Supplementary Data

Cardio-muscular biomarkers in the diagnosis and prognostication of immune checkpoint inhibitor myocarditis

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Appendix. List of collaborating centers in the international ICI myocarditis registry. *Collaborators with over 2 cases entered in the registry are displayed.*

Assistance publique Hôpitaux Universitaires de Marseille Nord	Paris, France	Franck Thuny
Barts Health NHS Trust	London, United Kingdom	Shanthini Crusz
Beth Israel Deaconess Medical Center	Boston, USA	Aarti Asnani
Bern University Hospital	Bern, Switzerland	
Cedars Sinai	Los Angeles, USA	Anja Karlstaedt
Centre Hospitalier Universitaire de Nice	Nice, France	Fanny Rocher
Centre Hospitalier Universitaire de Rennes	Rennes, France	Elise Paven
Centre hospitalier universitaire Vaudois	Lausanne, Switzerland	Michel Obeid
Charité – Universitätsmedizin Berlin	Berlin, Germany	
Chi Mei Medical Center	Tainam, Taiwan	Wei Ting Chan
Clínica Universidad de Navarra	Pamplona, Spain	
Dartmouth	Hitchcock Medical Center, Lebanon, USA	Danette L. Flint
East Carolina University	Greenville, USA	
Eisenhower Medical Center	Rancho Mirage, USA	
Emory University Hospital	Atlanta, USA	
Evangelische Lungenklinik Berlin	Berlin, Germany	
General Hospital of Chinese People's Liberation Army	Beijing, China	
Hôpital Bichat	Paris, France	Dimitri Arangalage
Hospices Civils de Lyon	Lyon, France	Courand Pierre Yves
Hôpital Lariboisière	Paris, France	Martin Nicol
Hôpital Rangueil	Toulouse, France	Cariou Eve
Hôpital Saint Antoine	Paris, France	Stephane Ederhy
Institut Bergonié : Centre Régional de Lutte Contre le Cancer	Bordeaux, France	
International University of Health and Welfare Mita Hospital	Tokyo, Japan	Yuichi Tamura
Japan Community Health Organization Kyushu Hospital	Kitakyushu, Japan	
Johns Hopkins University	Baltimore, USA	Roberta Florido
Kumamoto University	Kumamoto, Japan	
Maine Medical Center	Portland, USA	Sanjeev Francis
McMaster University	Hamilton, Canada	Darryl Leong
Memorial Sloan Kettering	New York City, USA	
Mitsui Memorial Hospital	Tokyo, Japan	
Monash University	Melbourne, Australia	
Nagoya University Graduate School of Medicine	Nagoya, Japan	
Nantes University Hospital	Nantes, France	Nicolas Piriou
National Cancer Institute, National Institutes of Health	Bethesda, USA	
New York University	New York City, USA	
Northwestern Memorial Hospital	Chicago, USA	Nausheen Akhter
Ohio State University Wexner Medical Center	Columbus, USA	

Peter MacCalum Cancer Centre	Melbourne, Australia	Sandhu Shahneen
Rabin Medical Center	Petah Tikva, Israel	Osnat Itzhaki
Rambam Medical Center	Haifa, Israel	Manhal Habib
Rosewell Park	Buffalo, USA	Pankit Vachhani
San Raffaele Hospital	Milan, Italy	Giovanni Peretto
Sorbonne University	Paris, France	Joe-Elie Salem
St. Luke's International Hospital	Tokyo, Japan	
Stanford University	Palo Alto, USA	Han Zhu
Teikyo University School of Medicine	Tokyo, Japan	
Tel Aviv Sourasky Medical Center affiliated to the Sackler School of Medicine	Tel Aviv, Israel	Michal Laufer Perl
Tokyo Women's Medical University	Tokyo, Japan	
UCSF Medical Center	San Francisco, USA	Mandar Aras
Université de Caen Basse	Normandie, Caen, France	Joachim Alexandre
University Hospital Basel	Basel, Switzerland	
University of Alabama	University Medical Center, Birmingham USA	Carrie Lenneman
University of Michigan	Ann Arbor, USA	Salim Hayek
University of Pittsburgh Medical Center	Pittsburgh, USA	Joshua Levenson
University of Texas MD Anderson Cancer Center	Houston, USA	Anita Deswal
University of Texas Southwestern Medical Center	Dallas, USA	Vlad Zaha
University of Tsukuba	Tsukuba, Japan	
University of Virginia	Charlottesville, USA	Elizabeth M Gaughan
University of Wisconsin	Madison, USA	Steven Ewer
Vanderbilt University Medical Center	Nashville, USA	Douglas Johnson
Yale University School of Medicine	New Haven, USA	Lauren A Baldassarre

Supplementary-Table-1. Diagnostic certainty criteria for ICI-myocarditis

Hierarchical criteria's accounting for different levels of evidence for ICI-myocarditis

For all, other diagnosis/explanations must be excluded (particularly cardiotoxicity of another liable drug used in combination). Overlap between ICI-myocarditis and acute coronary syndrome or venous thromboembolism has been described previously.

