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Doxorubicin effect on myocardial metabolism as a pre-requisite for subsequent development of cardiac toxicity: a translational ^{18}F-FDG PET/CT observation.

Running title: Doxorubicin myocardial metabolic impact

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ABSTRACT

The present translational study aimed to verify whether serial ^{18}F-fluoro-deoxyglucose Positron Emission Tomography/Computed Tomography (FDG-PET/CT), predicts doxorubicin cardiotoxicity.

Methods. Fifteen athymic mice were treated with intravenous administration of saline (n=5), doxorubicin 5 mg/Kg (n=5) or doxorubicin 7.5 mg/Kg (n=5) and submitted to dynamic microPET scan to estimate left ventricular glucose consumption (LV-MRGlu) before and after chemotherapy. Thereafter, we retrospectively identified 69 patients successfully treated with Adriamycin, Bleomycin, Vinblastine, and Dacarbazine (ABVD) regimen for Hodgkin's Disease (HD) and submitted to four consecutive FDG-PET/CT scans. Volumes of interest were drawn on LV myocardium to quantify mean standardized uptake value (LV-SUV). All patients were subsequently interviewed by telephone (median follow-up: 30 months); 36 of them accepted to undergo electrocardiogram and transthoracic echocardiography.

Results. In mice LV-MRGlu was 17.9±4.4 nMol x min^{-1} x g^{-1} at baseline. doxorubicin selectively and dose-dependently increased this value in the standard-dose (27.9±9 nMol x min^{-1} x g^{-1}, $p<0.05$ vs. controls) and in the high-dose subgroup (37.2±7.8 nMol x min^{-1} x g^{-1}, $p<0.01$ vs. controls; $p<0.05$ vs. standard-dose). In HD patients LV-SUV showed a progressive increase during doxorubicin treatment that persisted at follow-up. New onset cardiac abnormalities appeared in 11/36 (31%) patients. In these subjects, pre-therapy LV-SUV was markedly lower with respect to the remaining ones (1.53±0.9 vs 3.34±2.54, respectively, $p<0.01$. Multivariate analysis confirmed the predictive value of baseline LV-SUV for subsequent cardiac abnormalities.

Conclusion. Doxorubicin dose-dependently increases LV-MRGlu, particularly in presence of low baseline FDG uptake. These results imply that low myocardial FDG uptake prior to the initiation of doxorubicin chemotherapy in HD patients may predict the development of chemotherapy-induced cardiotoxicity suggesting that prospective clinical trials are warranted to test this hypothesis.

Key words: Doxorubicin, myocardial metabolism, FDG-PET/CT.

INTRODUCTION

Anthracycline cardiomyopathy represents a major drawback of chemotherapy for HD (*1,2*). Although underlying mechanisms have not been fully elucidated, drug interference on respiratory chain and consequent oxidative stress seem to play a major role (*2,3*). This effect is eventually followed by an enhanced glucose consumption (*2,4*) paralleled by an enhanced myocardial uptake of FDG (*5,6*). However, the potential association between this index and anthracycline cardiotoxicity has been only described in one case report (*6*) while its clinical potential remains uncertain.

The present translational study aimed to verify whether response of cardiac FDG uptake to doxorubicin might actually predict a late cardiotoxic effect. To this purpose, we first verified the dose-dependent nature of doxorubicin action on myocardial metabolism analyzing a series of cancer mouse models previously studied in our lab by microPET scanning (*7*). Concurrently, we evaluated the serial PET/CT scans obtained in a cohort of HD patients to define the time sequence of doxorubicin metabolic effect and to verify its possible clinical correlates.

MATERIALS AND METHODS

Animal Experiments

Experiments were conducted under the Guide for the Care and Use of Laboratory Animals (*8*) and approved by the local ethical committee. Animals were kept under the same dietary regimen and divided in three groups to be treated once a week for three weeks with intravenous saline (n=5), doxorubicin 5 mg/Kg (standard-dose, n=5) or doxorubicin 7.5 mg/Kg (high-dose, n=5). Dynamic microPET imaging (Albira, Bruker US) was performed soon before and six days after chemotherapy. Patlak graphical approach (*9*) was adopted to estimate LV-MRGlu and corresponding index in skeletal muscle (SM-MRGlu) according to the standard procedure previously validated in our lab (*7,10*). SUV were also estimated in LV myocardium (LV-SUV) and limb skeletal muscle (SM-SUV).

Clinical study

Searching the keyword HD in the database of all patients submitted to FDG-PET/CT in our lab between January 2007 and December 2015, we identified 587 patients. The study population was thus selected according to the following inclusion criteria: 1) no cardiovascular disease; 2) no diabetes; 3) normal baseline electrocardiogram and echocardiogram; 4) available staging FDG-PET/CT scan (PET1); 5) negative interim PET (PET2); 6) completion of ABVD chemotherapy scheme (doxorubicin dose: 40-50 mg/m^2 per cycle); 7) negative FDG-PET/CT evaluation both 4-6 weeks post-therapy (PET3) and at six-month follow-up (PET4); 8) no subsequent HD relapse at late clinical follow-up. This process narrowed the final population down to 69 patients (Supplemental Fig. 1). Myocardial metabolic pattern in these patients was compared with the corresponding findings in 69 sex- and age-matched subjects selected from our data base (*11*). The institutional review board approved this study and all subjects signed a written informed consent related to the imaging procedure, as part of our routine clinical care.

FDG intravenous injection was preceded by a minimum of six hours fasting and serum glucose level control. All FDG-PET/CT scans were acquired according to the conventional procedure, using a Hirez-16 PET/CT hybrid system (Siemens Medical Solutions). Two volumes of interest were manually drawn on LV myocardium and on SM (Longissimus thoracis) to estimate the LV-SUV and SM-SUV, respectively. CT images were used to identify the myocardium in case of absent cardiac uptake.

All 69 patients were interviewed by telephone; 36 of them accepted to undergo a clinical re-evaluation (14 women, mean age 39±14, age range 21-68). An experienced cardiologist, unaware of PET findings, confirmed the absence of interval development of palpitations, syncope, chest pain or dyspnea and complemented the physical examination with electrocardiogram and echocardiographic evaluation of wall thicknesses, LV diameters, ejection fraction and diastolic function by E/A wave ratio and E wave deceleration time (*12*).

Statistical Analysis

All data are presented as mean ± standard deviation or proportions. Differences between paired and unpaired continuous data were analyzed by Student t-test, as appropriate. Categorical variables were analyzed using the chi-square test. A probability value $p < 0.05$ was considered statistically significant. In instances of a skewed data distribution, values were transformed using a natural logarithmic transform. Finally, the ability of LV-SUV1 to

predict the occurrence of cardiotoxicity, while adjusting for various potential confounders, was tested by multivariate logistic regression analysis. Presence/absence of cardiac abnormalities were tested with respect to the following baseline covariates: age, gender, Ann-Arbor staging, mediastinal irradiation, cumulative administered doxorubicin dose, baseline myocardial FDG uptake. Since clinical assessment for the presence of cardiac abnormalities occurred at various times (from 8 to 26 months) after the administration of ABVD, also follow-up duration was included in the model as a covariate. Due to collinearity between Ann-Arbor staging and mediastinal irradiation, these two variables could not be included in the same model. Their role as confounders was tested separately with all the other covariates included. Since results of the two analyses were similar, only those of the first one are reported. The multivariate analyses proceeded by means of a backward stepwise procedure, based on the likelihood ratio test, with a p value for removal ≥0.1. The estimated coefficients with their standard errors were used to compute the Odds Ratios with 95% confidence intervals. The same model was also fitted separately for each covariate to estimate the univariate Odds Ratios with 95% CI. Statistical analyses were performed using a dedicated software application SPSS (version 21.0, IBM).

