VIA MEDICA

ORIGINAL ARTICLE

Cardiology Journal
2022, Vol. 29, No. 5, 739–750
DOI: 10.5603/CJ.a2022.0072

ISSN 1897–5593
eISSN 1898–018X

Ion channel inhibition with amiodarone or verapamil in symptomatic hospitalized nonintensive-care COVID-19 patients: The RECOVERY-SIRIO randomized trial

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Abstract

Background: *Ion channel inhibition may offer protection against coronavirus disease 2019 (COVID-19). Inflammation and reduced platelet count occur during COVID-19 but precise quantification of risk thresholds is unclear. The RECOVERY-SIRIO study aimed to assess clinical effects of amiodarone and verapamil and to relate patient phenotypes to outcomes.*

Methods: *RECOVERY-SIRIO is a multicenter open-label 1:1:1 investigator-initiated randomized trial with blinded event adjudication. A sample of 804 symptomatic hospitalized nonintensive-care COVID-19 patients, follow-up for 28 days was initially planned.*

Results: *The trial was stopped when a total of 215 patients had been randomized to amiodarone (n = 71), verapamil (n = 72) or standard care alone (n = 72). At 15 days, the hazard ratio (hazard ratio [HR], 95% confidence interval [CI]) for clinical improvement was 0.77 (0.52–1.14) with amiodarone and 0.97 (0.81–1.17) with verapamil as compared to usual care. Clinically relevant associations were found*

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Received: 20.02.2022 Accepted: 2.07.2022 Early publication date: 29.07.2022

between mortality or lack of clinical improvement and higher peak C-reactive protein (CRP) levels or nadir platelet count at 7, 10 and 15 days. Mortality rate increased by 73% every 5 mg/dL increment in peak CRP (HR 1.73, 95% CI 1.27–2.37) and was two-fold higher for every decrement of 100 units in nadir platelet count (HR 2.19, 95% CI 1.37–3.51). By cluster analysis, thresholds of 5 mg/dL for peak CRP and 187 × 10^3/mcL for nadir platelet count identified the phenogroup at greatest risk of dying.

Conclusions: *In this randomized trial, neither amiodarone nor verapamil were found to significantly accelerate short-term clinical improvement. Peak CRP and nadir platelet counts were associated with increased mortality both in isolation and by cluster analysis.* (Cardiol J 2022; 29, 5: 739–750)

Key words: amiodarone, verapamil, COVID-19, ion-channel inhibition, randomized trial

Introduction

Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is characterized by viral entry and replication within host cells that may lead to full-blown 2019-coronavirus disease (COVID-19). The SARS-CoV-2 spike protein mediates virus entry through receptor binding and membrane fusion. Ions promote viral membrane fusion and conformational changes and allow fusion peptide insertion into the lipid bilayer followed by endocytosis [1, 2]. Interaction of viral proteins with host cell ion channel activity may represent a crucial virus–host mechanism [3]. Pharmacological agents targeting ion channels may modulate SARS-CoV2's life cycle [3]. Preliminary reports have shown potential antiviral efficacy of ion channel inhibitors in COVID-19 [4, 5].

Two cardioprotective agents, amiodarone and verapamil, are ion channel antagonists. This multicenter randomized study in symptomatic hospitalized nonintensive-care COVID-19 patients was conducted to compare the effects of amiodarone or verapamil on top of usual care versus usual care alone on progression of clinical status.

Enhanced inflammation and reduced platelet count caused presumably by platelet consumption are reported during COVID-19 in association with adverse prognosis [6, 7]. Within this randomized trial, the relation between biomarkers and outcomes were quantitatively addressed following prespecified analyses of biomarkers both in isolation and by cluster analysis; cluster analysis is a machine learning method allowing identification of distinct COVID-19 phenotypic groups.

Methods

Trial design and patient population

RECOVERY-SIRIO (ClinicalTrials.gov number NCT04351763) is a multicenter, investigator-initiated, not-for-profit, open-label randomized trial with clinical events validated by an independent clinical events committee that was unaware of treatment allocation. Eligible patients were randomly assigned in a 1:1:1 ratio to receive either amiodarone + usual care, verapamil + usual care or usual care alone, and were followed for up to 28 days. The study was approved by an independent Ethical Committee of the Nicolaus Copernicus University of Poland. Written informed consent was obtained from all patients. Full rationale of the study was previously presented [3]. Briefly, viral proteins interact with host cell ion channel activity [3, 4]. In the "early entry" phase, the viral S protein-subunit S1 binds the angiotensin converting enzyme 2 (ACE2)-receptor on human cells, with transmembrane protease-serine 2 (TMPRSS2) facilitating virus-membrane fusion [1]. Ca2+ ions promote viral membrane fusion and S protein conformational changes which allow insertion of the fusion peptide into the lipid bilayer. In the "late entry" phase, SARS-CoV-2 is endocytosed and Ca2+ ions have a role in endocytic vesicle maturation [8]. This process ends with the release of the viral genome into the cytoplasm and subsequent viral replication. Amiodarone and verapamil block Ca2+ channels in the cell membrane and endosomal/lysosomal membranes, thereby potentially interfering with the coronavirus' life-cycle [3, 8]. Experimental studies indicate that amiodarone impairs endosomal transport in SARS-CoV-2-infected cells by blocking ion channels [9].

The study was additionally conceived to identify, through serial laboratory measurements, parameters quantitatively predicting disease progression and mortality in hospitalized non-intensive care COVID-19 patients. The full trial protocol is detailed in **Supplement material**. The authors take full responsibility for the design and conduct of the trial and vouch for the accuracy and completeness of the data, data analysis, and protocol adherence.

