SUPPLEMENTAL MATERIAL

Supplemental appendix

Supplemental data regarding the APACE control cohort

Cohort details

The Advantageous Predictors of Acute Coronary Syndromes Evaluation (APACE)[49, 50] is an ongoing prospective multicentre international diagnostic study including 12 centres in 5 countries in Europe aiming to advance the early diagnosis of AMI. Briefly, adult patients presenting to the ED with symptoms suggestive of AMI, such as acute chest discomfort or angina pectoris were recruited to the APACE study. Adjudication of the final diagnosis was performed centrally at the core laboratory (University Hospital Basel) according to the fourth Universal Definition of MI (UDMI)[3]. Two independent cardiologists reviewed all available medical records including cardiac imaging and serial hs-cTnT measurements. Blood was collected at four different time-points (enrolment/admission to the ED, 1hr, 2hr and 3hr), as long as the patient was staying in observation in the ED. 3314 patients adjudicated as presenting with chest pain of non-cardiac etiology were available for the derivation of bioequivalent ULN for the approved ULN of 14ng/L for hs-cTnT-Elecsys. Patients charts were reviewed to exclude cases presenting with a muscle disorder (n=22), leaving 3292 patients for analysis. Patients with a final adjudicated non-cardiac cause of acute chest pain were selected for comparing the prevalence of cTnT/I mismatches.

Derivation of bioequivalent cut-offs in the control cohort

The two available hs-cTnI (Architect or Access) assays concentrations were plotted against hs-cTnT-Elecsys concentrations for all patients of the APACE cohort where both biomarkers were available across all collection timepoints in the same sample (of 3292 patients, 8952 concomitant measurements available for hs-cTnT-Elecsys and hs-cTnI-Architect and 2878 concomitant measurements available for hs-cTnT-Elecsys and hs-cTnI-Access). We did not assume any given distribution for the relationship between hs-cTnT-Elecsys and the I-assays and therefore used a quantile regression with restricted cubic spline using 5 knots (placed at the 5^{th}, 27.5^{th}, 50^{th}, 72.5^{th} and 95^{th} percentile). The bioequivalent hs-cTnI ULN was predicted using the uniform approved ULN of 14ng/L for hs-cTnT-Elecsys (**figures S1 and S2**). The analysis was conducted in the R statistical software using the packages "rms"[32]

Sample size calculation

We planned to adjust for multiple testing for three comparison (each hs-cTnI assay with the hs-cTnT-Elecsys assay), leading to an adjusted two-sided type I error of 0.016 (0.05/3) according to the stringent correction for multiple testing by Bonferroni.

Based on a McNemar test (as observations are paired) with the proportion of patients to present an elevated hs-cTnT in the overall cohort to be 67% and the proportion of patients presenting an elevated hs-cTnI to be 10% and a selected power of 90% with an adjusted two-sided type I error of 0.016, we obtained a first sample size of 40 patients. Based on initial preliminary data, we conservatively predicted the proportion of patients without cardiac disease to be 25% in our cohort, leading to a total sample size of 160 patients. We estimated at 10% of the patients lacking at least one of the three hs-cTnI measurement (16 patients). Therefore, a total sample size of 176 patients for the overall cohort was calculated. This would allow us to detect a difference in proportion of hs-cTn between the two assays using a Fisher exact test and to allow sufficient power the population with no cardiac disease.

Table S1 – STROBE checklist for reports of cohort studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract **Page 1**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **Page 3.4**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported **Page 6-7**
Objectives	3	State specific objectives, including any prespecified hypotheses **Page 7**
Methods		
Study design	4	Present key elements of study design early in the paper **Page 8-12**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **Page 8**
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up **Page 8**
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed **Not applicable but control cohort presented page 11**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable **Page 10**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group **Page 8-12**
Bias	9	Describe any efforts to address potential sources of bias **Page 8-12**
Study size	10	Explain how the study size was arrived at **Page 8-12**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why **Page 11-13**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **Page 14**
		(*b*) Describe any methods used to examine subgroups and interactions **Page 14**
		(*c*) Explain how missing data were addressed **Page 14**
		(*d*) If applicable, explain how loss to follow-up was addressed **Not applicable**
		(*e*) Describe any sensitivity analyses **Page 14**
Results		

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed **Page 15**
		(b) Give reasons for non-participation at each stage **Not applicable**
		(c) Consider use of a flow diagram **Supp fig. 12**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders **Table 1**
		(b) Indicate number of participants with missing data for each variable of interest **Table 1**
		(c) Summarise follow-up time (eg, average and total amount) **Not applicable**
Outcome data	15*	Report numbers of outcome events or summary measures over time **Page 15-16**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included **Page 15-17**
		(*b*) Report category boundaries when continuous variables were categorized **Page 15-17**
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period **Not applicable**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses **Page 15-17**
Discussion		
Key results	18	Summarise key results with reference to study objectives **Page 18**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias **Page 21-22**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence **Page 18-22**
Generalisability	21	Discuss the generalisability (external validity) of the study results **Page 17-22**
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based **Page 29**

Table S2 – Subtypes for each diagnosis mainclass of muscle disorders

Type	Subtype	nr
n		211
Neuropathies (%)	Neuromyotonia	3 (14)
	Neuropathy	15 (71)
	Motoneuron disorders	3 (14)
Myasthenic syndromes (%)	Lambert Eaton Syndrome	1 (2)
	Myasthenia gravis	42 (98)
Myopathies (%)	FSHD	5 (10)
	LGMD	15 (29)
	Myotonic dystrophy	13 (25)
	Fatty acid oxidation disease	1 (2)
	Glycogen storage disease	4 (8)
	Mitochondrial disease	7 (13)
	Dynamin 2 Mutation	1 (2)
	Not further specified	4 (8)
	Ryanodin R Myopathy	1 (2)
Myositis (%)	Dermatomyositis	9 (17)
	Hereditary inclusion body myositis	3 (6)
	Immune mediated necrotizing myopathy	12 (23)
	Myositis with overlap with collagenous	8 (15)
	Polymyositis	9 (17)
	Sporadic inclusion body myositis	7 (13)
	Statin induced myositis	1 (2)
	Vasculitis	1 (2)
	Toxic	1 (2)
	Unclear	2 (4)
Muscle symptoms (%)	Unclear	8 (40)
	Muscle atrophy	3 (15)
	Myalgia	9 (45)
Autoimmune disease with muscle symptoms (%)	Polymyalgia rheumatica	21 (91)
	Rheumatoid arthritis	2 (9)

FSHD = Facioscapulohumeral muscular dystrophy, LGMD = Limb–girdle muscular dystrophy

Table S3 — Baseline characteristics of the control cohort of patients with adjudicated non-cardiac causes of acute chest pain

Number	Overall
n	3508
Sex: Female (%)	1287 (37)
Age (mean (SD))	54.9 (16.5)
Coronary artery disease (%)	761 (22)
Previous AMI (%)	552 (16)
Hypertension (%)	1585 (45)
Hypercholesterolemia (%)	1260 (36)
Diabetes Mellitus (%)	401 (11)
Previous PE (%)	73 (2)
Pacemaker, ICD or CRT (%)	55 (2)
Stroke (%)	121 (3)
Kidney failure (%)	183 (5)

RNA-seq experiment

Sample randomization

RNA extraction was performed in batches of up to 18 samples, library preparation and sequencing in batches of up to 30 samples. We performed block randomization using the R package OSAT (Optimal Sample Assignment Tool v1.40.0 (DOI: 10.18129/B9.bioc.OSAT) such that samples were equally distributed across the batches with regard to the case/control status and gender.

RNA extraction

RNA extraction was performed by CEGAT GmbH (Tübingen, Germany). Total RNA was extracted for 54 samples using the RNeasy Mini Kit (Qiagen, Netherlands). For samples with low RIN or insufficient RNA yield, RNA extraction was performed again by using RNeasy Fibrous Tissue Mini kit (Qiagen, Netherlands). Finally, four samples failed during RNA extraction. Of the remaining, RNA of 10 samples was with RNeasy Fibrous Tissue Mini kit and 39 samples was extracted with RNeasy Mini Kit. Because of the re-extraction for some of the samples, the predefined batches for RNA extraction could not be adhered to.

Library preparation and RNA sequencing

Purification of mRNA by poly(A)-selection, mRNA fragmentation and cDNA library preparation for RNA-Seq were performed using the TruSeq Stranded mRNA Library Preparation Kit (Illumina, USA) according to the manufacturer's protocol. cDNA libraries were sequenced as paired-end reads with 100 cycles and a depth of 80M clusters on a NovaSeq 6000 sequencing system (Illumina, USA). No sample failed during library preparation.

RNA-seq computational analysis

Mapping and pre-processing

We performed adaptor clipping and quality-trimming of sequences using Fastp v0.11.9 (https://github.com/OpenGene/fastp). In Fastp, we used the default adaptor clipping as well as sequence length filtering and disabled quality filtering. Moreover, since the two colour chemistry system of NovaSeq is not able to distinguish "G" and "no signal", we applied a polyG trail trimming.

Reads were aligned to the GENCODE v35 GRCh38 reference transcriptome using the transcript quantifier Salmon v1.3.0 (https://salmon.readthedocs.io/).

Subsequently, we used the R package tximeta to combine Salmon transcript quantifications and sample data and to summarize transcript quantifications on gene level.

Quality control

For assessing the data quality on a read and alignment level, we used FastQC v0.11.9 (www.bioinformatics.babraham.ac.uk/projects/fastqc/) and MultiQC v1.9 (https://multiqc.info/). before and after trimming.

