Discrepancies of cardio-muscular biomarkers in the diagnosis and prognostication of immune checkpoint inhibitor (ICI)-associated myocarditis

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Abstract

Background: Immune-checkpoint inhibitors (ICI) are approved for multiple cancers but can result in ICI-associated myocarditis, an infrequent but life-threatening condition. Elevations in cardiac biomarkers, troponin-I (cTnI), troponin-T (cTnT) and creatine-kinase (CK) are used for diagnosis. However, the temporal elevation of these biomarker elevations with course of disease and their association with outcomes have not been established.

Methods: We analyzed the diagnostic accuracy and prognostic performances of cTnI, cTnT and CK in ICI-myocarditis (n=61) in two cardio-oncology units (APHP.Sorbonne, France & Heidelberg, Germany). Major adverse cardio-myotoxic events (MACE) were defined as heart failure, ventricular arrhythmia, atrioventricular/sinus block requiring pacemaker, respiratory muscle failure requiring mechanical ventilation, and related death. Diagnostic performances of troponins were also assessed in an international ICI-myocarditis registry (n=244 independent cases, 13 countries).

Results: On presentation, cTnT, cTnI or CK were increased compared to upper reference limit (URL) in 51/52 (98%), 28/34 (82%, p=0.009 vs. cTnT), 33/48 (69%, p<0.0001 vs. cTnT), respectively. This higher rate of positivity for cTnT vs. cTnI was independently confirmed in an international registry. In patients surviving to 30 days, cTnI and CK had normalized in 20/34(59%) and 30/35(86%), respectively, while cTnT had reached normal values in only 5/42(12%), (p<0.0001). The highest value of cTnT/URL within the first 72h of admission performed best in predicting MACE (AUC:0.82) vs. CK/URL (AUC:0.74) and cTnI/URL (AUC:0.67), even after adjustment for age and sex. Maximal value of cTnT/URL≥32 within ≤72h of diagnosis was the best predictor cut-off for MACE (Hazard-ratio=9.4(95% CI 3.1, 28.3), p<0.0001) over a median follow-up of 4 months. cTnT was increased in all patients just before MACE (22/22, 100%) while cTnI and CK values were normal in 3/21(14%) and 6/24(25%) of patients (p<0.0001).

Conclusions. Significant discrepancies between cTnT (compared to cTnI, and CK) circulating levels exist in ICI-myocarditis. cTnT is the best predictor of MACE and most suitable for diagnosis and surveillance. A ratio of cTnT/URL<32 within ≤72h of diagnosis identifies a subgroup at low-risk of MACE.

Clinical Perspective

What is new?

- Circulating levels of cTnI and CK normalized much earlier in the course of immune checkpoint-inhibitor (ICI) myocarditis while cTnT levels continued to stay elevated. cTnT was increased in all patients at the time of the first major adverse cardiac and respiratory muscle failure events (MACE) while cTnI and CK were within normal ranges in up to one quarter of patients with MACE.
- Maximum increase in circulating cTnT within 3 days of initial diagnosis for ICI-myocarditis is a much better predictor of MACE as compared to cTnI and CK.

- Patients below 32x fold change of circulating cTnT levels over its upper reference limit within 3 days of diagnosis for ICI-myocarditis have a minimal risk of developing MACE.

What are the clinical implications?

- Circulating levels of cTnT are better predictors of MACE and more often elevated in patients presenting with ICI-myocarditis compared to CK and cTnI.
- Risk stratification of patients with ICI-myocarditis may rely on kinetic changes of circulating levels of cTnT within the first 72 hours of admission.
- Normal values of cTnI but not cTnT might be misleading in the course of ICI-myocarditis and for the ability to predict incidence of MACE including heart failure, ventricular arrhythmia, atrioventricular or sinus blocks requiring pacemaker implantation, respiratory muscle failure requiring mechanical ventilation support and related death.

Introduction

Immune-Checkpoint inhibitors (ICI) are a potent class of oncology therapies used to treat up to 50% of cancer types.[1, 2] Currently approved ICI are monoclonal antibodies targeting three inhibitory immune checkpoints: CTLA4 (Cytotoxic T-Lymphocyte Associated protein 4), PD1 (Programmed cell Death protein 1) and its ligand (PDL1).[3] By virtue of activating the adaptive immune system in fighting cancer, ICI can result in immune-related toxicities that can affect any organ.[1] ICI-induced myocarditis is one such toxicity which, although infrequent, can result in mortality in up to ~ 50% of patients.[4, 5] ICI-myocarditis often presents concurrently with other myotoxicities including myositis (~ 30–35% of the time) and may lead to fatal respiratory muscle failure.[4–7] Mechanistically, ICI-myocarditis is associated with macrophage and T-cell infiltration into muscles and associated muscle death.[8–11] The diagnosis of ICI-myocarditis is often challenging and a combination of biomarkers, cardiac imaging and endomyocardial biopsy is needed to properly make the diagnosis.[12] Cardiac biomarkers, including high-sensitive cardiac troponin-T (cTnT), cardiac troponin-I (cTnI) and creatine kinase (CK), are sensitive (though not specific) for the detection of myocarditis. However, there are no comparative data on cardiac biomarker performance for diagnosis of ICI-myocarditis.[13] Available data regarding using cardiac biomarkers for risk prediction of major adverse cardiac and respiratory muscle failure events (MACE) in patients with ICI-myocarditis are limited and mainly use appearance of pathological electrocardiographic features or signs and symptoms of clinical heart failure.[5, 14] Most ICI-myocarditis reports used blood analysis for cardiac troponins to detect cardiac injury, which is currently part of the most diagnostic criteria.[12, 13, 15] Increased levels of CK has also been used for ICI-myocarditis diagnosis.[11–13] However, the diagnostic and predictive performance of these different cardiac biomarker for the diagnosis and prediction of MACE in ICI-myocarditis is unknown. Herein, we investigated their diagnostic, prognostic, and surveillance value in ICI-myocarditis.

Methods

Patient cohort

We studied consecutive patients (n = 61) admitted for probable or definite ICI-myocarditis (having at least a histology of cardiac biopsy specimens and/or cardiac magnetic resonance imaging consistent with myocarditis and presentation not explained by other conditions)[13] into the Franco-German study at the university hospitals of Heidelberg (Heidelberg, Germany) or Pitié-Salpêtrière (AP-HP; Sorbonne, Paris, France) between 2018 and 2020. Initial laboratory tests were performed on the day of admission when ICI-myocarditis was suspected. Data from the initial hospital stay and subsequent one-year follow-up visits were prospectively gathered and analyzed. MACE events were prospectively collected and adjudicated and were defined as heart failure; ventricular arrhythmias; high-degree atrioventricular or sinus blocks requiring pacemaker implantation; respiratory muscle failure requiring mechanical ventilation support; and related death, termed as 'cardiomyotoxicity-related' death.

For an independent external validation of the diagnostic descriptive portion of this study, a previously described multicenter registry of ICI-myocarditis[14] was used to study 244 cases of definite or probable ICI-myocarditis where cTnI or cTnT were available. Cases were collected from over 60 institutions across 13 countries (See **Appendix** for full list of contributing centers) not including patients from university hospitals of Heidelberg or Pitié-Salpêtrière.