Definite myocarditis (any one of the below situations is sufficient)

- Definite cardiac pathology
- Definite CMR + syndrome + (biomarker or ECG or WMA)
- WMA + syndrome + biomarker + ECG + negative angiography
- Definite muscular pathology + (suggestive/definite CMR or WMA or ECG or suggestive cardiac pathology) + biomarker
- Suggestive (muscular or cardiac) pathologies + (suggestive/definite CMR or WMA or ECG) + biomarker + syndrome

Probable myocarditis (any one of below situations is sufficient)

- Definite CMR + (biomarker or ECG or WMA) and no syndrome
- Suggestive CMR + biomarker + (syndrome or ECG or WMA)
- WMA + syndrome + biomarker + ECG and no available angiography
- Suggestive muscular pathology + (suggestive CMR or WMA or ECG) + (biomarker or syndrome)
- Suggestive cardiac pathology + (biomarker or syndrome or ECG)
- Syndrome + biomarker + ECG + negative angiography + no WMA + normal CMR + normal cardiac pathology

Possible myocarditis (any one of the below situations)

- Suggestive CMR + biomarker with no (syndrome or ECG or WMA)
- Suggestive/aspecific lesions on muscular pathology + biomarker
- Suggestive cardiac pathology
- Biomarker + (syndrome or ECG) and no alternative diagnosis*

Abbreviations: Biomarker: identification of unknown increase of troponin assay above its 99th percentile upper reference limit; CMR: cardiac magnetic resonance imaging (analysis based on T1 & T2 properties of cardiac tissue); ECG: electrocardiogram (12-leads 10 seconds) identifying an unknown abnormality (atrio-ventricular or sinus blocks, bundle branch blocks, ST-T wave changes, micro-voltage, ventricular or supra-ventricular arrhythmias, pathological Q-waves); Syndrome: appearance on ICI of any of the following abnormalities (dyspnea, chest pain, diplopia, ptosis, myalgia, muscular weakness, fatigue, dysphonia, dysphagia, abdominal paradox, syncope, faintness, palpitations, ventricular hyperexcitability on electrocardiographic holter/telemetry (>5% of non-sinus ventricular rhythms), and respiratory muscle involvement probable or definite; WMA: identification of unknown wall motion abnormality or left ventricular ejection fraction<50% by cardiac imaging.

* Acute coronary syndrome and ICI-myocarditis may co-occur at presentation. ICI-myocarditis should be reassessed after revascularization particularly if this has not led to resolution of alteration in biomarkers, ECG or syndrome or if syndrome is including peripheral muscle involvement not explained by cardiac ischemia (diplopia, ptosis, myalgia, myalgia, muscle weakness, dysphagia, dysphonia).

Addendum for detailed criteria required depending on modality used for diagnosis of ICI-myocarditis :

- ***Cardiac pathology*** (endomyocardial or autopsy samples) :
    - **Definite** : Abnormal lympho-histiocytic inflammatory cells infiltrate ($CD3^+$ cells ≥7/mm² or $CD68^+$ cells ≥4/mm²) AND cardiomyocytes necrosis.
    - **Suggestive** (borderline): Abnormal lympho-histiocytic inflammatory cells infiltrate ($CD3^+$ cells ≥7/mm² or $CD68^+$ cells ≥4/mm²) WITHOUT cardiomyocytes necrosis.
- ***Cardiac magnetic resonance imaging*** (analysis based on T1 & T2 properties of cardiac tissue) :
    - **Definite** : Tissue imaging identifying unknown abnormalities in cardiac segments in both T1 (native T1 ± extracellular volume ± presence of late gadolinium enhancement) AND T2 derived maps and sequences.
    - **Suggestive** (borderline): Tissue imaging identifying unknown abnormalities in cardiac segments in either T1 OR T2 derived maps and sequences.
- ***Peripheral muscle pathology*** (skeletal muscles or autopsy samples) :
    - **Definite** : Abnormal endomysial lympho-histiocytic inflammatory cells infiltrate AND abnormal class 1 human leukocyte antigen overexpression on myocytes AND myocytes necrosis.
    - **Suggestive** (borderline): Abnormal endomysial lympho-histiocytic inflammatory cells infiltrate AND abnormal class 1 human leukocyte antigen overexpression on myocytes WITHOUT myocytes necrosis.
    - **Non-specific lesions**: Abnormal class 1 human leukocyte antigen overexpression on myocytes WITHOUT abnormal endomysial lympho-histiocytic inflammatory cells infiltrate WITHOUT myocytes necrosis.