For detecting outlier samples, we performed a principal component analysis on variance stabilizing transformed counts for the 500 genes with the highest variance using the R package DESeq2 v1.28.0. We calculated pairwise correlations of the first two principal components, patient data and technical variables obtained from MultiQC using (1) linear regression for pairs of nominal and numeric variables, (2) Spearman's rank correlation coefficient for pairs of numeric variables and (3) Cramer's V and Pearson's chi-squared test for pairs with nominal variables to assess for confounding variables and hidden associations. The effect of batch removal through identified variables was simulated with limma v3.46.0. We defined variables as confounders if they showed a correlation of > 0.5 at a P-value < 0.05 with either PC1 or PC2.

We also tested self-reported sex to correspond with sex inferred by the expression of *XIST*, a gene that encodes for long non-coding RNA in the sex-specific X-inactivation process. This gene is not or only marginally expressed in males. Accordingly we predicted samples to be male if transcripts per million (TPM) < 5 and female if not.

Differential gene expression

We performed differential gene expression (DGE) analysis on all sequenced samples using the summarized gene counts with DESeq2 v1.28.0.

In our first DGE analyses, we compared all cases versus all controls. In total, we tested six genes for DGE, namely *TNNT1-3* and *TNNI1-3*. Resulting P-values attained by the Wald test were corrected for multiple testing using the Benjamini and Hochberg (BH) method. We defined the significant level as $\alpha = 0.05$. Since there was no value overlap in the "muscle type" variable between cases and controls, we could not investigate the effect of this variable by adjusting for it (**Figure S7**).

Next, we performed a subgroup analysis on the major gene of interest, *TNNT2* (cardiac type), by forming three disease groups according to **Figure S8** and comparing them pairwise: (1) containing all samples of the disease class "Myopathy", (2) containing all samples from the disease class "Myositis" and (3) "Other SMD" combining all samples of the remaining disease classes ("Muscle symptom", "Myasthenic syndrome" and "Neuropathy"). Since the correlation analysis (next section) revealed a significant association of biopsy status and expression level, which cannot be solely explained by the disease groups, we also added the "biopsy status" to the model to correct for it. In this analysis, we corrected the statistical test of the three pairwise comparisons for multiple testing using BH.

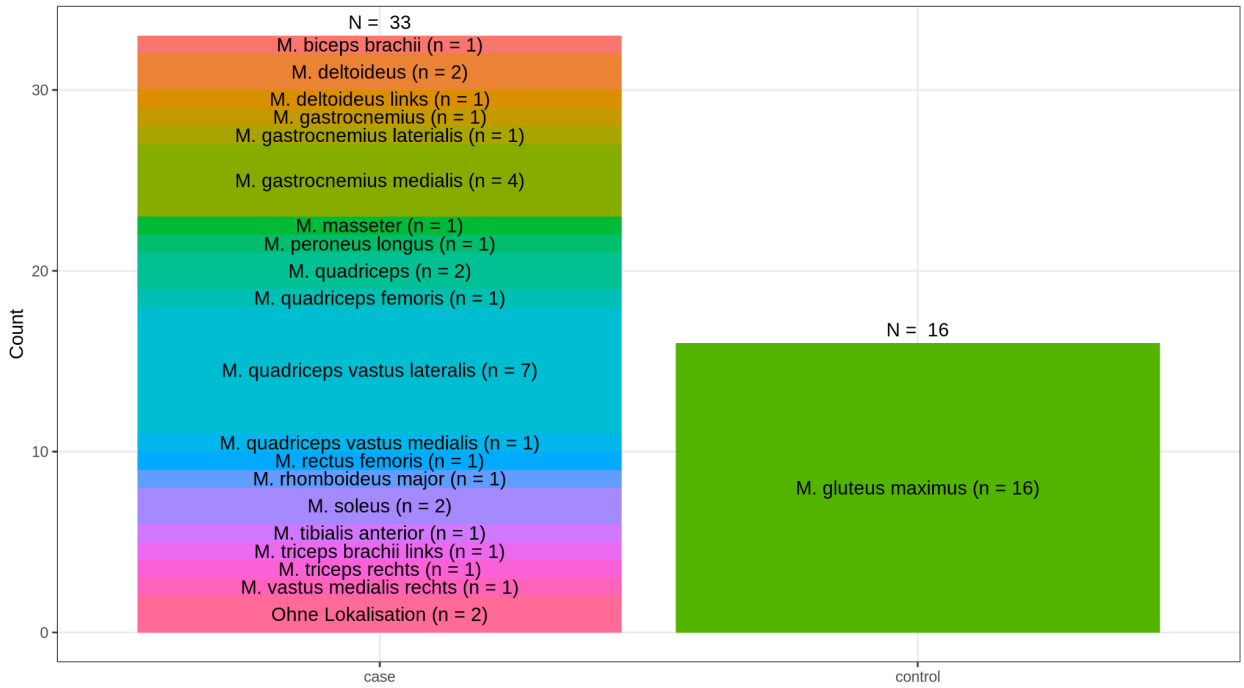


Figure S1. Biopsied muscle types in case and control cohorts.

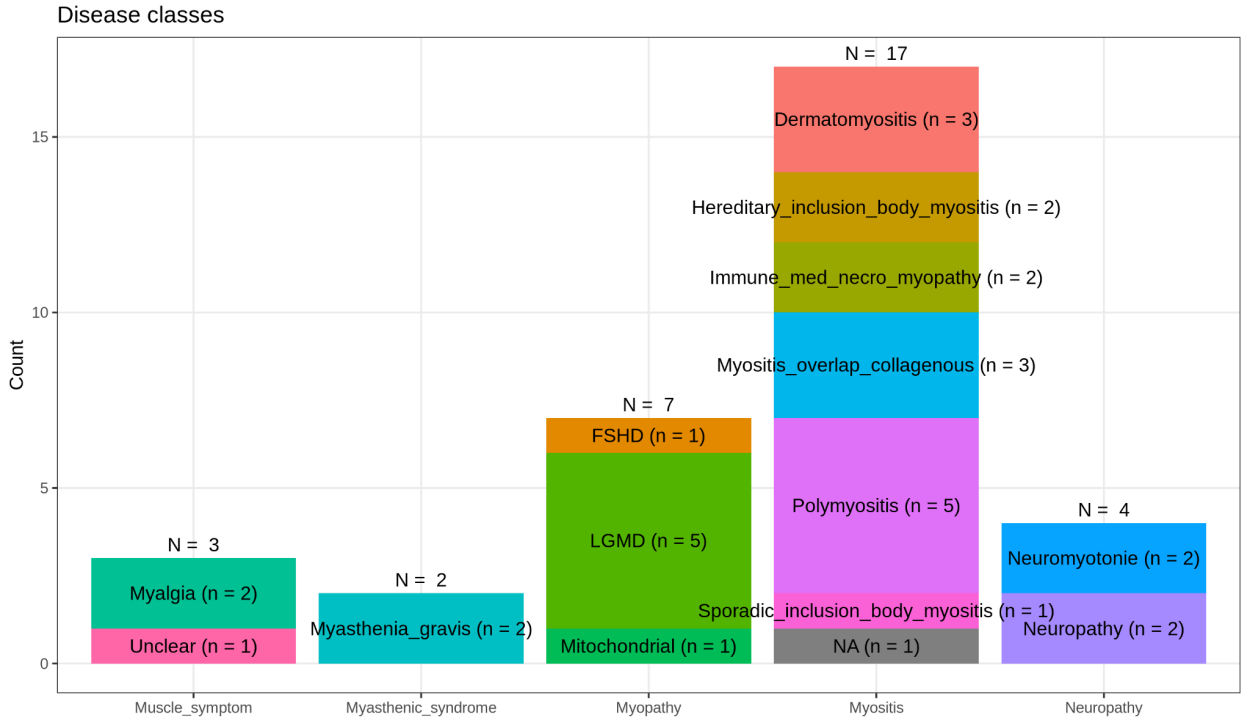


Figure S2. Case samples and assigned disease classes.

TNNT2 vs disease activity correlation analysis

To investigate the effect of disease activity in the biopsy samples on the gene expression of *TNNT2*, we calculated a disease activity score for the case cohort based on 18 ordinal marker variables describing the disease status of the biopsy samples.

Due to a substantial amount of missing values we applied a filtering by removing variables with more than 70% of missingness and subsequently case samples with more than 60% of missingness. 14 markers and 28 of 33 case samples passed the filtering. These markers were : Fiber caliber variability, central nuclei, endomysial connective tissue, Vakafett, atrophic fibers, myophagia and necrotic fibers, fiber splitting, rimmed vacuoles, ragged red fibers, COX or SDH positive fibers, fiber distribution pattern, neutral fat and inflammatory infiltrates.

Then we transformed the discrete values for each variable to the interval [0,1]. Then, we imputed missing values by setting them to the mean value of the respective variable. Finally, we summed up the resulting values for each case sample to form a disease activity score.

We then applied linear regression on the models

$$(1)\ \ log10(norm\_count) \sim disease\_activity\_score$$

$$(2)\ log10(norm\_count) \sim disease\_activity\_score + disease\_class$$

Dependent variable *norm_count* denotes the normalized counts calculated by DESeq2 and independent variable *disease_class* denotes the disease classes (“Myopathy” [n = 7], “Myositis” [n = 13], “Other SMD” [n = 8]).

Disease class differences

To investigate potential differences among the three disease classes, we performed a Likelihood ratio test with DESeq2 by adjusting for a disease activity score which is described in detail in the previous section. In this analysis we used subset of case samples (n = 28) for which disease activity score could be derived.