No other author contributed to the writing of the manuscript apart from those listed herein.

Patient enrollment was conducted between May 20, 2020 and May 13, 2021. The main patient inclusion criteria were: 1) Confirmed COVID-19 based on real-time polymerase chain reaction of naso- or oropharyngeal swabs, sputum or tracheal aspirates; 2) Symptomatic hospitalization initially not requiring intensive care; 3) Age >18 years; 4) Oxygenation index — defined as the quotient of arterial oxygen partial pressure (PaO2 in mmHg) to fraction of inspired oxygen (FiO2) — > 200; 5) Written informed consent was given prior to any trial-related procedure. The trial conduction followed local regulations, the Declaration of Helsinki, and the guidelines for Good Clinical Practice by the International Council for Harmonisation Committee for Medicinal Products for Human Use (GCP CHMP/ICH/135/95).

Endpoints

Clinical outcomes

The primary study endpoint was the first change in at least one category toward clinical improvement from enrollment (i.e., baseline) up to 15 days. Clinical categories were defined as per World Health Organization (WHO) classification used in COVID-19 trials [10]. An ordinal scale from 1 to 7 was used to define categories: 1) Death; 2) Hospitalized patients requiring mechanical ventilation, extracorporeal membrane oxygenation (ECMO) or both; 3) Hospitalized patients requiring high flow nasal oxygen therapy, noninvasive mechanical ventilation or both; 4) Hospitalized patients requiring oxygen therapy; 5) Hospitalized patients not requiring oxygen therapy; 6) Nonhospitalized patients, but unable to resume normal activities; 7) Nonhospitalized patients with resumption of normal activities. Improvement was considered as the increase of at least one point on the ordinal scale, lower scores indicating worse outcomes and higher scores more favorable ones. Main secondary endpoints included clinical category improvement at 28 days, 28-day mortality, days of hospitalization and of oxygen therapy, mechanical ventilation, and 15-day National Early Warning Score 2 (NEWS2) values [11, 12].

Biomarkers

Serum C-reactive protein (CRP, mg/dL) and high-sensitivity (hs) cardiac troponin (cTn, ng/mL) I, whole blood platelet count (per mcL) and plasma D-dimers (ng/mL) were measured at prespecified time points (baseline, 7, 10 and 15 days) using Siemens Healthineers, Germany, for CRP and hs-cTn I, and routine chemical hematology for platelets and D-dimers. The coefficient of variation was < 10% for all measures. Prespecified peak or nadir values were analyzed.

Interventions

Allocation to amiodarone, verapamil or usual care alone was performed after patient enrollment by investigator connection to a prespecified web-link. The random allocation sequence was generated by computer software. During hospitalization patients randomized to amiodarone received usual care plus 200 to 400 mg of amiodarone daily (oral administration) adjusting to age, heart rate, blood pressure, QT/QTc interval and heart rhythm. Patients randomized to verapamil received usual care plus 120 to 480 mg of verapamil administrated orally in 3 to 4 divided doses every 6–8 hours (adjusted to age, heart rate, blood pressure, QT/QTc interval and heart rhythm). Patients randomized to usual care received no additional treatment (control group). Further drug administration details are provided in the full study protocol (**Supplement material**).

Statistical analysis

Power calculation for the primary efficacy endpoint was based on the assumption of superior clinical improvement at 15 days in favor of amiodarone plus usual care or verapamil plus usual care versus usual care alone. On the basis of preliminary data [3–5, 10] we assumed clinical improvement would occur in 30% of the control group [10] and in 39% of the experimental group (amiodarone or verapamil) [3–5], resulting in an overall sample size of 804 subjects to achieve at least 80% power at a 0.05 significance level.

The primary efficacy analysis was on an intention-to-treat basis. Hazard point estimates with two-sided 95% confidence interval (CI) measured by the hazard ratio (HR) were calculated based on the Cox proportional hazards model. Probability of clinical improvement is presented using the Kaplan–Meier curves. Data distribution was checked by the Kolmogorov-Smirnov test with data presented as median with interquartile range (IQR) or mean ± standard deviation (SD) as appropriate. Baseline characteristics were compared by χ^2 or the Fisher exact test for categorical variables and by the Kruskal-Wallis, t-test or ANOVA for continuous variables. To determine independent predictors of mortality, the following routine laboratory values were prespecified, based on

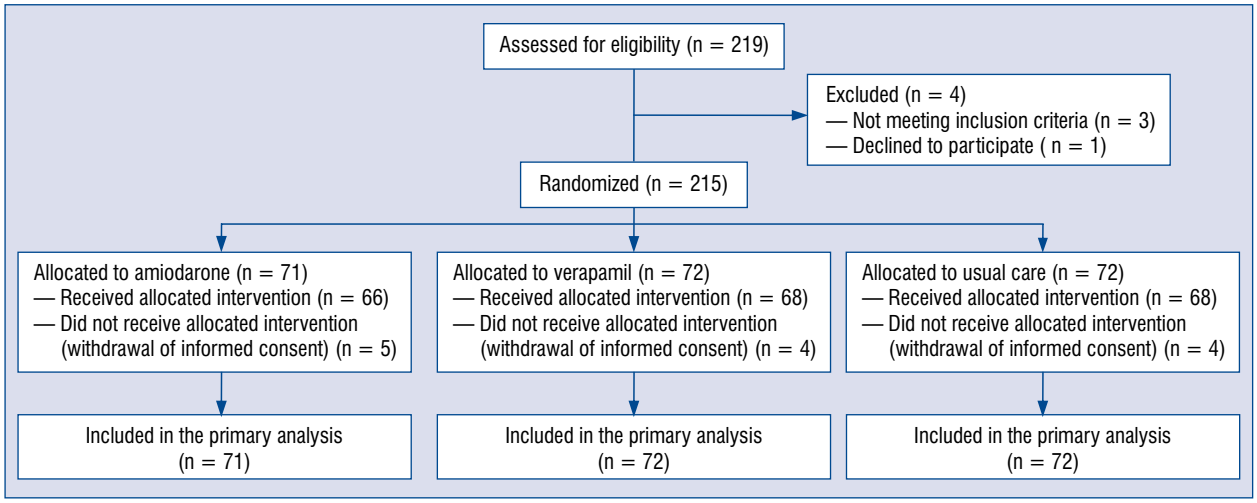

Figure 1. Randomization and treatment assignment.

