

## RESEARCH ARTICLE

# COVID19-associated cardiomyocyte dysfunction, arrhythmias and the effect of Canakinumab

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## Abstract

### Background

Cardiac injury associated with cytokine release frequently occurs in SARS-CoV-2 mediated coronavirus disease (COVID19) and mortality is particularly high in these patients. The mechanistic role of the COVID19 associated cytokine-storm for the concomitant cardiac dysfunction and associated arrhythmias is unclear. Moreover, the role of anti-inflammatory therapy to mitigate cardiac dysfunction remains elusive.

### Aims and methods

We investigated the effects of COVID19-associated inflammatory response on cardiac cellular function as well as its cardiac arrhythmogenic potential in rat and induced pluripotent stem cell derived cardiomyocytes (iPS-CM). In addition, we evaluated the therapeutic potential of the IL-1 $\beta$  antagonist Canakinumab using state of the art in-vitro confocal and ratio-metric high-throughput microscopy.

### Results

Isolated rat ventricular cardiomyocytes were exposed to control or COVID19 serum from intensive care unit (ICU) patients with severe ARDS and impaired cardiac function (LVEF 41 $\pm$ 5%; 1/3 of patients on veno-venous extracorporeal membrane oxygenation; CK 154 $\pm$ 43 U/l). Rat cardiomyocytes showed an early increase of myofilament sensitivity, a decrease of Ca<sup>2+</sup> transient amplitudes and altered baseline [Ca<sup>2+</sup>] upon exposure to patient serum. In addition, we used iPS-CM to explore the long-term effect of patient serum on cardiac

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electrical and mechanical function. In iPS-CM, spontaneous  $\text{Ca}^{2+}$  release events were more likely to occur upon incubation with COVID19 serum and nuclear as well as cytosolic  $\text{Ca}^{2+}$  release were altered. Co-incubation with Canakinumab had no effect on pro-arrhythmogenic  $\text{Ca}^{2+}$  release or  $\text{Ca}^{2+}$  signaling during excitation-contraction coupling, nor significantly influenced cellular automaticity.

## Conclusion

Serum derived from COVID19 patients exerts acute cardio-depressant and chronic pro-arrhythmogenic effects in rat and iPS-derived cardiomyocytes. Canakinumab had no beneficial effect on cellular  $\text{Ca}^{2+}$  signaling during excitation-contraction coupling. The presented method utilizing iPS-CM and in-vitro  $\text{Ca}^{2+}$  imaging might serve as a novel tool for precision medicine. It allows to investigate cytokine related cardiac dysfunction and pharmacological approaches useful therein.

## Introduction

The current Sars-CoV-2 pandemic affects health care systems worldwide in an unprecedented way, resulting in high morbidity and mortality. Cardiac injury occurs in almost 20% of patients during hospitalization and mortality is particularly high in these patients [1]. Troponin elevations are a frequently reported finding [2] and arrhythmias are linked to ICU admission in up to 12% of patients, while acute respiratory distress syndrome (ARDS) and sepsis occur in up to 29% of hospital admissions [3]. In addition, a considerable number of patients present with cardiovascular comorbidities (up to 15%) worsening their overall outcome. Systemic inflammation and cytokine release are a hallmark feature of the disease [4].

However, the role of COVID19 associated cytokine-storm for concomitant cardiac dysfunction [5] remains elusive. In addition, potential beneficial effects of immunomodulation to decrease circulatory cytokines with their assumed negative impact on cardiac contractile function have not yet been established. Cardiac mechanical and electrical function are disturbed during heart failure with reduced ejection fraction (HFrEF) leading to acute decompensation, ventricular tachyarrhythmias and sudden cardiac death. HFrEF is a feared complicator of septic conditions on the ICU and associated with increased mortality [6]. Direct and indirect triggers for HFrEF include infectious viral diseases. It is widely known that a variety of viruses causes inflammatory cardiomyopathy leading to impaired function [7].

However, besides direct infection of myocytes with cardiotropic viruses, septic cardiomyopathy is a common denominator of severely affected ICU patients that suffer from a variety of viral infections. Septic cardiomyopathy has been associated with an altered interleukin profile in peripheral blood samples [8] and interleukins like  $\text{TNF}\alpha$  and  $\text{IL-1}\beta$  have been shown to depress myocardial contractile function [9]. Clinical reports indicate that critically ill COVID19 patients develop sepsis and ARDS at days 10 and 12, which is paralleled by a surge of cytokines (i.e.  $\text{IL-1}$ ,  $\text{IL-2}$ ,  $\text{IL-6}$ ,  $\text{IL-7}$ ,  $\text{IL-10}$ , GSCF,  $\text{IP-10}$ , MCP1, MIP1A,  $\text{TNF}\alpha$ ) known to be involved in septic cardiomyopathy and clinical deterioration [3]. Several groups have suggested early-on to utilize immunosuppressant therapy for the treatment of COVID19 associated sepsis. While the  $\text{IL-6}$  receptor blocker Tocilizumab administered to COVID19 patients in cytokine release syndrome has not shown any benefit in terms of survival in a very recent multicenter randomized controlled trial [10–12], the  $\text{IL-1}$  receptor blockers Anakinra and

Canakinumab conferred a significant survival benefit in patients with septic hyperinflammation [10, 13, 14].