Table S4 — Cardiac imaging (transthoracic echocardiography and cardiac MRI) performed in the patients with skeletal muscle disease

rn	Overall	High risk	Medium risk	Low risk	p
n	211	59	44	108	
Echocardiography					
Examination conducted in (%)	118 (56)	44 (75)	26 (59)	48 (44)	0.001
LVEDD (median [IQR])	46.0 [42.0, 49.0]	48.0 [45.0, 50.3]	46.0 [41.0, 50.0]	44.0 [39.2, 48.0]	0.003
LVEF (median [IQR])	60.0 [55.0, 65.0]	59.0 [53.0, 65.5]	59.0 [56.0, 60.0]	61.0 [58.2, 65.0]	0.065
Reduced motility (%)	10 (8)	8 (18)	2 (8)	0 (0)	0.007
LVH (%)	12 (10)	11 (25)	1 (4)	0 (0)	<0.001
Any severe valvular defect (%)	14 (7)	14 (24)	0 (0)	0 (0)	<0.001
Cardiac MRI					
Examination conducted in (%)	47 (22)	19 (32)	8 (18)	20 (19)	0.097
LVEF (median [IQR])	61.0 [55.0, 64.0]	58.5 [51.8, 63.5]	63.5 [58.8, 65.0]	61.0 [55.5, 62.8]	0.461
motility LV reduced (%)	5 (2)	4 (7)	1 (2)	0 (0)	0.023
motility RV reduced (%)	1 (0)	0 (0)	1 (2)	0 (0)	0.149
Dilatation LV (%)	3 (1)	1 (2)	2 (5)	0 (0)	0.098
Dilatation RV (%)	1 (0)	0 (0)	1 (2)	0 (0)	0.149
hypertrophy LV (%)	5 (2)	3 (5)	2 (5)	0 (0)	0.067
hypertrophy RV (%)	0 (0)	0 (0)	0 (0)	0 (0)	
enhancement LV (%)	10 (5)	10 (17)	0 (0)	0 (0)	<0.001
enhancement RV (%)	3 (1)	3 (5)	0 (0)	0 (0)	0.020
scar LV (%)	4 (2)	4 (7)	0 (0)	0 (0)	0.005
scar RV (%)	0 (0)	0 (0)	0 (0)	0 (0)	
Any severe valvular defect (%)	3 (1)	3 (5)	0 (0)	0 (0)	0.020

RV = right ventricle, LV = left ventricle, LVEF = Left ventricular ejection fraction, LVEDD = Left ventricular end diastolic diameter, LVH = Left ventricular hypertrophy.

Table S5 — hs-cTnT/I concentrations in ng/L as measured by the different assays and comparison with the controls adjudicated to have non-cardiac causes of acute chest pain.

Assay	Classification of cardiac disease	Median	Lower IQR	Higher IQR	Wilcoxon p-value comparing with controls*
hs-cTnT Elecsys	Overall	16	7	32.5	<0.001
	No cardiac disease	9	5	25.1	<0.001
	Mild disease	19	10.8	32.5	<0.001
	Severe cardiac disease	26	16.5	44.5	<0.001
	Controls	5	3	9	
hs-cTnI Architect	Overall	2.5	1.2	6.2	0.265
	No cardiac disease	1.5	0.9	2.8	<0.001
	Mild disease	3.7	1.6	6.2	0.369
	Severe cardiac disease	6.8	3.2	18	<0.001
	Controls	2.9	1.8	5	
hs-cTnI Access	Overall	3.3	2.4	6.1	<0.001
	No cardiac disease	2.6	1.9	3.6	0.63
	Mild disease	5.5	3.2	7.6	<0.001
	Severe cardiac disease	6.1	3.3	14.5	<0.001
	Controls	2.7	1.6	5.0	
hs-cTnI Vista	Overall	7.4	5.2	13.4	0.833
	No cardiac disease	5.7	4.6	8.2	<0.001
	Mild disease	8.4	5.8	10.5	0.438
	Severe cardiac disease	14.7	7.6	34.1	<0.001
	Controls	7.5	6	10	

IQR = Interquartile range. *The p-values have been corrected for multiple testing using the Benjamini and Hochberg method.

Table S6 – Characterizations of patients above the rule-in hs-cTnT cut-off of the ESC 0/1hr and ESC 0/2hr algorithm (52ng/L) in the overall cohort and subgroup with no cardiac disease

Variable	Overall cohort	Subgroup with no cardiac disease
n	34	14
hs-cTnT (median [IQR])	80.8 [62.2, 199.1]	92.5 [68.2, 286.9]
hs-cTnI Architect (median [IQR])	6.2 [2.6, 20.3]	2.8 [1.3, 7.2]
hs-cTnI Access (median [IQR])	5.7 [3.0, 14.2]	3.1 [2.6, 4.5]
hs-cTnI Vista (median [IQR])	9.1 [5.5, 25.7]	6.3 [4.8, 8.0]
Sex: Female (%)	13 (38)	7 (50)
Clinical or ECG evidence of AMI or other acute cardiac disease (%)	0 (0)	0 (0)
Prior AMI (%)	4 (12)	0 (0)
eGFR (median [IQR])	103.5 [79.9, 112.5]	105.3 [103.3, 117.8]

AMI = Acute myocardial infarction, eGFR = estimated GFR, IQR = Interquartile range

Table S7 – Percentages of elevated hs-cTnT/I in the overall cohort and the cohort with no cardiac disease

assay1	assay2	number of patients with both assays	Assay 1 : Number above assay-specific ULN	Assay 2: Number Above assay-specific ULN	Assay 1: % above assay-specific ULN (% [95%-CI])	Assay 2 : % above assay-specific ULN (%[95%-CI])	p-value for comparison of percentages*
Overall cohort							
Elecsys TnT	Architect approved uniform ULN	211	116	19	54.9 [48.2; 61.5]	9.8 [5.8; 13.7]	<0.001
Elecsys TnT	Access approved uniform ULN	187	103	9	55 [47.9; 62]	5.8 [2.5; 9.1]	<0.001
Elecsys TnT	Vista approved uniform ULN	194	108	9	55.6 [48.6; 62.5]	5.6 [2.4; 8.7]	<0.001
Elecsys TnT	Architect bioequivalent ULN	211	116	48	54.9 [48.2; 61.5]	23.3 [17.6; 28.9]	<0.001
Elecsys TnT	Access bioequivalent ULN	187	103	41	55 [47.9; 62]	22.5 [16.6; 28.4]	<0.001
Elecsys TnT	Vista bioequivalent ULN	194	108	13	55.6 [48.6; 62.5]	7.6 [3.9; 11.3]	<0.001
Elecsys TnT sex-specific	Architect sex-specific ULN	211	130	18	61.4 [54.9; 67.9]	9.3 [5.4; 13.2]	<0.001
Elecsys TnT sex-specific	Access sex-specific ULN	187	116	12	61.8 [54.9; 68.7]	7.3 [3.6; 11]	<0.001
Elecsys TnT sex-specific	Vista sex-specific ULN	194	120	10	61.6 [54.8; 68.4]	6.1 [2.7; 9.4]	<0.001
Cohort with no cardiac disease							
Elecsys TnT	Architect approved uniform ULN	108	41	2	38.4 [29.4; 47.4]	3.6 [0.1; 7]	<0.001

assay1	assay2	number of patients with both assays	Assay 1 : Number above assay-specific ULN	Assay 2: Number Above assay-specific ULN	Assay 1: % above assay-specific ULN (% [95%-CI])	Assay 2 : % above assay-specific ULN (%[95%-CI])	p-value for comparison of percentages*
Elecsys TnT	Access approved uniform ULN	97	36	1	37.6 [28.2; 47.1]	3 [0; 6.3]	<0.001
Elecsys TnT	Vista approved uniform ULN	100	37	1	37.5 [28.2; 46.8]	2.9 [0; 6.1]	<0.001
Elecsys TnT	Architect bioequivalent ULN	108	41	8	38.4 [29.4; 47.4]	8.9 [3.6; 14.2]	<0.001
Elecsys TnT	Access bioequivalent ULN	97	36	3	37.6 [28.2; 47.1]	5 [0.7; 9.2]	<0.001
Elecsys TnT	Vista bioequivalent ULN	100	37	2	37.5 [28.2; 46.8]	3.8 [0.2; 7.5]	<0.001
Elecsys TnT sex-specific	Architect sex-specific ULN	108	46	1	42.9 [33.7; 52]	2.7 [0; 5.7]	<0.001
Elecsys TnT sex-specific	Access sex-specific ULN	97	41	1	42.6 [32.9; 52.2]	3 [0; 6.3]	<0.001
Elecsys TnT sex-specific	Vista sex-specific ULN	100	42	1	42.3 [32.8; 51.8]	2.9 [0; 6.1]	<0.001

CI = Confidence interval , ULN= Upper limit of normal. *The p-values have been corrected for multiple testing using the Benjamini and Hochberg method.

Table S8 - Percentages of elevated hs-cTnT/I in the APACE control cohort

assay1	assay2	number of patients with both assays	Assay 1 : Number above assay-specific ULN	Assay 2: Number Above assay-specific ULN	Assay 1: % above assay-specific ULN (% [95%-CI])	Assay 2 : % above assay-specific ULN (%[95%-CI])	p-value for comparison of percentages*
Elecsys TnT	Architect approved ULN	3243	425	134	13.2 [12; 14.3]	4.2 [3.5; 4.9]	<0.001
Elecsys TnT	Access approved ULN	1051	114	41	11 [9.1; 12.9]	4.1 [2.9; 5.3]	<0.001
Elecsys TnT	Architect bioequivalent ULN	3243	425	562	13.2 [12; 14.3]	17.4 [16.1; 18.7]	<0.001
Elecsys TnT	Access bioequivalent ULN	946	75	41	8.1 [6.4; 9.8]	4.5 [3.2; 5.8]	0.0016
Elecsys TnT sex-specific ULN	Architect sex-specific ULN	3243	515	149	15.9 [14.7; 17.2]	4.7 [3.9; 5.4]	<0.001
Elecsys TnT sex-specific ULN	Access sex-specific ULN	1051	153	44	14.7 [12.6; 16.8]	4.4 [3.1; 5.6]	<0.001

CI = Confidence interval , ULN= Upper limit of normal. *The p-values have been corrected for multiple testing using the Benjamini and Hochberg method.