their known clinical relevance in COVID-19: peak values of CRP, D-dimers, hs-cTn I and total white blood cell count, as well as nadir values of platelet and lymphocyte counts [6, 7]. Visual associations between continuous biomarkers and mortality were evaluated by restricted cubic splines with 3 knots at fixed percentiles in Cox regression models. A Wald-type test was applied to test for non-linearity of the models.

An unsupervised cluster analysis was conducted using a machine learning method that allows categorization of complex entities by segregating samples into homogenous groups based on each cluster's dissimilarities. For cluster analysis, the partitioning around medoids (PAM) algorithm was applied, which is less sensitive to outliers and more robust compared to k-means [13]. The number of clusters was selected on the basis of minimal total intra-cluster variation or minimal total within-cluster sum of squares (WSS). Total WSS measures the compactness of clustering. After allocating each patient to a cluster, cluster phenotypes and outcomes were compared by the Kaplan–Meier curves. A two-tailed p-value < 0.05 was considered statistically significant.

Results

Patient enrollment and characteristics

Enrollment began in May 2020. Owing to a slower than predicted recruitment caused by abatement of new COVID-19 cases in Poland in the second half of 2021, the trial was terminated prematurely by the Steering Committee at the prespecified interim analysis of May 2021 with a final sample size of 215 subjects. The CONSORT flow diagram of patient disposition through the study is illustrated in Figure 1: 71 patients were assigned to receive amiodarone (93% or 66 actually received the drug), 72 to verapamil (94% or 68 actually received the drug) and 72 to standard care alone. None of the patients were admitted to an intensive care unit at the time of enrollment.

Baseline characteristics (Table 1) were balanced among amiodarone, verapamil and control groups in terms of age (median 60, 62 and 63 years, respectively) and sex (69%, 58% and 64% men, respectively). Underlying cardiovascular disease was present in 35%, 40% and 33% ($p = 0.66$), and diabetes mellitus in 23%, 25% and 24%, respectively ($p = 0.94$). Median days from symptom onset to randomization were 7 (4–8) for amiodarone, 6 (3–8) for verapamil and 6 (4–9) for usual care ($p = 0.49$). At enrollment no significant intergroup differences emerged in other demographic or laboratory characteristics, clinical category ordinal scale or NEWS2 values (Table 1). During the trial, therapeutic measures against COVID-19 and its sequelae (including chloroquine, azithromycin, convalescent plasma, heparin and acetylsalicylic acid) were administered in a balanced way to the three treatment groups (Table 1).

Primary and secondary endpoints

Clinical outcomes

The rate of clinical category improvement at 15 days did not differ significantly among arms: it occurred in 56.3% with amiodarone, 68.1% with verapamil and 68.1% with usual care (Table 2). At 15 days the HRs (95% CI) for clinical improvement

Table 1. Baseline characteristics of the three randomized groups.