Supplementary-Table-2. Technical aspects of the different assays used to determine cardiac troponins and CK circulating levels

Analyzer, Assay (manufacturer)	*n/N, % of tested samples*	*Assay Range in ng/L*	*Limit of detection (LoD) in ng/L*	*Limit of blank (LoB) in ng/L*	*10% coefficient of variation ($^{10\%}$CV) in ng/L*	*99th percentile normal cut-off values in ng/L*
Cardiac Troponin-T (cTnT)						
Cobas Elecsys Troponin T hs (Roche)*	1751/1751 (100%)	3 – 10,000	3	2.5	13	14
Cardiac Troponin-I (cTnI)						
Cobas Elecsys Troponin I (Roche)*	40/920 (4%)	160 – 25,000	160	100	300	160
Atellica IM High-Sensitivity Troponin I (Siemens)*	512/920 (56%)	2.5 – 25,000	1.6	0.5	<6.0	34.11 (F) 53.48 (M)
Architect STAT High Sensitive Troponin-I (Abbott)*	101/920 (11%)	10 – 50,000	1.1 – 1.9	0.7 – 1.3	4.0 – 10.0	15.6 (F) 34.2 (M)
ADVIA Centaur TnI-Ultra (Siemens)	267/920 (29%)	6 – 50,000	3	NA	30	40
Analyzer, Assay (manufacturer)	***n/N, % of tested samples***	***Assay Range in U/L***	***Limit of detection (LoD) in U/L***	***Limit of blank (LoB) in U/L***	***10% coefficient of variation ($^{10\%}$CV) in ng/L***	***95th percentile normal cut-off values in U/L***
Creatine Kinase (CK)						
Atellica CH Creatine Kinase (CK L) (Siemens)	22/1191 (2%)	15 – 1,300	6	1		34 – 145 (F) 46 – 171 (M)
Cobas Creatine Kinase (CK) (Roche)	820/1191 (69%)	7 – 2,000	7	7		26 – 192 (F) 39 – 308 (M)
Dimension Vista Creatine Kinase (CKI) (Siemens)	2/1191 (<1%)	7 – 1,000	7	2		26 – 192 (F) 39 – 308 (M)
ADVIA Chemistry XPT (Siemens)	340/1191 (29%)	15 – 1,300	6	3		34 – 145 (F) 46 – 171 (M)
ARCHITECT Creatine Kinase (CK) (Abbott)	7/1191 (<1%)	7 – 4,267	5.1	NA		29 – 168 (F) 30 – 200 (M)

Abbreviations: CV, coefficient of variation; CI, confidence interval; F, female; LoB, Limit of Blank; LoD, Limit of Detection; LoQ, Limit of Quantitation; M, male; NA, not available.

* high-sensitive assays (hs)

Supplementary-Table-3. Detailed results of non-linear mixed effects models. In model-A, we studied if the ratio of cTnT/URL over cTnI/URL (as a proxy of their divergence, n=761 timepoints) was influenced by the following fixed effects variables (time-period after admission for ICI-myocarditis, inclusion center, age and sex), integrating the following random effects (patients' identity, and cTnI assay types). In model-B, we added to model-A the presence of detectable IgG or IgM anti-cTnI antibodies as a fixed effect (n=493 time-points). Inclusion center was dropped for this latter model because IgG/IgM anti-cTnI antibodies status was only available in the French cohort. A serum sample identifying detectable IgG or IgM anti-cTnI (at least >1/40 for each) was considered positive for a 10days time period, except if plasmapheresis was preformed within this time-period.