The specific effects of anti-inflammatory treatment on the concomitant cardiac contractile and electric function [5] in the context of COVID19 remain elusive. We investigated the effect of the COVID19 associated cytokine-release on cardiac function in-vitro using a rat model and induced pluripotent stem-cell derived cardiomyocytes. For that purpose, we established an in-vitro setup to study potential effects of anti-inflammatory treatment on cardiac dysfunction as exemplified by the IL-1 $\beta$  inhibitor Canakinumab.

## Material and methods

### Chemicals and solutions

Chemicals were obtained from Sigma Aldrich (St. Louis, MO, USA) if not noted otherwise. Fluorescent dyes Fluo-4 and Fura-2 AM were obtained from Thermo Fisher Scientific (Waltham, MA, USA). Normal Tyrode (NT) solution consisted of (in mM): 30 NaCl, 4 KCl, 2 CaCl, 1 MgCl<sub>2</sub>, 10 Glucose, 10 HEPES and was pH adjusted to 7.4 with NaOH.

### Patient serum

The study was conducted at the Charité Campus Virchow Hospital between 04/2020 and 12/2020 in accordance with the local ethics committee approval (EA2/066/20; pa-COVID19/Charité; "Ethikausschuss am Campus Virchow Klinikum"; Head: PD Dr. E. Kaschina). Patients provided informed written consent to have data from their medical records used in research and to participate in this study. Patient serum was acquired from either healthy control donors (n = 3) or SARS-CoV-2 positive patients (n = 12) with severe acute respiratory distress syndrome (i.e. Horowitz index <300; see Table 1 for patient characterization) immediately upon intensive care unit (ICU) admission. Serum was obtained using a serum separator tube upon immediate cooled centrifugation and stored at -80°C. Patient characteristics are shown in Table 1 and ELISAs were performed for interleukin(IL)-1 $\beta$ , IL-6 and TNF $\alpha$  as per kit instructions (Invitrogen/Thermo Fisher Scientific, USA). C-reactive protein (CRP), procalcitonin (PCT), creatinine kinase (CK) and hemoglobin (Hb) concentrations were obtained using the clinical routine laboratory infrastructure at Charité University Medicine Berlin.

### Animal in-vitro experiments

All animal experiments were approved by local authorities and performed in accordance with local guidelines (local ethical committee approval T0060-15, LAGeSo Berlin). Ventricular cardiomyocytes were isolated from 12 to 16 weeks old, male Sprague Dawley rats as previously described [17]. In short, rats were sacrificed using cervical dislocation, hearts were excised, the aorta was cannulated and mounted on a Langendorff apparatus. Perfusion with a calcium (Ca<sup>2+</sup>) free solution for 2 min was followed by enzymatic digestion in a solution containing 20  $\mu$ M Ca<sup>2+</sup> and 75 $\mu$ g/ml Liberase (Sigma-Aldrich (St. Louis, MO, USA) for 12 to 15 min. The left ventricle was removed from the heart, the tissue was dissected, filtered and washed and Ca<sup>2+</sup> was introduced stepwise. Isolated cardiomyocytes were kept in NT solution between experiments.

Isolated adult rat ventricular cardiomyocytes were used for two different sets of experiments: First, we investigated effects upon ultra-short term (i.e. 15 min) and second upon short-term (i.e. 60 min) incubation in either patient or control serum. For the ultra-short term study, aiming at acute receptor mediated effects, a subset of ventricular cardiomyocytes was loaded with Fura-2 AM at 1 $\mu$ M for 15 min and plated on laminin-coated coverslips in NT

**Table 1. Patient characteristics of the study cohort. [15, 16].**

	COVID19 (n = 12)	Control (n = 3)	Reference Range
Sex (%)	♂: 75 / ♀: 25	♂: 100/ ♀: 0	-
Age (ys)	64±4	35±6	-
LVEF (%)	41±5	58±2	55–70
VV-ECMO (%)	33	0	-
CVVHD (%)	58	0	-
Anticoagulation (%)	100	0	-
Glucorticoids (%)	67	-	-
Antibiotics (%)	92	-	-
Hb (mg/dl)	9.1±0.3	-	♂: >13.5 / ♀: >12
CK (U/l)	154±43	-	♂: <170 / ♀: <145
CRP (mg/dl)	173±30	-	<5
PCT (µg/l)	6.6±2.4	-	<0.5
Catecholamines (µg/kg/h)	0.06625±0.03	0	-
Horowitz-Ratio	220±24	-	>500
IL-6 (pg/ml)	12±7	0.5±0.1	<4.5 (Todd et al; 15)
IL-1β (pg/ml)	2±1	< 0.1	0.3–1.4 (Iorio et al; 16)
TNFα (pg/ml)	23±1	18±8	<2.5 (Todd et al; 15)

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solution. The coverslips were mounted on a computer-controlled-microscope (CytoCypher MultiCell System, CytoCypher BV, Netherlands), cells were electrically stimulated (1 and 3 Hz field-stimulation; Myopacer, Ionoptix) and fluorescence (excitation at 340 nm and 385 nm, emission collected at 510±40) and sarcomere shortening were recorded at 37°C during steady state. Subsequently, NT solution was replaced by solution containing either patient or control serum diluted to 10% and cells were incubated for 15 min. Fluorescence signal and contractility were measured in a paired fashion (cells individual position was stored digitally) while cells remained in 10% serum solution.