RESULTS

In vivo animal experiments

In animal models, doxorubicin increased cardiac glucose disposal (Fig. 1A) without affecting either body weight or serum glucose level (data not shown). At compartmental analysis of dynamic PET scans (Fig. 1B), LV-MRGlu remained stable in control mice (from 17.9±4.4 to 18.9±4.8 nMol x min^{-1} x g^{-1}; p=ns). Standard dose increased LV-MRGlu from 17.5±3.7 to 27.9±9.1 nMol x min^{-1} x g^{-1} ($p<0.05$ vs. controls; $p<0.05$ vs corresponding baseline). This effect was significantly more evident at the high drug dose that augmented LV-MRGlu from 16.7±5.1 to 37.2±7.8 nMol x min^{-1} x g^{-1} ($p<0.01$ vs. controls and corresponding baseline; $p<0.05$ vs. standard-dose). As expected, the analysis of LV-SUV strictly reproduced all these findings (Supplemental Fig. 2A and 2B).

Cardiac selectivity of doxorubicin metabolic effect was confirmed by the absent response of SM in all groups. In fact, both SM-MRGlu (Fig. 1C) and SM-SUV (Supplemental Fig. 2B) remained remarkably stable

before and after treatment. Consequently, the ratio LV-SUV/SM-SUV remained stable at baseline values in sham mice (3.28±0.9), while it increased in a dose-dependent fashion after chemotherapy (to 4.42±1.02 and to 6.43±1.96 in standard and high doxorubicin dose, $p<0.05$ and $p<0.01$, respectively).

Overall doxorubicin effect on cardiac metabolism in humans

Clinical data and time intervals between the four PET/CT studies of patient population and controls are reported in Table 1. Baseline LV-SUV was similarly distributed in controls, in the 69 studied subjects and in the 35 excluded patients because of positive PET2 (Fig. 2A). Similarly, no difference in age, gender and Ann-Arbor staging could be observed between the 69 recruited patients and the 138 excluded ones either because of positive PET2 (n=35) or other reasons (n=103, Supplemental Fig. 1, Supplemental Table 1), suggesting a low likelihood of selection bias.

In HD population, LV-SUV was 2.37±1.6 at baseline, showed a progressive increase during doxorubicin treatment up to PET3 and remained persistently elevated at PET4 (Fig. 2B). By contrast, overall cardiac uptake remained unchanged in controls subjects throughout the study period. Accordingly, HD patients showed significantly higher LV-SUV values with respect to controls both at PET3 and PET4 (Fig. 2B).

Again, the selective nature of cardiac response was confirmed by the divergent behavior of SM metabolism that was only scarcely and transiently affected by doxorubicin (Fig. 2C). Therefore, the ratio between LV- and SM-SUV remained stable during treatment, while it significantly increased during follow-up (Fig. 2D) to values significantly higher than those observed in control subjects.

Despite this trend in average values, patients' response to chemotherapy only partially reproduced the repeatability of doxorubicin action on myocardial metabolism observed in animal experiments. Although no patient showed a progressive reduction in myocardial tracer retention during or after therapy, doxorubicin effect on FDG myocardial uptake was heterogeneous and largely independent from the cumulative drug dose (Supplemental Fig. 3).

Myocardial Function Assessment

During the interval between PET4 and cardiological interview (median time from PET4: 30 months; range: 3-96 months), none of the 69 patients reported any hospitalization potentially related to cardiac disorders. In the 36

patients who accepted to undergo a clinical evaluation, median time elapsed from treatment start to visit was 27 months (range: 8-96 months). Electrocardiogram or echocardiogram documented new onset abnormalities in 11 patients (31%, 4 females, mean age 44±17, age range 21-66, Table 2). Signs of possible cardiotoxicity were: dyspnea associated with a decrease in LV ejection fraction (n=2); atrial fibrillation (n=1); appearance of negative T waves in the anterior leads (n=2); alteration in diastolic mitral flow profile (inversion of E/A wave ratio) at Doppler examination (n=6).

According to inclusion criteria, pre-therapy LV dimensions and function were normal and were remarkably similar between the two subgroups. By contrast, average LV ejection fraction significantly decreased after doxorubicin in the 11 patients with cardiotoxic response and became significantly lower with respect to the remaining 25 ones (Table 2, Fig. 3).

Appearance of cardiac abnormality was not related to differences in gender, age, mediastinal radiotherapy, total drug dose or follow-up duration (Table 2). By contrast, it was associated with markedly lower LV-SUV values at baseline with respect to both the remaining 25 ones (1.53±0.9 vs 3.34±2.54, respectively $p<0.01$, Fig. 4A and 4B) and control subjects (3.2±1.7, $p<0.01$ vs abnormal and p=ns vs negative clinical follow-up). This difference tended to progressively disappear over time (Fig. 4C). In fact, FDG uptake significantly and progressively increased in the 11 patients with late cardiac abnormalities (Fig. 4D). This trend persisted even after doxorubicin discontinuation as opposed to the remaining 25 subjects with negative follow-up in whom cardiac metabolic pattern remained relatively stable during and after ABVD (Fig. 4E). Multivariate analysis confirmed that baseline FDG uptake was strongly associated with the subsequent development of cardiac abnormalities, providing an additive predictive power with respect to conventional risk stratification (Table 3).

DISCUSSION

The present study documents that doxorubicin influences myocardial glucose consumption. The dose-dependent nature of this metabolic effect is well evident and reproducible in animal experiments, while it is largely heterogeneous in patients according to baseline metabolic pattern. Relatively preserved cardiac FDG retention predicted a virtually absent response of cardiac metabolism to doxorubicin. By contrast, patients with

low baseline tracer uptake displayed a systematic metabolic reaction to doxorubicin: myocardial SUV progressively increased during the therapy; this state of increased glucose consumption persisted for months after treatment. This metabolic pattern correlated with the onset of late electrocardiographic or echocardiographic abnormalities. Multivariate analysis confirmed the low myocardial FDG uptake before therapy as the most potent predictor of subsequent cardiac alterations whose power even overcame the predictive value of acknowledged risk factors such as mediastinal irradiation or total doxorubicin dose (*2*).

Doxorubicin effect on cardiac metabolism

The analysis of doxorubicin effect on myocardial FDG uptake has been hampered by the large variability of myocardial metabolic pattern under fasting conditions (*13*), commonly attributed to the dietary regimen in the days before FDG-PET/CT (*14*). The experimental part of our study ruled out this confounding interference to document that doxorubicin dose-dependently and rapidly increased myocardial SUV in mice fed with a standardized diet. No data about cardiac function was available in these animals and thus the present study does not elucidate whether the increased FDG uptake was a marker of an early contractile impairment. Nevertheless, this effect occurred in all animals and was induced by the same drug doses able to trigger the late appearance of both electrocardiographic abnormalities and contractile impairment (*15*). In this line, it is conceivable that the observed metabolic response might represent an early marker of cardiac toxicity at least partially able to predict the development of clinically evident anthracycline-related cardiac dysfunction.