Measurement of cardiac and muscular biomarkers

In the index Franco-German cohort, cTnT was measured in 1625 samples (n = 61 patients with at least one measurement), cTnI in 801 samples (n = 56) and CK in 1331 samples (n = 61) over a median follow-up of 113 days, interquartile range [47–304]. Blood samples were collected as clinically indicated up to one year after ICI-myocarditis diagnosis and were subsequently analyzed at different time intervals in days (d) after first hospital admission for ICI-myocarditis: 0-3d (i.e diagnosis phase), 4-7d, 8-14d, 15-30d, 31-90d, 91-180d and 181-360d. Across the whole surveillance period, the median available number of CK/cTnI/cTnT samples per patient was 16[11–34], 12 [7–17], and 19[14–58]; respectively. For a detailed list of the different assays used and their individual characteristics including limit of detection, 10% coefficient of variation, and 99th and 95th percentile (for troponins, and CK, respectively) upper reference limit of normal population values (URL); refer to **Table-1**. In the international ICI-myocarditis registry, cTnI and cTnT and their URL were entered by contributors but data regarding the assays used were not collected.

Statistical analysis

Data were analyzed using R (Version 4.0.4). Quantitative data were presented as median and interquartile range [IQR], or mean (± standard deviation, SD) as appropriate. Biomarker measurements were compared to their respective 99th and 95th percentiles URL for troponins and CK, respectively. Calculated ratios (cTnT/URL, cTnI/URL, CK/URL) were subsequently normalized by logarithmic transformation. Kaplan Meier analyses and multivariate logistic Cox regression models were computed using the survival package (Version 3.1-8). For model comparisons, missing values were imputed using Amelia II package

using the median of 1000 imputations.[16] Nested multivariate Cox hazard models were again calculated and compared using an analysis of deviance (ANDEVA). Wilcoxon-Rank-Sum tests were used to test for significant differences for normalized biomarkers evolution between different time points. P-values were adjusted for multiple testing's using the Holm-Bonferonni method. ROC (Receiver-Operator curves) and AUC (Area under the curve) analysis were calculated by the plotROC package (version 2.2.1). AUCs and its confidence intervals were calculated with using the pROC package with 2000 stratified bootstrap replicates each. The study protocol was approved by the Ethics Committee / institutional review board of both institutions (Heidelberg University: S-286/2017, 390/2011; APHP-Sorbonne: APHP-CSE-20-37_JOCARDITE; *NCT04637672*). International ICI-myocarditis ethical approval has already been described elsewhere (*NCT04294771*).[14] The investigation conforms with the principles outlined in the Declaration of Helsinki.

Results

Population studied

Our cohort included 61 consecutive patients admitted with ICI-myocarditis. Mean age was 68±13 years, 34% were female and median follow-up was 113 [47-304] days. All patients were promptly hospitalized upon suspicion of ICI-myocarditis with diagnosis confirmed via endomyocardial biopsy or cardiac MRI. Each patient was serially assessed for circulating biomarker (CK/cTnI/cTnT) of myotoxicity; multiple biomarkers were assessed within the first 3 days (median number[IQR] of tests/patient for CK: 3[2-4], cTnI: 3[1-4], cTnT: 4[3-5]). The clinical and demographic characteristics of this cohort are displayed in **Table-2**. Most patients had definite ICI-myocarditis (49/61, 80%) as determined by a diagnostic level of certainty; the rest had probable myocarditis.[12] A total of 64% of patients received anti-PD1 monotherapy, 21% anti-PDL1 monotherapy and 15% received a combination of anti-PD1 and anti-CTLA4. Most patients suffered from non-small lung cancer (39%) or malignant melanoma (21%). Most patients were symptomatic at initial presentation (44/61; 72%) contrasting with a small subset being identified asymptomatically as part of systematic screening strategy (17/61; 28%). A total of 24/61 (39%) patients developed at least one MACE (MACE events detailed in **Table-2**). Overall mortality and ICI cardio-myotoxicity related death occurred in 26/61 (43%) and 10/61 (16%) patients, respectively. Cardio-myotoxicity related deaths occurred earlier after ICI-myocarditis diagnosis versus non-related deaths (11[9-29] vs. 132[85-344] days, p<0.0001). Causes of death are detailed in **Table-2**.

Time course of cTnT, TnI and CK in patients with ICI-myocarditis

At index admission, cTnT, cTnI and CK were increased in most patients: 51/52 (98%), 28/34 (82%, p=0.009 vs. cTnT), 33/48 (69%, p<0.0001 vs. cTnT), respectively. Within 72h after admission, maximum blood concentrations expressed as multiples of URL were higher for cTnT (median=28[10-64]) compared to cTnI (median=12[5-59]; p=0.03 vs. cTnT) and CK (median=6[1-23]; p<0.0001 vs. cTnT). These biomarkers were serially tested during hospitalization and time-dependent concentration changes in the different time periods following presentation are displayed in **Figure-1**. Peak values were observed for

cTnT on day 7[3-15], for cTnI on day 4[2-9] (p=0.02 vs. cTnT, paired Wilcoxon-test), and for CK on day 1[1-6] (p=0.05 vs. cTnT, paired Wilcoxon-test) after initial ICI-myocarditis diagnosis. **Figure-1** shows a rapid decline in maximal (**Figure-1A**), minimal (**Figure-1B**) and median (**Figure-1C**) CK and cTnI levels during the early phase of the ICI-myocarditis (weeks) in contrast to a more prolonged elevation of TnT lasting several months. Minimal circulating levels of cTnT, cTnI and CK were below URL between day 15-30 after ICI-myocarditis diagnosis in 2%, 66% and 83% of cases; and in 11%, 84%, 98% between day 31-90 (p<0.0001 at all times, more extended follow-up data are shown in **Figure-1E**), respectively. In patients in which measured biomarkers normalized during the follow-up, the median time to first value below URL was longer for cTnT (55[39-141]days), compared to cTnI (23[9-34]days), and CK (9[5-15]days). Maximum discrepancy between ratio of cTnT/URL over cTnI/URL (maximal ratio= 19.88 [5.7-77.6]) during follow-up was identified between day 15-30 after diagnosis (**Figure-1D**).