Table S9 – Percentages of elevated hs-cTn in the overall cohort and subgroup with no cardiac disease of patients with a myositis or a non-inflammatory myopathy

assay1	assay2	number of patients with both assays	Assay 1 : Number above assay-specific ULN	Assay 2: Number Above assay-specific ULN	Assay 1: % above assay-specific ULN (%)	Assay 2 : % above assay-specific ULN (%)	p-value for comparison of percentages*
Overall cohort							
Elecsys TnT	Architect approved uniform ULN	104	77	15	73.1 [64.8; 81.5]	15.7 [8.9; 22.6]	<0.001
Elecsys TnT	Access approved uniform ULN	94	71	7	74.5 [65.9; 83.1]	9.2 [3.5; 14.9]	<0.001
Elecsys TnT	Vista approved uniform ULN	95	72	7	74.7 [66.2; 83.3]	9.1 [3.4; 14.8]	<0.001
Elecsys TnT	Architect bioequivalent ULN	104	77	29	73.1 [64.8; 81.5]	28.7 [20.2; 37.2]	<0.001
Elecsys TnT	Access bioequivalent ULN	94	71	23	74.5 [65.9; 83.1]	25.5 [16.9; 34.1]	<0.001
Elecsys TnT	Vista bioequivalent ULN	95	72	10	74.7 [66.2; 83.3]	12.1 [5.7; 18.6]	<0.001
Elecsys TnT sex-specific	Architect sex-specific ULN	104	85	14	80.6 [73.1; 88]	14.8 [8.1; 21.5]	<0.001
Elecsys TnT sex-specific	Access sex-specific ULN	94	78	9	81.6 [74; 89.3]	11.2 [5; 17.5]	<0.001
Elecsys TnT sex-specific	Vista sex-specific ULN	95	79	8	81.8 [74.2; 89.4]	10.1 [4.2; 16]	<0.001
No cardiac disease							

assay1	assay2	number of patients with both assays	Assay 1 : Number above assay-specific ULN	Assay 2: Number Above assay-specific ULN	Assay 1: % above assay-specific ULN (%)	Assay 2 : % above assay-specific ULN (%)	p-value for comparison of percentages*
Elecsys TnT	Architect approved uniform ULN	51	35	2	67.3 [54.9; 79.7]	7.3 [0.4; 14.1]	<0.001
Elecsys TnT	Access approved uniform ULN	45	31	0	67.3 [54.2; 80.5]	4.1 [0; 9.6]	<0.001
Elecsys TnT	Vista approved uniform ULN	46	32	0	68 [55.1; 80.9]	4 [0; 9.4]	<0.001
Elecsys TnT	Architect bioequivalent ULN	51	35	6	67.3 [54.9; 79.7]	14.5 [5.2; 23.9]	<0.001
Elecsys TnT	Access bioequivalent ULN	45	31	0	67.3 [54.2; 80.5]	4.1 [0; 9.6]	<0.001
Elecsys TnT	Vista bioequivalent ULN	46	32	1	68 [55.1; 80.9]	6 [0; 12.6]	<0.001
Elecsys TnT sex-specific	Architect sex-specific ULN	51	36	1	69.1 [56.9; 81.3]	5.5 [0; 11.5]	<0.001
Elecsys TnT sex-specific	Access sex-specific ULN	45	32	0	69.4 [56.5; 82.3]	4.1 [0; 9.6]	<0.001
Elecsys TnT sex-specific	Vista sex-specific ULN	46	33	0	70 [57.3; 82.7]	4 [0; 9.4]	<0.001

CI = Confidence interval , ULN= Upper limit of normal. *The p-values have been corrected for multiple testing using the Benjamini and Hochberg method.

Table S10 — Correlations between CK and hs-cTn assays

a) Overall cohort	Equation for the linear regression	Correlation coefficient (Kendall Tau)	R-squared	P-values from linear regression analysis*
hs-cTnT Elecsys	y=0.4+(0.469x)	0.3319	0.2669	<0.001
hs-cTnI Architect	y=0.6+(0.105x)	0.04724	0.008242	0.189
hs-cTnI Access	y=0.9+(0.0965x)	0.05136	0.01713	0.1069
hs-cTnI Vista	y=2+(0.0894x)	0.04	0.01586	0.1069
b) Subgroup with no cardiac disease				
hs-cTnT Elecsys	y=-0.5+(0.562x)	0.4305	0.3557	<0.001
hs-cTnI Architect	y=-0.2+(0.132x)	0.05021	0.0194	0.301
hs-cTnI Access	y=0.9+(0.0164x)	0.08613	0.0006851	0.7991
hs-cTnI Vista	y=2+(0.0266x)	0.004477	0.002964	0.7875

Equations were derived using the logarithm of CK and hs-cTn concentrations, as both biomarkers were not normally distributed. *The p-values have been corrected for multiple testing using the Benjamini and Hochberg method.

Table S11 — Correlations between CK-MB and hs-cTnT/I assays

a) Overall cohort	Equation for the linear regression	Correlation coefficient (Kendall Tau)	R-squared	P-values from linear regression analysis*
hs-cTnT Elecsys	y=1+(0.762x)	0.4533	0.4599	<0.001
hs-cTnI Architect	y=0.05+(0.35x)	0.1735	0.06671	0.067
hs-cTnI Access	y=0.8+(0.282x)	0.1773	0.09357	0.049
hs-cTnI Vista	y=2+(0.111x)	0.07315	0.01322	0.3987
b) Subgroup with no cardiac disease				
hs-cTnT Elecsys	y=1+(0.785x)	0.5207	0.5676	<0.001
hs-cTnI Architect	y=-0.9+(0.461x)	0.2775	0.1729	0.0322
hs-cTnI Access	y=1+(0.0354x)	0.1161	0.007338	0.7355
hs-cTnI Vista	y=2+(-0.041x)	0.04555	0.003997	0.7355

Equations were derived using the logarithm of CK-MB and hs-cTn concentrations, as both biomarkers were not normally distributed. *The p-values have been corrected for multiple testing using the Benjamini and Hochberg method.

Table S12 — Baseline characteristics of patients providing a biopsy for mRNA analysis and controls patients

Variable	Cases	Controls	p
n	33	16	
Sex : Female (%)	13 (39)	7 (44)	1.000
Age (mean (SD))	59.0 (17.2)	68.4 (12.9)	0.061
Hospitalized (%)	1 (3)	0 (0)	<0.001
Coronary artery disease (%)	2 (6)	1 (6)	1.000
Previous AMI (%)	2 (6)	1 (6)	1.000
Hypertension (%)	13 (39)	12 (75)	0.032
Hypercholesterolemia (%)	12 (36)	4 (25)	0.526
Diabetes Mellitus (%)	5 (15)	5 (31)	0.261
History of atrial fibrillation (%)	1 (3)	2 (12)	0.245
Previous DVT or PE (%)	1 (3)	4 (25)	0.034
Heart failure: NYHA II (%)	0 (0)	1 (6)	0.327
Pacemaker (%)	0 (0)	0 (0)	
ICD (%)	0 (0)	0 (0)	
Stroke (%)	2 (6)	1 (6)	1.000
Muscle manifestations: Upper or lower body (%)			
Lower body	9 (27)	0 (0)	
Upper body	5 (15)	0 (0)	
Lower and upper body	8 (24)	0 (0)	
Not localized	11 (33)	0 (0)	
Muscle manifestations : proximal or distal (%)			
proximal	2 (6)	0 (0)	
distal	12 (36)	0 (0)	
proximal and distal	8 (24)	0 (0)	
Not localized	11 (33)	0 (0)	
Begin of symptoms >1 year (%)	21 (66)	0 (0)	
Muscle pain (%)	17 (52)	0 (0)	
Muscle cramps (%)	6 (18)	0 (0)	
Muscle atrophy (%)	10 (30)	0 (0)	
Muscle stiffness (%)	6 (18)	0 (0)	
Muscle weakness (%)	21 (64)	0 (0)	
Clinical evaluation (%)			
Planned follow-up visit	28 (85)	0 (0)	
First evaluation	4 (12)	0 (0)	
Relapse	1 (3)	0 (0)	