	Amiodarone (n = 71)	Verapamil (n = 72)	Usual care alone (n = 72)	P
Median age [years]	60 (51.5, 71)	62 (50.75, 72.25)	62.5 (52, 72)	0.58
Male sex	49 (69%)	42 (58%)	46 (64%)	0.41
Cardiovascular disease	25 (35%)	29 (40%)	24 (33%)	0.66
Diabetes	16 (23%)	18 (25%)	17 (24%)	0.94
Cancer	7 (10%)	5 (7%)	3 (4%)	0.37
COPD	4 (6%)	5 (7%)	5 (7%)	1
Median body mass index [kg/m^2]	28.25 (25.85, 32.94)	30.45 (27, 32.8)	29.36 (26.74, 32.32)	0.32
Median days from illness onset to randomization	7 (4, 8)	6 (4, 9)	6 (3, 8)	0.494
PO2/FiO2	324.10 ± 98.48	317.76 ± 94.90	325.69 ± 92.60	0.87
Requiring O2 therapy	49 (69%)	52 (72%)	49 (68%)	0.85
Cough	45 (63%)	45 (62%)	46 (64%)	0.98
Dyspnea	53 (75%)	48 (67%)	44 (61%)	0.22
Muscle or joint pain	19 (27%)	10 (14%)	17 (24%)	0.14
Diarrhea	21 (30%)	12 (17%)	14 (19%)	0.14
Fatigue	59 (83%)	54 (75%)	62 (86%)	0.20
Chest pain	11 (15%)	11 (15%)	19 (26%)	0.15
Fever	55 (77%)	50 (69%)	54 (75%)	0.53
Median body temperature [°C]	36.7 (36.6, 36.95)	36.7 (36.5, 37.23)	36.8 (36.6, 37.5)	0.39
Median pulse rate [bpm]	81 (73, 92.5)	85.5 (76.75, 96)	84 (76, 92.25)	0.31
Respiratory rate [/min]	16.46 ± 2.56	16.50 ± 2.32	16.53 ± 2.33	0.98
Median NEWS2	3 (2, 4)	2 (2, 4)	3 (2, 4)	0.80
Platelet count [10^3/mcL]	182.48 ± 2.17	210.70 ± 87.36	200.77 ± 92.29	0.13
WBC count [×10^3/mcL]	5.96 ± 2.23	6.50 ± 3.13	6.16 ± 2.36	0.45
Median lymphocytes count [×10^3/mcL]	0.96 (0.73, 1.42)	1.04 (0.8, 1.5)	1 (0.65, 1.39)	0.86
Serum creatinine [mg/dL]	0.95 ± 0.30	1.02 ± 0.99	1.03 ± 1.15	0.82
Median ALT [mg/dL]	29.32 (22.85, 44.89)	31.64 (20.94, 51.31)	28.09 (20.09, 54.5)	0.97
Median D-dimer [ng/mL]	500.16 (398.48, 989.51)	619.88 (458.44, 924.25)	659.5 (473.44, 943.59)	0.34
Median CRP [mg/dL]	5.75 (2.43, 10.61)	6.32 (2.22, 9.74)	4.34 (1.56, 9.41)	0.56
Median hs-Tn I [ng/mL]	0.007 (0.005-0.01)	0.006 (0.04-0.11)	0.008 (0.005-0.02)	0.15
Median creatine kinase [IU/mL]	128.4 (70.15, 326)	115.45 (66.3, 194.52)	103.4 (73.75, 198.25)	0.47
Median MB-creatine kinase [IU/mL]	1.1 (0.4, 2.42)	1.19 (0.63, 2.06)	1.25 (0.5, 2.2)	0.79
Chloroquine	2 (3%)	2 (3%)	1 (1%)	0.87
Azithromycin	3 (4%)	3 (4%)	7 (10%)	0.31
Remdesivir	3 (4%)	0 (0%)	1 (1%)	0.13
Convalescent plasma	2 (3%)	2 (3%)	0 (0%)	0.47
Supplemental oxygen	12 (17%)	11 (15%)	10 (14%)	0.88
Fluids	9 (13%)	8 (11%)	10 (14%)	0.88
Heparin	11 (15%)	8 (11%)	9 (12%)	0.72
Acetylsalicylic acid	8 (11%)	11 (15%)	12 (17%)	0.62
Noninvasive mechanical ventilation	0 (0%)	2 (3%)	1 (1%)	0.77
ACE-inhibitors	16 (23%)	15 (21%)	16 (22%)	0.96
Beta-blockers	27 (38%)	28 (39%)	19 (26%)	0.21
Statins	13 (18%)	25 (35%)	20 (28%)	0.08
Antidiabetic medications	14 (20%)	16 (22%)	12 (17%)	0.70
Other antiplatelet agents	6 (3%)	1 (1%)	4 (6%)	0.37
Diuretics	43 (20%)	14 (19%)	14 (19%)	0.95
Sartans	37 (17%)	14 (19%)	7 (10%)	0.10

Data are shown as mean (interquartile range) or mean ± standard deviation (SD) or number (percentage). ACE — angiotensin-converting enzyme; ALT — alanine transaminase; bpm — beats per minute; COPD — chronic obstructive pulmonary disease; CRP — C-reactive protein; hs — high sensitivity; MB — myocardium brain; mcL — microliters; ng/mL — nanograms per milliliter; NEWS2 — National Early Warning Score 2; PO2/FiO2 — arterial partial oxygen pressure in mmHg to fraction of inspired oxygen ratio; Tn — troponin; WBC — white blood cell

Table 2. Outcomes in the intention-to-treat population.

	Amiodarone (n = 71)	Verapamil (n = 72)	Usual care (n = 72)	P
Median time to clinical improvement [days]	9 (6.5, 13)	9 (5, 12)	9 (6, 12.5)	0.65
Clinical category improvement at 15 days	40 (56.3%)	49 (68.1%)	49 (68.1%)	0.41
Clinical category improvement at 28 days	54 (76.4%)	51 (70.45)	50 (69.4%)	0.60
Death	6 (8.5%)	3 (4.2%)	3 (4.2%)	0.43
Median days of oxygen therapy	7 (2, 11)	6 (2, 10.75)	6 (2.25,10.75)	0.90
Median days of hospitalization	14 (10, 15.25)	13 (10.25, 17)	13 (11,15.75)	0.96
Hospitalization in intensive care unit	3 (4%)	4 (6%)	1 (1%)	0.45
Mechanical ventilation	9 (12.6%)	7 (9.72%)	6 (8.33%)	0.68
NEWS2 ≤ 2 at 28 days	56 (78.8%)	61 (84.7%)	61 (84.7%)	0.47

Data are shown as mean (interquartile range) or number (percentage); NEWS2 — National Early Warning Score 2