Unfortunately, the retrospective nature of data selection prevents any possible evaluation of the mechanisms underlying the accelerated glucose consumption. However, Hrelia et al. previously reported that 1 μM doxorubicin almost doubles the uptake of the FDG analogue 2-deoxyglucose enhancing the expression of GLUT-1 in neonatal rat ventricular cardiomyocytes (*16*). This response might reflect the acknowledged doxorubicin capability to inhibit fatty acid oxidation and mitochondrial function (*17*) thus increasing glucose intake via the phosphorylation of AMP-activated protein kinase (*18*). However, the direct relationship between this metabolic shift and energy depletion remains largely elusive mostly because the increase in glucose

consumption has been found to be transient (*16*) and followed by a late decrease in FDG uptake, at least in rats treated with doxorubicin doses lower with respect the one employed in the present study (*19*).

Besides energy metabolism, the evident doxorubicin capability to induce myocardial lipid peroxidation might contribute to increase glucose intake since glutathione oxidation inevitably accelerates glucose flux through pentose phosphate pathway. This hypothesis is in accordance with recent indication of the role of endoplasmic reticulum oxidative stress as a major determinant of doxorubicin cardiotoxicity (*20*). Similarly, recent evidences by our lab suggest that FDG uptake in cancer cells is relatively independent from overall glucose consumption selectively tracking a peculiar pathway located within the endoplasmic reticulum, dedicated to preserve redox equilibrium (*10*).

Doxorubicin effect in HD patients

In the clinical setting, several confounding factors hamper a precise definition of the link between FDG uptake trend and doxorubicin cardiotoxic effect. On one side, obvious ethical considerations prevented an accurate definition of dose-dependency of this drug action. On the other hand, changes in cardiac energy expenditure (e.g. changes in LV rate-pressure product) were not assessed as part of the clinical imaging procedure and therefore it is uncertain if a portion of the observed increases in LV-SUVs might have reflected changes in myocardial workload. However, no patient complained symptoms suggesting a progressive increase in heart rate or arterial pressure to levels able to induce measurable increase in LV-MRGlu during treatment and after its discontinuation (*21*). Similarly, follow-up cardiological examination did not report tachycardia or new onset hypertension in any of the 36 tested patients suggesting that changes in workload are unlikely to have a major impact on the observed cardiac metabolic responses to doxorubicin. Finally, we only evaluated patients with negative interim PET to avoid the interference of changes in chemotherapy regimen. This procedure was adopted because this condition predicts the highest prevalence of disease remission and thus identify patients in whom doxorubicin cardiotoxicity is a major drawback. This decision might have introduced possible selection biases. However, clinical picture and LV-SUV were similarly distributed in enrolled population, excluded HD patients and control subjects.

Together with its systematic nature, the prolonged duration of cardiac FDG response to doxorubicin intrinsically implies that the known variability of myocardial FDG uptake cannot be simply attributed to the dietary regimen of the days immediately preceding PET study (*14*). Rather, it identifies a direct and selective doxorubicin action on the myocardium. In fact, the increase in cardiac FDG uptake persisted for six months after ABVD discontinuation when SM had turned back to baseline metabolic pattern. The different time trend documented in our patient cohort nicely reproduces the different time course in respiratory impairment in the two tissues (*22*) and the selective nature of mitochondrial damage induced by doxorubicin in the myocardium (*23*) of experimental models.

Differently from PET data, cardiac evaluation was available in only 36 out of the 69 selected HD patients. This limitation was obviously caused by the retrospective nature of our analysis and implies that the only information available for the remaining 33 patients is the absence of hospital admissions. Similarly, only two and one patients developed symptomatic LV dysfunction or atrial fibrillation, respectively. However, applied criteria identified a decrease in LV ejection fraction that, though modest, is an acknowledged predictor of subsequent cardiotoxic evolution (*24*). Unfortunately, the present data do not elucidate whether baseline FDG uptake predicts a progression of contractile impairment in high-risk HD patients with previous cardiovascular diseases. However, this task would have requested a multicenter approach and large patient sample to account for the inevitable interference of heterogeneities in the progression rate of pre-existing cardiac disorder and in the necessary adaptation of chemotherapy scheme.

CONCLUSION

The present study highlights a dose-dependent action of doxorubicin on myocardial metabolism. In the clinical setting, this response seems to be present only (or mainly) in the subset of patients in whom a low baseline myocardial FDG uptake predicts a progressive increase in cardiac glucose consumption during and after chemotherapy as well as a higher incidence of cardiac abnormalities.

The potential impact of these findings and their correct placement into the clinical practice ask for further prospective studies. Nevertheless, their relevance relies on the observation of a predictive value of cardiac FDG

uptake in the PET session performed, under fasting condition, for staging purpose. Should these data be confirmed, the selection of candidates to alternative therapeutic protocols or to cardio-protective treatments might be possible without the need of a further scan adopting specific procedures to optimize the study of myocardial metabolism.

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FIGURE LEGENDS

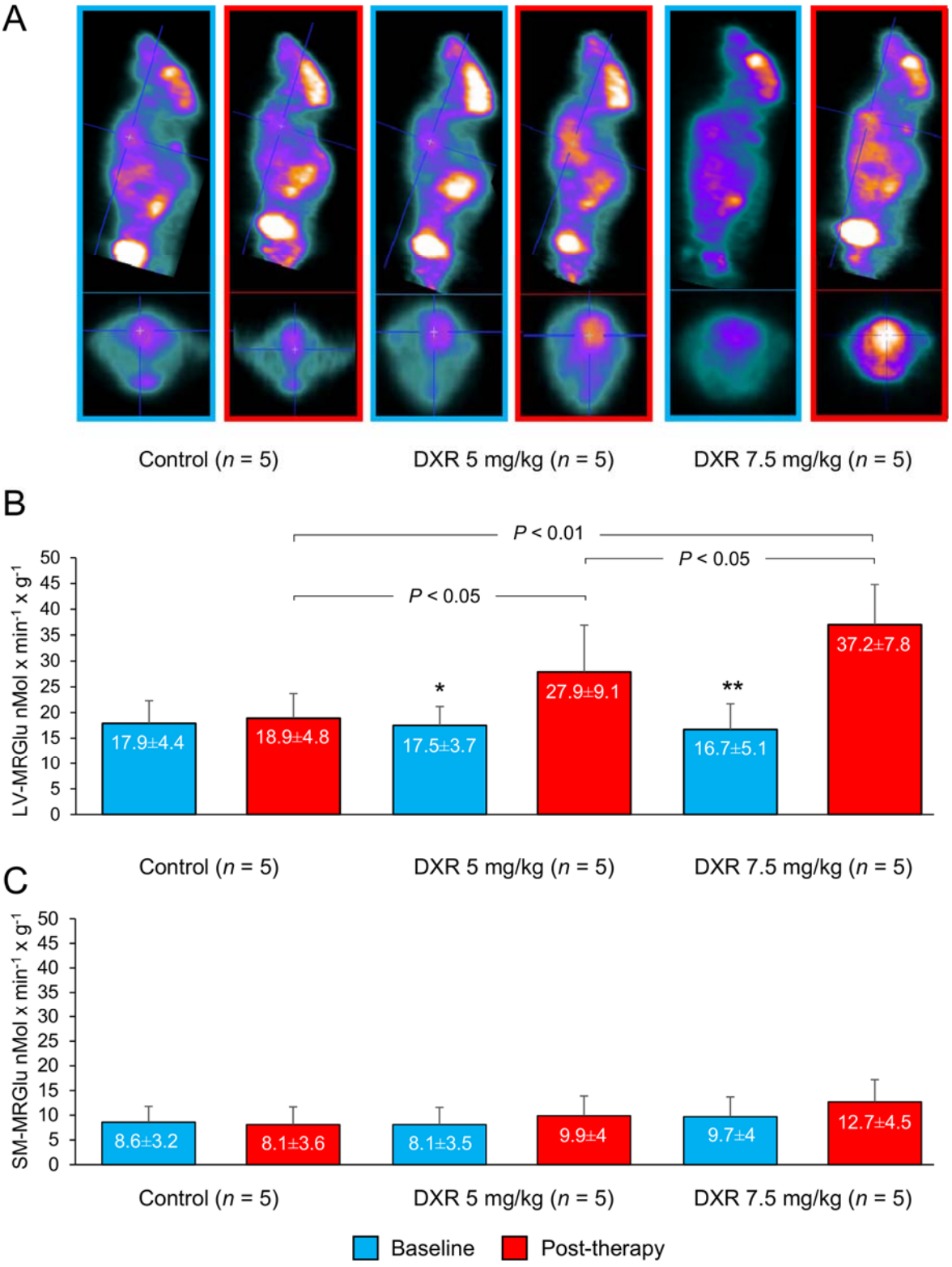


FIGURE 1: Dose-dependent doxorubicin effect on LV-MRGlu.