Predictors of MACE in ICI-myocarditis

We next investigated the prognostic value of cTnT, cTnI and CK at index admission and during the course of their surveillance. Characteristics of ICI-myocarditis patients with MACE compared to patients without MACE during follow-up are shown in **Table-2**. The maximal cTnT/URL value measured within 72h upon diagnosis performed best in predicting MACE (AUC=0.82) during follow-up compared to CK/URL (AUC=0.74) and cTnI/URL (AUC=0.67) (**Figure-2A**). An analysis of deviance showed consistency and significant superior performance of models including cTnT/URL with no improvement by addition of CK/URL, age, sex, and cTnI/URL (**Figure-2B**). Consistently, the addition of cTnT/URL to models containing cTnI alone, CK alone, and both cTnI/CK significantly increased their performance (p=0.0003, p=0.0004, and p=0.003, respectively). Using best threshold detection based on ROC analysis, we found a maximal cTnT/URL value within ≤72 hours of admission for ICI-myocarditis above >32 to be the best predictor for MACE during the first 100 days (71% vs. 13%, p<0.0001, Cox regression hazard-ratio: 9.4, 95% confidence interval: 3.1,28.3, **Figure-2C**). Results were similar when missing data were imputed (see methods, **Figures-3A,B**)**.** MACE in the 4/31 patients with cTnT/URL<32, were non-fatal and occurred after hospitalization discharge except for one ventricular tachycardia diagnosed at the time of admission for ICI-myocarditis. Notably, cTnT/URL values (58[28-187]) were abnormal in all patients before the occurrence of first MACE (22/22 patients) while cTnI/URL (14[2-38]) and CK/URL (4[1-13]) values were normal in 3/21 (14%) and 6/24 (25%) of patients, respectively (p<0.0001 Fisher's exact test) (**Figure-4B**). The time interval from last blood sample to occurrence of first MACE were 1[1-5], 3[1-4], 1[1-6] days before MACE for cTnT, cTnI and CK, respectively. Selected kinetic changes of declining or normalizing CK and cTnI despite persistently high or even increasing cTnT levels at the time of first MACE in 4 ICI-myocarditis patients are shown in **Figure-4A**. These cases highlight the discrepant prognostic information of kinetic changes of cTnI/CK versus cTnT in ICI-myocarditis (**Table-2**).

External validation of cTnT, cTnI diagnostic value in patients with ICI-myocarditis

The external validation cohort comprise 35 patients from an international registry (cases described in the Franco-German cohort were not included) who had both cTnI and cTnT measurements within 72 hours of

admission **(Figures-5A & B)**. While 66% patients (23/35) with ICI-myocarditis had an increased cTnI/URL on admission, the respective percentage was 91% (32/35) for cTnT/URL (p=0.003, **Figure-5E**). This discrepancy also persisted for peak troponin values (cTnI/URL>1 in 21/30 (70%) vs. TnT/URL>1 in 29/30 (97%) (p= 0.001, **Figure-5F**). When this external validation cohort was queried for cases with either cTnI or cTnT available; initial cTnT was increased in 115/126 (91%) vs. 127/153 (83%) for cTnI (p=0.04, **Figure-5C**).

Discussion

Herein, we leveraged a cohort of 61 ICI-myocarditis patients from two cardio-oncology programs where cTnT, cTnI and CK were prospectively collected as clinically indicated during the first year of follow-up after diagnosis. This cohort is unique given the frequency of measurements of cardio-muscular biomarkers, particularly within 72 hours after admission. Our study allowed for direct comparison of the value of each biomarker as a diagnostic and prognostic tool. At the time of initial diagnosis, cTnT was more often elevated compared to cTnI and CK. This higher sensitivity for ICI-myocarditis of cTnT compared to cTnI or CK was also observed in an independent international cohort of over 200 cases. These data are in contrast with current ICI-myocarditis diagnostic guidelines recommending cTnI.[12] Our data also highlight an early increase of cTnI and CK to peak levels within hours of initial presentation, followed by a normalization within days. In contrast, cTnT peaked within days after the initial presentation, but this increase persisted for months. Importantly, we identified a significant difference in the association of each biomarker elevation and kinetics with MACE with cTnT being a stronger prognosticator than cTnI or CK. Therefore, cTnT should be the preferred biomarker to be used in ICI-myocarditis for risk of MACE assessment and surveillance objectives. Repeated measurement of cTnT within the first few days of presentation may help to capture the peak value of cTnT; allowing for identification of a subgroup of patients at low-risk of event; when cTnT/URL is <32.

Troponins as a diagnostic tool for ICI-myocarditis

To date, most ICI-myocarditis cases reported in the literature were identified using cTnI, because cTnI is widely available with multiple vendors and often preferred over cTnT given the recent recommendations for diagnosis of ICI-myocarditis.[12] cTnI is considered by some to be more cardiac specific than cTnT and therefore more suitable for diagnosis of ICI-myocarditis.[17-19] However, in the few reported cases where ICI-myocarditis was diagnosed despite negative troponins, troponin assay used was cTnI.[20] Those findings are in line with our results showing that ~10% of our cases lack an increase of cTnI on admission, despite cTnT being positive. The reason of the discrepancy between cTnT and cTnI is unclear. It has been reported in patients developing cardiotoxicity on ICI that 2/4 patients developed anti troponin-I antibodies vs none in 4/4 ICI treated control patients.[21] Those findings mirror what was observed in preclinical models of myocarditis including transgenic mice with PD-1 deletion where antibodies against cardio-muscular antigens, including troponin-I were felt to cause myocardial damage.[22-26] Interferences between cTnI and such antibodies (e.g., targeting the troponin I epitope) have previously and consistently been

reported in humans, while such antibody production resulting in biomarker detection interferences has been rarely reported with cTnT.[27, 28]

Troponins as a prognosticator of MACE in ICI-myocarditis

Discrepancies between cTnT and cTnI blood kinetics and prognostic implications has been assessed in various research settings including cardiac ischemia,[29-35] cardiac hypertrophy,[36] diabetes,[37] general population,[38] and patients affected by neuromuscular disorders.[17-19, 39] In these studies, cTnT was shown to be associated with overall and cardiovascular mortality while cTnI was more associated with cardiovascular specific mortality.[36] In addition, cTnT can be elevated due to coexisting non-cardiac pathologies including muscular disorders with regenerating muscle expressing cTnT, impacting non-cardiac death.[38, 40, 41] In our study, this discrepancy was even greater with cTnI only marginally capturing MACE. Furthermore, cTnI normalized within days while cTnT remained elevated for over three months in ~90% of patients. These differences in troponin blood concentration cannot be explained by their plasma half-life, which is only slightly longer for cTnT compared to cTnI, but still within a range of few hours for both types of troponins.[29, 30, 35] The fact that skeletal muscle damage is often concomitant with ICI-myocarditis trigger-events such as muscular respiratory failure may have contributed to our results.

Study limitations

While careful attention was paid to prospectively collect cTnT, cTnI and CK biomarkers in the standard of care of our Franco-German index cohort, some timepoints were missing given the prolonged follow-up. Extended follow-up may have occurred and biomarkers collected in clinics closer to patient's main residence, which explains an additional confounder of different cTnI assay measurements (various providers, variable sensitivity detection, **Table-1**); this concern was less of an issue with cTnT, given a single vendor (almost all using the Elecsys kit by Roche®, >99%, **Table-1**). While these latter points may be seen as limitations of our study, they reflect use of these biomarkers in the real-life setting. Subgroup analysis by type of cTnI kit assays used, of cancer or ICI drugs may be worth pursuing but our cohort was too small and heterogenous to allow for such analysis. Another important limitation is that these findings reflect the biomarker use and MACE evolution of the first cases of ICI-myocarditis, which is an emerging and very recently described disease.[5, 7] Given the better recognition of the disease by oncologists and cardiologists, we expect an identification of patients at a much earlier stage or even while asymptomatic during systematic troponin/CK screening strategies in ICI treated patients. Therefore, our findings and conclusion mostly apply to symptomatic patients upon initial diagnosis. Lastly, our troponin prognostic cut-off threshold needs to be validated in independent cohorts, completed prospectively to evaluate if the low-risk population can be managed in an outpatient setting.