Model-A, Random Effects			
- Patients identity (n=55) - cTnI assay types (n=4)			
Fixed effects			
	Estimate	Standard-error	p-value
Intercept	-51.4	28.7	0.11
Age (years)	**0.7**	**0.3**	**0.02**
Sex (Male)	**16.3**	**8.0**	**0.05**
Center (France)	5.9	21.8	0.82
Days after diagnosis (4-7)	-6.3	6.5	0.34
Days after diagnosis (8-14)	2.8	6.8	0.63
Days after diagnosis (15-30)	**45.9**	**5.9**	**<0.001**
Days after diagnosis (31-90)	**21.4**	**5.9**	**<0.001**
Days after diagnosis (91-180)	6.5	7.0	0.35
Days after diagnosis (181-360)	1.1	7.9	0.89

Model-B, Random Effects			
- Patients identity (n=29) - cTnI assay types (n=2)			
Fixed effects			
	Estimate	Standard-error	p-value
Intercept	-48.7	31.0	0.14
Age (years)	**0.9**	**0.4**	**0.02**
Sex (Male)	**27.5**	**12.1**	**0.03**
IgG anti-cTnI (detectable)	-9.6	9.2	0.30
IgM anti-cTnI (detectable)	-1.1	7.6	0.89
Days after diagnosis (4-7)	-17.1	11.6	0.14
Days after diagnosis (8-14)	-10.4	9.9	0.29
Days after diagnosis (15-30)	**40.6**	**9.1**	**<0.001**
Days after diagnosis (31-90)	15.5	9.5	0.10
Days after diagnosis (91-180)	-3.2	11.0	0.77
Days after diagnosis (181-360)	-11.6	12.1	0.34

Abbreviations: cTnI: cardiac troponin-I; cTnT: cardiac troponin-T; Ig: immunoglobulin ; ICI: immune-checkpoint inhibitor ; URL: upper reference limit (99^{th} percentile)

Supplementary-Table-4. Detailed results of non-linear mixed effects models by subgroups of patients with a same cTnI assay performed. We studied if the ratio of cTnT/URL over cTnI/URL (as a proxy of their divergence) was influenced by the following fixed effects variables (time-period after admission for ICI-myocarditis, age and sex), integrating the patients' identity as a random effect for Architect STAT High sensitive Troponin-I (ABBOTT, n=37 timepoints; A), Atellica IM TnIH (SIEMENS, n= 458 timepoints; B) and TnI-Ultra/ADVIA Centaur XPT (SIEMENS, n=265; C).