For the short term study and to mimic in-vivo conditions as closely as possible, ventricular cardiomyocytes were incubated for 60 min in either patient or control serum diluted to 50% at room temperature. Cells were then removed from the serum solution, washed twice, plated on laminin-coated coverslips and loaded with Fura-2 AM at 1 µM for 15 min. The additional wash-step was introduced to mitigate interferences by the serums autofluorescence and heterogeneity in local fluorescence.

Fluorescence and contractility were assessed as described above. Recorded fluorescence and contractility data were analyzed using the software Cytosolver Desktop (CytoCypher MultiCell System, CytoCypher BV, Netherlands).

### Human induced pluripotent stem cells experiments

iPS-derived cardiomyocytes (iPS-CM) were generated from the previously established hiPSC line BIHi004-A generated from cells of a healthy donor (see Hossini et al. [18] and <https://hpscereg.eu/cell-line/BIHi004-A> for further details) using an in-house optimized protocol established at the BIH Stem Cell Core that is based on the protocol described by Lian et al [19]. IPS-CM were incubated for 24 hours in either patient or control (healthy donors) serum diluted to 10% with maintenance medium (RPMI 1640 Thermo Fisher Scientific, USA with B27 Supplement (50X) Thermo Fisher Scientific, USA) at 37.5°C. Incubation was performed with or without 10µg/ml of the monoclonal anti-IL-1β antibody Canakinumab (Novus Biologicals, Colorado, USA) present. Cells were subsequently loaded with Fluo-4-AM and

fluorescence was measured using confocal line-scan imaging (Zeiss LSM 800, excitation at 488 nm, emission collected at  $> 515$  nm). Clusters of cells were identified and the scan line was placed along the maximal diameter of at least one cell with a pixel-size of  $0.12 \mu\text{m}$ . Cells were paced at 1 Hz (field-stimulation) and images were acquired either during steady-state or immediately upon cessation of electrical stimulation as indicated. Local  $\text{Ca}^{2+}$  transients were calculated as  $F / F_0$  from regions that were identified to be cytosolic or nuclear using 2D confocal images of cell-clusters. Spontaneous  $\text{Ca}^{2+}$  release measurements were obtained during a 10 sec period upon termination of electrical stimulation.

## Statistical analysis

Experiments with serum were performed in a non-pooled fashion for each individual patient. All data is presented as mean  $\pm$  standard error mean and analysis was performed in a blinded-fashion. Wherever feasible, individual data points are displayed, otherwise sample sizes are provided. Parts of the data analysis were conducted in R (version 3.6.1, R Core Team (2018)), scripts are fully available upon request. GraphPad Prism was used for statistical inference and plotting (GraphPad Software, San Diego, California USA). To test for group differences, for data with two groups student's t-test or Kruskal-Wallis One Way ANOVA on Ranks in non-normal distributed data was used. For data with more than two groups ANOVA analysis was performed. A  $p < 0.05$  indicates significant statistical difference.