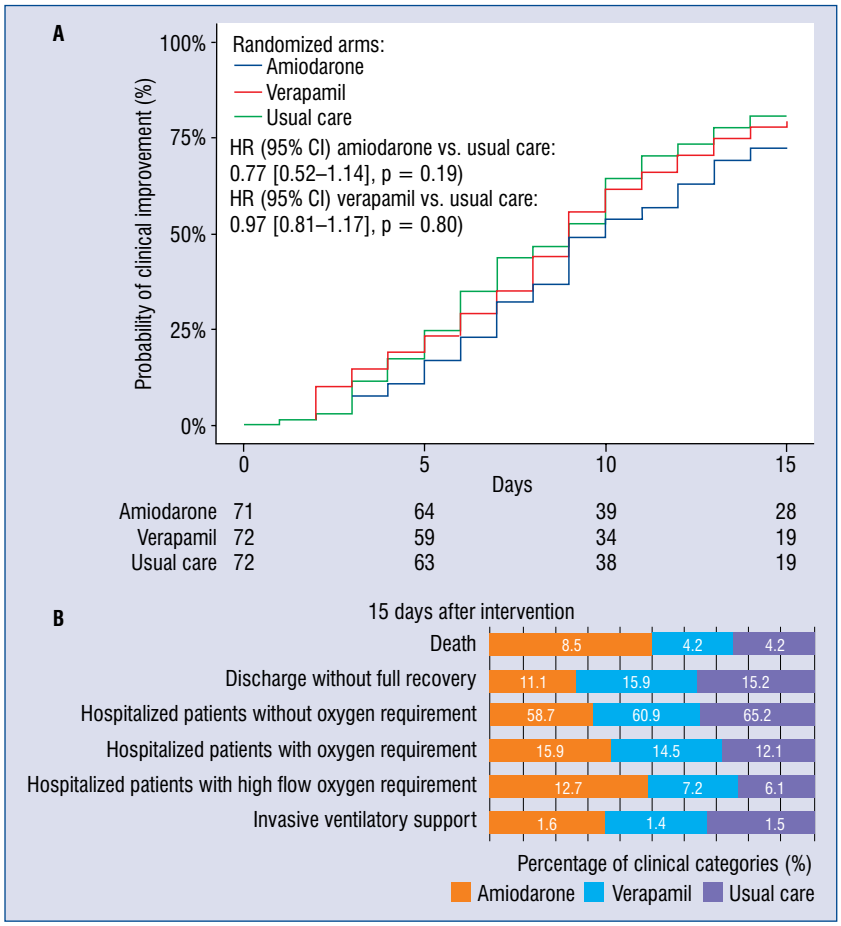

Figure 2. Clinical improvement at 15 days among patients treated with amiodarone or verapamil versus usual care alone; **A.** Kaplan-Meier curves of the time to clinical improvement in the intention-to-treat population; **B.** Distribution of clinical status according to the percentage of clinical categories; CI — confidence interval; HR — hazard ratio.

were 0.77 (0.52–1.14, p = 0.19) with amiodarone and 0.97 (0.81–1.17, p = 0.80) with verapamil as compared to usual care (Fig. 2A); at 28 days, the respective HRs were 0.81 (0.57–1.16, p = 0.26) and 0.81 (0.57–1.16, p = 0.26). At 15 and 28 days, no significant differences were observed among

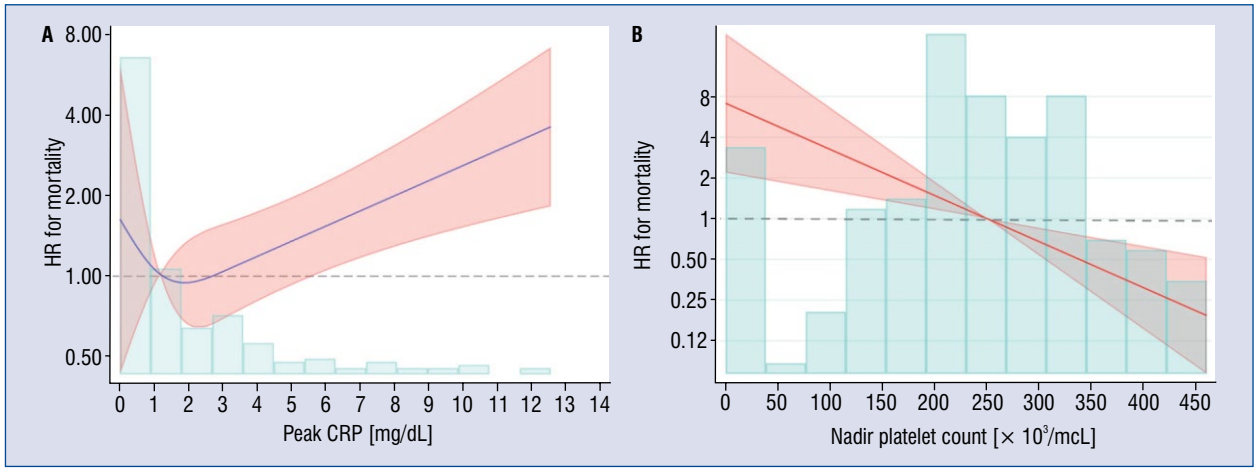

Figure 3. A. Mortality hazard ratios (HRs) according to peak C-reactive protein (CRP). Data were fitted with a restricted cubic spline Cox regression model. The background histograms in light blue represent the percent of density distribution of peak CRP in the study population. Heavy central lines represent HRs with shaded ribbons denoting 95% confidence intervals. The value of 1 (median) served as reference value in presenting the estimated mortality HRs; **B.** Mortality HRs according to nadir platelet count. Data were fitted with a restricted cubic spline Cox regression model. The background histograms in light blue represent the percent of density distribution of nadir platelet count in the study population. Heavy central lines represent HRs with shaded ribbons denoting 95% confidence intervals. The value of 250 (median) served as reference value in presenting the estimated mortality HRs.

groups in clinical outcome ordinal scale categories (Fig. 2B, Table 2). Similarly, hospitalization days were not significantly different among the amiodarone (14 [10–15.3]), verapamil (13 [10.3–17]) and usual care (13 [11–15.8]) arms (Table 2).