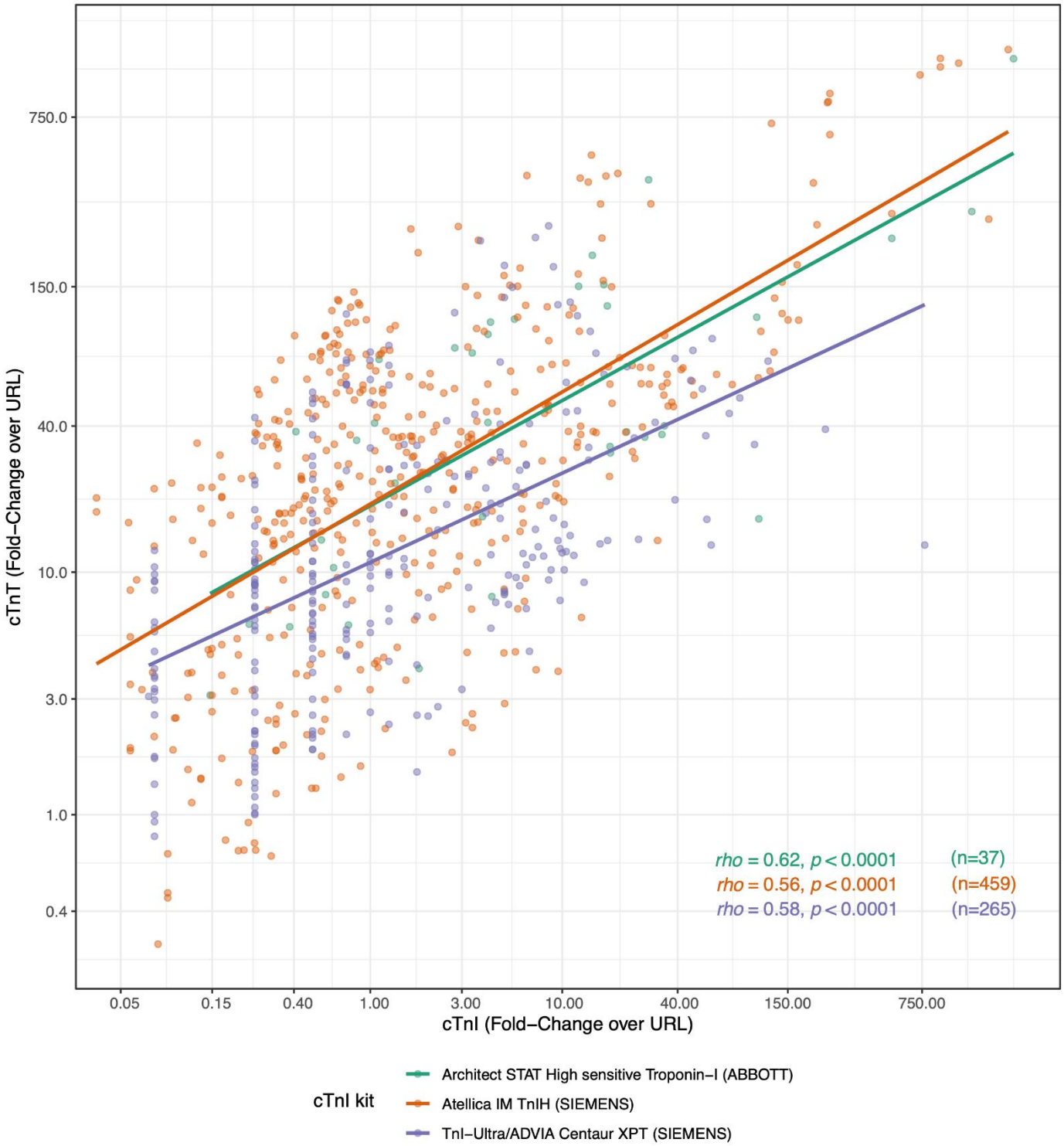
Model-A, Random Effects			
- Patients identity (n=13)			
Fixed effects			
	Estimate	Standard-error	p-value
Intercept	**-48.2**	**10.1**	**<0.001**
Age (years)	**0.7**	**0.2**	**<0.001**
Sex (Male)	**18.9**	**4.9**	**<0.001**
Days after diagnosis (4-7)	11.4	14.9	0.45
Days after diagnosis (8-14)	0.5	10.3	0.96
Days after diagnosis (15-30)	10.5	8.1	0.21
Days after diagnosis (31-90)	**17.1**	**7.9**	**0.04**
Days after diagnosis (91-180)	-3.2	9.9	0.75
Days after diagnosis (181-360)	-7.0	15.8	0.66

Model-B, Random Effects			
- Patients identity (n=29)			
Fixed effects			
	Estimate	Standard-error	p-value
Intercept	-48.2	30.6	0.13
Age (years)	**1.0**	**0.4**	**0.03**
Sex (Male)	**27.4**	**13.0**	**0.05**
Days after diagnosis (4-7)	-19.2	12.2	0.12
Days after diagnosis (8-14)	-9.3	10.5	0.38
Days after diagnosis (15-30)	**45.6**	**10.0**	**<0.001**
Days after diagnosis (31-90)	15.5	9.9	0.12
Days after diagnosis (91-180)	0.2	11.6	0.99
Days after diagnosis (181-360)	-8.6	12.5	0.49