Conclusion

Discrepancies between cTnT, cTnI circulating levels exist at diagnosis but are even more important clinically for surveillance and prognostication of ICI-myocarditis patients. Our data indicate that cTnT is

the best predictor of MACE and the most suitable for surveillance among cTnI, cTnT and CK in symptomatic patients. Within the first 72h of initial ICI-myocarditis diagnosis, cTnT/URL < 32 identifies a subgroup of patients at low-risk of MACE.

Declarations

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Conflict of interest

L.H.L. has served on the advisory board for Daiichi Sankyio, Senaca, and Servier as an external expert for Astra Zeneca and received speakers' honoraria from Novartis and MSD. JES has served as consultant for BMS, AstraZeneca, BeiGene, Novartis and had received grants from BMS, and Novartis. NLP is a Cancer Prevention Research Institute of Texas (CPRIT) Scholar and Andrew Sabin Family Foundation Fellow. NLP is supported by CPRIT RP200670 and by NIH/NCI 1P01CA261669-01.

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Tables

Table 1. Technical aspects of the different assays used to determine cardiac troponins and CK circulating levels

ay	*n/N, % of samples analyzed*	*Range of detection in ng/L*	*Limit of detection (LoD) in ng/L*	*Limit of blanc (LoB) in ng/L*	*10% coefficient of variation ($^{10\%}$CV) in ng/L*	*99th percentile normal cut-off values in ng/L*
diac Troponin-T (cTnT)						
csys (Roche nostics)*	1623/1625 (>99%)	3 – 10,000	3	2.5	13	14
T90 Flex munoassay lyzer (Radiometer)*	2/1625 (<1%)	10 – 25,000	10	5	30	17
diac Troponin-I (cTnI)						
csys (Roche nostics)*	40/801 (5%)	160 – 25,000	160	100	300	160
llica IM H (SIEMENS thineers)*	208/801 (26%)	2.5 – 25,000	1.6	0.5	2.5	LH: 34.11 (F) 53.48 (M) S: 38.64 (F) 53.53 (M)
/IA taur (SIEMENS thineers)*	14/801 (2%)	2.5 – 25,000	1.6	0.5	2.5	LH: 36.99 (F) 57.27 (M) S: 39.59 (F) 58.05 (M)
ension (SIEMENS thineers)*	2/801 (<1%)	17 – 40,000	2.7	1.1	4.0	56
ension a (SIEMENS thineers)*	3/801 (<1%)	3 – 25,000	2.0	1.0	10.0	45
hitect T (ABBOTT)*	177/801 (22%)	10 – 50,000	1.1 – 1.9	0.7 – 1.3	1.3	15.6 (F) 34.2 (M)
ity T (ABBOTT)*	35/801 (4%)	10 – 50,000	0.7 – 1.6	0.1 – 1.0	2.1	15.6 (F) 34.2 (M)
CESS (Beckman ter, CA)*	11/801 (1%)	10 – 2,000 000	2.3	1.7	2.3	17.5
uTnI ay (Beckman ter, CA)*	3/801 (<1%)	10 – 60,000	<10	NA	0.03	40
AS (Biomerieux)*	8/801 (1%)	4.9 – 40,000	1.3 – 3.2	0 – 1.9	2.9 – 4.9	11 (F) 25 (M)
ROS munodiagnostic ducts (Ortho cal Diagnostics)	8/801 (1%)	1.23 – 30,000	12	7.0	34.0	34
-Ultra/ADVIA taur (Siemens)	292/801 (36%)	NA	3	NA	30	40

Table 1. (continued)

ssay	*n/N, % of samples analyzed*	*Range of detection in U/L*	*Limit of detection (LoD) in U/L*	*Limit of blank (LoB) in U/L*	*95th percentile normal cut-off values in U/L*
reatine Kinase (CK)					
tellica	38/1331	15 –	6	1	34 – 145 (F)
H (SIEMENS)	(3%)	1,300			46 – 171 (M)
obas (Roche)	809/1331	7 – 2,000	7	7	26 – 192 (F)
	(61%)				39 – 308 (M)
imension	4/1331	7 – 1,000	7	2	26 – 192 (F)
ista (SIEMENS	(<1%)				39 – 308 (M)
ealthineers)					
DVIA (SIEMENS	397/1331	15 –	6	3	34 – 145 (F)
ealthineers)	(30%)	1,300			43 – 171 (M)
LINITY (ABBOTT)	38/1331	7 – 4,267	5	3	29 – 168 (F)
	(3%)				30 – 200 (M)
RCHITECT (ABBOTT)	8/1331	5 – 4,267	5	NA	29 – 168 (F)
	(<1%)				30 – 200 (M)
U 5800 (Beckman	6/1331	10 –	NA	NA	30 – 223
oulter)	(<1%)	2,000			
itros (Ortho Clinical	31/1331	20 –	NA	NA	30 – 135 (F)
iagnostics)	(2%)	1,600			55 – 170 (M)

Abbreviations: CV, coefficient of variation; CI, confidence interval; F, female; LoB, Limit of Blank; LoD, Limit of Detection; LH, lithium heparin sample; LoQ, Limit of Quantitation; M, male; S, serum sample; NA, not available.
* high-sensitive assays (hs)