Biomarker analyses and phenomapping

CRP, platelet count and mortality. At 28-day follow-up, 12 (5.6%) of 215 patients had died. Based on the estimated restricted cubic spline model, an overall linear association between peak CRP levels and mortality rates was observed (Wald test = 0.78, p for non-linearity = 0.37). In particular, a possible departure from linearity was limited to values below the median peak CRP level of 1 mg/dL, but the confidence intervals were wide (Fig. 3A). Every 5 mg/dL increment in peak CRP was estimated to increase mortality rates by 73% (HR 1.73, 95% CI 1.27–2.37, p = 0.001; Fig. 3A). Data were in agreement with an overall linear association between nadir platelet count and mortality rates (Wald test = 1.43, p for non-linearity = = 0.23), with every 100 × 10^3 per mcL decrement in nadir platelet count increasing the risk of dying by two-fold (HR 2.19, 95% CI 1.37–3.51, p = 0.001; Fig. 3B). By stratified analysis, CRP levels were markedly higher at all time points after randomization in patients who died compared to survivors (Fig. 4A) and in patients without clinical improvement compared to those who improved (**Suppl. Fig. 1**). Nadir platelet counts were lower in subjects who died in comparison to survivors at all time points (Fig. 4B). No statistically significant associations were found between other explored biomarkers and mortality, with the exception of median peak D-dimer: 753 (500–946) ng/mL in nonsurvivors versus 665 (443–700) ng/ml in survivors (p = 0.03) (**Suppl. Table 1**).

Phenomapping. An artificial intelligence-driven variable selection algorithm was applied to a total of 46 clinical and biomarker variables (**Suppl. Table 2**) with retainment of peak CRP and nadir platelet count as the most informative features. On the basis of minimal intra- and within-cluster variation, an optimal number of 4 clusters was selected. The population was then divided into 4 phenotypes, the 4th of which had the greatest peak CRP values (median 5 mg/dL) and lowest nadir platelet counts (median 187 × 10^3/mcL), that in turn was associated with significantly higher 28-day mortality in comparison to the other 3 clusters (p = 0.02, Fig. 5A, B). A cluster plot with 4 phenotypes was generated (**Suppl. Fig. 2**).

Safety

At day 15 no significant increase of serious adverse events was observed in the amiodarone or verapamil arms compared to the control group,

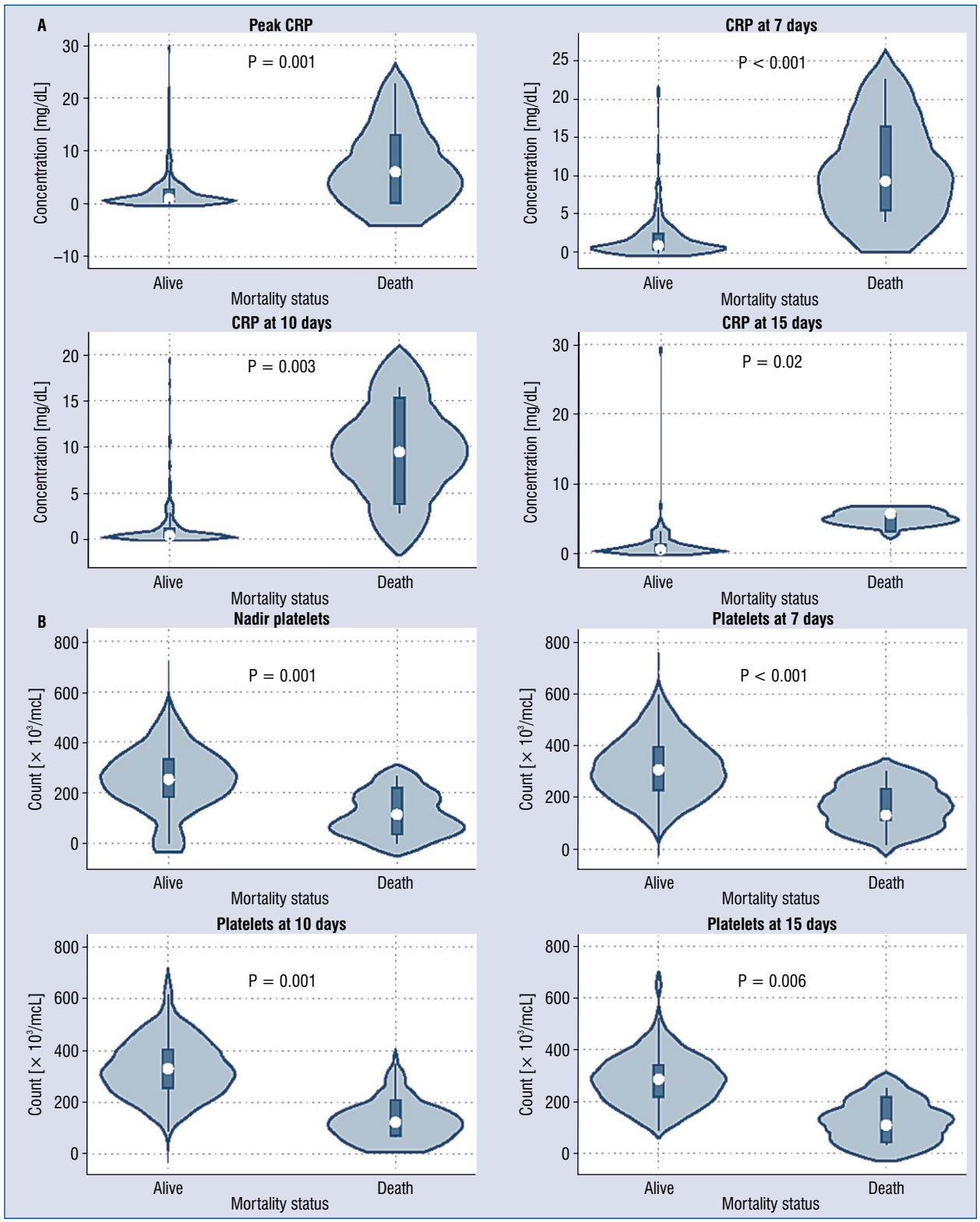

Figure 4. A. Violin plots of peak C-reactive protein (CRP) values and CRP levels at 7, 10 and 15 days after randomization in patients who survived or died during the study. The width of each region corresponds to the frequency of data points in each part of the violin. Densities are accompanied by an overlaid box plot to provide additional information. The circle denotes the median and the box limits the 25th and 75th percentiles; **B.** Violin plots of nadir platelet count values and platelet counts at 7, 10 and 15 days after randomization in patients who survived or died during the study. The width of each region corresponds to the frequency of data points in each part of the violin. Densities are accompanied by an overlaid box plot to provide additional information. The circle denotes the median and the box limits the 25th and 75th percentiles.