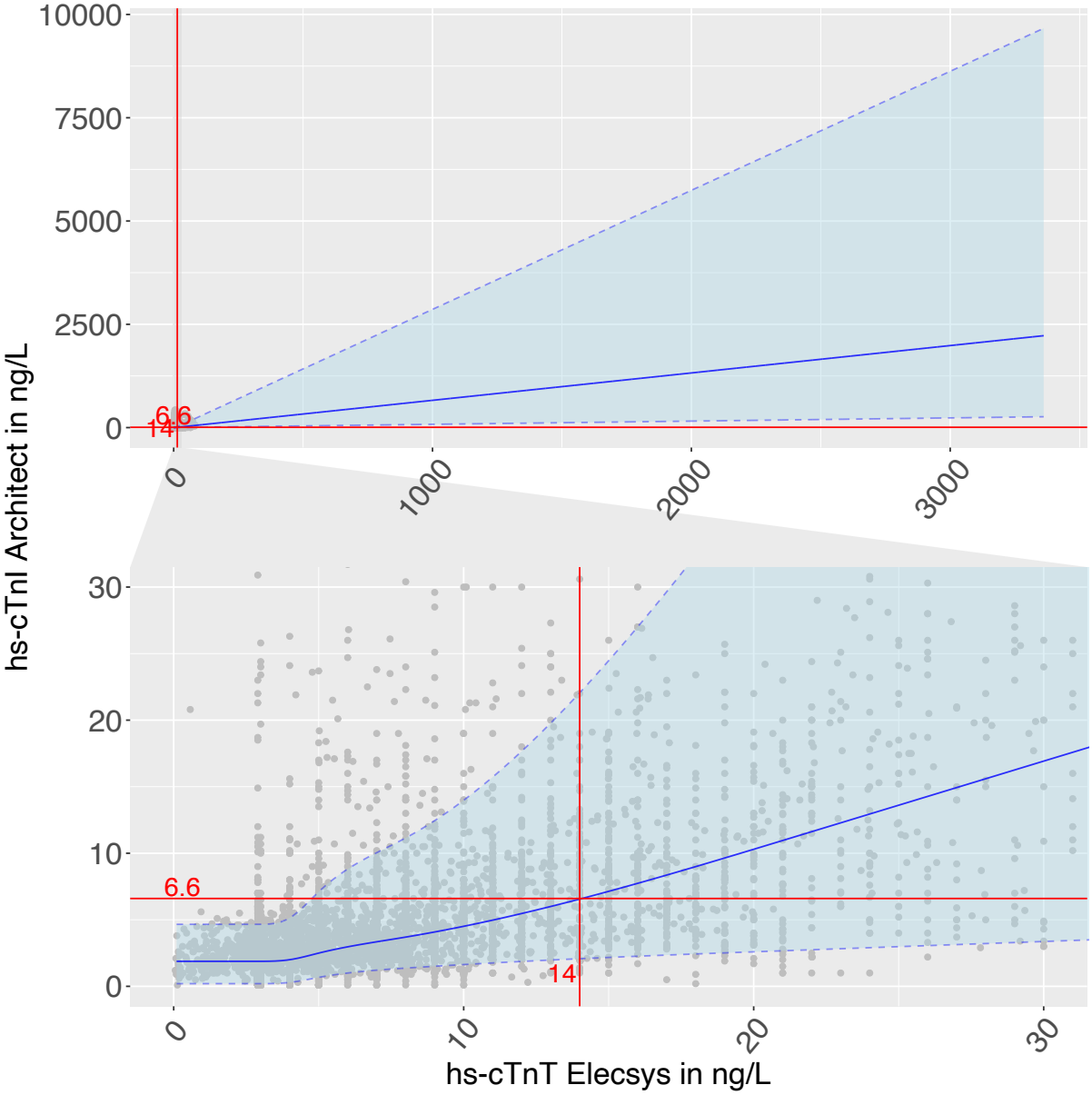
Variable	Cases	Controls	p
Final diagnosis			
Non-inflammatory myopathy	7 (21)	0 (0)	
Muscle symptoms	3 (9)	0 (0)	
Neuropathy	4 (12)	0 (0)	
Myasthenic syndrome	2 (6)	0 (0)	
Myositis	17 (52)	0 (0)	

Table S13 — Correlation between circulating log(hs-cTnT) concentrations and TNNT genes expression

Gene	Equation for the linear regression	Correlation coefficient (Kendall Tau)	R-squared	P-values from linear regression analysis*
TNNT1 (Skeletal slow)	y=5+(-0.149x)	-0.2154	0.03262	0.3145
TNNT2 (Cardiac)	y=1+(0.239x)	0.2574	0.1928	0.0317
TNNT3 (Skeletal fast)	y=8+(-0.342x)	-0.1277	0.05625	0.2759

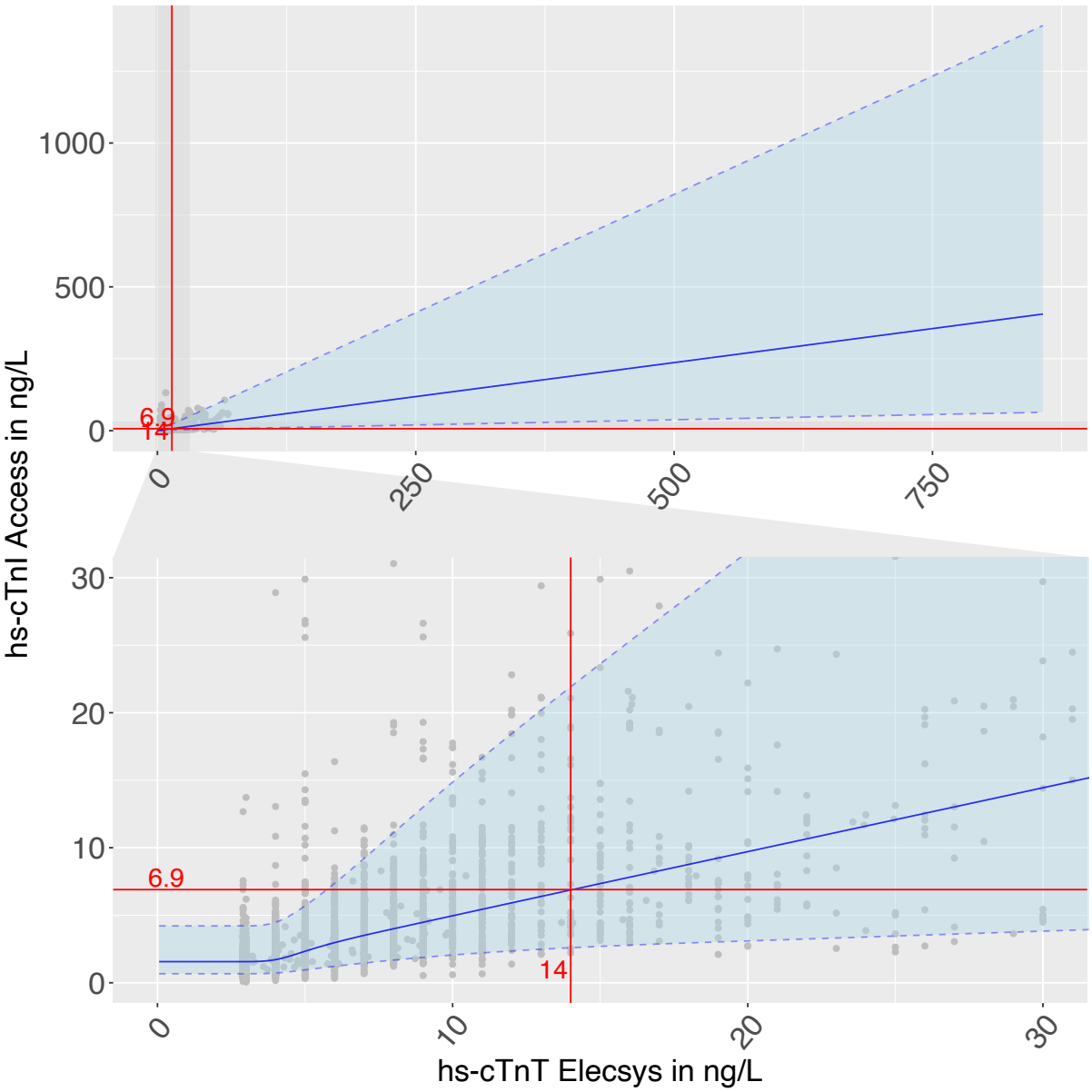
Equations were derived using the logarithm of hs-cTn concentrations and normalized gene expression, as both parameters were not normally distributed. *The p-values have been corrected for multiple testing using the Benjamini and Hochberg method.

Figure S3 – Derivation of a bio-equivalent Upper Limit of Normal for the hs-cTnI-Architect assay to the ULN of 14ng/L for the hs-cTnT Elecsys assay in the APACE cohort using



The blue line represents a median regression using a restricted cubic spline with 5 knots. The light blue ribbon represents 95% prediction intervals.

Figure S4 – Derivation of a bio-equivalent Upper Limit of Normal for the hs-cTnI-Access assay to the ULN of 14ng/L for the hs-cTnT Elecsys assay in the APACE cohort



The blue line represents a median regression using a restricted cubic spline with 5 knots. The light blue ribbon represents 95% prediction intervals.