## Results

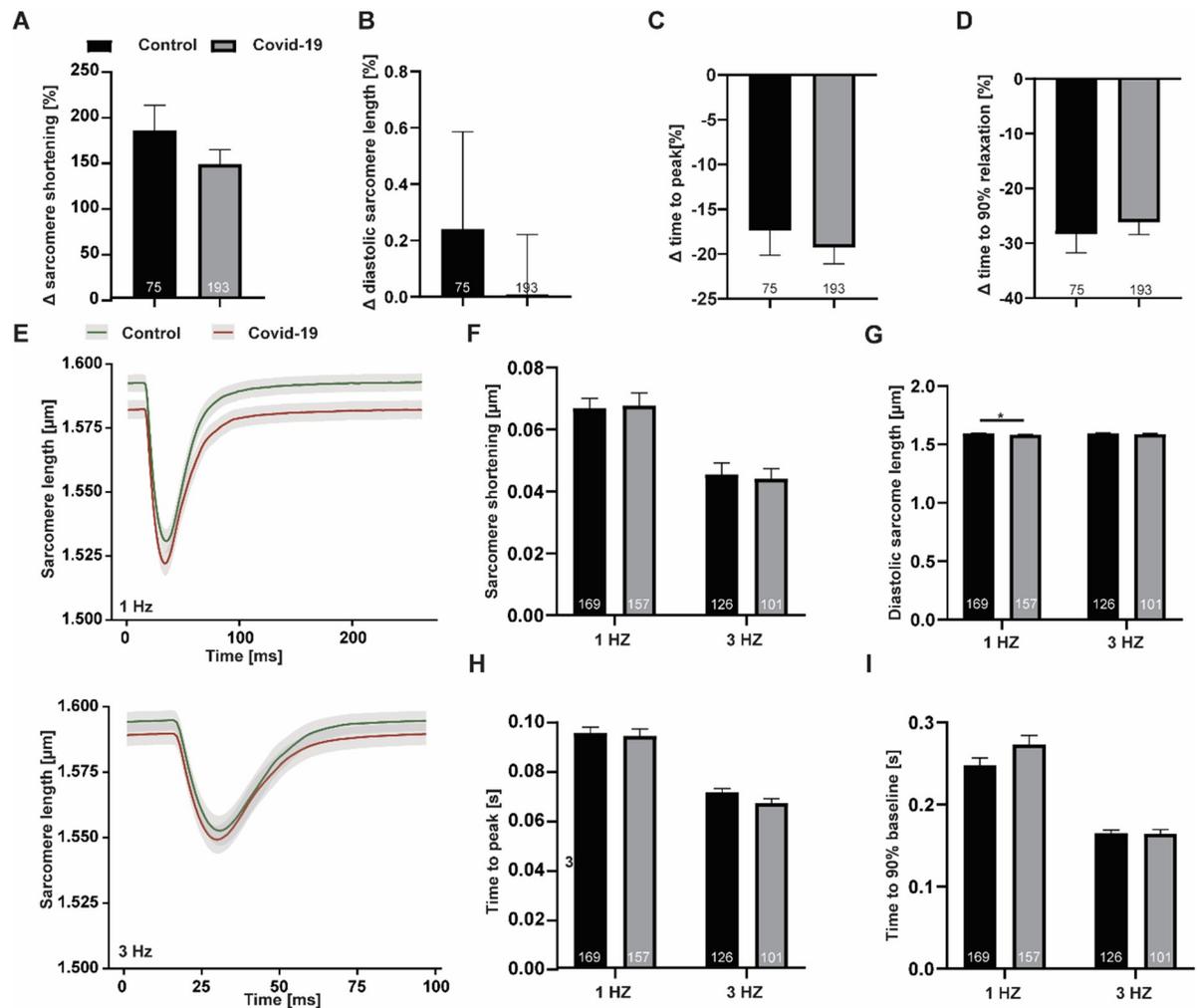
Patients included in the study were mainly male ( $n = 12$ ; 75% male), at an age of  $64 \pm 4$  years and showed an impaired left ventricular ejection fraction of  $41 \pm 5\%$ . In this severely diseased patient cohort, 33% of patients were treated with veno-venous ECMO therapy and markers for cardiac injury and inflammation were increased (mean CK  $154 \pm 43$  U/l, mean CRP  $173 \pm 30$  mg/dl; [Table 1](#)).

To test the effects of patient serum on cardiac excitation-contraction coupling mimicking different clinical scenarios of severe COVID19 mediated ARDS, cardiomyocytes were incubated for an ultra-short, short or long term period in either patient or control serum.

First we performed experiments using ratiometric imaging to determine ultra-short term (15 minutes incubation) and short term (60 minutes incubation) effects on rat ventricular cardiomyocyte contractile function during excitation-contraction coupling. When exposing isolated adult rat ventricular cardiomyocytes to COVID19 serum the amplitude and kinetics of sarcomere shortening were not affected at 15 minutes. Interestingly, in these paired measurements, we observed an augmentation of contractile function upon addition of control and patient serum alike (difference of amplitude after and before incubation:  $+183\% \pm 27\%$  vs  $+145\% \pm 16\%$  (control vs patient),  $n = 75$  vs  $193$ ,  $p = 0.23$ ). After 60 minutes at either 1 Hz or 3 Hz maximum sarcomere shortening was still unaffected (1Hz:  $0.06 \pm 0.003$  vs  $0.06 \pm 0.004$  (control vs patient),  $\mu\text{m}$ ,  $n = 167$  vs  $157$ ,  $p = 0.87$ ; 3 Hz:  $0.045 \pm 0.003$  vs  $0.044 \pm 0.003$  (control vs patient),  $\mu\text{m}$ ,  $n = 126$  vs  $101$ ,  $p = 0.78$ ). Although we did see a slight shortening of diastolic sarcomere length with patient serum at 1 Hz, this was not seen at 3 Hz (1 Hz:  $1.59 \pm 0.003$  vs  $1.58 \pm 0.003$  (control vs patient),  $\mu\text{m}$ ,  $n = 169$  vs  $157$ ,  $p = 0.03$ ; 3 Hz:  $1.59 \pm 0.003$  vs  $1.58 \pm 0.004$  (control vs patient),  $\mu\text{m}$ ,  $n = 126$  vs  $101$ ,  $p = 0.36$ ). In addition, contraction and relaxation kinetics were unaltered ([Fig 1](#)).

In a next step, we determined  $\text{Ca}^{2+}$  transient amplitudes upon short term exposure to COVID19 serum.