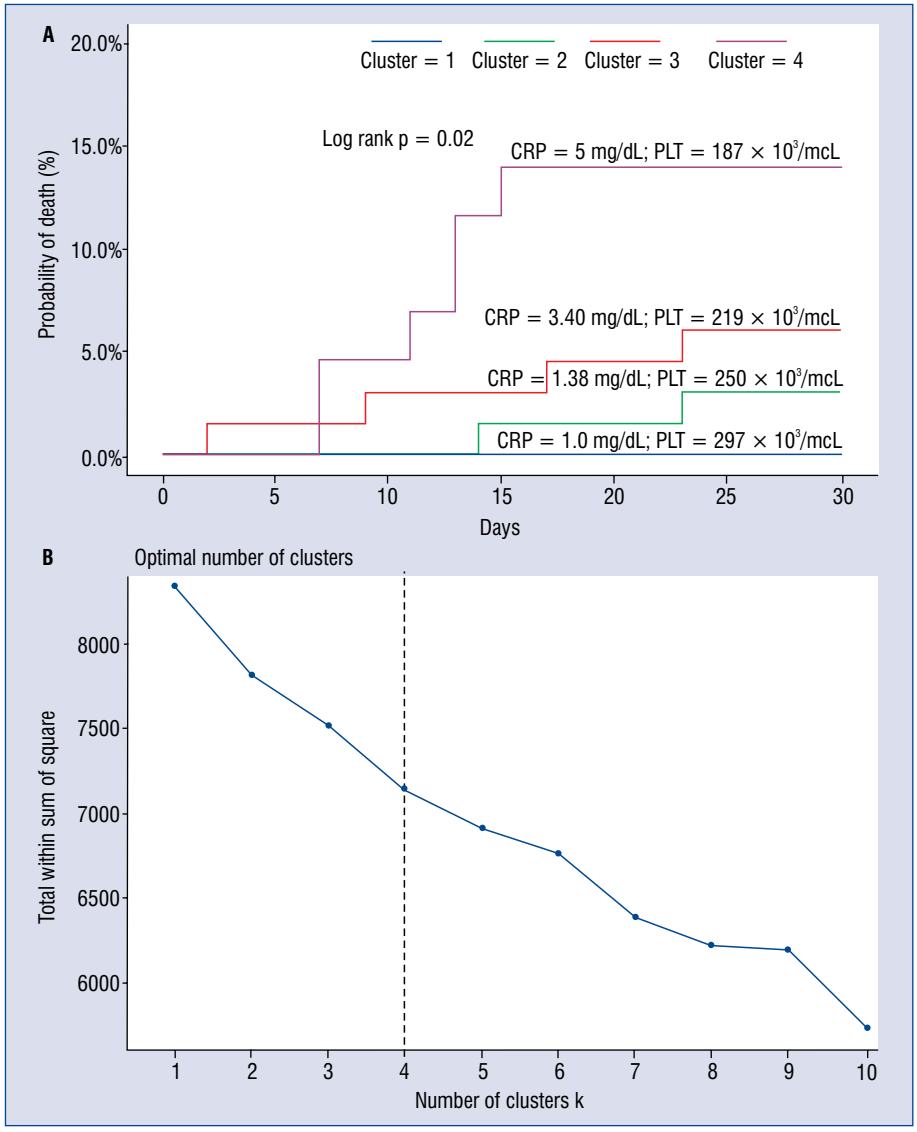

Figure 5. A. Kaplan-Meier mortality curves of patients belonging to 4 distinct biomarker phenotypes generated by cluster analysis. Patient median values of peak C-reactive protein (CRP) and nadir platelet count are shown stratified by phenogroup; **B.** Algorithm plot of the optimal number of clusters using the sum of squares method. The location of a bend (knee) in the plot is generally considered an indicator of the appropriate number of clusters; PLT — platelet count.

including second- or third-degree atrioventricular blocks, other bradyarrhythmias or ventricular tachyarrhythmias (**Suppl. Table 3**). No significant prolongation of the QT or corrected QT intervals was recorded in patients treated with amiodarone and verapamil versus usual care alone (**Suppl. Table 3**).

Discussion

This multicenter randomized trial enrolling symptomatic hospitalized nonintensive-care patients with COVID-19 did not detect any significant differences in the rates of clinical improvement among patients randomized to amiodarone or verapamil on top of usual supportive care as compared to patients randomized to usual care alone. However, the trial was underpowered, given the slow enrollment and recruitment of 215 out of 804 planned patients (26.7%). Thus, although no apparent trend was noted by adding an ion channel inhibitor on top of usual care, the findings should be considered preliminary.

In contrast, the prespecified individual and cluster laboratory-based analyses showed: 1) Significantly increased mortality across levels of peak CRP and nadir platelet counts; 2) An inverse association between CRP and clinical

improvement; 3) Cluster-analysis identification of distinct phenotypes with highest mortality in the cluster with higher CRP and lowest platelet count (median 5 mg/dL CRP and median 187 × 10^3/mcL, respectively); 4) Patterns of increased mortality for increasing CRP and decreasing platelet count modeled on serial measurements at 7, 10 and 15 days after randomization.