Figure S5 – Study chart flow

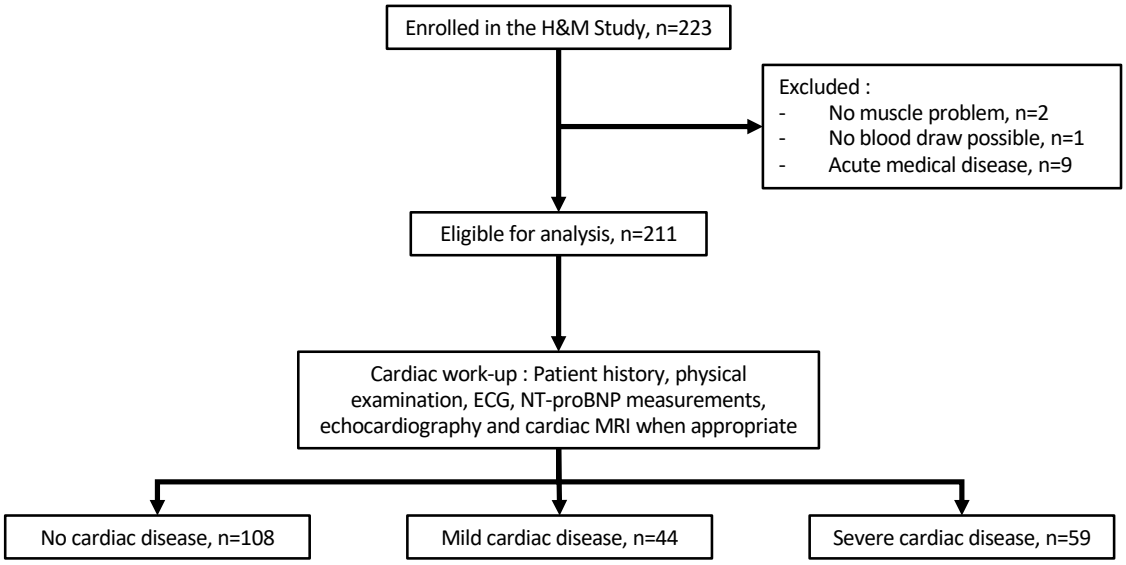
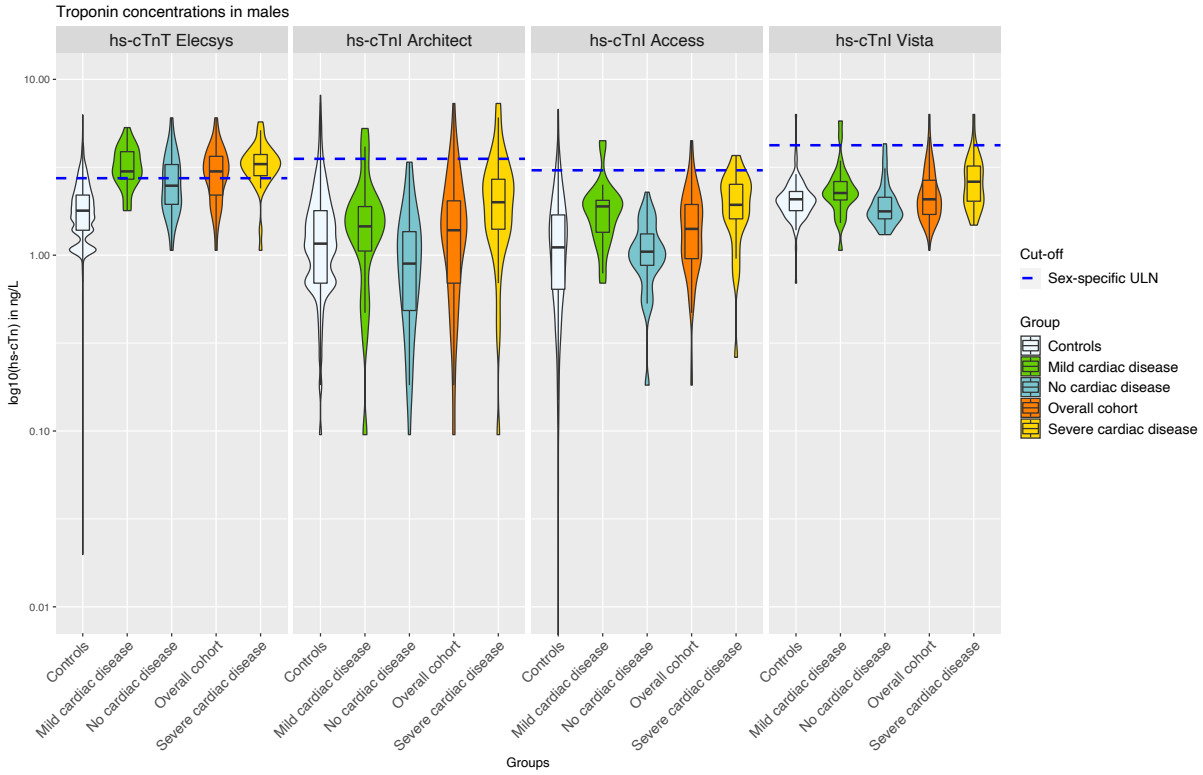
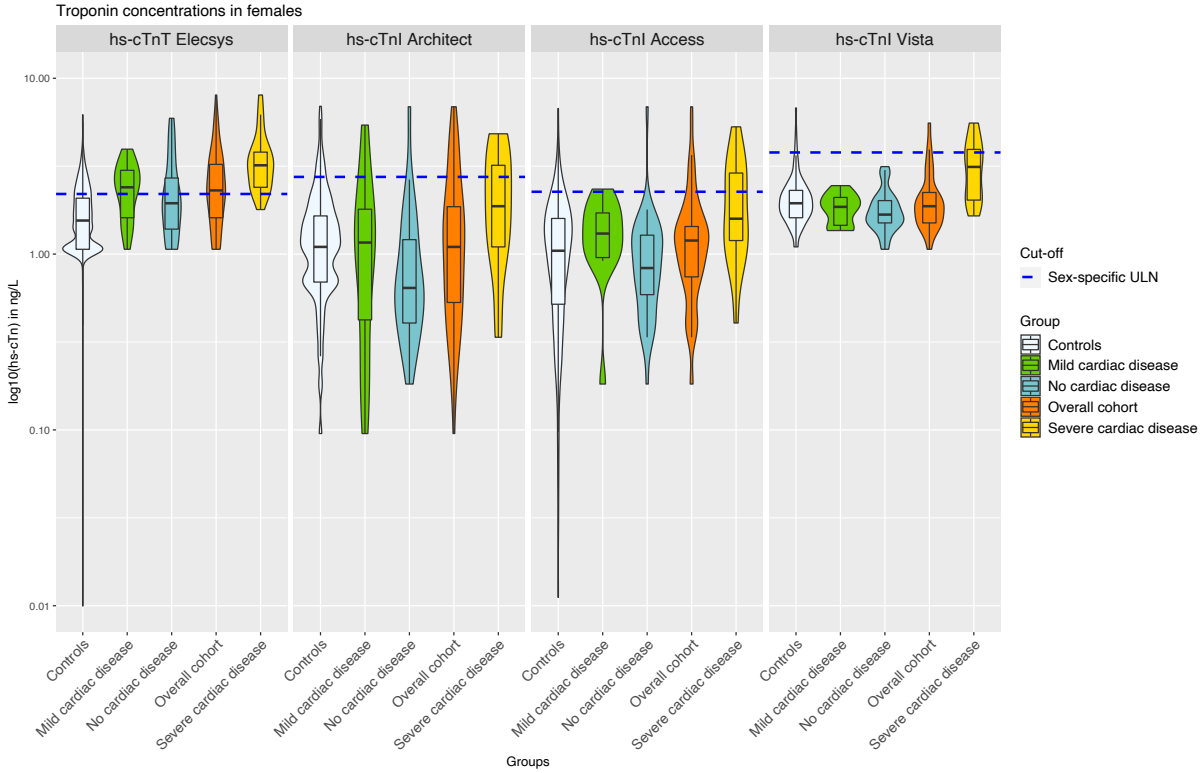


Figure S6 – hs-cTnT/I concentrations in males as measured by the different assays and comparison with the controls from the APACE cohort.



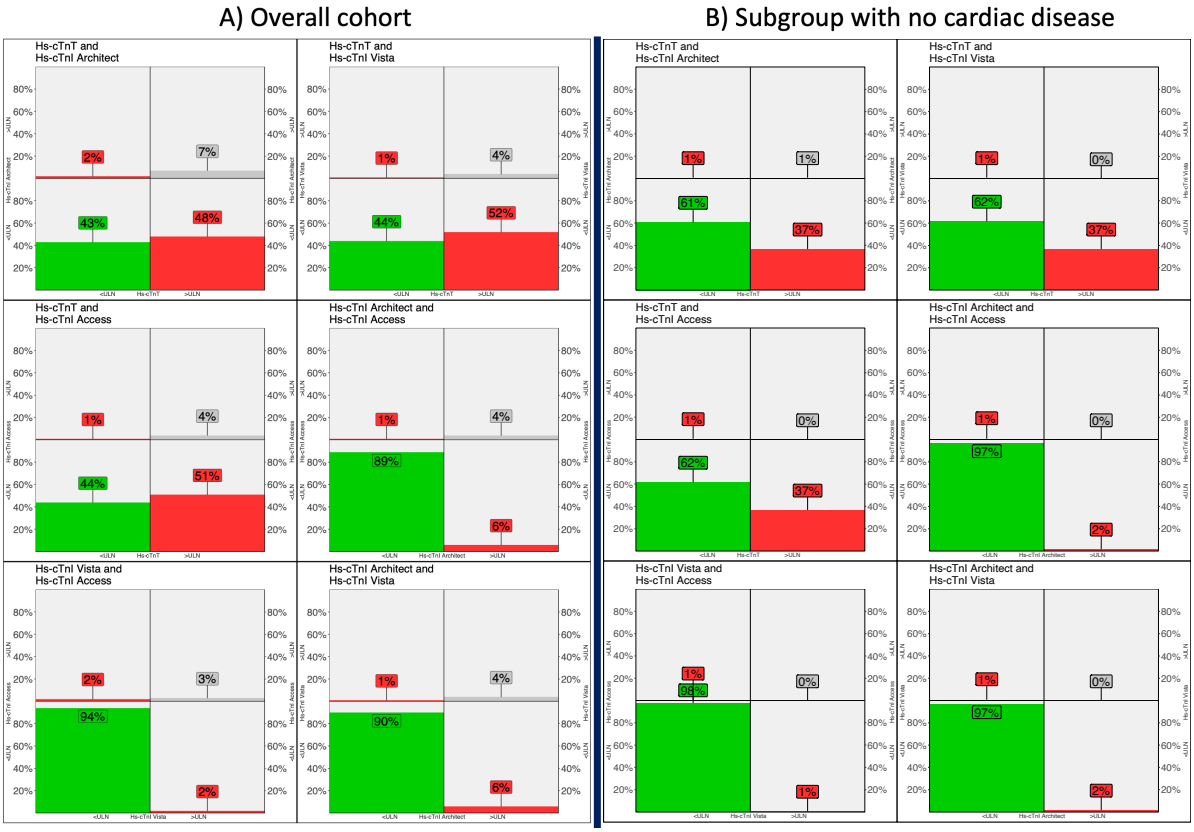
Violine plots representing the distribution of hs-cTnT/I concentrations for the four tested assays and across categories of cardiac disease in males. Sex-specific ULN are represented as broken lines.

