Myocardial fibrosis in hypertrophic cardiomyopathy: clinical and epigenetic assessment

Background

In patients with hypertrophic cardiomyopathy (HCM), myocardial fibrosis is primarily involved in arrhythmogenesis, representing one of the main electrophysiological substrates of life-threatening arrhythmias and sudden cardiac death (SCD) (1,2). Although the magnitude and distribution of fibrosis as assessed with cardiac magnetic resonance (CMR) and late gadolinium enhancement (LGE) has been reported to predict SCD in HCM patients, cardiac remodelling is often unpredictable (3). Indeed, standard CMR follow-up is of limited value in revealing rapid changes in myocardial collagen accumulation. Moreover, LGE reveals areas of patchy fibrosis, while is unable to detect patterns of diffuse fibrosis, a putative substrate for major cardiac arrhythmias. Recently, a new CMR technique (post-contrast myocardial longitudinal relaxation time - T1- mapping) has been described with the potential of accurate estimation of areas of diffuse fibrosis (4). However, whether the magnitude and distribution of diffuse fibrosis as assessed with T1-mapping reflect major arrhythmic substrates and predict SCD is presently unknown. Furthermore, while multiple electrocardiographic markers of inhomogeneity in ventricular depolarization (QRS fragmentation, late potentials on signal averaged ECG -SAECG- and microvolt T-wave
alternans) have been described in association with patchy fibrosis, it has never been assessed whether they may also reflect patterns of diffuse fibrosis in LGE negative patients (5,6,7). Finally, myocardial fibrosis has been shown to be regulated at the epigenetic level. Indeed, specific microRNAs may affect collagen accumulation and, thus, putatively predict the development of overt myocardial fibrosis as assessed with CRM.

**Study aims**

This study will assess myocardial fibrosis with (a) CMR LGE/T1-mapping techniques, (b) electrocardiographic markers of ventricular depolarization inhomogeneity, and (c) circulating microRNA acting as epigenetic modifiers of collagen expression/accumulation in patients with HCM. Moreover, we will investigate whether these anatomical and functional substrates of electrical instability can be used to predict major cardiac arrhythmias.

**Methods**

**CMR and LGE assessment:** CMR imaging will be performed with a 1.5-T scanner using steady-state, free-precession breath-hold cines in 3 long-axis planes and sequential short-axis slices from the atioventricular ring to the apex. LGE images will be acquired 10 to 20 minutes after intravenous administration of 0.2 mmol/kg gadolinium-DTPA with breath-hold 2-dimensional segmented inversion-recovery sequence or phase-sensitive inversion-recovery sequences in identical planes as in cine images. Inversion time will be optimized to null normal myocardial signal. For phase-sensitive sequences, uncorrected magnitude images will be used. The acquisition of a non-contrast T1 (native T1) and a corresponding post-contrast T1 map will provide extracellular volume (ECV) fraction mapping. This latter represents the volume of the extracellular tissue components reflecting changes in
collagen volume fraction and thus provides a quantitative assessment of diffuse myocardial fibrosis.

**Microvolt T-wave alternans**: MTWA will be assessed at rest and during exercise test. If the alternans voltage (V alt) will be $\geq 1.9$ V and the alternans ratio (R) $\geq 3$ with an onset heart rate $\leq 110$ beats/min during exercise, the test will be considered positive. If alternans will be absent during a sustained interval of exercise without an artifact at a heart rate $\geq 105$ beats/min, the test will be considered negative. If a result will not meet the positive or negative criteria, it will be considered indeterminate.

**Fragmented QRS**: QRS fragmentation will be evaluated on resting 12-lead ECG recorded at a paper speed of 25 mm/sec (filter range 0.15-100 Hz, AC filter, 10 mm/mV). QRS fragmentation will be established by the presence of $\geq 1$ additional R wave ($R'$) or notching in the nadir of the R or S wave in at least two leads reflecting the same myocardial area.

**Late potentials**: for the standard time-domain analysis we will use three orthogonal leads (X,Y,Z). For the X lead (horizontal) the electrodes will be positioned at the fourth intercostal space in both midaxillary lines. The Y lead (vertical) will be recorded between the manubrium and on either the upper left leg or left iliac crest. The Z lead will record an antero-posterior vector recorded between two electrode placed at the fourth intercostal space (V2-like position) and on the left side of the vertebral column. Positive electrodes will be considered left, inferior, and anterior. Electrocardiographic signals will be recorded with a low-noise amplifier with a minimum band pass from 0.5 Hz to 250 Hz (most of the measures will be performed using a 40Hz high-pass bidirectional filter). SAECG abnormal pattern will include a filtered QRS duration > 114 ms, a root-mean-square voltage of the terminal 40 msec of the filtered QRS (RMS40) < 20 microvolt and a duration of the low-amplitude signal ($< 40$ microvolt) > 38 msec.

**Statistical analysis**: baseline characteristics of patients will be presented as percentage for
dichotomous variables and mean±SD or median (range) for continuous variables. Differences among continuous variables will be assessed using Student's t-test or ANOVA after verification of normal data distribution. Categorical variables will be assessed among groups by the chi-square test or the Fisher exact test, as appropriate. All tests will be two-sided, and a P value of less than 0.05 will be considered statistically significant. All calculations will be generated using SPSS, version 21.0.

Enrolment and preliminary results

From an initial cohort of 600 patients with hypertrophic cardiomyopathy followed in our clinic, we selected 156 patients with identified sarcomeric gene mutation and a follow-up of at least 4 years. Among these, we analyzed 76 subjects that underwent to cardiac magnetic resonance for the evaluation of presence and extension of fibrosis with late-gadolinium enhancement technique. In the first phase of our project we focused on the presence of QRS fragmentation (QRSf) on resting 12-lead ECG following the above reported criteria.

We analyzed baseline surface ECG at first visit to verify the presence of QRSf and observed its evolution during the follow-up. Particularly, for patients without QRSf at baseline, we analyzed if there was a development of fragmentation at the most recent available visit. We identified three populations: a group of patients without QRSf at baseline and no development of fragmentation at follow-up (n=50 subjects, group A), a group of patients without QRSf at baseline who develop fragmented QRS at follow-up (n=13 subjects, group B) and patients with QRSf already at baseline evaluation (n=13 subjects, group C). Then we evaluated cardiac magnetic resonance (CMR) performed at baseline. All the subjects had a CMR exam at the same time of ECG evaluation (within the same year). 25 of 50 subjects (50%) of group A had positive LGE at CMR. Among group B, 9 of 13 patients (69%) had positive LGE at CMR and 9 of 13 patients (69%) in the group C had positive LGE at CMR.

With concern to QRSf localization (at least two leads of the same cardiac region), we
found that inferior QRSf was present in 19 out of 27 ECGs (70%), anterior QRSf was present in 9 out of 27 ECGs (33%) and lateral QRSf was present in 3 out of 27 ECGs (11%).

In the next phases of the project we will perform CMR to evaluate the evolution of cardiac substrate and correlate it to QRSf at follow-up and to other electrocardiographic markers of ventricular depolarization inhomogeneity (i.e. late potentials). Furthermore we will investigate if fibrosis-specific circulating microRNA are correlated to fibrosis assessed by CMR.

REFERENCES: