated with severe functional limitation, mainly related to the extreme diastolic dysfunction, with reduced diastolic filling and stroke volume, and a poor prognosis [31].

ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY

ARVC is characterized by fibrofatty replacement of the right ventricular myocardium and ventricular arrhythmias [32]. In the most common right-dominant form, structural changes may be absent or confined to a localized region of the right ventricle (inflow and outflow tract, right ventricular apex, known as the 'triangle of dysplasia') at an early stage [Fig. 5]. Progression to more diffuse right ventricular disease and LV involvement (typically affecting the posterior lateral wall), associated with ventricular systolic dysfunction, is common at later stages [33]. Ventricular arrhythmias are the clinical hallmark of the disease, but atrial fibrillation may also occur. The diagnosis of ARVC is often challenging for the cardiologist, in particular during the early 'concealed phase', when individuals are still asymptomatic. Predominant LV disease has also been recognized. New diagnostic criteria with higher sensitivity and specificity have recently been published [32,34]. ARVC is generally a familial disease with autosomal dominant inheritance but it may be recessive when associated with woolly hair and palmopalmar hyperkeratosis (eg, Naxos disease, Carvajal syndrome). Mutations in desmosomal and non-desmosomal genes have been identified, but interpretation of their pathogenicity is often challenging in the affected individual [Table 4].
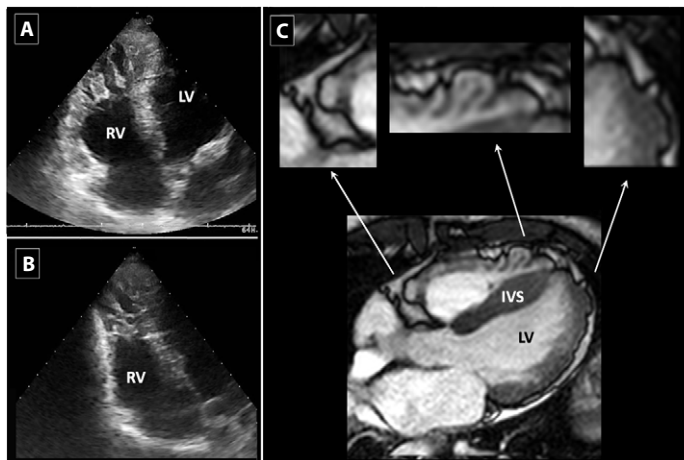

Figure 5. Arrhythmogenic right ventricular cardiomyopathy. 38 year old female with diagnosis of ARVC, resuscitated from out-of-hospital cardiac arrest. She has family history of ARVC (mother) and sudden death (brother, 28 year old). CMR images show clearly wall aneurysms within the so-called "triangle of dysplasia" (panel A–C, white arrows: evident systolic bulging in infundibular, apical, and subtricuspid regions of the RV). Abbreviations: LV = left ventricle, RV = right ventricle.

UNCLASSIFIED CARDIOMYOPATHIES

Isolated LV non-compaction (LVNC) is characterized by prominent LV trabeculae and deep inter-trabecular recesses, that can be associated with LV dilatation and systolic dysfunction [Fig. 6]. LVNC is familial, with 25% of asymptomatic first-degree relatives having some echocardiographic abnormalities [Table 5]. Of note, this rather mysterious disease shows substantial phenotypic overlap with other cardiomyopathies (in particular HCM and DCM, which often exhibit limited areas of non-compaction in the left ventricle), as well as a common genetic substrate [8]. Furthermore, LVNC may be associated with congenital cardiac disorders (such as Ebstein's anomaly or complex cyanotic
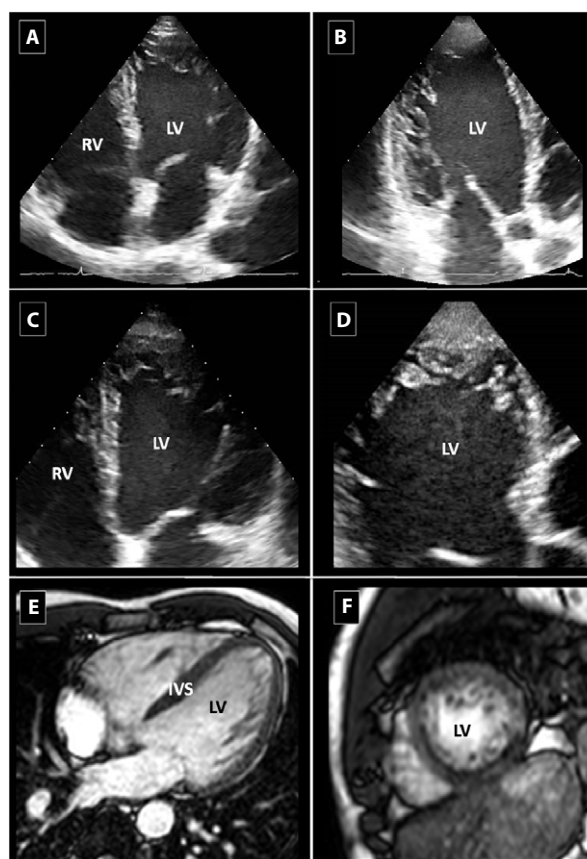
Figure 6. Unclassified cardiomyopathy. Isolated left ventricular non-compaction in a 45 year-old male, with mild systolic dysfunction (EF 48%), ventricular arrhythmias and normal LV diameters. Multiple trabeculations and recesses are evident, particularly in the apex and the free wall of the LV (panels A and C: apical 4 chambers view; panels B and D: apical 3 chambers view). CMR confirmed the diagnosis (panels E–F). Abbreviations: LV = left ventricle, RV = right ventricle IVS = inter-ventricular septum.

heart disease) and some neuromuscular diseases. Therefore, it is still debated whether isolated LVNC should be considered a separate clinical and genetic entity, or a morphological trait shared by many distinct cardiomyopathies. As a result of the difficult comprehension of this clinical entity, the real prevalence of LVNC and its outcome remain largely unknown.

Takotsubo cardiomyopathy, also known as LV apical ballooning or stress-induced cardiomyopathy, is characterized by transient regional systolic dysfunction involving the apex and/or mid-ventricle in the absence of obstructive coronary artery disease on angiogram [8]. The condition is reported all over the world, and most reported cases occur in post-menopausal women following physical or psychological stress, but it has been described also in patients with intracranial haemorrhage or other acute cerebral accidents (so-called "neurogenic myocardial stunning"). Typically, takotsubo cardiomyopathy has a sudden onset, with chest pain, diffuse T-wave inversion and mild cardiac enzyme elevation. Symptoms are often preceded by emotional or physical stress. If the patient survives the acute phase of disease, the prognosis is almost invariably favourable, with a normalization of LV function over a period of days to weeks; recurrence is possible, but rare.

LIMITATIONS OF CURRENT CLASSIFICATIONS AND NOVEL PERSPECTIVES

As more families with cardiomyopathies are genotyped, and new diseases are being described, the paradigm "one gene, one disease" appears no longer sustainable. The same mutation can be expressed at a different age and give rise to hugely different phenotypes within the same family, due to environmental factors and modifier genes. Different phenotype patterns may originate from the same genetic substrates, in a spectrum encompassing HCM, DCM, RCM and LVNC (all associated with sarcomere genes), or ARVC/DCM (associated with desmosomal genes).

Furthermore, the recent introduction of next generation sequencing has started what promises to be a revolution in molecular diagnostics, allowing rapid and affordable testing of hundreds of genes, or even whole genomes. As an example, a wide range of truncating gene mutations encoding Titin, a very large cytoskeleton gene which could not be assessed by traditional sequencing techniques, has recently been discovered to represent a prevalent cause of familial DCM, up to 25% [13].