Table 2. Characteristics of the Franco-German cohort

		Overall cohort (n=61)	MACE (n=24)	No MACE (n=37)	p-value
Age (years; mean ± SD)		68 ± 13	69 ± 13	68 ± 13	0.88
Sex (female)		22/61 (36%)	8/24 (33%)	14/37 (38%)	0.79
Follow-up after diagnosis (days, median [IQR])		113 [47, 304]	83 [25, 211]	127 [74, 341]	0.13
Patients with MACE		24/61 (39%)			NA
- Respiratory failure (mechanical ventilation)			8/24 (33%) 12/57* (21%)		
- Ventricular arrythmias			8/24 (33%) 15/57* (26%)		
- Pacemaker implantation			7/24 (29%) 7/57* (12%)		
- Cardiogenic shock			5/24 (21%) 5/57* (9%)		
- Symptomatic heart failure			7/24 (29%) 8/57* (14%)		
- Cardiomyotoxic related death			10/24 (42%) 10/57* (17%)		
Overall mortality		26/61 (43%)	16/24 (66%)	10/37 (27%)	0.002
Cause of death:					
- Cancer progression		10/26(38%)	1/16 (6%)	9/10 (90%)	<0.0001
- Cardio-respiratory failure		8/26 (31%)	8/16 (50%)	0/10 (0%)	0.01
- Infection		4/26 (15%)	3/16(19%)	1/10 (10%)	1.0
- Sudden cardiac death		2/26 (8%)	2/16 (13%)	0/10 (0%)	0.51
- Cerebral hemorrhage		1/26 (4%)	1/16 (6%)	0/10 (0%)	1.0
- Myocardial infarction		1/26 (4%)	1/16 (6%)	0/10 (0%)	1.0
Time to first MACE (days, median [IQR])		NA	4 [1, 16]		NA
cTnT/URL ratio at diagnosis (median [IQR])		28 [10, 64]	50 [40, 130]	14 [4, 29]	<0.0001
cTnI/URL ratio at diagnosis (median [IQR])		12 [5, 59]	16 [11, 109]	8 [3, 50]	0.07
CK/URL ratio at diagnosis (median [IQR])		6.1 [1, 23]	11 [6, 35]	2 [1, 12]	0.003
cTnT/URL ratio before MACE (median [IQR]) †		NA	58 [28, 87]	NA	NA
cTnI/URL ratio before MACE (median [IQR]) †		NA	14 [2, 38]	NA	NA
CK/URL ratio before MACE (median [IQR]) †		NA	4.0 [1, 14]	NA	NA
Drugs	Anti-PD1	39/61 (64%)	17/24 (71%)	22/37 (59%)	0.5
	Anti-PD1 + Anti-CTLA4	9/61 (15%)	2/24 (8%)	7/37 (19%)	
	Anti-PDL1	13/61 (21%)	5/24 (21%)	8/37 (22%)	
Tumor	NSC lung cancer	24/61 (39%)	9/24 (38%)	15/37 (41%)	0.35
	Melanoma	13/61 (21%)	3/24 (13%)	10/37 (27%)	
	Renal cell carcinoma	6/61 (10%)	2/24 (8%)	4/37 (11%)	
	Hepatocarcinoma	4/61 (7%)	1/24 (4%)	3/37 (8%)	
	SC carcinoma	3/61 (5%)	2/24 (8%)	1/37 (3%)	
	other‡	11/61 (18%)	7/24 (29%)	4/37 (11%)	

Abbreviations: CTLA4 (Cytotoxic T-Lymphocyte Associated protein 4); IQR: interquartile range; MACE, major adverse cardiomyotoxic event; NA, not applicable; NSC, Non-small cell; PD1 (Programmed cell Death protein 1) and its ligand (PDL1); SC, Squamous cell; SD, standard deviation; URL: upper reference limit being upper 99^{th} percentile of normal values for troponins and 95^{th} for CK.
Statistics: Proportions were compared using Fisher's exact test. Quantitative values were compared using a Wilcoxon test.

* One patient may develop more than one MACE (n=57, total Number of events).
† The closest measured value after diagnosis before the occurrence of MACE was selected.
‡ Other cancers involved thymoma (3), sarcoma (2), colorectal carcinoma (2), urothelial carcinoma (1), pleural mesothelioma (1), endometrial carcinoma (1), cancer of unknown primary (1)

Appendix

Appendix. List of collaborating centers in the international ICI myocarditis registry

Assistance publique Hôpitaux Universitaires de Marseille Nord	Paris, France	Jennifer Cautela
Barts Health NHS Trust	London, United Kingdom	Shanthini Crusz
Beth Israel Deaconess Medical Center	Boston, USA	Aarti Asnani
Bern University Hospital	Bern, Switzerland	Eva Haegler-Laube
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	Karl Stangl
Chi Mei Medical Center	Tainam, Taiwan	Wei Ting Chan
Clínica Universidad de Navarra	Pamplona, Spain	Nicolas Martinez Calle
Dartmouth	Hitchcock Medical Center, Lebanon, USA	Lauren Gilstrap
East Carolina University	Greenville, USA	Melissa Y.Y. Moey
Eisenhower Medical Center	Rancho Mirage, USA	Yazeed Samara
Emory University Hospital	Atlanta, USA	Ajay K. Nooka
Evangelische Lungenklinik Berlin	Berlin, Germany	Christian Grohe
General Hospital of Chinese People's Liberation Army	Beijing, China	Hongbin Liu
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	Marie Claire Zimmer
International University of Health and Welfare Mita Hospital	Tokyo, Japan	Yuichi Tamura
Japan Community Health Organization Kyushu Hospital	Kitakyushu, Japan	Masahiro Mohri
Johns Hopkins University	Baltimore, USA	Roberta Florido
Kumamoto University	Kumamoto, Japan	Toshihiro Kimura
Maine Medical Center	Portland, USA	Sanjeev Francis
McMaster University	Hamilton, Canada	Darryl

		Leong
Memorial Sloan Kettering	New York City, USA	Vicky Makker
Mitsui Memorial Hospital	Tokyo, Japan	Kazuyuki Yahagi
Monash University	Melbourne, Australia	Andrew Haydon
Nagoya University Graduate School of Medicine	Nagoya, Japan	Ryota Morimoto
Nantes University Hospital	Nantes, France	Nicolas Piriou
National Cancer Institute, National Institutes of Health	Bethesda, USA	Cecilia Monge
New York University	New York City, USA	Benjamin P. Geisler
Northwestern Memorial Hospital	Chicago, USA	Kambiz Ghafourian
Ohio State University Wexner Medical Center	Columbus, USA	Sergey Brodsky
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
St. Luke's International Hospital	Tokyo, Japan	Ryosuke Imai
Stanford University	Palo Alto, USA	Han Zhu
Teikyo University School of Medicine	Tokyo, Japan	Nobuhiko Seki
Tel Aviv Sourasky Medical Center affiliated to the Sackler School of Medicine	Tel Aviv, Israel	Michal Laufer Perl
The Angeles Clinic and Research Institute	Los Angeles, USA	Lawrence D. Piro
Tokyo Women's Medical University	Tokyo, Japan	Kitagawa Kazuo
UCSF Medical Center	San Francisco, USA	Mandar Aras
Université de Caen Basse	Normandie, Caen, France	Joachim Alexandre
University Hospital Basel	Basel, Switzerland	Alfred Zippelius
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	Kazuko Tajiri
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

