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Journals	20490801	2012-2021	Homepage How to publish in this journal annalsjournal@elsevier.com

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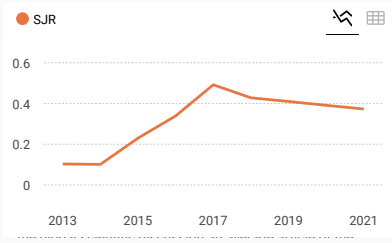

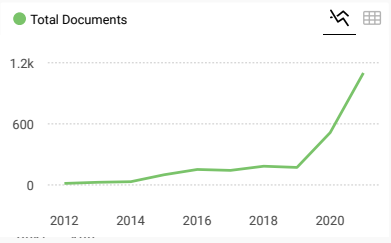

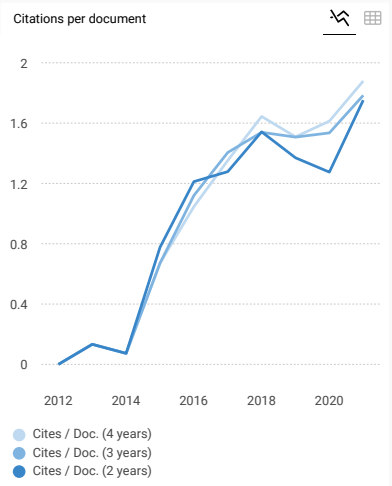

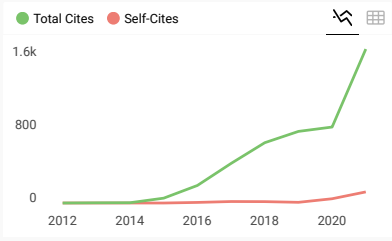

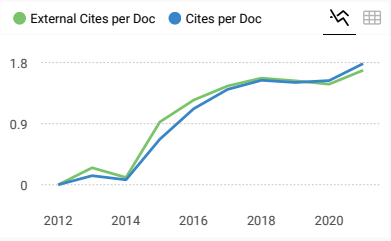

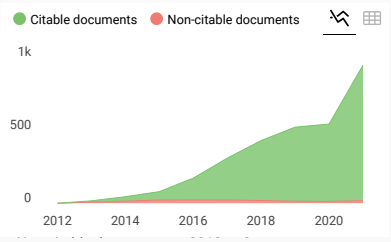

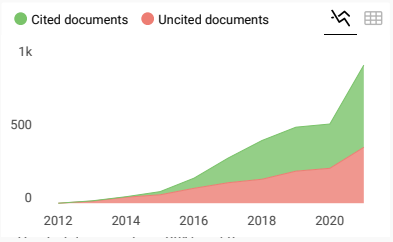

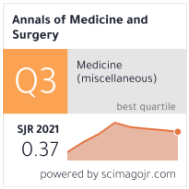

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Case-controlled Study

The role of C-Reactive protein as an inflammatory marker to predict prolonged QTc interval in rifampicin-resistant tuberculosis patients: A case-control study

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ABSTRACT

Background: long-term use of anti-tuberculosis drugs (ATD) increases the risk of QTc prolongation, while C-reactive protein (CRP) can be used as an inflammatory marker of *Mycobacterium tuberculosis* infection.
Objective: correlation of CRP on the QTc interval in Rifampicin-resistant tuberculosis (RR-TB) patients with the short regimen.
Methods: An observational study was conducted in Rifampicin-resistant tuberculosis (RR-TB) patients from 2 groups, patients on intensive phase and patients on continuation phase. CRP levels were measured from blood samples and measured automatically using the immunoturbidimetric assay. QTc interval was calculated using electrocardiography. Levels of CRP levels and QTc interval between the 2 groups were analyzed. The statistical analysis used includes the independent *t*-test, Mann Whitney test, and Rank Spearman test with $p = 0.05$.
Results: Forty-five eligible RR-TB patients were included in this study. CRP levels and QTc intervals between 2 groups (intensive and continuation phase) showed significant difference with $p < 0.001$ but found no significant correlation of CRP levels and QTc interval in both intensive and continuation phase with $p = 0.226$ and 0.805, respectively. A higher level of CRP strongly indicated the inflammation caused by RR-TB infection at the early phase of the disease, but not correlated with QTc interval in RR-TB patients.
Conclusion: Levels of CRP and QTc interval do not correlate in RR-TB patients and can not be used to be the marker of QTc prolongation in RR-TB Patients.