In the near future, the list of causative genes will therefore likely require an update. This should ideally become an ongoing process, under the auspices of the ESC Working Group on Myocardial and Pericardial Diseases and AHA experts. The focus for researchers will necessarily shift from analyzing single mutations in candidate genes, to interpreting the hundreds of variants of unknown significance in putative causative as well as modifier genes, requiring entirely new skills and significant interaction with biophysicists and computer scientists. At present, and in the foreseeable future, however, clinical classifications of cardiomyopathies based on clinical presentation and morphological criteria represent an important tool for clinicians involved with these complex diseases. While calling for constant improvement and update in the light of advances provided by imaging genetics and basic science, individual patient phenotypes continue to represent the core of any classification in clinical medicine, something that has not changed with time.

LIST OF ABBREVIATIONS:
ESC: European Society of Cardiology
AHA: American Heart Association
ECHO: echocardiography
CMR: Cardiac magnetic resonance
LV: left ventricular
EF: ejection fraction
HCM: hypertrophic cardiomyopathy
DCM: dilated cardiomyopathy
RCM: restrictive cardiomyopathy
ARVC: arrhythmogenic right ventricular cardiomyopathy
LVNC: left ventricular non compaction

COMPETING INTERESTS
The authors have no competing interests.

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Rilevanza clinica del test genetico nella cardiomiopatia ipertrofica

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Riassunto. Sono trascorsi più di 20 anni dalla scoperta del primo gene sarcomerico associato all'insorgenza della cardiomiopatia Ipertrofica (CMI). Durante tale periodo lo svilupparsi di moderne tecnologie per il sequenziamento del genoma umano, unitamente alla crescita delle conoscenze sulla genetica, hanno reso disponibile un test genetico specifico per la CMI effettuabile a scopo diagnostico e diffuso in molti paesi. Nel frattempo, per l'intensificarsi dello scambio reciproco di conoscenze tra cardiologi e genetisti, è emersa la necessità di discutere sulla vera utilità clinica del test genetico. La prescrizione del test ha incontrato spesso difficoltà da parte del cardiologo perché ritenuto di scarsa utilità nella pratica clinica anche a fronte del costo elevato. In realtà tale resistenza è in contrasto con una serie di evidenze che supportano la necessità del test genetico nella gestione del paziente con CMI. Tali ragioni sono passate in rassegna nel presente lavoro e spaziano dall'importanza dell'identificazione dei genotipi complessi perché prognostici di un decorso più sfavorevole della malattia, alla possibilità di effettuare diagnosi nei casi dubbi mediante l'identificazione della mutazione, fino a porre diagnosi differenziale con le forme non sarcomeriche. In ultimo, ma non per importanza, vale la pena sottolineare che il test genetico permette di stabilire la natura ereditaria della malattia, rendendo possibile la diagnosi talvolta presintomatica nei familiari del probando.

Parole chiave. Cardiomiopatia ipertrofica, consulenza genetica, mutazioni sarcomeriche, test genetico specifico per la cardiomiopatia ipertrofica.

Clinical relevance of genetic testing in hypertrophic cardiomyopathy.

Summary. More than two decades have elapsed since the discovery that sarcomere gene defects cause familial hypertrophic cardiomyopathy (HCM). Since then, genetic testing in HCM has developed, and become an important tool in clinical practice for diagnosis and prognosis overall in the Western countries. However its practical benefits are still understimated and clinicians often question about cost-effectiveness of genic testing in HCM patients and their families. This resistance is in contrast with considerable evidence supporting the role of genetics in tailoring management for HCM patients. Several current clinical uses of genetic testing in HCM, ranging from diagnosis in ambiguous situations, identification of disease phenocopies and HCM complex genotypes and confirmation of inherited disease in family members are reviewed.
In the near future it is hoped that next generation sequencing will provide further diffusion of genetic testing in HCM and improvement in care.

Key words. Genetic counselling, genetic test in hypertrophic cardiomyopathy, sarcomeric mutation.

Introduzione

La cardiomiopatia ipertrofica (CMI) è la più frequente malattia genetica del muscolo cardiaco, con una prevalenza di 1:500 nella popolazione generale, pari a oltre 100.000 pazienti stimati in Italia. La patologia è caratterizzata da espressione morfologica e decorso clinico eterogenei[1], e si identifica per la presenza di ipertrofia ventricolare sinistra, generalmente asimmetrica, in assenza di malattie cardiache o sistemiche in grado di produrre tale ipertrofia. Nel 1869, Liouville e Hallopeau descrissero per la prima volta quadri di ipertrofia asimmetrica del setto interventricolare, ma solo negli anni '50 la CMI

venne descritta come entità clinica a sé stante, denominata «ostruzione funzionale del ventricolo sinistro» e «ipertrofia settale asimmetrica» grazie alle osservazioni pubblicate dal patologo Teare. Nel 1957, in seguito ad esami autoptici effettuati su nove pazienti (di cui otto morti di morte improvvisa), Teare individuò una spiccata ipertrofia del muscolo cardiaco e ne intuì la natura familiare[2]. Poco dopo, nel 1959, venne effettuata per la prima volta una diagnosi di CMI *in vivo*, presso il Clinical Center of National Institutes of Health, negli USA[3]. Negli anni '70, l'introduzione della metodica ecocardiografica e i suoi continui progressi hanno consentito di caratterizzare con precisione il grado e la sede del-

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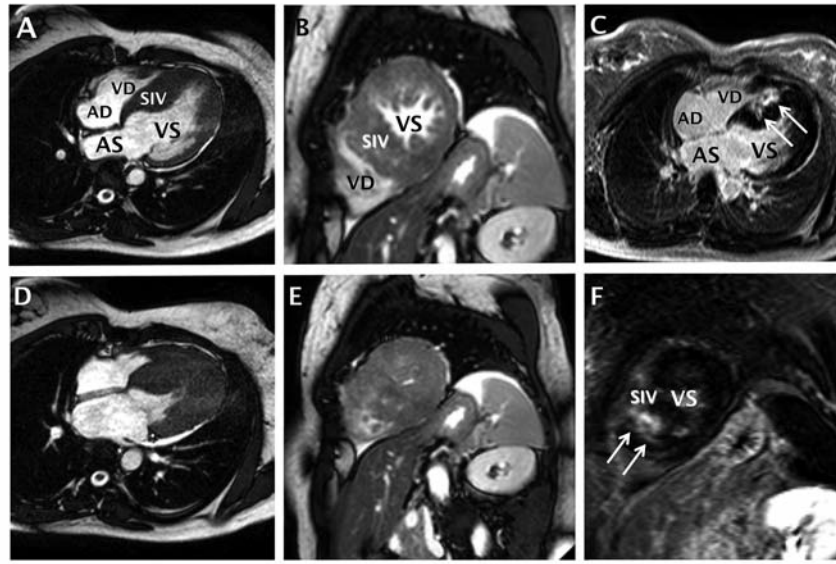[2]Centro di Riferimento per le Cardiomiopatie, Firenze.

Figura 1. Paziente di 32 anni affetta da cardiomiopatia Ipertrofica con ipertrofia di grado marcato prevalente a livello medio-apicale. Immagini di risonanza magnetica nucleare ottenute con sequenze cine in telediastole (A,B,C) ed in telesistole (D,E,F). A,D: Le immagini in 4 camere mostrano l'importante ispessimento del setto interventricolare e della parete anteriore medio-apicale, e dilatazione biatriale. B,E: Sezione coronale asse corto a livello medio-ventricolare: è evidente l'obliterazione sistolica di cavità a questo livello. C,F: Immagini ottenute rispettivamente in 4 camere ed asse corto, dopo somministrazione di mezzo di contrasto: sono evidenti le aree di impregnazione tardiva del setto interventricolare (frecce), espressione di fibrosi intramiocardica. AD atrio destro; AS atrio sinistro; SIV setto interventricolare; VD ventricolo destro; VS ventricolo sinistro

lo scompenso cronico, che può divenire refrattario, fino ad una fase ipocinetico-dilatativa definita "end-stage". Sfortunatamente, predire il decorso clinico e l'esito della malattia nei singoli pazienti con CMI si è rivelato molto difficile. Le prime grandi casistiche di pazienti con CMI, raccolte presso i Centri di riferimento internazionali negli anni '70, presentavano la CMI come una condizione ad alto rischio, soprattutto nei giovani, con una mortalità annuale del 3-6%[11,12]. Studi più recenti, in popolazioni con minor grado di selezione, hanno mostrato che la CMI presenta in realtà un decorso relativamente stabile, una prognosi complessivamente benigna ed una mortalità globale assai minore di quanto precedentemente riportato (intorno all'1% all'anno)[1,13,14]. La complicanza più temibile, la morte improvvisa aritmica, si osserva in una minoranza di pazienti; purtroppo, l'identificazione dei soggetti a rischio resta molto difficile, in assenza di marker specifici per la prevenzione primaria.