Figures

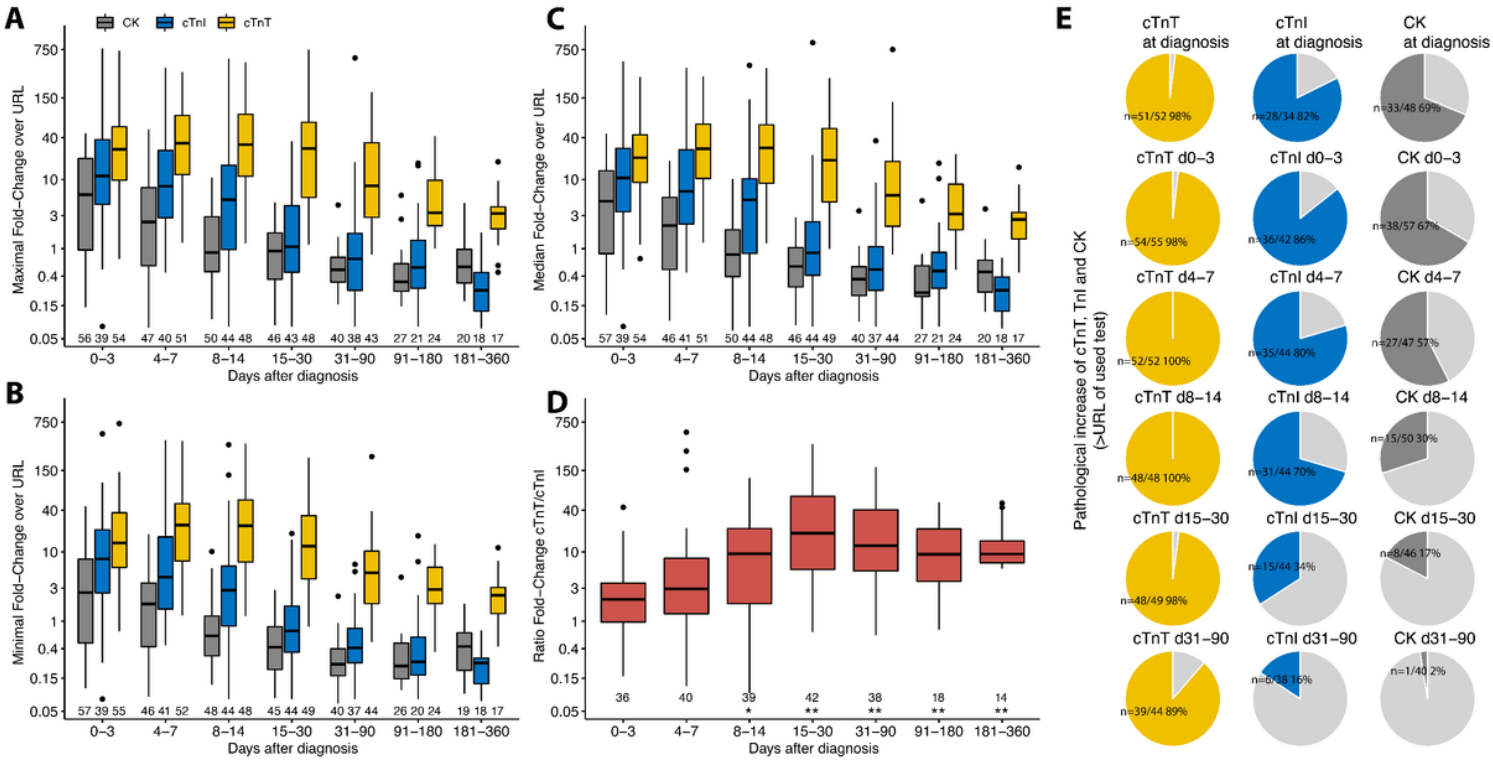

Figure 1

Time course of troponins and creatine kinase (CK) after admission for ICI-myocarditis. URL stands for 99th percentile upper reference limit for troponins and 95th percentile for CK. Evolution of maximal (**A**), minimal (**B**), and median (**C**) values (median, IQR in the boxplots) of cardiac troponin-T (cTnT)/URL, cardiac troponin-I (cTnI)/URL and CK/URL ratios over time after initial diagnosis of ICI-myocarditis within specific timeframes (x-axis) in follow-up. (**D**) Ratio of maximum cTnT/URL over cTnI /URL over time after initial diagnosis of ICI-myocarditis within specific timeframes (x-axis) in follow-up. Adjusted p-values for multiple testing's when comparing these ratios versus baseline are shown (*<0.001, **<0.0001). For **A-D**, n

available for each biomarker at each time frame is just above the x-axis. (**E**) Proportion of patients with biomarkers above URL over time after diagnosis are displayed, numbers indicate patients with abnormal values. Light grey area represents the proportion of patients with biomarker levels below URL. Minimal values within the indicated time period were used for figure E (d for days).

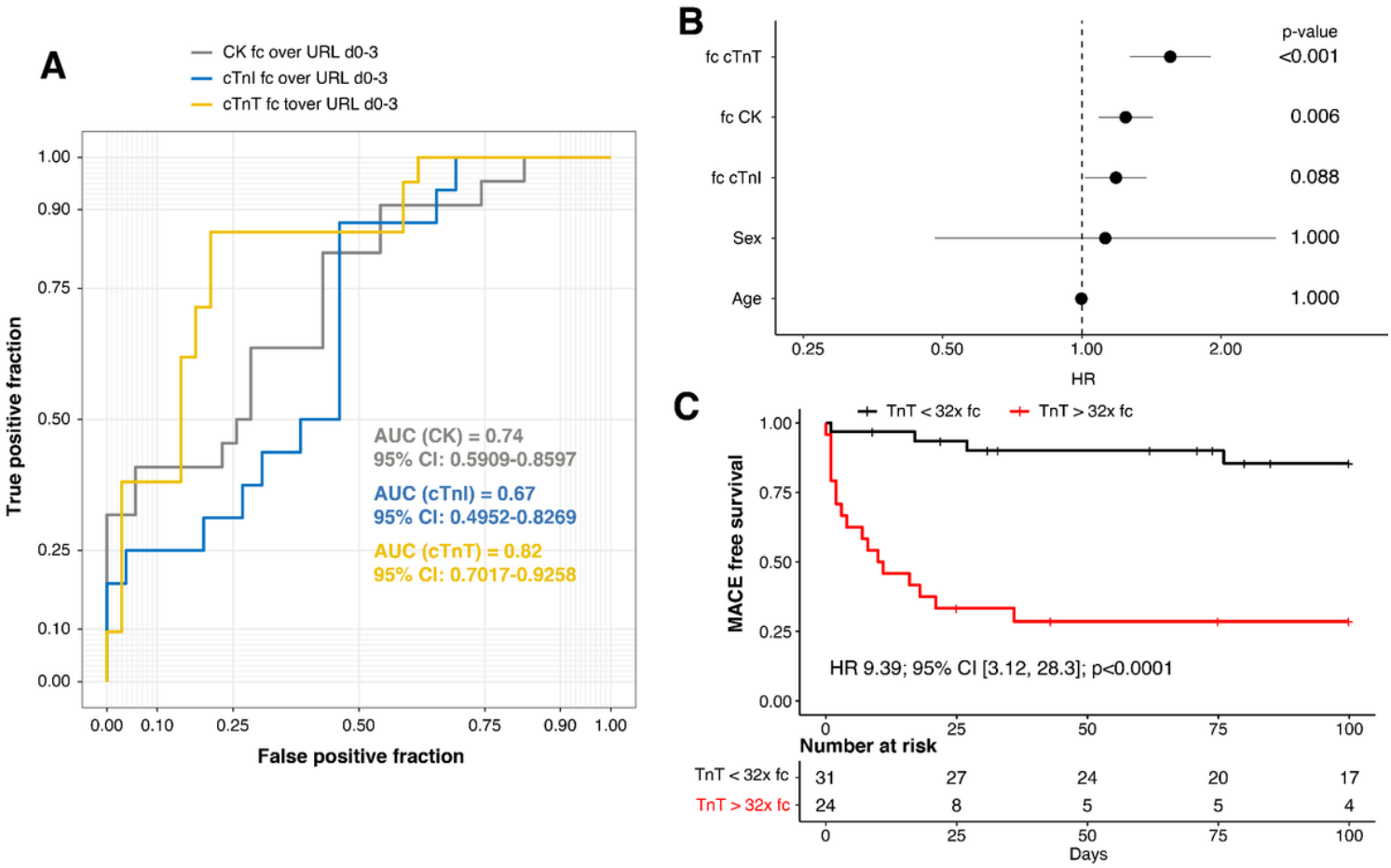

Figure 2

Maximal cardiac biomarkers values within 72h of ICI myocarditis diagnosis as a predictor of MACE. (**A**) Receiver operating curve of cTnT/URL, cTnI/URL, and CK/URL to predict MACE during a follow-up of 100 days. (**B**) Univariate logistic regression of maximal cTnT/URL, cTnI/URL and CK/URL within 72h after ICI-myocarditis diagnosis to predict MACE in 100 days follow-up. (**C**) MACE over a time-course of 100 days after diagnosis as a function of cTnT/URL value above and below 32 (n=55).