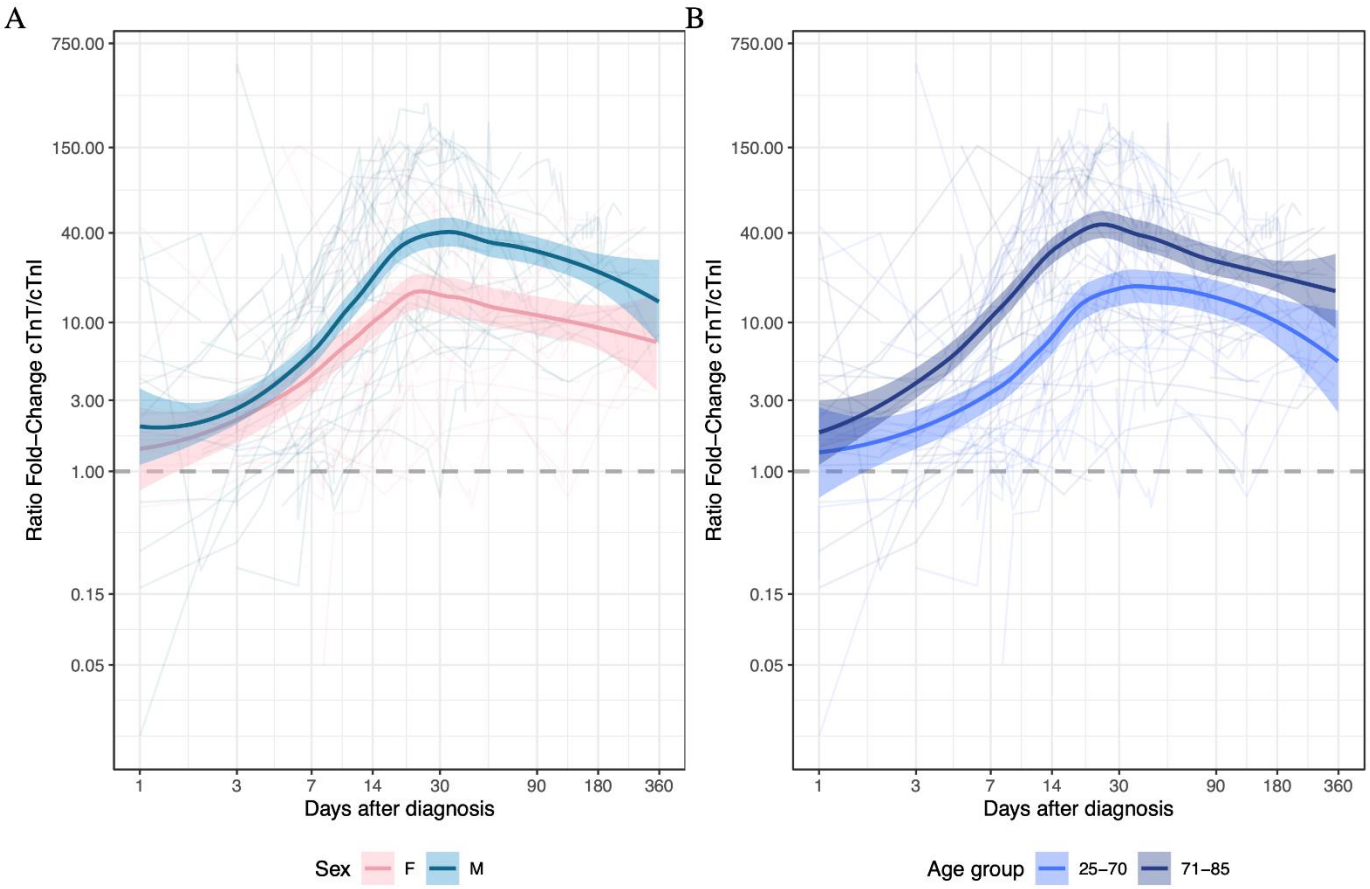
Model-C, Random Effects			
- Patients identity (n=26)			
Fixed effects			
	Estimate	Standard-error	p-value
Intercept	29.3	33.7	0.39
Age (years)	-0.3	0.4	0.48
Sex (Male)	-3.7	8.3	0.66
Days after diagnosis (4-7)	3.4	5.2	0.51
Days after diagnosis (8-14)	**14.9**	**5.1**	**0.004**
Days after diagnosis (15-30)	**46.9**	**5.5**	**<0.001**
Days after diagnosis (31-90)	**23.5**	**5.6**	**<0.001**
Days after diagnosis (91-180)	12.2	6.8	0.07
Days after diagnosis (181-360)	**-11.6**	**12.1**	**0.05**

Abbreviations: cTnI: cardiac troponin-I; cTnT: cardiac troponin-T; Ig: immunoglobulin ; ICI: immune-checkpoint inhibitor ; URL: upper reference limit (99^{th} percentile)

Supplementary-Figure-1. Correlation (rho; spearman's test) **between concomitant cTnT and cTnI values as a function of cTnI assay used**. There was no significant difference in the correlations observed between the different cTnI assay used and cTnT as obtained with the Fisher's z test for independent correlations (p=0.58, p=0.68, p=0.76, respectively for Architect vs. Atellica, Architect vs. Centaur and Atellica vs. Centaur). *URL stands for 99th percentile upper reference limit. Cobas Elecsys® Troponin I assay is not shown because only one value was concomitantly available with cTnT evaluation.*