l'ipertrofia, di visualizzare in tempo reale il contatto sistolico del lembo anteriore della mitrale (SAM) con il setto interventricolare, e di misurare i gradienti sistolici intraventricolari e i flussi intracavitari[4-7]. L'ecocardiografia ha inoltre permesso di osservare l'evoluzione nel tempo dei pazienti con CMI, portando alla luce i diversi profili evolutivi della malattia[8,9] e di eseguire screening familiari su ampia scala. Recenti contributi nella diagnosi di CMI sono stati portati dall'introduzione della RMN cardiaca che permette una migliore definizione anatomica rispetto all'ecocardiografia, ma soprattutto la valutazione della presenza ed estensione della fibrosi intramiocardica[10] (figura 1).

Le manifestazioni morfofunzionali e cliniche della malattia sono molto eterogenee. I pazienti con CMI possono rimanere asintomatici per tutta la vita; nella maggioranza dei casi, tuttavia, sono presenti sintomi che possono comparire a qualsiasi età e che comprendono la dispnea, l'astenia, il dolore toracico tipico o atipico per *angina pectoris*, le palpitazioni e la sincope. Il quadro sintomatologico è in genere di entità lieve o moderata, e risulta compatibile con uno stile di vita normale: nei bambini e negli adolescenti la malattia viene spesso riconosciuta solo nel corso di studi di screening familiari. In circa un terzo dei pazienti, tuttavia, la CMI progredisce verso

La cardiomiopatia ipertrofica come malattia del sarcomero

La scoperta delle basi genetiche della CMI è scaturita dalla collaborazione tra clinici, genetisti, ricercatori e famiglie di pazienti. La natura familiare della malattia era nota fin dagli anni '50; tuttavia l'identificazione della prima mutazione genetica associata a CMI, nel gene codificante la catena pesante della β-miosina (MYH7), risale al 1987, in seguito a studi di linkage su famiglie estremamente ampie[15]. Oggi la CMI è universalmente conosciuta come una malattia autosomica dominante del sarcomero, causata da varianti in almeno 13 geni codificanti proteine dell'apparato contrattile del cardiomiocita. (figura 2 e tabella 1). Tra i geni sarcomerici, 8 sono quelli comunemente analizzati a scopo diagnostico: il gene della proteina C legante la miosina, la cui sigla internazionale è MYBPC3; il gene della catena pesante della β-miosina, o MYH7; il gene della catena essenziale leggera 1 della miosina, o MYL3; il gene della catena regolatrice leggera 2 della miosina, o MYL2; il gene della troponina T cardiaca, o TNNT2;
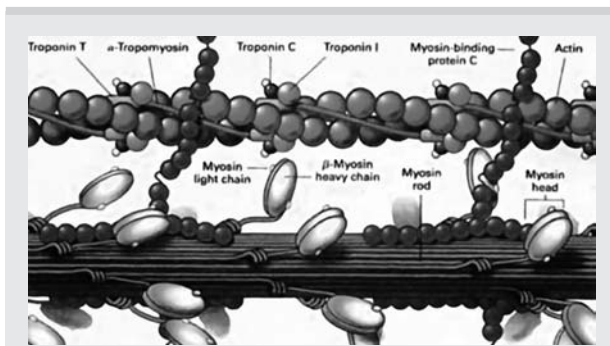
Figura 2. Il Sarcomero cardiaco. (Immagine modificata da Watkins, 2003).

il gene della troponina I, o TNNI3; il gene dell'alfa-actina, o ACTC; ed il gene dell'alfa-tropomiosina, o TPM1. Una mutazione in questi geni viene identificata in circa i due terzi dei pazienti sottoposti a screening genetico. I difetti di MYH7 e MYBPC3 sono i più frequenti, caratterizzando circa il 50% delle diagnosi genetiche; gli altri 6 geni coprono una percentuale che va da un 3% ad un 15% del totale a seconda dei Centri[16].

Inoltre, dal 3% al 6% dei soggetti con CMI presenta un genotipo complesso, caratterizzato cioè da più mutazioni coesistenti, sia nello stesso gene (eterozigote composto) sia in geni diversi (eterozigote doppio); nell'1% dei pazienti sono addirittura descritte triple mutazioni[17].

Al contrario di altre malattie genetiche quali la fibrosi cistica o l'emofilia, in cui le mutazioni genetiche sono ricorrenti, la CMI è caratterizzata da estrema variabilità nelle mutazioni riscontrate. Nei geni sarcomerici sono state infatti descritte oltre 1000 varianti diverse, la maggior parte delle quali identificate in una sola famiglia e perciò definite "private" Eccezionalmente sono descritte mutazioni con "effetto founder" e perciò tipiche di una precisa area geografica[18,19]. Le varianti identificate, soprattutto nel gene MYH7, sono di tipo missense, ossia caratterizzate dalla sostituzione di una singola base di DNA che dà luogo a sua volta alla sostituzione di un singolo aminoacido nella struttura proteica. Nel gene MYBPC3, invece, circa la metà delle varianti sono di tipo frameshift, dette anche mutazioni da scivolamento.

Tabella 1. *Geni coinvolti nella cardiomiopatia Ipertrofica.*

Gene	Simbolo	Locus	Prevalenza (%)
• Proteine sarcomeriche			
Catena pesante della β-miosina	MYH7	14q11.2-q12	25-30
Proteina C legante la miosina	MYBPC3	11p11.2	25-30
Troponina T	TNNT2	1q32	5
Troponina I	TNNI3	19p13.4	~5
α-tropomiosina	TPM1	15q22.1	<5
Catena leggera della miosina			
Essenziale	MYL3	3p21.2-p21.3	<1
Regolatrice	MYL2	12q23-q24.3	<1
Catena pesante dell'α-miosina	MYH6	14q11.2-q12	rara
Troponina C	TNNC	15q14	rara
Actina	ACTC	3p21	rara
• Altre proteine			
Proteina LIM	CSRP3	10q22.2-q23.3	<2
α-actina 2	ACTN2	1q42-q43	rara
Titina	TTN	2q13-33	rara
Fosfolambano	PLN	6q22.1	rara
Caveolina-3	CAV3	3p25	rara
Teletonina	TCAP	17q12-q21.1	rara
Giuntofilina	JPH2	20q12	rara
LIM binding domain 3 (ZASP)	LBD3	10q22.2-q23.3	rara

Queste sono caratterizzate dall'aggiunta (inserzione) o eliminazione (delezione) di uno o pochi nucleotidi alterando tutta la struttura della proteina a valle (in inglese: frame). Molto frequentemente tale meccanismo porta ad un arresto della sintesi proteica e conseguentemente alla produzione di una proteina tronca. Per le forme di CMI associate a mutazioni frameshift di MYBPC3, è stato ipotizzato un meccanismo patogenetico di aploinsufficienza, per cui l'allele non mutato (wild-type) non riesce a compensare la carenza di proteina determinata da quello mutato. Per le mutazioni missense, la maggior parte dei modelli transgenici indica che la CMI deriva da effetti di dominanza negativa (cioè da un effetto "tossico" della proteina mutata), più che da aploinsufficienza[1]. Ad oggi, tuttavia, la patogenesi della CMI a partire dalla mutazione sarcomerica resta largamente ignota, e verosimilmente sono coinvolti più meccanismi, come suggerito dalla marcata eterogeneità di espressione interindividuale della malattia[20].