Ion channels have been recently suggested as a potential important target for present and future major viral infections owing to the emerging role of ions in viral membrane entry and fusion [14, 15]. RECOVERY-SIRIO is the first dedicated randomized trial to have addressed the effect of ion-channel inhibition in COVID-19. It was found that amiodarone and verapamil, two cardiovascular agents with ion-channel inhibitor actions, did not improve the clinical status of hospitalized nonintensive-care COVID-19 patients. The prespecified serial laboratory assessments in the present trial offered the opportunity to conduct an in-depth investigation of a set of candidate predictive parameters in relation to clinical outcomes. In the current study, both peak CRP and nadir platelet count were most significantly related to increased mortality and peak CRP alone to lack of clinical improvement in hospitalized initially nonintensive-care patients with COVID-19. The notion that systemic inflammatory response to severe SARS-CoV-2 infection contributes to disease severity has been confirmed in several reports [16]. During the advanced stages of COVID-19 a cytokine storm response can be triggered which is, in turn, associated with high mortality. The released cytokines stimulate hepatocytes to produce CRP [17].

Prior retrospective reports showed increased CRP trends in COVID-19 patients who eventually died compared to survivors [18, 19]. Similar CRP, although less robust, is a significant association between mortality and nadir platelet count found in the present study. Thrombocytopenia has been detected in 58–95% of severe cases of COVID-19 [20]. Additionally, nonsurvivors have been reported to have lower platelet count than survivors [21]. The current study extends these earlier results in the context of a randomized trial conducted with balanced patient characteristics and prospective serial laboratory assessments at predefined time points. The extent to which CRP could serve as a quantitative reliable prognostic marker during the relatively early phases of COVID-19 among symptomatic hospitalized nonintensive-care patients, particularly when combined with platelet count, remains incompletely known.

According to available research, this is the first study to prospectively address by a quantitative serial approach to the combined predictive role of inflammation and platelets during the early stages of nonintensive-care COVID-19 patients and to have applied an artificial intelligence algorithm to the randomized trial population that contributed to unveil meaningful phenotypes within COVID-19 based on distinct values of peak CRP and nadir platelet count. The laboratory-focused analytical approach applied in the current trial provided a more nuanced appraisal between CRP level and mortality risk in COVID-19. The analyses conducted allowed for the identification of a significant gradient for mortality across levels of peak CRP and nadir platelet counts.

More specifically, a relation was found between CRP and mortality with progressive risk increments when peak CRP was above the 1 mg/dL threshold. When CRP values exceeded 4–5 mg/dL the risk of mortality became approximately three-fold greater in comparison to patients with CRP values below 1 mg/dL. Recent advances in artificial intelligence, namely machine learning-based clustering methodologies, explicitly model the inherent nature of data directly. Accordingly, unsupervised clustering was applied to the laboratory data of this study that ultimately provided a phenotypic stratification. The two most important variables retained were peak CRP and nadir platelet count. These two factors allowed the identification of four distinct phenotypic subgroups, of which the 4^{th} (median 5 mg/dL peak CRP and median 187 × 10^3/mcL nadir platelet count) was associated with the greatest mortality risk.

In contrast, no significant associations were found between other explored biomarkers and mortality, with the exception of peak D-dimer that was however of lower magnitude than peak CRP and nadir platelet count. These findings trigger arguments for prioritization of the assessment of the latter two biomarkers to attain optimal early risk stratification in COVID-19. In addition to their significant association with mortality and disease progression, one practical advantage to track CRP and platelet count is that they are routine laboratory tests.

Clinical improvement, based on a clinical severity scale, has been widely implemented as a standardized clinical endpoint in COVID-19 trials. However, the appropriate summary measure for severity scores has been a matter of debate, particularly given the variable time course of COVID-19, the heterogeneous clinical presentation of the disease [22] and the subjectiveness of clinical

interventions and categorization [23]. In the current study, tracking practical laboratory parameters allowed precise early stratification of the risk of dying and prediction of disease progression in COVID-19. Sensitive biomarkers measured as continuous variables, such as peak CRP and nadir platelet count, may offer a reliable outcome prediction and avoid the loss of statistical power that occurs instead when categorical variables such as clinical improvement ordinal scales or a binary 'recovered' versus 'not recovered' status is used. Further dedicated randomized studies are warranted to test the hypothesis of biomarker-based endpoints.

Limitations of the study

The trial was stopped prematurely because of significant abatement of COVID-19 cases in the country in 2021 and slow-enrollment. Therefore, it was not possible to exclude that differences in improvement rates could have emerged had the trial been larger. The study was conducted with an open-label design; however, the lack of a blind placebo control arm is mitigated by the adjudication of events performed by an independent event committee not involved in the study.

Conclusions

In this randomized trial ion channel inhibition with amiodarone or verapamil was not found to significantly accelerate short term clinical improvement in symptomatic hospitalized nonintensive-care COVID-19 patients, although the study was underpowered for this endpoint owing to premature trial conclusion. In contrast, the trial allowed to quantitatively and serially assess the prognostic role of the combination of peak C-reactive protein and nadir platelet count which were significantly associated with mortality and disease progression. Whether early risk stratification with these practical laboratory tests can modify prognosis and guide therapies can be tested in dedicated studies.

Acknowledgments

We are deeply grateful to all patients. We also thank the physicians, nurses and research staff for their intensive work in enrolling patients for this trial.

Funding

The trial received a grant from the Nicolaus Copernicus University, Poland (grant ID 20134).

Conflict of interest: None declared

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