Panel A displays axial and sagittal planes of microPET studies in mice before and after treatment with saline, doxorubicin 5mg/kg and 7.5 mg/kg, respectively. As displayed in Panel B, doxorubicin administration was followed by a significant increase in LV-MRGlu, as opposed to stable values in untreated mice. Moreover, the dose-dependent nature of doxorubicin metabolic effect was confirmed by the significant difference between post-therapy scans in animals treated with standard or high doses. Doxorubicin administration did not affect SM-MRGlu (Panel C). *=$p<0.05$ vs after treatment; **=$p<0.01$ vs after treatment.

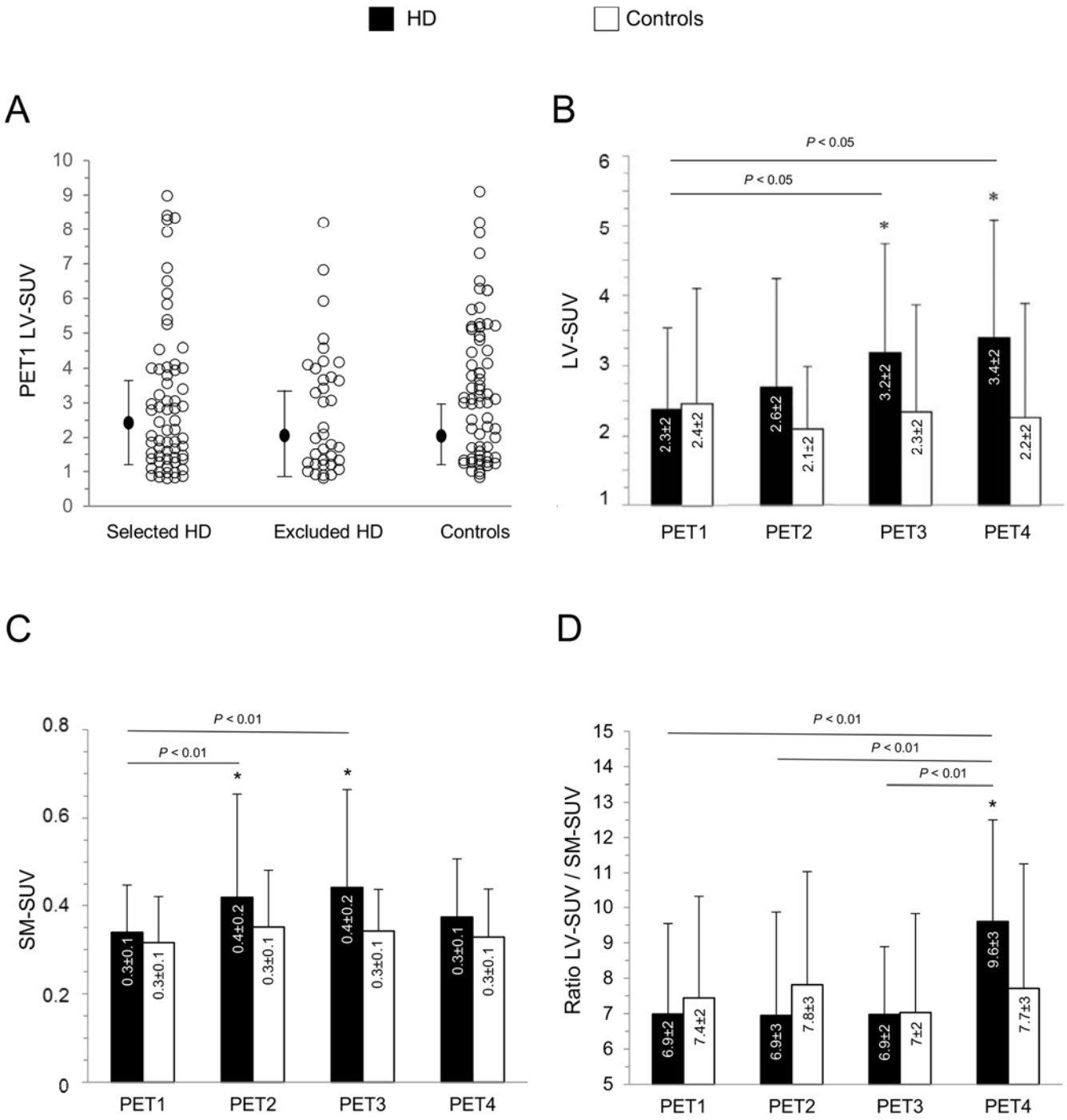


FIGURE 2: Myocardial and skeletal muscle divergent metabolic pattern in HD population.

The distribution of baseline LV-SUV was similar between the 69 enrolled subjects, excluded patients and controls (Panel A). In HD patients LV-SUV progressively increased from PET1 to PET3, remaining relatively stable at PET4 (Panel B). By contrast, it remained unchanged in controls. The divergent nature of SM doxorubicin effect can be appreciable at PET4 (Panel C and D). *=p<0.05 vs control.

