Correlations Between Cardiac Magnetic Resonance and Myocardial Histologic Findings in Fabry Disease

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Funding: The work reported in this publication was also funded by the Italian Ministry of Health, RC-2022-2773270 project

Word count=986

Fabry disease (FD) causes myocardial native T1 lowering on cardiac magnetic resonance (CMR), left ventricular hypertrophy (LVH), and late gadolinium enhancement (LGE). LVH is thought to be due to a mix of myocyte lipid storage and compensatory sarcomeric increase and potentially fibrosis – the explanation for why T1 lowering is related to LV mass until overt LVH after which the relationship strength falls. However no histological correlations/validations have been provided.

We aimed to understand the histological basis of clinical FD - in particular 3 processes: storage, hypertrophy and fibrosis, by comparing CMR with quantitative histological analysis. Written informed consent was obtained with ethics committee approval. Fifteen FD patients (60% females; 49years [IQR39-63]) undergoing standard CMR (1.5T Philips Ingenia: cines-T1/T2 mapping-LGE) and either right ventricular endomyocardial biopsy (n=11) or septal myectomy (n=4). T1/T2 was measured in the basal/midseptum ROI. LVH was defined as maximum wall thickness (MWT) ≥12mm or increased indexed LV mass (LVMi) above sex-specific reference ranges.[1] Myocardial samples were conventionally prepared[2] with quantitative analysis performed on Haematoxylin-Eosin and Azan-Mallory trichrome (AMT) stained sections (AXIO-ImagerM2-microscope). Myocyte diameter was measured on cross section with the nucleus centrally located (increased when >15μm)[3]. Myocardial fibrosis was recorded on AMT-stained sections as scar-like or interstitial. Storage quantification was semi-automated (ZEISS-ZENBlue software) and reported in two ways: as percentage of vacuolated myocytes (%VM) and percentage vacuolated myocyte area (%VMA: vacuolated area/total myocyte area×100%). Electron microscopy (EM) storage quantification and assessment of other features was performed (PhilipsCM100 at 13500×, using ImageJ software - NIH Image; Bethesda, USA, for averaged area of autophagolysosomal lipid accumulation). Data were expressed as counts/percentages, median[IQR]. Fisher's exact test used for categorical data comparison. Correlations analyzed using Pearson(r) or Spearman's rho(r_s). Statistical significance was considered for $p<0.05$. Analyses performed with STATA-V.13.0 (Texas, USA).

Histologically, using EM, all patients showed autophagolysosomes filled with finger-print/zebra bodies osmiophilic lamellar inclusions. In patients without LVH autophagolysosomes were mainly scattered in the sarcoplasm, whereas in LVH patients extended progressively associating with myofibrillar displacement/loss, mitochondrial hyperplasia/degeneration, and lipofuscin accumulation. The three vacuolization measurement methods were correlated (light microscopy %VMA and %VM r=0.938, p<0.00001; EM autophagolysosome area and %VMA: r=0.622, p=0.023). Vacuolization extent varied (%VM: ≤30% in 4, intermediate in 2, ≥80% in 9; %VMA: <10% in 4, intermediate in 2, >20%(max 38%) in 9). Myocyte hypertrophy was common for both vacuolated and non-vacuolated myocytes, with vacuolated myocytes typically larger (35μm[27-43] vs 18μm[17-20];p<0.001), and diameters correlated with vacuolation (vs %VM, r=0.688, p=0.004; vs %VMA r=0.618, p=0.014). By histology, fibrosis was almost ubiquitous (14/15 patients – both early and advanced disease): 12(80%) interstitial and 2(16%) scar-like.

By CMR, 2(13%) patients had LV dilatation, 3(20%) LVEF<50% and 10(67%) had LVH (5 increased MWT and 5 both increased MWT and LVMi). T1 lowering and LGE were more common in LVH positive patients: 6 had low T1 (with 4 suspected pseudonormal T1) and 7 had infero-lateral LGE (3 also apical-septal). In those without LVH, 1(20%) had low T1 and none had LGE. Septal T2 was high in 1 (59ms), normal in the rest of the cohort (50ms[47-52]).

Histology/CMR correlations: in patients with normal LVMi (n=10, 5 with normal MWT and 5 with increased MWT), T1 inversely correlated with MWT (r=−0.680, p=0.03) and LVMi (r=−0.793, p=0.006), whereas in patients with increased LVMi there was no correlation (MWT r=0.433, p=0.466; LVMi r=0.142, p=0.820). Myocytes' diameter, %VM and %VMA positively correlated with LVH (MWT r=0.645, r=0.780 and r=0.859; LVMi r=0.534; r_s=0.823 and r_s=0.847; p<0.05 for all). All patients with increased MWT or elevated LVMi had a minimum 45% and 80% of %VM, and 18% and 22% %VMA respectively. The relationship between LVMi and vacuolization was exponential, panel 1.

In patients with normal LVMi (n=10), T1 values fell as %VMA increased (r=-0.883, p<0.001), panel 2. This trend was lost in those with increased LVMi, (r=-0.501, p=0.389). Low T1 always had a %VM >45% and %VMA ≥10%. Histological fibrosis correlated with LVH (at least moderate fibrosis more frequent in patient with LVH: 70% vs 0%; p=0.025), but histologic fibrosis was far more common than CMR septal LGE (14/15 vs 3/15).

We summarize these data in 5 ways: 1)histological changes precede in-vivo imaging changes: myocyte hypertrophy pre-detectable LVH, storage pre-detectable T1 lowering, and fibrosis pre-detectable LGE; 2)myocyte size increases with storage and LVH; 3)significant storage is necessary for LVH and low T1; 4)the relationships between storage and clinical LVH is non-linear, and 5)T1 falls with storage until detectable LVH, when the relationship is lost.

Our findings support the hypothesis that LVH in FD is storage related, but other factors are at play. Myocyte hypertrophy precedes clinical hypertrophy. Whilst vacuolization correlates with and is necessary for clinical LVH, apparently unaffected cells with no storage have also hypertrophy suggesting additional mechanisms (pro-hypertrophic paracrine or systemic factors or perhaps compensation for impaired function of regional myocytes with storage). The presence of histologic fibrosis before LGE is common, but all patients had myocyte hypertrophy so the order in which fibrosis and myocyte hypertrophy occur cannot be inferred. Other factors may also be at play: myocyte loss (fewer but larger residual myocytes) remains possible, and there are other less well defined processes histologically – intracellular optical free spaces may not just be storage (autophagolysosomal glycosphingolipid accumulation) but also myofibrinolysis, hyperplastic/degenerate mitochondria, lipofuscines etc. Study limitations are small sample size, histologic sampling bias, single timepoint observations and lack of controls. Nevertheless, these data do validate clinical CMR measurements of LVH, LGE and T1 in FD, highlighting however that all changes occur and are detectable histologically before in-vivo imaging, with potential implications for the timing of drug therapy.

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