Nell'ambito delle casistiche cliniche di CMI, è stata inoltre accertata in tempi recenti la presenza delle cosiddette "fenocopie", cioè di patologie con espressione morfologica molto simile o indistinguibile dalla CMI sarcomerica, ma causate da meccanismi molecolari diversi e associate a significative differenze sul piano prognostico e terapeutico. Sono state, ad esempio, identificate cardiomiopatie a fenotipo ipertrofico associate a mutazioni nei geni del metabolismo energetico e della produzione di ATP. Rientrano in questo gruppo le cardiomiopatie secondarie a deficit della catena respiratoria mitocondriale, le cardiomiopatie da alterazioni del metabolismo lipidico e un gruppo geneticamente eterogeneo rappresentato dalle cardiomiopatie da accumulo. Tra queste ultime troviamo due malattie X-linked quali la sindrome di Anderson Fabry (da difetto del gene per l'alfa-galattosidasi A) in cui le manifestazioni cardiologiche si associano spesso a un quadro clinico con interessamento multiorgano, e la malattia di Danon (da difetto del gene LAMP2)[21,22], caratterizzata da aumento estremo della massa cardiaca in età pediatrica. Infine, una cardiomiopatia di incerta classificazione è quella secondaria a mutazioni nella subunità catalitica 2 della proteina chinasi AMP-attivata (PRKAG2), in cui il fenotipo ipertrofico si associa a frequente e precoce comparsa di pre-eccitazione ventricolare[23].

Ipotesi patogenetiche

L'incorporazione di proteine mutate nel sarcomero porta alla compromissione del normale funzionamento delle proteine wild-type: è la principale causa di sviluppo dell'ipertrofia nella CMI[24]. Inizialmente si pensava che mutazioni nei geni sarcomerici fossero la causa di una depressione della funzione contrattile, con conseguente deplezione energetica del cardiomiocita determinando così un'ipertrofia di tipo compensatorio[25]. Tuttavia, studi successivi hanno dimostrato che mutazioni a carico della miosina possono addirittura portare ad un incremento dell'attività contrattile e ad un aumento della sensibilità al Ca2+ delle proteine regolatorie dei filamenti sottili[26,27]. Per questo, alcuni autori hanno ipotizzato che la CMI rappresenti non tanto il risultato di un deficit contrattile, quanto della deplezione energetica del cardiomiocita, a causa di un inefficiente impiego dei depositi di energia cellulare. Ashrafian, nel 2003, ha dimostrato che la presenza di proteine mutate nel sarcomero porta ad un incremento della richiesta energetica causata da un inefficiente utilizzo dell'ATP. Questa alterazione comporta una ridotta capacità del cardiomiocita di mantenere i livelli energetici nel compartimento responsabile della contrazione e compromette importanti funzioni omeostatiche come il re-uptake del calcio intracellulare. Il conseguente, prolungato transiente di calcio stimola i processi trascrizionali, attraverso l'attivazione di messaggeri cellulari (protein chinasi calcio-calmodulina-dipendente; via delle MAP-chinasi; fattori di trascrizione): l'insieme di tutti questi segnali può indurre lo sviluppo dell'ipertrofia, come meccanismo di compenso.

Nuove ipotesi sullo sviluppo della CMI riguardano la possibilità che le mutazioni sarcomeriche interferiscano con lo sviluppo embriologico del cuore, in particolare con i meccanismi di migrazione e differenziazione delle cellule pluripotenti che derivano dal pro-epicardio. Durante le fasi iniziali dello sviluppo embrionale, cellule pro-epicardiche migrano a costituire l'epicardio, e da qui migrano diffusamente all'interno del miocardio e si differenziano in varie linee cellulari, che danno luogo ai fibroblasti interstiziali, alla muscolatura liscia vascolare, alle cellule avventizie, alle valvole atrioventricolari. Le mutazioni del sarcomero e l'alterata contrattilità del cuore primordiale all'epoca di questa colonizzazione, potrebbero influenzare l'espressione genica delle cellule epicardiche, mediante un meccanismo di meccano-trasduzione. Infatti, al momento della migrazione di queste cellule dall'epicardio al miocardio, il cuore ha già iniziato a contrarsi e le mutazioni causa di CMI sono già espresse[28]. Tale meccanismo potrebbe spiegare le molte espressioni fenotipiche della CMI che riguardano tessuti diversi dal miocardio, in cui le mutazioni non sono espresse: come, ad esempio, le alterazioni della valvola mitralica, il rimodellamento del microcircolo e la fibrosi interstiziale.

Correlazioni genotipo-fenotipo

La CMI è caratterizzata da una grande eterogeneità genetica, fenotipica e clinica, con scarsa correlazione tra i vari aspetti nel singolo individuo.

Per tale motivo, una volta identificata la mutazione causante CMI in un paziente, non è in genere possibile trarne informazioni prognostiche o implicazioni riguardo allo sviluppo di un fenotipo più o meno marcato nei familiari. La stessa mutazione può determinare un ampio spettro di fenotipi perfino nell'ambito della stessa famiglia, e suggerisce l'importanza di geni modificatori (varianti in cis o in trans, polimorfismi, varianti rare), fattori epigenetici (grado di metilazione del DNA, di acetilazione degli istoni, e modificazioni post trascrizionali), e fattori ambientali (età, attività sportiva, fumo di sigaretta, ecc.) nel determinare le manifestazioni morfologiche e cliniche della malattia. Sebbene labili, correlazioni genotipo-fenotipo sono state ricercate attivamente in passato, soprattutto nei primi anni dopo la scoperta dei geni coinvolti nella CMI. Ad esempio, in alcuni studi le mutazioni nel gene MYH7 venivano associate ad insorgenza precoce della malattia, elevata penetranza ed ipertrofia di grado marcato, mentre le mutazioni nel gene TNNT2 sembravano comportare una ipertrofia di grado modesto ma un alto rischio di morte improvvisa, e mutazioni del gene MYBPC3 si correlavano ad insorgenza tardiva di malattia, penetranza incompleta e prognosi favorevole[1]. Inoltre, specifiche mutazioni, identificate nei geni MYH7 e TNNT2, fortunatamente rare, sono state correlate con un alto rischio di morte improvvisa in giovane età e perciò definite "maligne"[29]. Tuttavia, tali associazioni genotipo-fenotipo si basavano su piccoli numeri di pazienti, appartenenti a famiglie molto ampie, altamente selezionate per rischio aritmico. In realtà, studi successivi su grandi casistiche hanno spesso contraddetto tali assunti. Un esempio eclatante è quello delle mutazioni di TNNT2, che in alcuni Centri venivano considerate di per sé un fattore di rischio maggiore per morte improvvisa, indipendentemente dal profilo clinico. L'esperienza del nostro e di altri Centri ha dimostrato che la CMI da TNNT2 può associarsi a profili estremamente diversi di malattia, che spaziano da forme lievi e asintomatiche fino a forme con ipertrofia marcata e sintomi invalidanti, con un rischio di morte improvvisa non diverso dagli altri geni[30].

Ruolo clinico del test genetico

Fino a poco tempo fa considerata un mero strumento di ricerca, la diagnostica genetica è oggi un momento estremamente importante dell'inquadramento di pazienti con CMI e delle loro famiglie, e riveste un ruolo pratico che è ancora poco apprezzato dai cardiologici, anche per le limitate opportunità di interazione con i genetisti in ambito cardiologico. Il contributo del test genetico si riflette su vari momenti dell'*iter* clinico dei pazienti con CMI.

CONFERMA DELLA DIAGNOSI NEI FENOTIPI DUBBI

Nell'ambito della CMI, sono frequenti i cosiddetti casi "grigi": il grado di ipertrofia è talmente lieve da non consentire una diagnosi si-

cura, e i restanti reperti strumentali (soprattutto l'ECG) sono nella norma. Questa situazione è ricorrente ad esempio nel caso degli atleti. In questi individui, l'identificazione di una mutazione in uno dei geni sarcomerici consente di confermare diagnosi di CMI[31]. Tale possibilità ha consentito di svelare gli aspetti più lievi della malattia e di caratterizzare meglio lo spettro fenotipico, soprattutto in ambito di screening familiare; questo ha permesso di validare le manifestazioni ancillari della malattia (dilatazione atriale sinistra, aspetti di non compattazione del VS, anomalie della mitrale) che vengono descritte frequentemente con le tecniche di imaging oggi disponibili.

DIAGNOSI DIFFERENZIALE

Il test genetico può essere utile per distinguere una CMI primitiva, cioè causata da mutazioni in geni sarcomerici, dalle forme cosiddette pseudo-ipertrofiche (fenocopie). Particolarmente importante è la possibilità di distinguere forme autosomiche dominanti da forme X-linked e perciò la possibilità di stabilire un preciso rischio di ricorrenza e di trasmissibilità familiare[32].

IDENTIFICAZIONE DEI GENOTIPI COMPLESSI E RISCHIO DI PROGRESSIONE DI MALATTIA

La possibilità di identificare genotipi complessi rappresenta un elemento prognostico importante. La presenza di due o più mutazioni in uno stesso paziente è solitamente associata ad insorgenza precoce della malattia, decorso sfavorevole e rischio aumentato di morte improvvisa o per scompenso[16]. Questi dati sono importanti nell'identificazione del paziente a rischio. In un recente lavoro del nostro gruppo[17], si descrivono quattro famiglie con CMI, in cui il soggetto indice presenta un genotipo complesso con tre mutazioni in vari geni. Anche se la presenza di tre mutazioni si riscontra raramente (0,8%), essa costituisce un rischio aumentato di progressione verso la fase end-stage e di aritmie ventricolari, suggerendo un'associazione tra genotipo complesso e progressione sfavorevole di malattia. Escludendo questi casi estremi, è comunque regola generale che il gruppo di pazienti con test genetico positivo presenti un aumentato rischio di decorso sfavorevole della malattia rispetto al gruppo dei pazienti negativi al test[16].

RIVALUTAZIONE CRITICA DELLA DIAGNOSI NEI PAZIENTI GENOTIPO-NEGATIVI

I pazienti negativi al test genetico rappresentano un gruppo composto, in cui la malattia è verosimilmente causata da geni non ancora identificati, oppure è ad eziologia non genetica[1,16].

Nella pratica clinica, ciascun paziente con fenotipo CMI e test genetico negativo deve essere rivalutato, sottoponendolo ad ulteriori esami. In particolare, è importante escludere una CMI associata a malattie neuromuscolari, o mutazione nei geni che possono produrre fenocopie. Soprattutto se il soggetto è un giovane, è fondamentale andare alla ricerca di manifestazioni extra-cardiache quali dimorfismi facciali, deficit neurologico, coinvolgimento renale etc. Queste evidenze, che spesso sfuggono al cardiologo, possono invece essere utili per orientare la diagnosi nella giusta direzione. Una consulenza genetica clinica di II livello può permettere di distinguere le forme sindromiche associate a cardiomiopatia, come ad esempio le sindromi di Noonan e di Leopard, la malattia di Anderson-Fabry, ecc.[16,21,22,30]. Perciò nei protocolli standard per l'esecuzione del test genetico deve essere inclusa la consulenza pre test e post test eseguita in team multidisciplinare.

Diagnosi pre-natale

In situazioni in cui la CMI familiare è associata ad un'insorgenza precoce ed in forma particolarmente severa, con più soggetti morti improvvisamente in giovane età, la diagnosi prenatale potrebbe essere effettuata allo scopo di identificare la presenza della mutazione nel feto[33].

Tuttavia a causa delle incerte correlazioni genotipo-fenotipo, dovute alla variabile espressione clinica, non si ha indicazione ad effettuare la diagnosi prenatale nel caso della CMI primitiva.

La consulenza genetica è stata definita come "Processo di comunicazione che concerne i problemi psicologici, etici e sociali correlati all'occorrenza o al rischio di ricorrenza di una patologia genetica in una famiglia"[32]. Durante la consulenza genetica pre-test vengono raccolte informazioni e quesiti posti dal consultando, viene costruito l'albero genealogico della famiglia, si effettua una prima valutazione del rischio di ricorrenza, si discute con l'interessato di ciò che è possibile fare per meglio precisare l'entità del rischio, chiarendo il significato e i limiti del test genetico; infine viene raccolto uno specifico consenso informato.

La consulenza genetica ed il consenso informato sono parti integranti di un test genetico poiché permettono la discussione preliminare di tutte le possibili implicazioni dei diversi risultati ottenuti dal test e devono fornire gli strumenti per la comprensione della malattia genetica; per questo implicano l'utilizzo di particolari procedure atte ad affrontare le problematiche psicologiche, etiche e sociali inerenti all'utilizzo dei test genetici[32].

Segue una fase analitica durante la quale viene effettuata la ricerca di mutazioni nei geni implicati nella CMI mediante sequenziamento diretto, a partire da DNA estratto da un prelievo di sangue periferico.

Nella fase post-analitica vengono effettuate la verifica dei risultati, la preparazione del referto e la consulenza genetica post-test. La consegna del referto al paziente viene fatta dal cardiologo insieme al genetista, secondo una procedura multidisciplinare che prevede in particolare la spiegazione delle ricadute pratiche del test.

Procedura seguita per effettuare il test genetico

Il rapporto del paziente (e del cardiologo) con la genetica va ben oltre il semplice test, e comporta un vero e proprio *iter* con diverse tappe di significato diverso (figura 3). La procedura seguita presso il Centro di Riferimento per le Cardiomiopatie in Firenze negli ultimi 10 anni prevede una consulenza pre-test (fase pre-analitica), la fase analitica durante la quale viene eseguito il test in laboratorio ed una consulenza post-test (fase post-analitica). Le procedura è la stessa per probandi e familiari. Durante la fase pre-analitica, il cardiologo propone al paziente di concordare una consulenza genetica con il genetista. Per tale colloquio si impiegano circa 30-45 minuti.
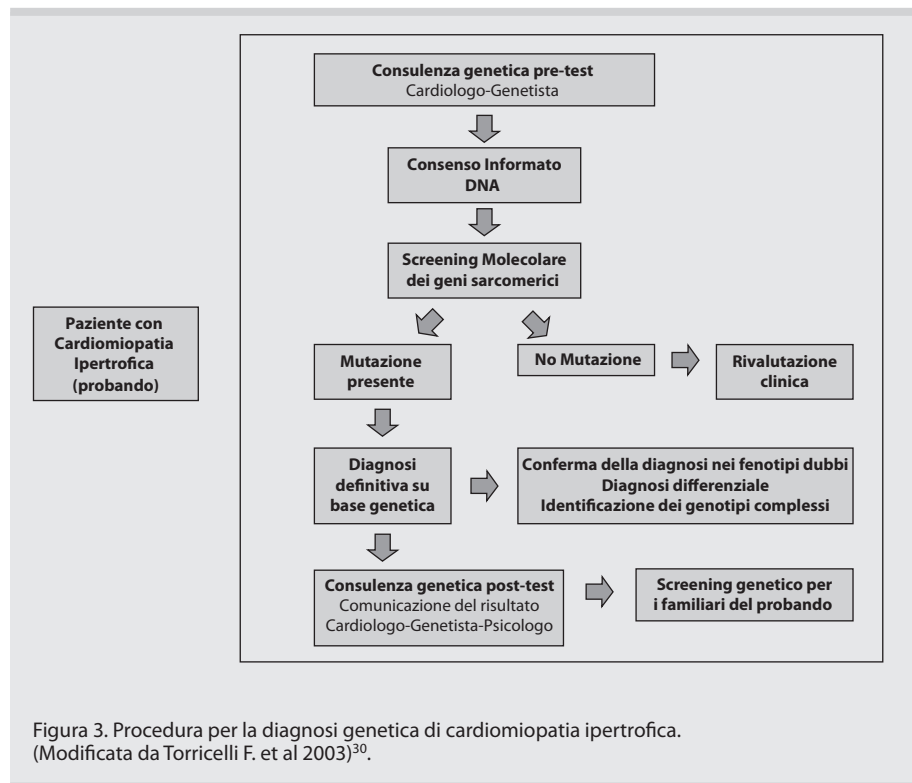

Figura 3. Procedura per la diagnosi genetica di cardiomiopatia ipertrofica. (Modificata da Torricelli F. et al 2003)[30].

Talvolta è presente anche uno psicologo. È in questa fase che si apre la possibilità di far accedere al test genetico i familiari del probando.

Il test genetico effettuato in un paziente con CMI può dare tre risultati diversi:

1. Identificazione di una mutazione sicuramente patogenetica già descritta in letteratura; in questo caso la diagnosi genetica è certa, per cui il genotipo osservato è compatibile con la diagnosi clinica e viene sempre proposto il test ai familiari.

2. Non identificare alcuna mutazione nei geni studiati a scopo diagnostico; in questo caso viene precisato che l'eventuale causa genetica della CMI potrebbe trovarsi o in geni non analizzati (più rari) oppure in geni al momento ancora non identificati. Ovviamente non può essere esclusa la diagnosi clinica sulla base di un risultato negativo del test.

3. Identificazione di una variante non classificabile (UVs), ossia di una alterazione precedentemente non descritta, per la quale è necessario definire una probabilità di associazione con la malattia tramite ulteriori approfondimenti[34].

Test presintomatico

In questa situazione il probando è clinicamente sano, con un ecocardiogramma ed un elettrocardiogramma normali, appartenente ad una famiglia che presenta uno o più casi di CMI. Il paziente richiede il test genetico perché vuole sapere se ha ereditato o meno la mutazione responsabile della malattia nella sua famiglia. Il rischio teorico di presentare la mutazione è del 50% (probabilità *a priori*), nel caso in cui il soggetto sia familiare di primo grado del paziente con CMI. Tuttavia la penetranza è incompleta (70% nell'adulto), incrementa con l'età ed è legata al sesso, oltre che a fattori ambientali (abitudini di vita quali fumo, alcol, attività sportiva). La ragione per cui un soggetto richiede il test è frequentemente duplice: il rischio di essere "portatore" della mutazione causa della malattia e di sviluppare la malattia stessa, e il rischio di trasmettere la malattia alla discendenza.

Tuttavia non è noto nessun trattamento medico in grado di prevenire o ritardare l'insorgenza della malattia. Il solo trattamento efficace nel prevenire la morte improvvisa è un defibrillatore impiantabile, ma esso viene proposto solo per i pazienti con CMI ad alto rischio di morte cardiaca improvvisa[1]. Nei pazienti sani, portatori della mutazione, può essere raccomandata la restrizione dell'attività fisica, tuttavia l'effetto di tale provvedimento nel prevenire la morte improvvisa è puramente speculativo[32].

Nel caso in cui il soggetto risulti positivo, sarà necessario un follow-up medico, volto alla diagnosi precoce dell'espressione fenotipica della malattia e alla valutazione – se possibile – del rischio di morte improvvisa. Questo soggetto sarà, inoltre a rischio di trasmettere la mutazione.

Nel caso, invece, di negatività al test, il paziente avrà un notevole beneficio a livello psicologico, e con l'interruzione dei controlli clinici periodici, si avrà un vantaggio anche per il sistema sanitario[30,32].

Il test genetico nei minori a scopo presintomatico

L'applicazione del test genetico a scopo presintomatico nei bambini è un argomento controverso: alcuni studi hanno enfatizzato i potenziali benefici, specialmente perché la morte improvvisa può essere il primo sintomo della malattia; altri, per mancanza di efficaci trattamenti per prevenire l'insorgenza della malattia e la morte improvvisa, considerano deleterio il test presintomatico nei bambini, per i possibili risvolti psicologici negativi[32]. Attualmente molti gruppi hanno riserve nell'effettuare il test genetico presintomatico prima della maggiore età, riferendosi al decreto del Garante della Privacy, dove si dice che: «I trattamenti di dati connessi all'esecuzione di test genetici presintomatici possono essere effettuati sui minori non affetti, ma a rischio per patologie genetiche, solo nel caso in cui esistano concrete possibilità di terapie o di trattamenti preventivi prima del raggiungimento della maggiore età» (Autorizzazione generale del trattamento dei dati genetici, pubblicato nella Gazzetta Ufficiale della Repubblica Italiana n. 159 dell'11 luglio 2011[30].

Conclusioni

Il test genetico rappresenta un punto di forza per il clinico allo scopo di inquadrare correttamente i pazienti con cardiomiopatia ipertrofica. Risulta fondamentale al fine di confermare la diagnosi nei fenotipi dubbi, riveste un ruolo importante nella diagnosi differenziale ed è utile nell'identificazione dei genotipi complessi. Inoltre, stabilendo la natura ereditaria della malattia, interessa anche i familiari del probando, nei quali può essere impiegato a scopo presintomatico.

Lo sviluppo di nuove tecnologie per l'analisi simultanea di molti geni, quali le piattaforme di Next-Generation Sequencing, unitamente alla sempre più stretta collaborazione tra clinici e genetisti, permetterà di ampliare sempre più le conoscenze sulla genetica di questa malattia.

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Quantitative Contrast-Enhanced Cardiovascular Magnetic Resonance Predicts Sudden Death in Patients with Hypertrophic Cardiomyopathy

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Running title: LGE and sudden death in HCM

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ABSTRACT

Background. Hypertrophic cardiomyopathy (HCM) is the most common cause of sudden cardiac death (SCD) in the young, although not all patients eligible for SCD prevention with the implantable cardioverter-defibrillator (ICD) are identified. Contrast-enhanced cardiovascular magnetic resonance (CMR) with late gadolinium enhancement (LGE) has emerged as an *in vivo* marker of myocardial fibrosis, although its role in stratifying SCD risk in subgroups of HCM patients remains unresolved.

Methods. We prospectively assessed the relation between LGE (as % of left ventricular mass) and cardiovascular outcomes in 1293 consecutive HCM patients followed for a median of 3.3 years.

Results. Sudden cardiac death events occurred in 37 (3%) patients. A continuous relationship was evident between %LGE (p=0.001) and SCD risk in HCM patients. Extent of LGE was independently associated with increased risk of SCD (HR_{adj} 1.4/10% increase in LGE; p=0.002), even after adjustment for other relevant disease variables. LGE of ≥15% of LV mass demonstrated a 2-fold increase for SCD in those patients otherwise considered to be at low risk, with an estimated likelihood for SCD of 6% at 5 years. Performance of the SD risk model was enhanced by LGE (net reclassification index of 12.9%; 95% CI: 0.3-38.3% with a relative integrated discrimination improvement of 0.565; 95% CI: 0.019-3.564). Absence of LGE was associated with lower risk for SD (HR_{adj} 0.39; p=0.02). Extent of LGE also predicted development of the end-stage of HCM with systolic dysfunction (HR_{adj} 1.8/10% increase in LGE; p<0.03).

<u>Conclusions.</u> Extensive LGE measured by quantitative contrast-enhanced CMR is an independent predictor of SCD in HCM and a new marker for judging high-risk status, identifying patients otherwise considered to be at low risk and who may benefit from ICD therapy.

CHAGAS DISEASE AS A CAUSE OF HEART FAILURE AND VENTRICULAR ARRHYTHMIAS IN PATIENTS LONG REMOVED FROM ENDEMIC AREAS: AN EMERGING PROBLEM IN EUROPE

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ABSTRACT

Chagas disease (CD) is a parasitic disease caused by the protozoan Trypanosoma cruzi. In endemic areas (South and Central America), CD represents a relevant public health issue, and is the most frequent cause of cardiomyopathy. In non-endemic areas such as Europe, CD represents an emerging problem following the establishment of sizeable communities from Brazil and Bolivia. Chagas cardiomyopathy represents the most frequent and serious complication of chronic CD, affecting about 20-30% of patients, potentially leading to heart failure, arrhythmias, thromboembolism, stroke and sudden death. Because late complications of CD may develop several years or even decades after the acute infection, it may be extremely challenging to reach the correct diagnosis in patients long removed from the countries of origin. We report two examples of Chagas cardiomyopathy in South American women permanently residing in Italy for >20 years, presenting with cardiac manifestations ranging from left ventricular dysfunction and heart failure to isolated ventricular arrhythmias. The present review emphasizes that CD should be considered as a potential diagnosis in patients from endemic areas presenting with "idiopathic" cardiac manifestations, even when long removed from their country of origin, with potential implications for treatment and control of CD transmission.