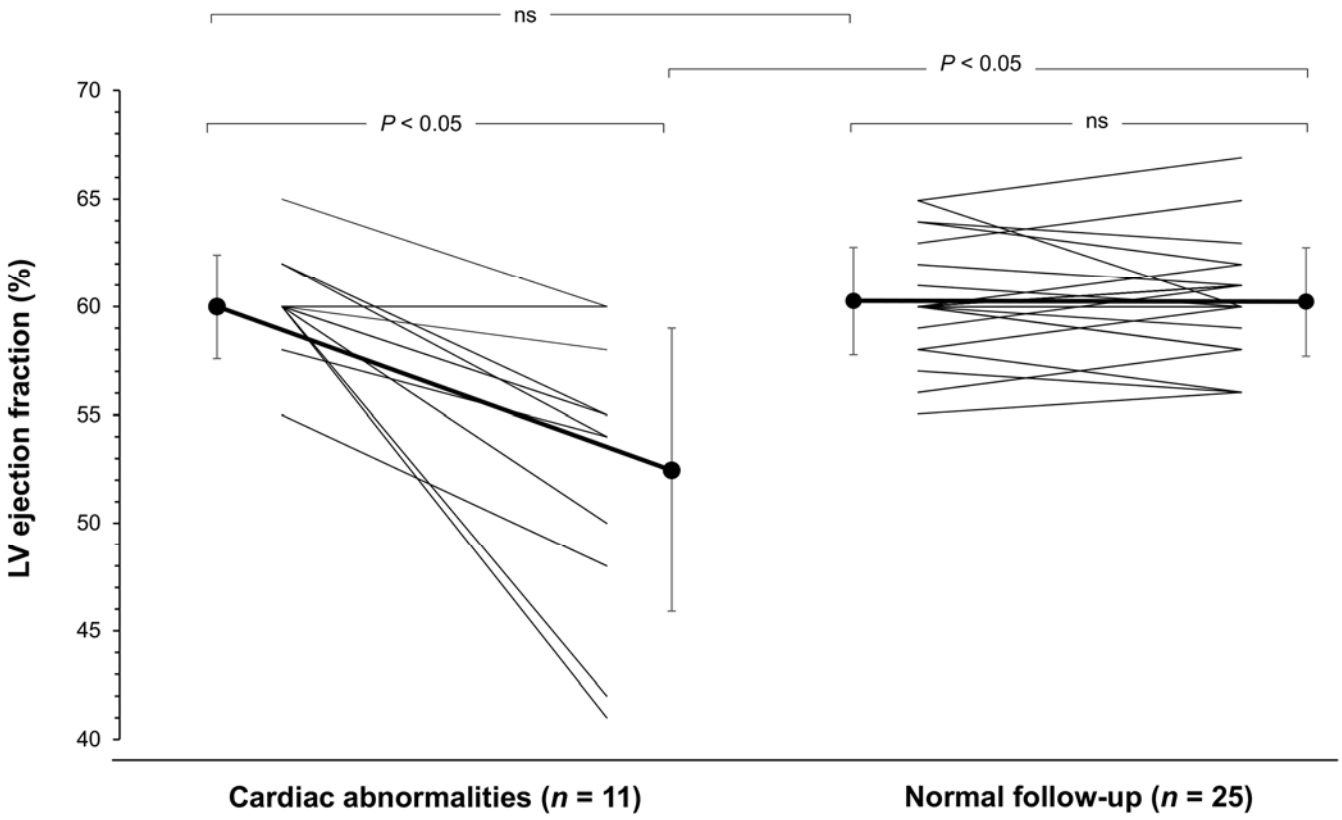


FIGURE 3: LV ejection fraction in patients with normal and abnormal cardiac follow-up.

Individual and average values of LVEF in the two subgroups of patients.

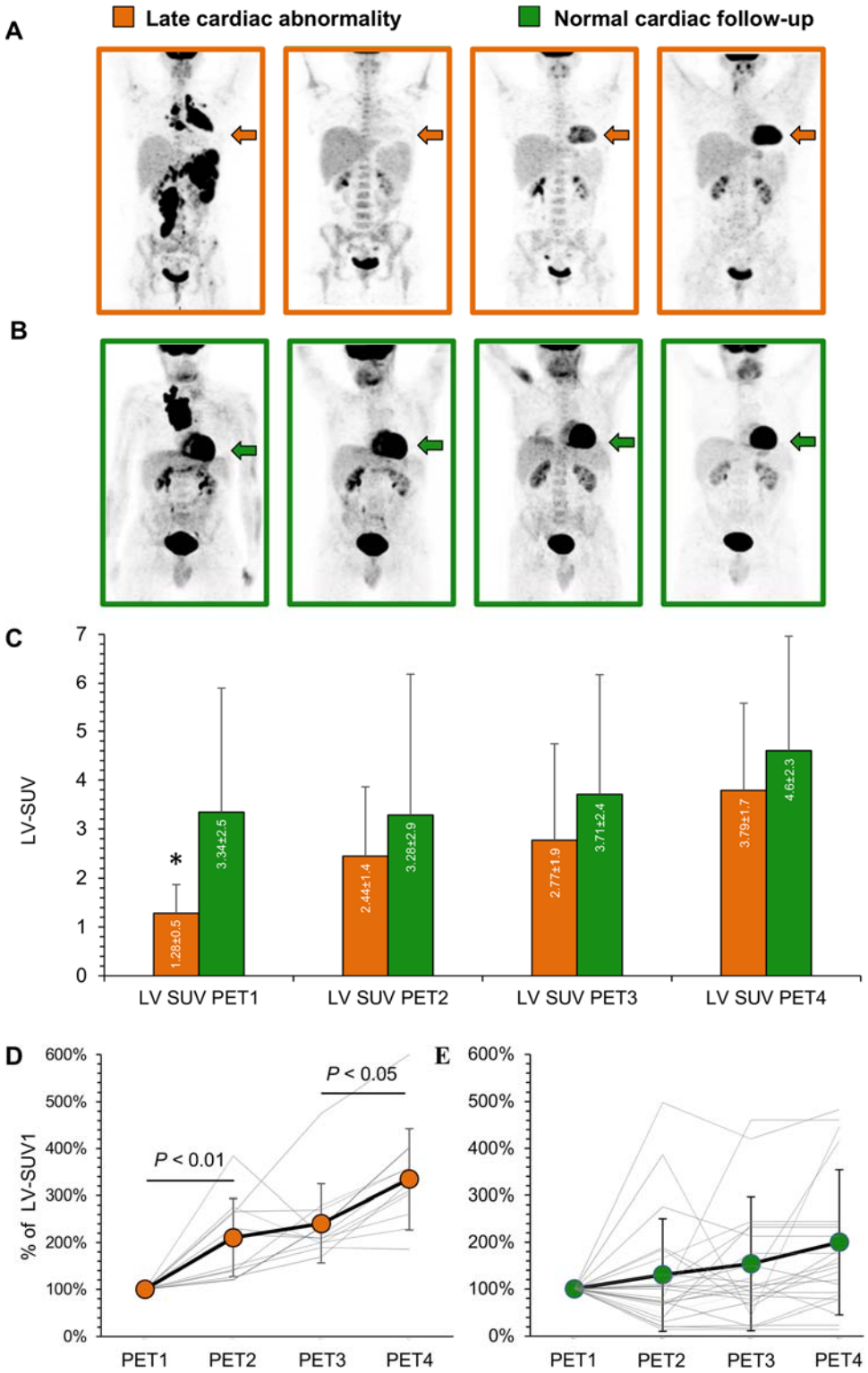


FIGURE 4: Divergent myocardial metabolic pattern between patients with normal and abnormal cardiac follow-up.

Example cases of abnormal and normal cardiac follow-up HD patients (Panel A and B, respectively). Panel C displays FDG uptake at the four PET/CT scans in the two subgroups, baseline LV-SUV was markedly lower in patients with late cardiac abnormalities with respect to the remaining 25 ones (* = $p<0.01$, Panel C). FDG uptake significantly and progressively increased in the 11 patients with late cardiac abnormalities (Panel D). This trend persisted even after doxorubicin discontinuation, as opposed to the remaining 25 subjects (Panel E).

Table 1: Baseline clinical characteristics of HD and control enrolled patients.