Incubation with patient serum significantly reduced  $\text{Ca}^{2+}$  transient amplitudes at 1 Hz in comparison to control serum ( $0.79 \pm 0.04$  vs  $0.68 \pm 0.04$  (control vs patient), arbitrary units,

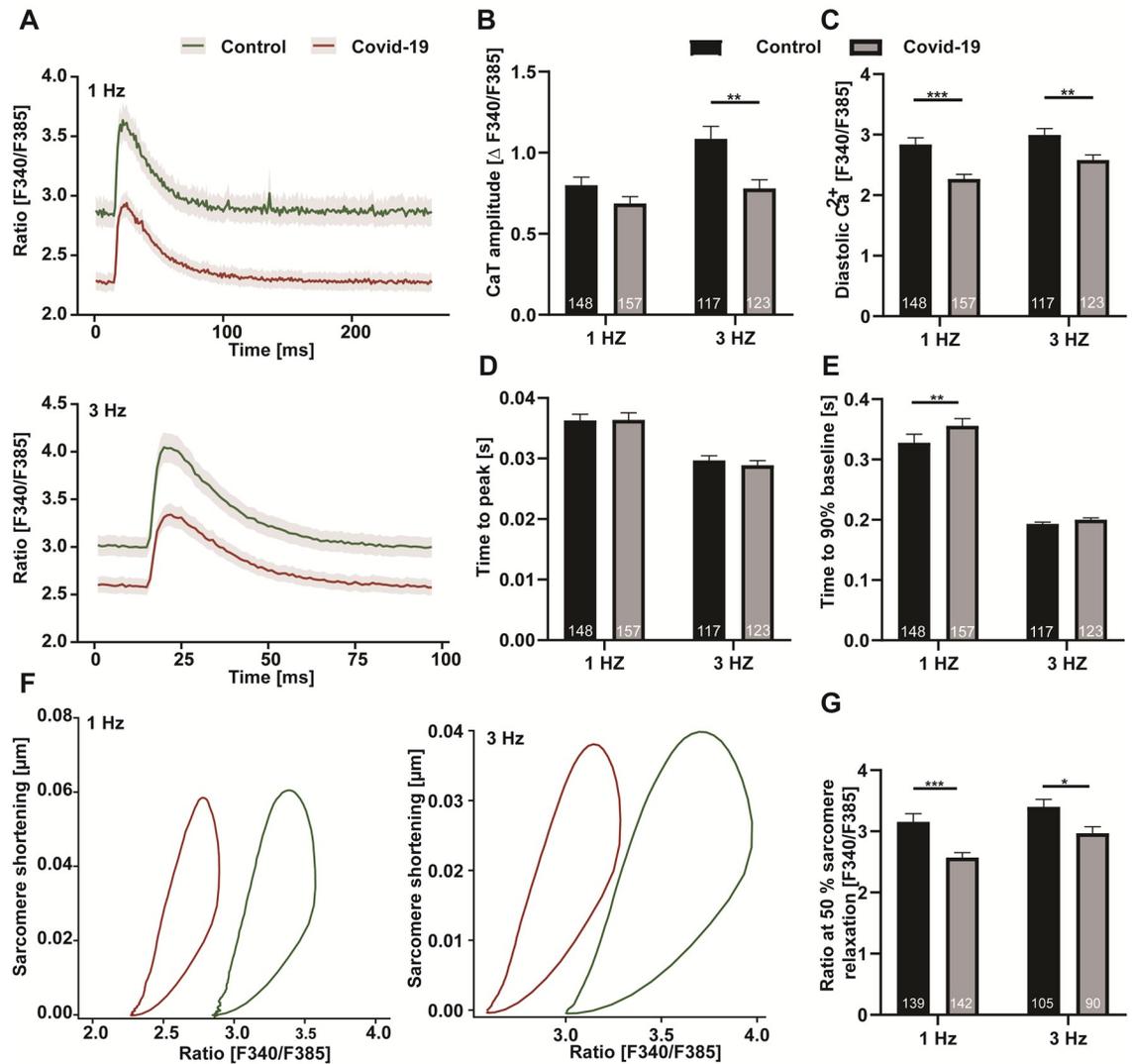


**Fig 1.** Excitation-contraction coupling in rat cardiomyocytes after 15 (A-D) and 60 (E-I) minutes incubation in either control (black) or COVID19 (grey) plasma, respectively. (A-D) Depicted as change after incubation in percent of initial value. (A) relative sarcomere shortening amplitude. (B) diastolic sarcomere length, (C) time to peak of sarcomere shortening and (D) time to 90% of relaxation. (E) Sarcomere shortening averaged over all cells per group at 1 Hz (upper) and 3 Hz (lower) electric stimulation and 1 mM extracellular  $[Ca^{2+}]$ . Related data of (F) sarcomere shortening amplitude, (G) diastolic sarcomere length, (H) time to sarcomere shortening peak and (I) time to 90% sarcomere relaxation. \* $p < 0.05$ .

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$n = 148$  vs  $157$ ,  $p = 0.08$ ) with an even more pronounced effect at 3 Hz ( $1.08 \pm 0.07$  vs  $0.77 \pm 0.05$ , (control vs patient), arbitrary units,  $n = 117$  vs  $123$ ,  $p = 0.01$ ). The positive inotropic cellular response to the increase in stimulation frequency was almost completely blunted with patient serum (difference of amplitude 3 Hz to 1 Hz:  $+0.29$  vs  $+0.09$  (control vs patient)). The decrease in amplitude was accompanied by a reduction of diastolic  $Ca^{2+}$  at both frequencies (1Hz:  $2.83 \pm 0.11$  vs  $2.26 \pm 0.07$  (control vs patient), arbitrary units,  $n = 148$  vs  $157$ ,  $p < 0.001$ ; 3 Hz:  $2.99 \pm 0.1$  vs  $2.58 \pm 0.08$  (control vs patient), arbitrary units,  $n = 117$  vs  $123$ ,  $p = 0.002$ ) (Fig 2).