Figure S7 – hs-cTnT/I concentrations in females as measured by the different assays and comparison with the controls from the APACE cohort.



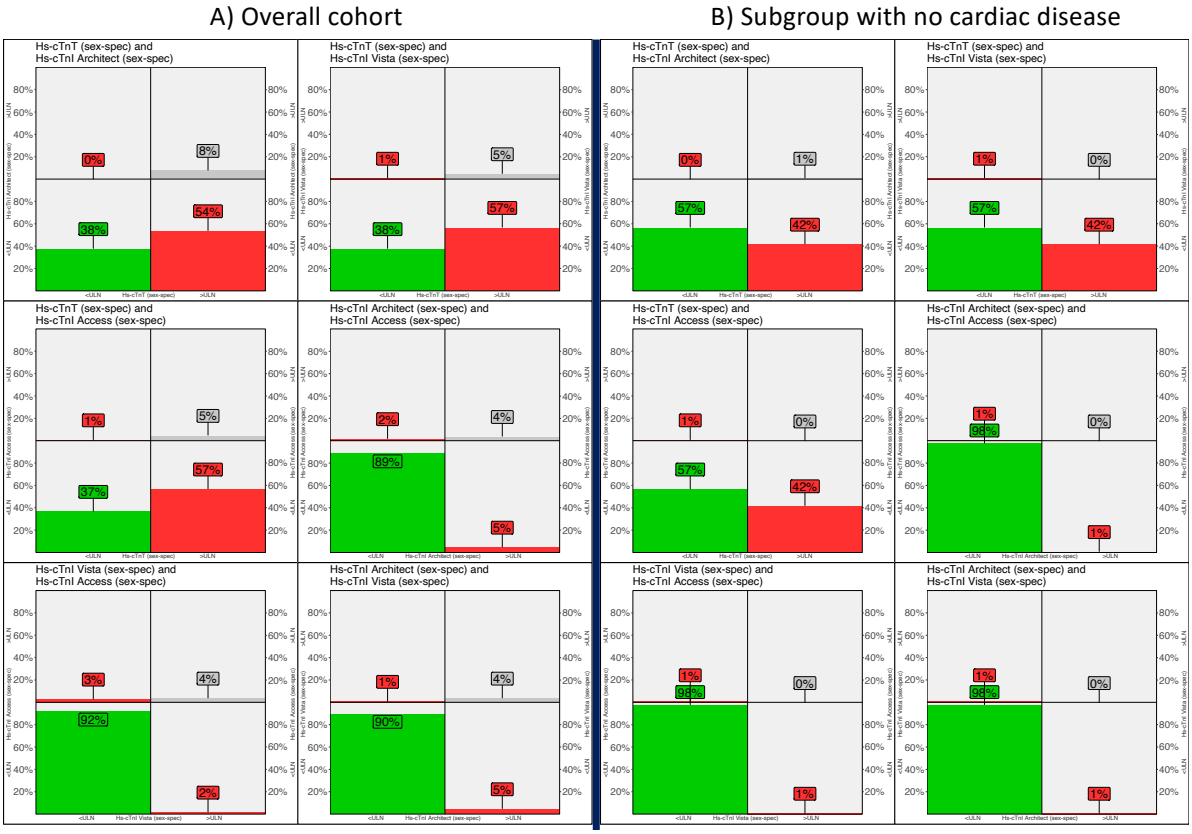
Violine plots representing the distribution of hs-cTnT/I concentrations for the four tested assays and across categories of cardiac disease in females. Sex-specific ULN are represented as broken lines.

Figure S8 – Inter-assay hs-cTnT/I mismatches using approved overall upper limits of normal (ULN)



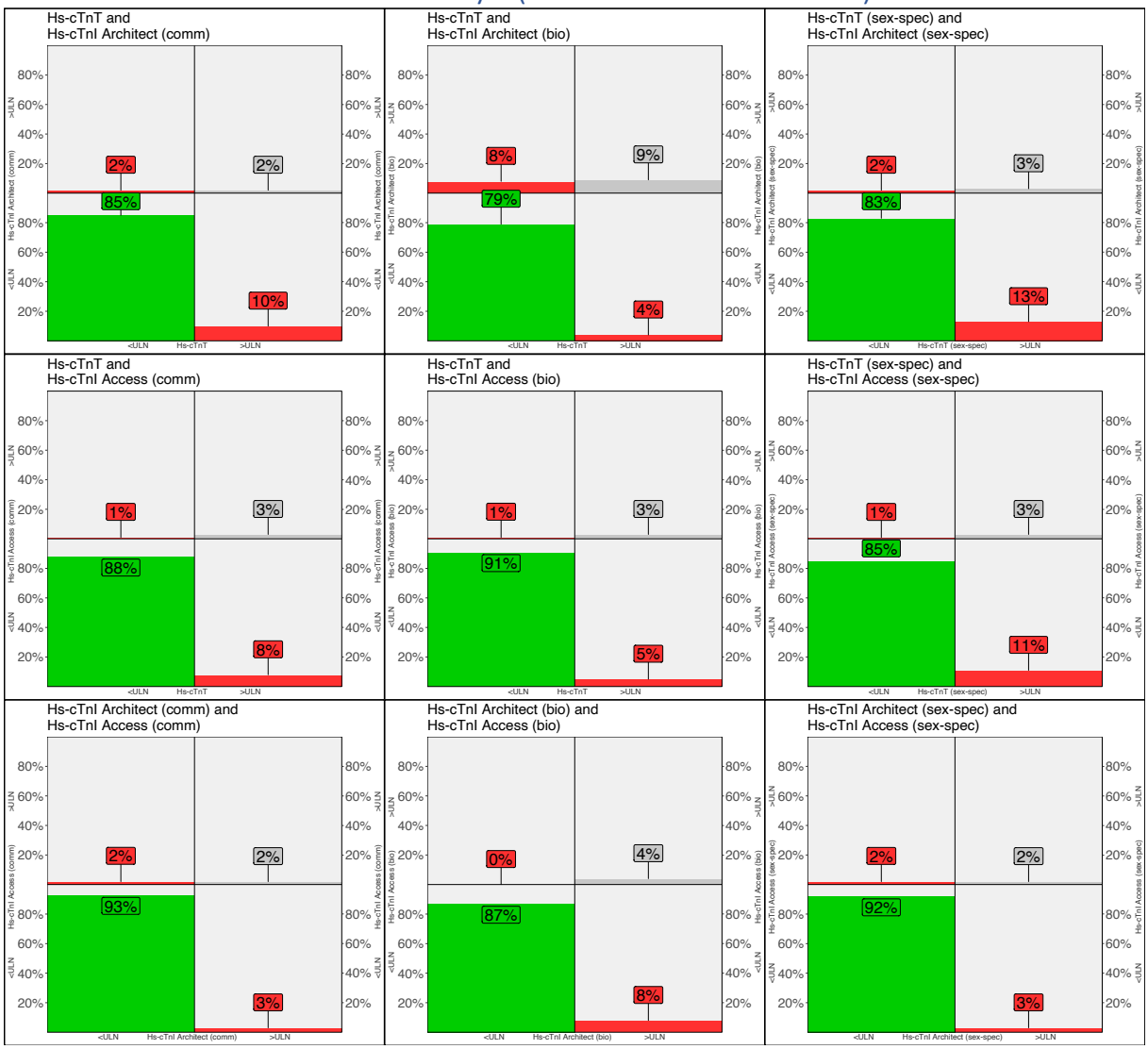
Inter-assay hs-cTnT/I mismatches using overall uniform approved upper limits of normal (ULN). For each subpanel, two hs-cTnT/I assay are represented with their uniform approved ULNs. In each panel, the four quadrants represent the percentage of patients with the following constellations : green when both hs-cTnT/I assays were below the ULN, grey when both were above the ULN, and red when there was a hs-cTnT/I mismatch (with one of the assay above and one of the assay below the ULN). A) overall cohort, B) Subgroup without cardiac disease.

Figure S9 – Inter-assay hs-cTnT/I mismatches using sex-specific upper limits of normal (ULN)