	HD	**Controls**	*p*
Age	39 ± 13 (range 19-58)	41 ± 8 (range 20-72)	ns
Male Sex	37/69 (53%)	35/69 (50%)	ns
Weight (Kg)	67.1 ± 12	76.5 ± 7	<0.05
Gycemia at FDG injection (mg/dl)	79 ± 7 (range 61-101)	83 ± 11 (range 62-94)	ns
Cardiovascular Risk Profile			
Hypertension	6/69 (8%)	15/69 (21%)	<0.01
Tobacco Use	19/69 (27%)	30/69 (43%)	<0.05
Total Cholesterol	183.7 ± 30	188 ± 53	ns
LDL	114.5 ± 32	120 ± 25	ns
Triglycerides	121.3 ± 49	129.7 ± 57	ns
Creatinine	0.8 ± 0.1	0.85 ± 0.2	ns
Family hystory of CAD	7/69 (10%)	5/69 (7%)	ns
Time intervals between PET studies			
PET1 - PET2 (days)	73.7 ± 21	99 ± 90	<0.05
PET2 - PET3 (days)	148 ± 70	167 ± 98	ns
PET3 - PET4 (days)	195 ± 92	229 ± 100	ns
Overall PET1 - PET4 (days)	427±198	448±141	ns
Baseline Ann-Arbor Staging			
I Stage	7/69 (10%)	-	-
II Stage	42/69 (60%)	-	-
III Stage	8/69 (12%)	-	-
IV Stage	12/69 (17%)	-	-
B symptoms	10/69 (14%)	-	-
Mediatinic Radiotherapy	35/69 (55%)	-	-
Total administered doxorubicin dose (mg)	456.6 ± 103	-	-

Table 2: Demographic and clinical data of normal with respect to abnormal follow-up HD patients.

	Normal FU (n=25)	Abnormal FU (n=11)	*p*
Age	36.8 ± 12	44.5 ± 17	ns
Male Sex	14 (56%)	7 (63%)	ns
Weight (Kg)	68.9 ± 13	70.7 ± 12	ns
Gycaemia at PET1 (mg/dl)	79.2 ± 5	82.4 ± 4	ns
Cardiovascular Risk Profile			
Hypertension	2 (8%)	1 (9%)	ns
Tobacco Use	5 (20%)	2 (18%)	ns
Total Cholesterol	186 ± 28	181 ± 33	ns
LDL	119.6 ± 28	113.3 ± 31	ns
Triglycerides	117.2 ± 56	122.9 ± 49	ns
Creatinine	0.8 ± 0.1	0.7 ± 0.1	ns
Family hystory of CAD	2 (8%)	1 (9%)	ns
Baseline Ann-Arbor Staging			
I Stage	3 (12%)	2 (18%)	ns
II Stage	15 (60%)	5 (45%)	ns
III Stage	3 (12%)	2 (18%)	ns
IV Stage	4 (16%)	2 (18%)	ns
B symptoms	5 (20%)	2 (18%)	ns
Mediastinic Radiotherapy	13(52%)	5 (45%)	ns
Total administered doxorubicin dose (mg)	430.9 ± 109	421.3 ± 107	ns
Clinical follow-up duration (days)	1121 ± 874	860 ± 665	ns
Follow up clinical data			
Chest pain	-	-	-
Dyspnea	-	2 (18%)	-
Syncope	-	-	-
Palpitations	-	2 (18%)	-
Follow-up ECG abnormalities	-	3 (27%) *	-
Baseline echocardiography			
End-diastolic diameter (EDD) (mm)	49.1 ± 2	48 ± 3	ns
End-systolic diameter (ESD) (mm)	29 ± 3	31 ± 2	ns
Fractional shortening (%)	44% ± 4%	46% ± 3%	ns
LV ejection fraction	59.8 ± 2.1	59.3 ± 1.7	ns
Distolic dysfunction	0 (0%)	0 (0%)	ns
Follow up echocardiography			
End-diastolic diameter (EDD) (mm)	49.2 ± 1	49.3 ± 5	ns
End-systolic diameter (ESD) (mm)	27.2 ± 3	33.6 ±11	ns
Fractional shortening (%)	44% ± 4%	38% ± 5% †	<0.05
LV ejection fraction	60.3 ± 2	55 ± 7 †	<0.05
Distolic dysfunction	0 (0%)	5 (45%)	<0.01

* 1 patient developed atrial fibrillation, 2 patients developed negativization of T waves in the anterior leads.

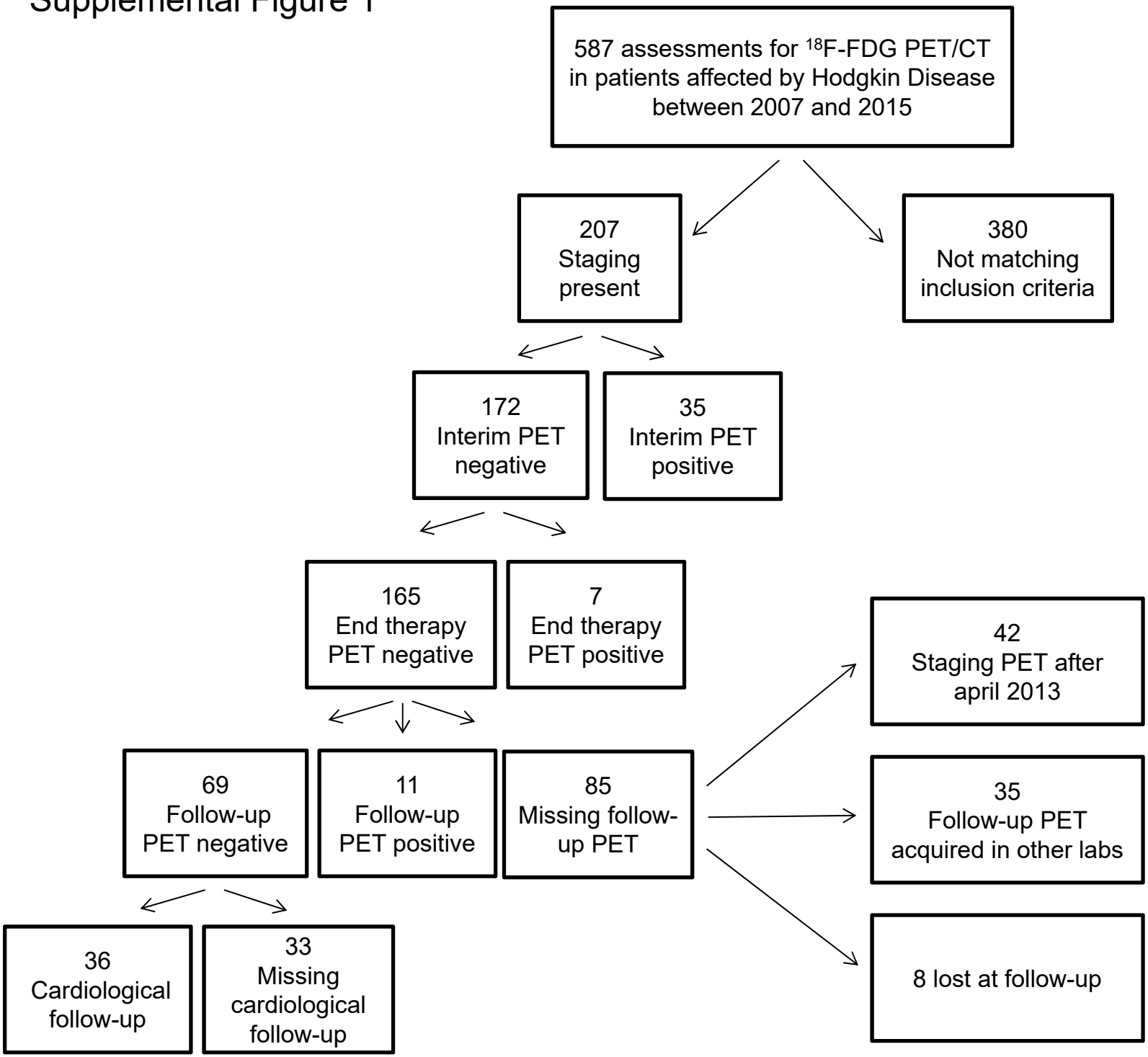
† $p<0.05$ with respect to corresponding baseline