Abbreviations: AUC, area under the curve; MACE, major adverse cardiomyotoxic event; fc, fold-change over URL; URL: upper reference limit being upper 99th percentile of normal values for troponins and 95th for CK; 95%CI, 95% confidence interval; HR, hazard ratio.

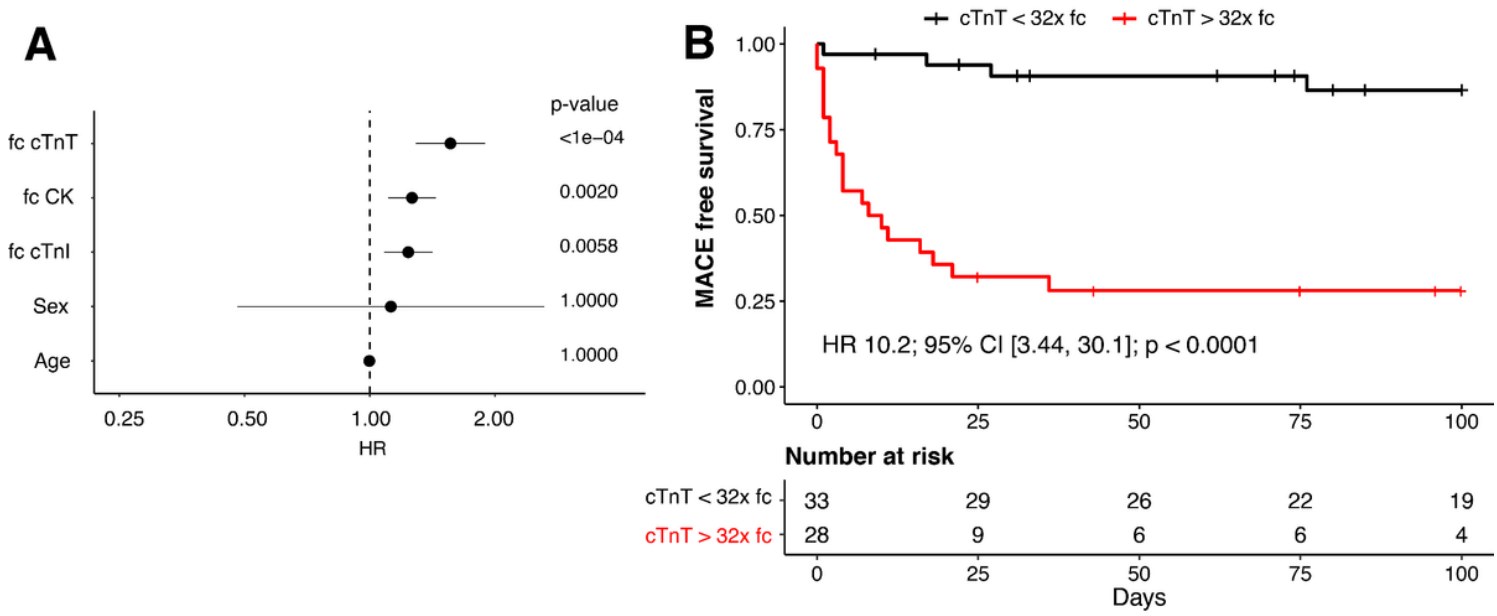

Figure 3

Peak cardiac biomarkers values within 72h of ICI myocarditis diagnosis as a predictor of MACE on imputed data. Univariate logistic regression of maximal cTnT/URL, cTnI/URL and CK/URL within 72h after ICI-myocarditis diagnosis to predict MACE in 100days follow-up by using imputed data **(A)**. MACE over a time-course of 100 days after diagnosis as a function of cTnT/URL value above and below 32 by using imputed data (n=61, see methods for details) **(B)**.

Abbreviations: MACE, major adverse cardiomyotoxic event; fc, fold-change over URL; URL: upper reference limit being upper 99th percentile of normal values for troponin; 95%CI, 95% confidence interval; HR, hazard ratio.