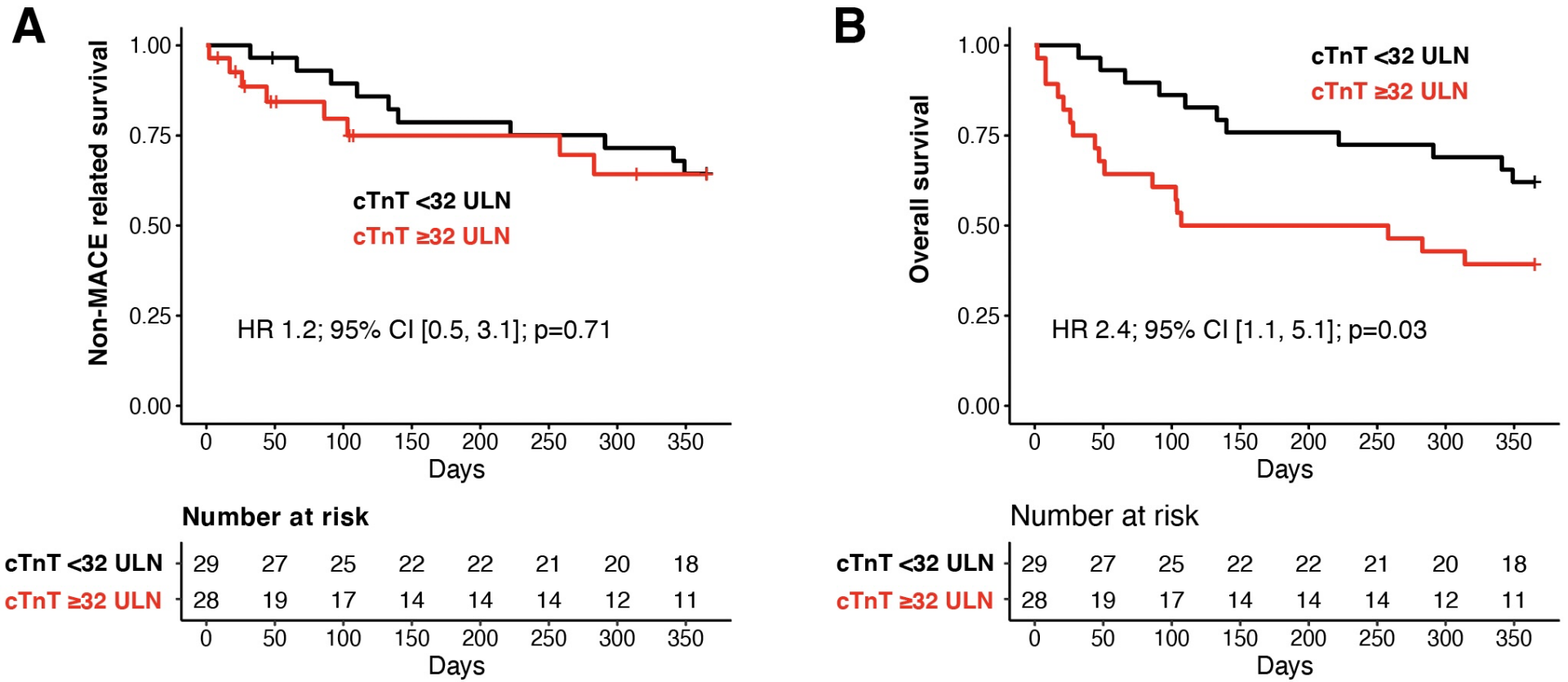


Supplementary-Figure-2. LOESS (Locally Estimated Scatterplot Smoothing) of the mean (and standard-error) of the ratio of cTnT/URL over cTnI /URL over time after initial admission for ICI-myocarditis in a one-year follow-up as a function of sex (A) and age median (B)