Table 3: Prediction of cardiac abnormalities: uni- and multivariate analysis

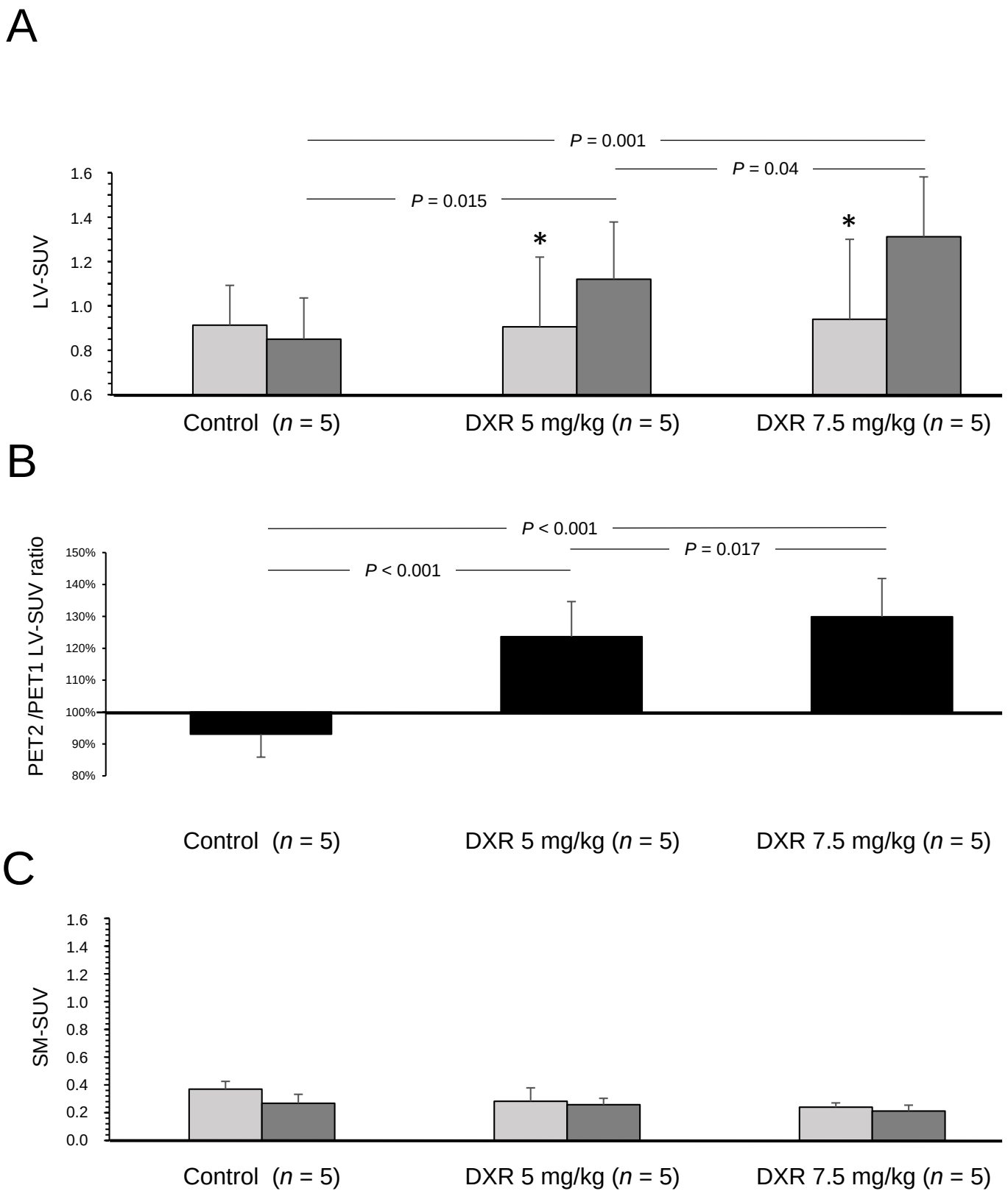
		Univariate analysis			Multivariate analysis		
	df	p	O.R.	95% CI	p	O.R.	95% CI
Sex	1	0.760	0.8	0.19 - 3.35	0.142	*	
Age (years)	1	0.140	1.04	0.99 - 1.09	0.339	*	
Anna Arbor Stage	1	0.882	0.94	0.41 - 2.12	0.694	*	
Mediastinal Radiotherapy	1	0.718	1.30	0.31 - 5.39	0.711	*	
Cumulative doxorubicin dose (mg)	1	0.963	1.00	0.99 - 1.01	0.486	*	
Baseline LV-SUV	1	0.030	0.18	0.03 - 0.85	<0.001	0.065	0.006 - 0.74
Follow up time (days)	1	0.364	1.00	0.99 - 1.001	0.408	*	