To further investigate the impact of COVID19 serum on short-term cardiomyocyte function, we computed  $Ca^{2+}$ -contractility loops and calculated the  $Ca^{2+}$  concentration at 50% sarcomere relaxation, a phase of the loop where the  $Ca^{2+}$ -contractility relation is almost linear (Fig 2). This parameter has previously been described as a measure of microfilament  $Ca^{2+}$

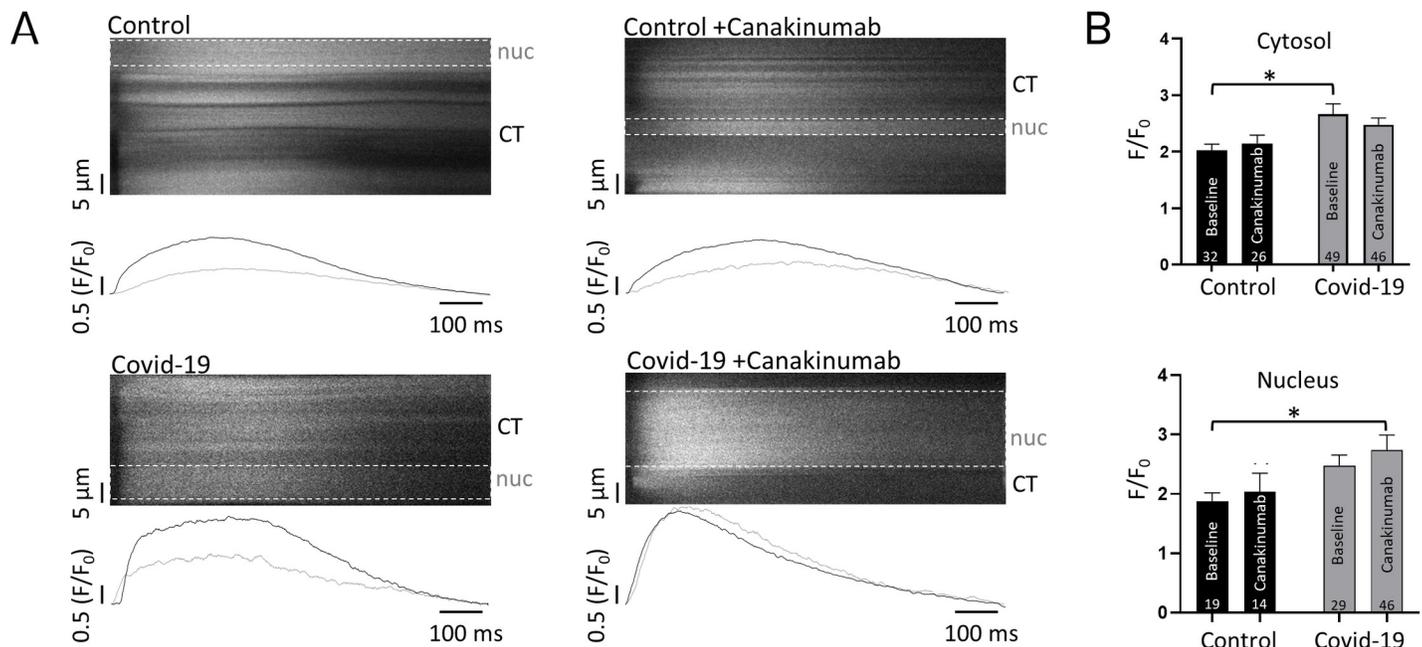


**Fig 2. Ca<sup>2+</sup> signaling during excitation-contraction coupling (rat) after 60 minutes incubation with control (black) or COVID19 (grey) serum.** (A) Ca<sup>2+</sup> transients averaged over all cells per group at 1 Hz (upper) and 3 Hz (lower) electric stimulation and 1 mM extracellular [Ca<sup>2+</sup>]. Related data of (B) Ca<sup>2+</sup> transient amplitude, (C) diastolic Ca<sup>2+</sup>, (D) time to Ca<sup>2+</sup> transient peak and (E) time to 90% relaxation. (F) Cardiomyocyte Ca<sup>2+</sup>-sarcomere loop; all-cell/group average at 1 Hz (left) and 3 Hz (right). (G) [Ca<sup>2+</sup>] at 50% sarcomere relaxation. \*p<0.05, \*\* p < 0.005, \*\*\* p < 0.001.

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sensitivity [20]. With patient serum, it decreased significantly, indicating an increased Ca<sup>2+</sup> sensitivity (1 Hz: 3.15 ± 0.13 vs 2.57 ± 0.07 (control vs patient), arbitrary units, n = 139 vs 142, p < 0.001; 3 Hz: 3.58 ± 0.16 vs 2.97 ± 0.10 (control vs patient), arbitrary units, n = 105 vs 90, p = 0.01) [21].