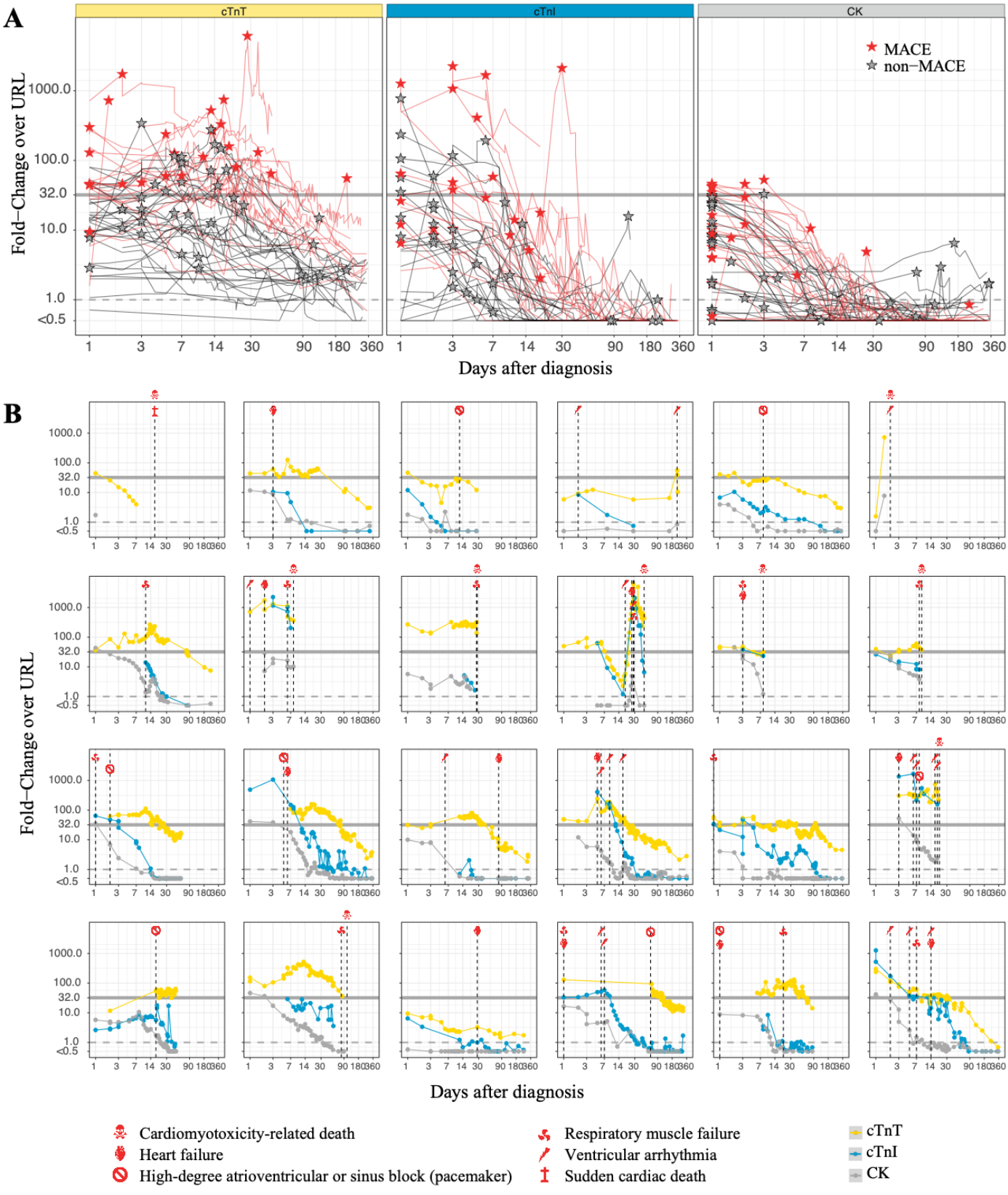
Abbreviations: cTnI: cardiac troponin-I; cTnT: cardiac troponin-T; F: female ; ICI: immune-checkpoint inhibitor ; M: male; URL: upper reference limit (99th percentile)

Supplementary-Figure-3. Cardiac biomarkers as predictors of non-MACE related death vs. overall mortality: (**A**) Non-MACE related death, and overall mortality (**B**) over one-year follow-up after admission for ICI-myocarditis as a function of cTnT/URL value above and below 32 (n=57) using maximal cTnT values within 72h of admission.



Abbreviations: MACE, major adverse cardio-myotoxic event; URL, upper reference limit being upper 99th percentile of normal values for troponins; HR, hazard ratio; CI, confidence interval; Tn, Troponin as indicated. CI, 95% confidence interval

Supplementary-Figure-4. ICI-myocarditis cases and their biomarker evolution over time as a function of MACE incidence or not in the overall cohort (A); and at the time of MACE in patients having developed a MACE (B). URL (upper reference limits) of cardiac troponin T (cTnT), cardiac troponin I (cTnI), and creatine kinase (CK) are represented by a grey dotted line. The stars represent the peak value of biomarkers (A). MACE is represented by dotted vertical lines in B (see icon legends for types of MACE).



Abbreviations: MACE, major adverse cardio-myotoxic event; URL: upper reference limit being upper 99th percentile of normal values for troponin and 95th percentile of normal values for creatine kinase