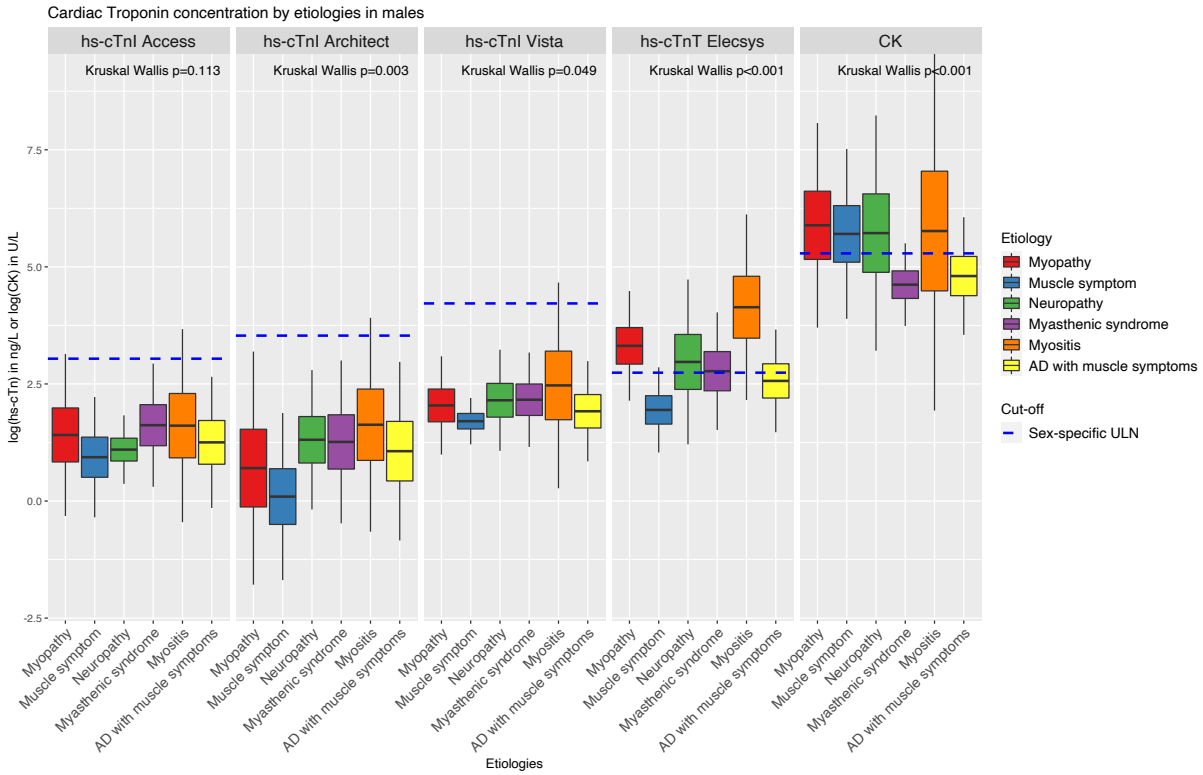
Inter-assay hs-cTnT/I mismatches using sex-specific upper limits of normal (ULN). For each subpanel, two hs-cTnT/I assay are represented with their sex-specific ULNs. In each panel, the four quadrants represent the percentage of patients with the following constellations : green when both hs-cTnT/I assays were below the ULN, grey when both were above the ULN, and red when there was a hs-cTnT/I mismatch (with one of the assay above and one of the assay below the ULN). A) overall cohort, B) Subgroup without cardiac disease.

Figure S10 – Inter-assay hs-cTn mismatches in the APACE cohort with no cardiac disease using uniform, bio-equivalent and sex-specific ULNs for the available hs-cTnI Assays (Architect and Access)



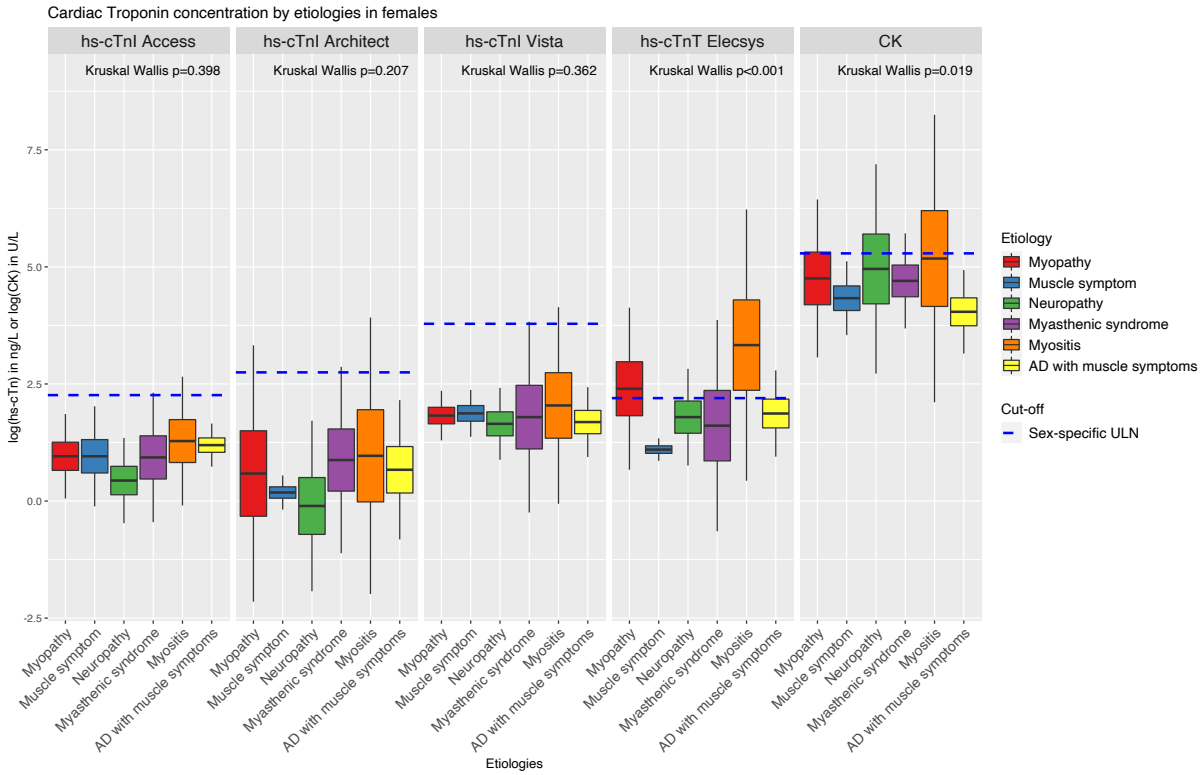
For each subpanel, two hs-cTnT/I assay are represented with their approved assay-specific 99th-percentile, bioequivalent or sex-specific Upper Limit of Normal (ULN). In each panel, the four quadrants represent the percentage of patients with the following constellations : green when both hs-cTnT/I assays were below the ULN, grey when both were above the ULN, and red when there was a hs-cTnT/I mismatch (with one of the assay above and one of the assay below the ULN).

Figure S11 – hs-cTn concentrations and muscle disorder etiologies in men



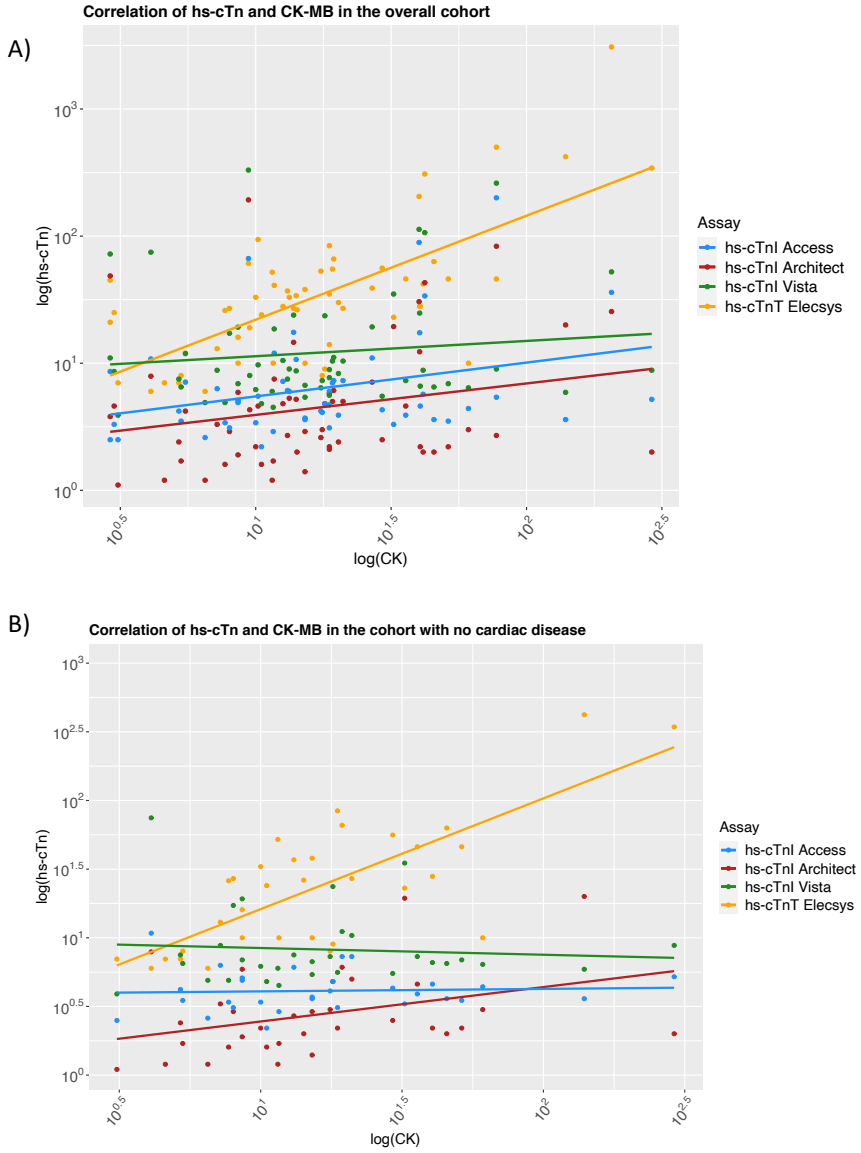
The different etiologies of the skeletal muscle disorders are represented on the X-Axis and the concentrations of the biomarkers are represented on the Y-axis using a logarithmic scale. Boxplots represents the interquartile range (IQR) and whiskers ± 1.5*IQR. The p-values have been corrected for multiple testing using the Benjamini and Hochberg method.

Figure S12 – hs-cTn concentrations and muscle disorder etiologies in women



The different etiologies of the skeletal muscle disorders are represented on the X-Axis and the concentrations of the biomarkers are represented on the Y-axis using a logarithmic scale. Boxplots represents the interquartile range (IQR) and whiskers ± 1.5*IQR. The p-values have been corrected for multiple testing using the Benjamini and Hochberg method.

Figure S13 – Correlation of CK-MB and hs-cTn



Correlation between CK-MB and hs-cTn in the A) overall cohort and in B) patients with no cardiac disease. Biomarkers have been logged to approximate normal distribution

Appendix

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