* = excluded from the final model

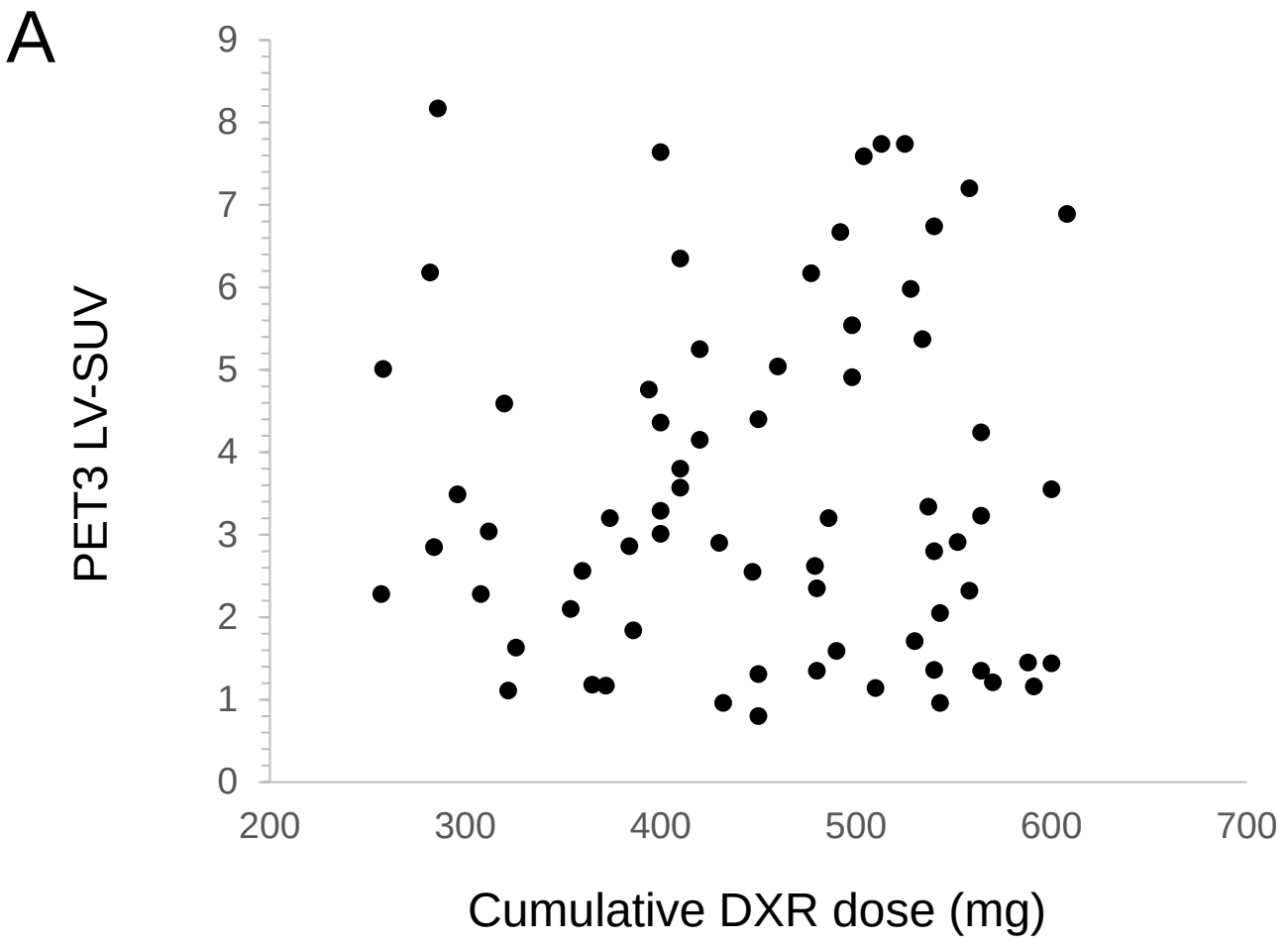
Supplemental Figure 1

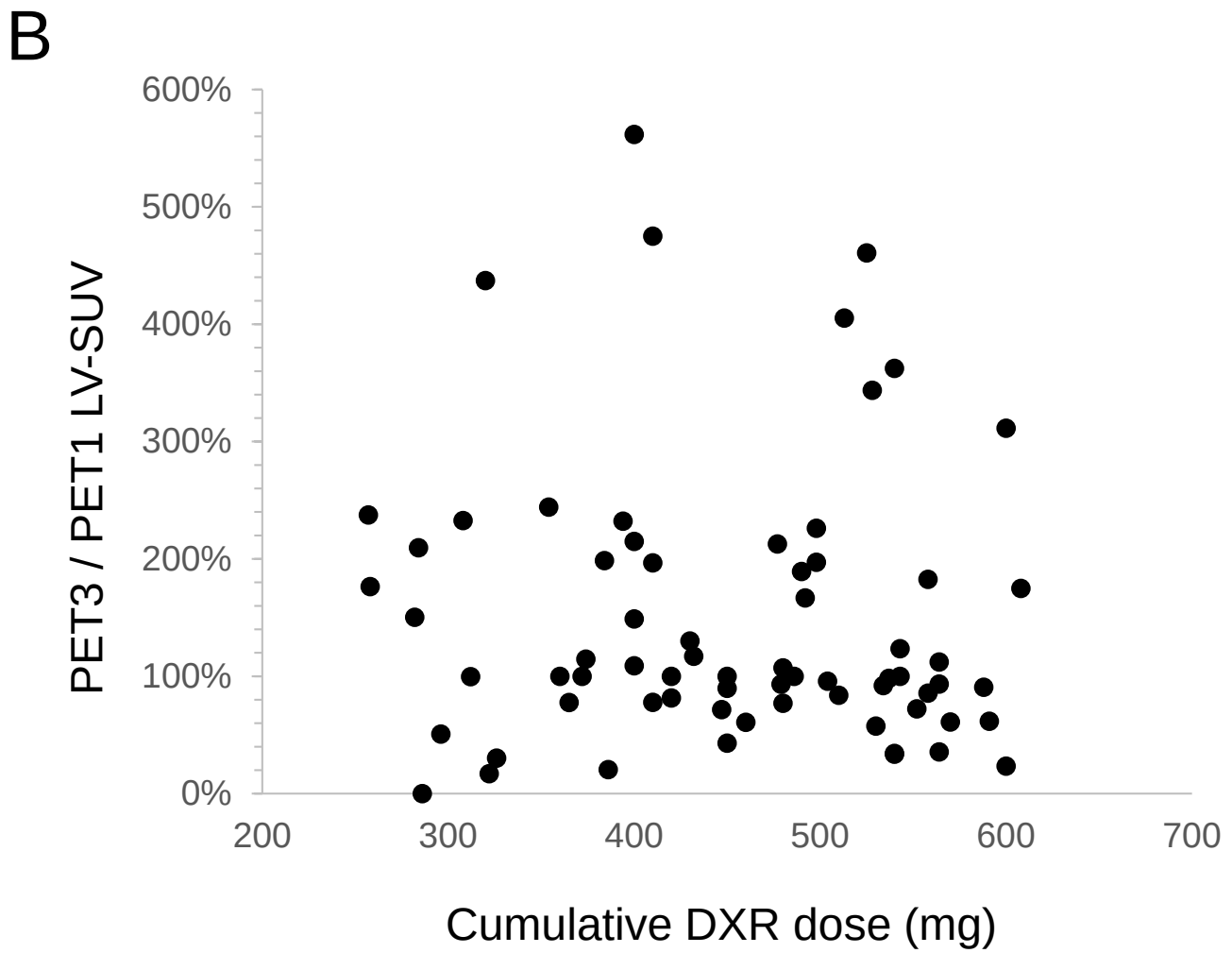


Supplemental Figure 2



Supplemental Figure 3





Supplementary Table 1: Baseline clinical characteristics of enrolled HD and excluded patients.

	Enrolled HD (n=69)	**Excluded HD (n=138)**		
		PET2 positive (n=35)	**PET3 or PET4 positive (n=18)**	**Missing PET4 (n=85)**
Age	39 ± 13 (range 19-58)	34 ± 9 (range 17-64)	36 ± 8 (range 23-48)	52 ± 21 (range 27-66)
Male Sex	37/69 (53%)	24/35 (68%)	12/18 (66%)	45/85 (52%)
Weight (Kg)	67.1 ± 12	64.6 ± 8	72.8 ± 14	75.2 ± 9
Gycemia at FDG injection (mg/dl)	79 ± 7	71 ± 10	67 ± 19	82 ± 15
Cardiovascular Risk Profile				
Hypertension	6/69 (8%)	5/35 (14%)	2/18 (11%)	29/85 (34%)
Tobacco Use	19/69 (27%)	10/35 (28%)	4/18 (22%)	14/85 (16%)
Total Cholesterol	183.7 ± 30	173.4 ± 19	201.4 ± 62	196.8 ± 54
LDL	114.5 ± 32	109 ± 15	107 ± 47	132.1 ± 52
Triglycerides	121.3 ± 49	115.9 ± 55	140 ± 76	191.6 ± 103
Creatinine	0.8 ± 0.1	1.1 ± 0.4	0.9 ± 0.2	1.1 ± 0.4
Family hystory of CAD	7/69 (10%)	7/35 (20%)	2/18 (11%)	14/85 (16%)
Time intervals between PET studies				
PET1 - PET2 (days)	73.7 ± 21	69.9 ± 32	79.3 ± 15	76.1 ± 22
PET2 - PET3 (days)	148 ± 70	117.5 ± 101	156.1 ± 91	141.2 ± 71
PET3 - PET4 (days)	195 ± 92	116.5 ± 143	122.9 ± 134	-
Baseline Ann-Arbor Staging				
I Stage	7/69 (10%)	1/35 (2%)	0/18 (0%)	21/85 (24%)
II Stage	42/69 (60%)	12/35 (34%)	9/18 (50%)	31/85 (36%)
III Stage	8/69 (12%)	12/35 (34%)	7/18 (38%)	19/85 (22%)
IV Stage	12/69 (17%)	10/35 (28%)	2/18 (11%)	14/85 (16%)
B symptoms	10/69 (14%)	4/35 (11%)	6/18 (33%)	33/85 (38%)