Word count = 195

NOVEL ALPHA-ACTININ-2 VARIANT ASSOCIATED WITH

FAMILIAL HYPERTROPHIC CARDIOMYOPATHY AND JUVENILE

ATRIAL ARRHYTHMIAS:

A MASSIVELY PARALLEL SEQUENCING STUDY

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(Short title: ACTN2-related HCM identified by deep sequencing)

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ABSTRACT

Aims. Next generation sequencing (NGS) analysis might be particularly advantageous in genetically heterogeneous conditions, such as familial hypertrophic cardiomyopathy (HCM), in which a considerable proportion of patients remain undiagnosed following conventional Sanger sequencing testing. In the present study we present an Italian family with atypical HCM in which a novel disease-causing variant in alpha-actinin2 (ACTN2) was identified by NGS.

Methods and Results. A large family spanning four generations was examined, exhibiting an autosomal dominant cardiomyopathic trait comprising a variable spectrum of a) mid-apical HCM with restrictive evolution with marked bi-atrial dilatation, b) early onset atrial fibrillation and atrioventricular block, and c) left ventricular (LV) noncompaction. In the proband, 36 candidate genes for HCM (based on published reports) were studied by targeted resequencing with a customized enrichment system. Following bioinformatics analysis, four likely pathogenic variants were identified: TTN NM_003319.4:c.21977G>A (p.Arg7326Gln); TTN NM_003319.4:c.8749A>C (p.Thr2917Pro); ACTN2 NM_001103.2:c.683T>C (p.Met228Thr) and OBSCN NM_052843.2:c.13475T>G (p.Leu4492Arg). The novel variant ACTN2 p.Met228Thr, located in the Actin–binding domain, proved to be the only mutation fully co-segregating with the cardiomyopathic trait in 18 additional family members (of whom 11 clinically affected). ACTN2_Met228Thr was absent in 570 alleles of healthy controls and in 1000 Genomes Project, and was labelled as *"Causative"* by *in silico* analysis using PolyPhen-2, as *"Deleterious"* by SIFT and as *"Disease-Causing"* by MutationTaster.

Conclusions. A targeted NGS approach allowed the identification of a novel ACTN2 variant associated with mid-apical HCM and juvenile onset of atrial fibrillation, emphasizing the potential of this technique in HCM diagnostic screening.

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CLINICAL PHENOTYPE AND OUTCOME OF

HYPERTROPHIC CARDIOMYOPATHY ASSOCIATED WITH

SARCOMERE THIN FILAMENT GENE MUTATIONS

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ABSTRACT

Purpose: HCM may be associated with a variety of causal genes. In a distinct subgroup of patients, HCM is caused by mutations of sarcomere thin filament protein genes, including cardiac troponin T and I (*TNNT2*, *TNNI3*), tropomyosin (*TPM1*) and cardiac actin (*ACTC*). The phenotype and clinical course of thin filament HCM, compared to the more prevalent thick filament form, is still unresolved. We sought to characterize the phenotypic correlates and outcome of 84 patients with thin filament HCM, followed for an average of 5 years, compared to 157 HCM patients with thick filament-related disease. **Results.** Compared with thick filament patients, patients with thin filament HCM showed: (1) lesser maximal LV wall thickness values (18±5 vs 24±6 mm, p<0.001), with larger prevalence of atypical distribution of hypertrophy; (2) higher rate of adverse events, including cardiovascular death, resuscitated cardiac arrest, appropriate ICD shock, nonfatal stroke or progression to NYHA class III/IV (annual event rate 4.6% vs 2.7%, p=0.032); (3) higher likelihood of progression towards LV systolic dysfunction (EF<50%) and/or restrictive diastole, the so-called end-stage of HCM (30% vs 18%, p=0.02). Furthermore patients with thin filament HCM had 2.2 fold higher prevalence of triphasic LV filling pattern (26% vs 12% in thick filament HCM, p<0.001). **Conclusions.** HCM related to thin filament mutations is characterized by significant differences in phenotype and clinical course compared to the more prevalent thick-filament HCM, including higher risk of adverse events related to ventricular arrhythmias as well as progressive LV dysfunction. Triphasic LV filling is distinctively common in thin filament HCM, pointing to peculiar molecular determinants of diastolic dysfunction in this genetic subset

Outcome of Hypertrophic Cardiomyopathy associated with sarcomere protein gene mutations: impact of the Implantable Cardioverter-Defibrillator

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Purpose

To date, large clinical studies on hypertrophic cardiomyopathy (HCM) have assessed outcome irrespective of genetic background. However, the large proportion of patients with no detectable sarcomere myofilament gene mutations, possibly including phenocopies, may confound our perception of the the natural history of HCM due to sarcomeric myofilament mutations. We therefore investigated the clinical features and outcome of 250 HCM patients followed 6±3 years after genetic identification of such mutations. The impact of the implantable cardioverter-defibrillator (ICD), both in terms of appropriate intervention rates and adverse effects, was specifically assessed.

Results

Overall, 16 pts (6%; incidence 1% per year) died of cardiovascular causes, including progressive heart failure (n=7), SCD (n=5), ischemic stroke (n=1) and other non-cardiac diseases (n=3). Of these, 9(5%) occurred in the subset of patents without ICD (group 1) including 3 sudden deaths; while 7 (11%) occurred among pts with ICD (group 2), of whom 3 had prior appropriate interventions. Two of the deaths in group 2 were sudden, occurring despite the device: unfortunately, neither an autopsy nor an ICD interrogation was performed in these pts, and the exact causes of death could not be ascertained. At final evaluation, 6±3 years after genetic testing, 34 pts (14%) were in NYHA class III/IV and 25 (10%) had overt LV systolic dysfunction (LVEF <50%). A total of 53 (21%) pts had received invasive management of LV obstruction by surgical myectomy (n=33) or alcohol septal ablation (n=20). Survival in the two groups was comparable (p=0.15) despite more severe clinical profile and greater prevalence of end-stage in group 2. No difference in survival was observed based on the affected gene. Among the 64 pts with ICD, only 7 (11%; 2 in primary and 5 in secondary prevention) experienced appropriate shocks for VT of VF, with an

overall annual incidence of 2%. However, 16 pts (25% of the ICD subset, including 2 with appropriate shocks) experienced device-related complications such as inappropriate ICD interventions (n=10; including 4 with electric storms due to lead fracture), infections (n=4) and lead dislocation (n=6).

Conclusions

In HCM patients due to sarcomere myofilament mutations, cardiovascular mortality and sudden cardiac death was low even in the presence of multiple risk factors. End-stage progression appeared to be common, supporting the hypothesis of long-term disease progression to heart failure. The ICD allowed favourable survival rates in a subset of high risk HCM patients, at the cost of considerable complication rates, even when no appropriate interventions were recorded. Sudden cardiac death may occur even after ICD implantation.

A novel desmoplakin dominant mutation responsible for Carvajal/Naxos syndrome identified by exome sequencing

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Purpose

Naxos and Carvajal syndrome are rare forms of recessive Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC), characterized by ventricle dysplasia/dilated cardiomyopathy and ventricular arrhythmias, associated with palmoplantar keratoderma and woolly hair.

We report the case of an Italian 37-year-old woman with a form of ARVC characterized by phenotypic features overlapping Naxos/Carvajal syndrome, with a dominant model of inheritance. Whole Exome Sequencing (WES) allowed the identification of a novel mutation in the desmoplakin gene (DSP), which was previously missed by traditional Sanger sequencing.