To overcome issues with longevity of isolated adult cardiomyocytes and to test our pharmacological approach with IL-1β inhibition in a setting that resembles human cardiomyocytes more closely, we used iPS-CM for our long term experiments. As shown in Fig 3, after 24 hours incubation Ca<sup>2+</sup> signaling during excitation-contraction signaling was significantly augmented in the cytosolic compartment, indicating profound changes of the Ca<sup>2+</sup> release machinery. However, addition of Canakinumab had no significant effect on Ca<sup>2+</sup> transient amplitudes (Fig 3).



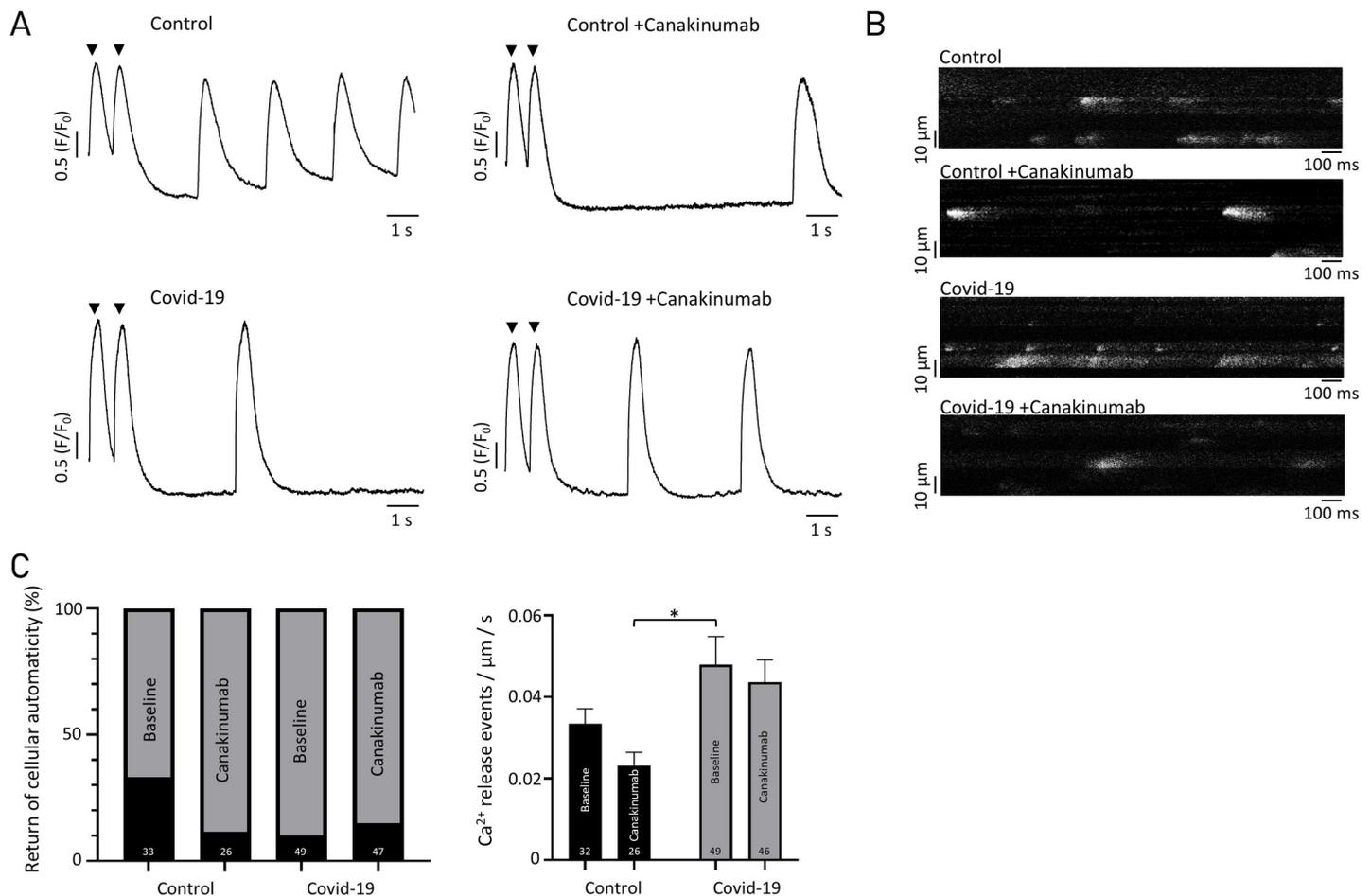
**Fig 3.  $\text{Ca}^{2+}$  transients during electrical stimulation of iPS-CM upon addition of COVID19 serum.** (A) Example  $\text{Ca}^{2+}$  transients as obtained after 24 hours incubation with serum +/- Canakinumab; iPS-CM during 1Hz electrical field stimulation. (B) Quantification of cytosolic (top) and nuclear (bottom) peak  $\text{Ca}^{2+}$  transient amplitudes. \* $p < 0.05$ .