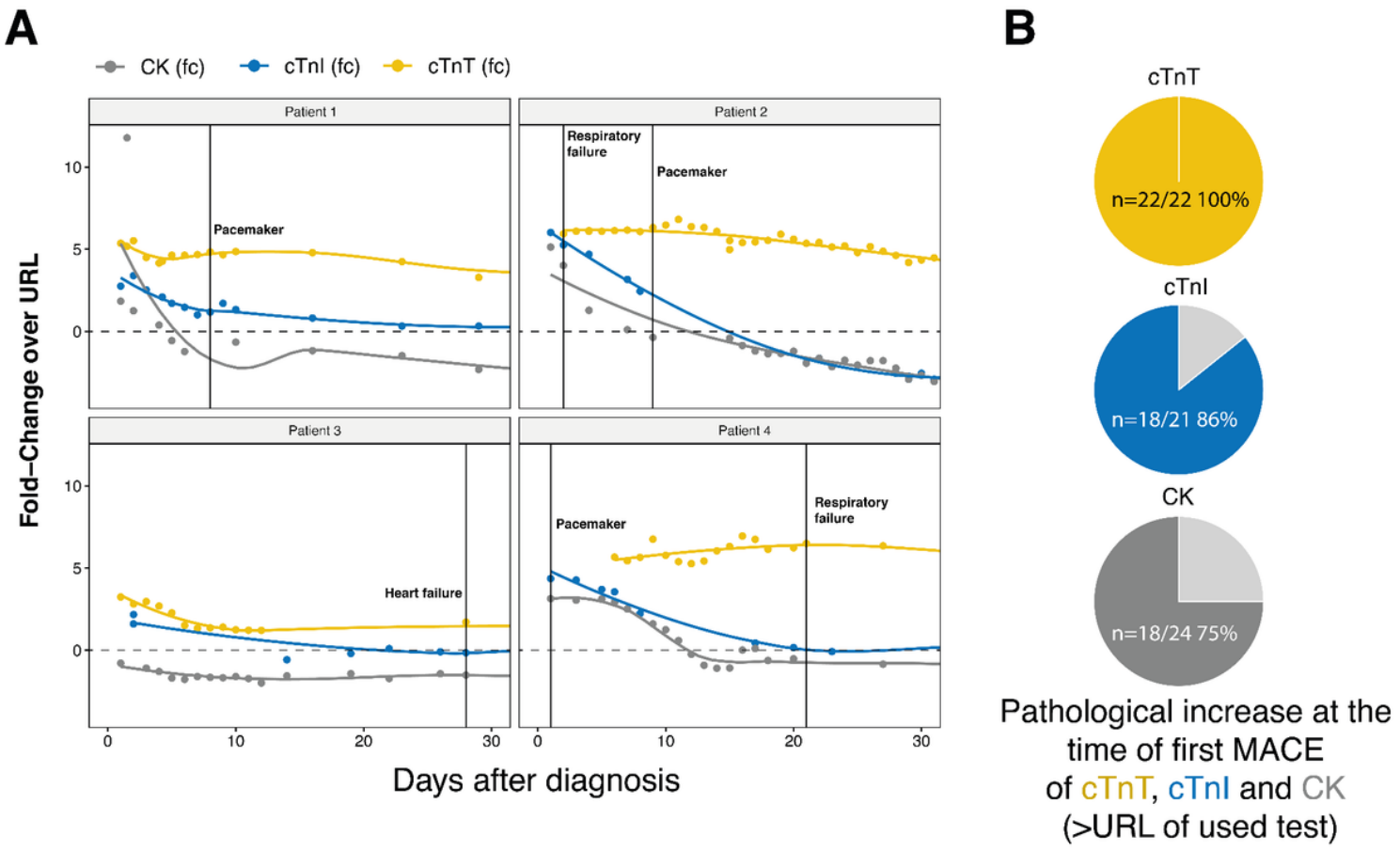

Figure 4

Examples of ICI-myocarditis cases and their biomarker evolution over time and at the time of MACE. (**A**) Time-course of cardiac troponin T (cTnT)/URL, cardiac troponin I (cTnI)/URL, and creatine kinase (CK)/URL with URL being represented by the black horizontal dotted line during the first month of the ICI-myocarditis diagnosis in 4 exemplified patients presenting a MACE (back vertical line). (**B**) Proportion of patients with biomarkers above URL before first MACE are displayed in yellow (cTnT, n=22/22), blue (cTnI, n=18/21) and dark grey (CK, n=18/24). Light grey area represents the proportion of patients with biomarker levels below URL. The biomarkers measured represent the last value measured before MACE within a time frame of 1[1-5], 3[1-4], 1[1-6] days before MACE for cTnT, cTnI and CK in median [IQR].

Abbreviations: MACE, major adverse cardiomyotoxic event; fc, fold-change over URL; URL: upper reference limit being upper 99th percentile of normal values for troponin and 95th percentile of normal values for creatine kinase.

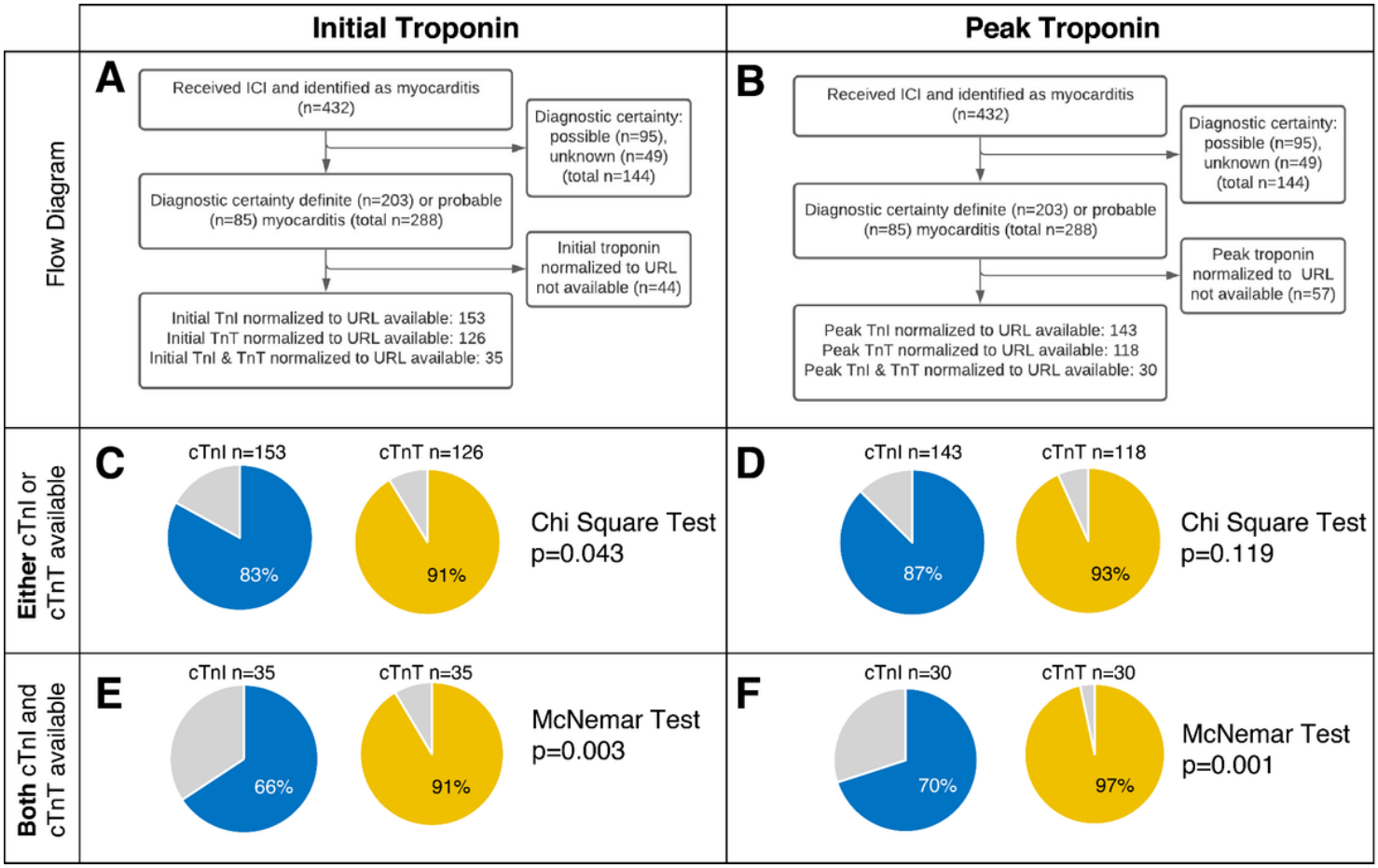

Figure 5

cTnI versus cTnT values in ICI-myocarditis patients in an external validation cohort (international registry).15 Flow chart of the analyzed patients with available initial (**A**) and peak (**B**) troponins assessed. Results in patients with either initial (**C**) or peak (**D**) cTnI/URL or cTnT/URL available; and in the subset of patients with both initial (**E**) and peak (**F**) cTnI/URL and cTnT/URL available.