Methods

The woman was admitted to the hospital after resuscitated cardiac arrest. Clinical and instrumental evaluations showed mild biventricular systolic dysfunction, with dilated ventricles and areas of bulging in the lateral wall of the right ventricle. She also had palmoplantar keratoderma and family history of juvenile sudden cardiac death (brother, 28 year-old). Traditional Sanger sequencing failed to identify any mutations in the genes usually associated with ARVC (transforming growth factor beta-3, ryanodine receptor 2, desmoplakin, plakophilin-2, desmoglein-2, desmocollin-2 transmembrane protein 43, junction plakoglobin protein). Whole exome sequencing was therefore performed on the Illumina HiScan SQ

platform. After bioinformatics analysis, data were filtered by frequency, function, conservation scores and predicted deleteriousness by means *in silico* 3D modeling. Prioritization of variants was performed by Gene Prioritization Portal and then cosegregation was tested in the family.

Results

Family's pedigree showed a dominant model of inheritance. 65993 variants were individuated by bioinformatics analysis; after data filtering and integration of filtered results with patient's clinical features, 4 potential mutations were selected. Only two of those variants cosegregated with the disease in the family: c.878A>T, p.Glu293Val in the DSP gene and c.626A>C, p.Tyr209Ser in the cytochrome *c* oxidase assembly gene (SCO2). Since the cardiomyopathy related to SCO2 gene mutations is usually associated with encephalopathy and is fatal in children, we excluded the causative role of such gene in our family. Therefore, we considered the DSP-Glu293Val variant as disease-causing and we hypothesized a modifier role for the SCO2 variant.

Conclusions

We identified a novel DSP gene variant associated to a rare dominant form of Carvajal/Naxos syndrome, by Whole Exome Sequencing. Our study confirmed the high efficiency of NGS, both in terms of accuracy and sensitivity, when compared to traditional sequencing. However, due to the huge amount of data arising from NGS analysis, bioinformatics management becomes crucial and traditional genetic rules are still needed to understand and interpret the real significance of new variants.

PREVALENCE AND CLINICAL SIGNIFICANCE
OF HEART FAILURE SYMPTOMS
IN PATIENTS WITH NONOBSTRUCTIVE HCM

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Background. One-third of patients with HCM do not have left ventricular outflow tract (LVOT) obstruction and the natural history of this subgroup remains undefined. We therefore sought to evaluate clinical features and outcome of 416 HCM patients, followed for 6.3±2.1 yrs. Furthermore, we assessed the prevalence of heart failure (HF) symptoms (NYHA class III/IV), with regard to the presence or absence of LVOT obstruction, in order to evaluate its clinical implications.

Results. At baseline, LVOT obstruction (≥30 mmHg at rest or following exercise) was present in 436 (64%), while 242 (36%) were non-obstructive. At the end of follow-up, 231 patients (34%) developed progressive HF symptoms; of these, 201 were obstructive and 30 nonobstructive (87 vs 13%, p<0.001). However, nonobstructive patients who developed HF symptoms, were more likely to require heart transplant compared to obstructive patients (0.7 vs 2.1%; p=0.02). Furthermore, nonobstructive HCM patients showed increased risk for end-stage progression (EF<50%; 8% vs 4% for patients with obstruction; p=0.05). Overall mortality and HCM-related event rate were comparable in the two subgroups.

Conclusions: Development of HF symptoms in nonobstructive patients, although less common than in the obstructive group, was associated with higher risk of end-stage progression and the need of heart transplant. While HF occurring in the context of LVOT obstruction, usually associated with little or no fibrosis within the LV, is possibly reversible following surgery, HF in nonosbtructive patients is often subtended by huge LGE and it is usually progressive and irreversible. This observation suggests the need for close surveillance, even though systolic function is preserved, and potentially early initiation of antiremodeling treatment.

MYBPC3-Glu258Lys RELATED HYPERTROPHIC CARDIOMYOPATHY:

A FOUNDER EFFECT IN TUSCANY

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ABSTRACT

Background. MYBPC3 is the most common gene involved in HCM. Even if the majority of mutations identified are novels, it is possible to find some recurrent pathogenic mutations. A few founding mutations have been defined, and the most of them are in MYBPC3 gene. The occurrence of a founder effect has never been reported in Italy so far.

Objectives. The aim of the present study was to describe a founder effect in Tuscany caused by MYBPC3-Glu258Lys mutation.

Methods and Results. A total 635 Italian unrelated index HCM patients underwent screening for myofilament gene mutations by direct sequencing of 8 genes, including MYBPC3, MYH7, TNNI2, TNNI3, ACTC, TPM1, MYL2 and MYL3. In 211/635 (33%) was identified a MYBPC3 mutation and 54/635 (8.5%) consecutive unrelated probands, all were born in Tuscany, harboured the same MYBPC3 Glu258Lys pathogenic mutation. Disease haplotypes analysis examined in 4 families using 9 intragenic loci,

showed a unique haplotype extended for a 2Mb genomic region. To confirm these results haplotype analysis was extended to all index cases and in 100 healthy adult subjects were born in the same geographical region of Tuscany.

Conclusions. MYPC3-Glu258Lys mutation is a founder effect in Tuscany HCM population. Further studies in family members are currently ongoing, in order to assess the cardiac phenotype in mutation carriers and potential modifier SNPs or genes.

SURVIVAL OF CHEMOTHERAPY-INDUCED CARDIOMYOPATHY

IN THE LAST TWO DECADES.

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Benedetta Tomberli, Iacopo Olivotto, Gian Franco Gensini.

Background: cardio-oncology is an emerging field with an increasing amount of patients suffering from cardiotoxic effects of antineoplastic drugs. In this context chemotherapy-induced cardiomyopathy (CI-CM) is a challenging problem and has been considered one of the most life-threatening conditions so far.

Aim: to analyze the actual survival of a cohort of CI-CM pts in comparison to a population of pts with idiopathic dilated CM (IDCM), both followed at our Center in the last two decades.

Methods: we consecutively enrolled 62 pts (32,3% male, age 49±14 y) presenting at our Center with CI-CM from 1990 to 2012. Global follow up was 70±62 months. At baseline mean left ventricular (LV) ejection fraction (EF) was 39.5±10%, mean LV end diastolic index 32.1±5 mm/m^2, mean NYHA class 2.6±1.2. Hypertension was present in 15%, diabetes in 7% and hypercholesterolemia in 10% of pts; 10% of pts were smokers. The majority of pts received chemotherapy (mainly anthracyclines) due to Non-Hodgkin's Lymphoma (64%); 12% were treated because of Hodgkin's disease, 10% due to breast cancer while a minority of pts were treated because of leukemia, osteosarcoma, hepatocellular carcinoma and glioblastoma. Twenty-three pts received a previous antineoplastic treatment regimen, while 41% had a history of mediastinum radiotherapy (RT). 63% of pts showed symptoms of heart failure (HF). Two or more antineoplastic treatment and RT related complications, beyond LV dilatation and dysfunction, were evident in 21% of pts. Mean time from last antineoplastic treatment dose and CI-CM diagnosis was 39±64 months with most

cases reporting a late-onset cardiotoxicity with LV dilatation/dysfunction occurring even after 23 years. At the end of follow up 64,5% of pts were receiving ACE-inhibitors, 18% ARBs (ACEI+ARBs: 82%) and 90% betablockers (BB) with a mean dose of 67%, 75% and 39% of the international HF-guidelines recommended dosage, respectively. Mineral corticoid receptor antagonists (MRA) were administered in 45% of pts; 76% of pts received a combination of ACEI/ARBs plus BB while only 12% of cases received a combination of ACEI/ARBs+BB+MRA.

Results: twenty-five patients (40,3%) reached the combined end-point of all cause death/heart transplantation. Causes of death were refractory HF in 23.8%, cancer-related in 14.3% and other (non cardiac) in 61.9% of pts. Overall survival was 72,6% at 60 months, 47,7% at 120 months, considerably lower compared to a matched population of 451 patients with IDCM, enrolled and followed at our Center from 1990 (86,1% and 68,8%, respectively), but far better in respect to data available in current literature.

Conclusions: CI-CM, even when treated with optimal HF therapy, portends a severe prognosis, especially when compared to IDCM. Our data show anyway an improvement in survival with respect to data available in current literature.

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"Let us be grateful to people

who make us happy;

they are the charming gardeners

who make our souls blossom"

Marcel Proust

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Love for medicine, research and cardiomyopathies

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