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Long-term incubation with COVID19 patient serum also had effects on cellular arrhythmogenic potential:  $\text{Ca}^{2+}$  waves showed a trend to occur more often upon incubation with patient serum as compared to control ( $0.22 \pm 0.04$  vs  $0.37 \pm 0.04$  (control vs patient), waves/sec,  $n = 32$  vs  $49$ ,  $p = 0.079$ ) and spontaneous  $\text{Ca}^{2+}$  release events (i.e.  $\text{Ca}^{2+}$  sparks) were increased upon addition of COVID serum (Fig 4). Spontaneous action potential activity of iPS-CM is dependent on the proper function of membrane ion channels and cell-cell interactions [22]. We measured the return of spontaneous action potential generation upon prolonged (i.e.  $>5$  min) electrical field-stimulation. Cells incubated with control serum had a significantly more frequent return to cellular automaticity after the end of stimulation than those incubated with patient serum (long term incubation). However, Canakinumab did not affect arrhythmogenic potential nor the return of cellular automaticity (Fig 4C).

## Discussion

The present study used a non-selective approach to study the effect of COVID19 associated cytokine release on cardiac in-vitro function in ICU patients. Indeed, serum derived from COVID19 patients was associated with acute cardio-depressant and chronic pro-arrhythmogenic effects in both, rat and iPS-CM. This impairment of  $\text{Ca}^{2+}$  release might be associated with the serum protein and cytokine profile of our set of ICU COVID19 patients: It has been shown that levels of IL-1,  $\text{TNF}\alpha$ , IL6 and other cytokines are significantly increased in patients resembling our clinical collective with COVID19 [23–25]. However, of note, 92% of the patients included in this study received antibiotic treatment at the time of sample acquisition. This indicates bacterial superinfection and might be a confounding factor in this collective. 67% of the included patients also received glucocorticoids potentially altering the cytokine profile. IL-1 and  $\text{TNF}\alpha$  are known pro-inflammatory cytokines with negative effects on cellular  $\text{Ca}^{2+}$  signaling during excitation contraction coupling in septic cardiomyopathy [26]. Even



**Fig 4. Arrhythmogenic Ca<sup>2+</sup> release in iPS-CM upon addition of COVID19 serum and treatment with Canakinumab.** (A) Example for stimulated and spontaneous ("cellular automaticity") Ca<sup>2+</sup> transients in iPS-CM as obtained after 24 hours incubation with serum +/- Canakinumab; Electrical field-stimulation with 1 Hz as indicated (black arrows). (B) Spontaneous Ca<sup>2+</sup> release events in iPS-CM upon prolonged 1 Hz electrical stimulation. (C) Quantification of cellular automaticity and Ca<sup>2+</sup> release events in iPS-CM. \* p<0.05.

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though poorly correlated to survival in COVID19 [27], especially IL-1 $\beta$  has been shown to decrease the beta-adrenergic responsiveness of L-type Ca<sup>2+</sup> channels in a cAMP-independent mechanism, potentially decreasing cellular inotropic response [28]. This is in line with our observed altered cytosolic [Ca<sup>2+</sup>] at 60 minutes serum incubation and further supported by results from Mitrokhin et al [29], associating electrical abnormalities and action potential prolongations with IL-1.

We detected a significant difference in Ca<sup>2+</sup> sensitivity upon cellular exposure to COVID19 serum. Notably, among critically ill patients with COVID19, ventricular malign arrhythmias were found in 2.5% of patients and especially prevalent in non-survivors (RR: 3.8) [30]. As demonstrated by Huke et al., regional slowing of impulse propagation, which can subsequently trigger reentrant activation and arrhythmias, is associated with altered Ca<sup>2+</sup> sensitivity [31]. Our observed changes in Ca<sup>2+</sup> sensitivity might contribute to the arrhythmogenic potential in severe COVID19 as this relation has been previously described for other models of septic cardiomyopathy [32].

Arrhythmic Ca<sup>2+</sup> release was observed especially after long-term exposure to COVID19 serum. In the present study, the IL-1 $\beta$  blocker Canakinumab was used to potentially mitigate

these in-vitro effects, as Canakinumab has been shown to improve outcomes in mild or severe COVID19 pneumonia in early clinical trials with limited numbers of patients [33]. However, chronic co-incubation with Canakinumab had no beneficial effect on cellular  $\text{Ca}^{2+}$  signaling during excitation-contraction coupling or on electrical dysfunction in our iPS-CM model. This missing effect might be linked to our heterogeneous patient collective and sample acquisition in a real-life scenario—yet it is supported by a very recent randomized clinical trial with over 450 patients, indicating only little effect of Canakinumab on overall patient outcome (CAN-COVID; trial identifier: NCT04362813). Further clinical trials like the currently conducted three C study [34] are going to assess potential benefits of Canakinumab treatment in COVID19 patients limited to cardiac effects. The presented method utilizing iPS-CM and  $\text{Ca}^{2+}$  imaging allows to study acute and chronic cardiac effects in conditions associated with cytokine release. As exemplified in the current study using Canakinumab, this approach might also serve as an in-vitro “assay” to assess the effectiveness of different drugs in this particular setting.

In conclusion, serum derived from COVID19 patients exerts acute cardio-depressant and chronic pro-arrhythmogenic effects in rat and iPS-derived cardiomyocytes, respectively. Chronic co-incubation with Canakinumab had no beneficial effect on cellular  $\text{Ca}^{2+}$  signaling during excitation-contraction coupling. The presented method utilizing iPS-CM to screen for the effectiveness of different pharmacological approaches in the context of conditions associated with cytokine release might serve as a novel method for precision medicine.

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