1. Introduction

Drug-resistant tuberculosis (DR-TB) remains a major public health concern in many countries. The World Health Organization (WHO) reported that 3.3% of new TB cases and 18% of previously treated cases had multidrug-resistant/rifampicin-resistant (MDR/RR) TB. In 2020 there were an estimated 465,000 new cases of Rifampicin-resistant tuberculosis (RR-TB), of which 78% had MDR-TB. Globally, treatment success rates of MDR/RR-TB are only 57%, while in Indonesia are 48% [1,2]. MDR/RR-TB requires treatment with second-line drugs, which have many more adverse effects than first-line anti-tuberculosis drugs (ATD) [1,3]. Adverse effects are expected as part of the normal course of treatment, which should be diagnosed and managed appropriately by the clinician [4].

A standardized shorter MDR/RR-TB regimen is the preferred treatment option for eligible patients [5]. However, the occurrence of a serious adverse effect of the shorter regimen such as QTc prolongation may need to be switched to an individual regimen. Soedarsono et al. reported 7% of MDR/RR-TB patients switched their regimens from shorter regimen to individual regimens due to the presence of prolonged QT [6]. A study in DR-TB patients who received shorter regimens reported that 21/98 (21.4%) patients experienced the incidence of ΔQTc

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>30 ms, while 10/98 (10.2%) patients experienced the incidence of ΔQTc >60 ms [7]. QTc prolongation may predispose some patients to *torsade de Pointes* (TdP), which may result in sudden death [8]. The use of certain markers for early detection and risk factors assessment of QTc prolongation may be beneficial to prevent unfavorable outcomes, in addition to performing regular electrocardiography (ECG) for QTc monitoring [9].

The correlation of increased blood level of C-reactive protein (CRP) as inflammation parameter and interval of the heart rate-corrected QT time (QTc) has been reported in many studies [10–12]. The correlation between CRP and QTc was strongest in patients who had prolonged QTc at baseline [13]. Studies in patients with rheumatoid arthritis (RA), hypertensive, and cardiovascular disease have revealed that CRP is a predictor for QTc interval and presence of QT prolongation [13–15].

CRP is an ideal inflammation marker due to the response precision and easy examination [16]. In recent years, CRP is used mainly as an inflammatory marker of *Mycobacterium tuberculosis* infection. The advantages of measuring CRP levels are to supplement the diagnosis of acute TB infection and monitoring for TB treatment response [17,18], but the association of CRP and QT prolongation in RR-TB patients has rarely been examined. CRP as a predictor of QTc prolongation in RR-TB patients who received regimens needs further study. We hypothesized that inflammation in the intensive phase of treatment is still high. QTc intervals may increase after treatment along with high CRP as a marker in the incidence of prolonged QTc intervals, especially in the continuation phase of treatment. Thus, this study was conducted to analyze the correlation between CRP levels and QTc interval in RR-TB patients.

2. Methods

2.1. Participant

The participants of this study were patients diagnosed with RR-TB [6,7,19]. The inclusion criteria in this study were RR-TB patients with age 18–65 years who are on the intensive phase of treatment with a shorter regimen and who are in the continuation phase of the shorter regimen. Patients with any risk factors for QTc prolongation were excluded to minimize their effects in the results of this study. The exclusion criteria were RR-TB patients with baseline QTc >500 ms, potassium <3.5 mmol/L, magnesium <1.7 mmol/L, calcium <8 mmol/L, creatinine clearance <30 cc/m, aspartate aminotransferase - alanine aminotransferase (AST-ALT) >5x upper limit normal (ULN), body mass index (BMI) < 18 kg/m2, on anti-arrhythmia therapy, on anti-depressant therapy, with bradycardia, on anti-fungal treatment (azoles), on erythromycin therapy, and phenytoin therapy [9,12,20].

2.2. Ethical approval

We have conducted ethical approval base on the Declaration of Helsinki with the registration of research at the Health Research Ethics Committee in the Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (1444/KEPK/VIII/2019). Informed consent was obtained for all participants.

2.3. Study design

This study used a case-control design with consecutive sampling from September 2019 to February 2020. The number of participants in this study was 45 TB patients who were obtained by consecutive sampling method. Participants were divided into two groups, such as the intensive phase (n = 29) and the continuation phase (n = 16). RR-TB patients who are on intensive phase were defined as RR-TB patients who are diagnosed with RR-TB and start intensive phase of treatment with the shorter regimen. RR-TB patients on the continuation phase of the shorter regimen were defined as RR-TB patients who have culture conversion. Participants CRP and QTC examinations after the short treatment regimen. RR-TB is a form of TB resistant to rifampicin according to a molecular rapid test using GeneXpert MTB/RIF [21]. Standardized shorter regimens were as recommended by the WHO in 2016 [3,5]. This study was reported by the Strengthening the Reporting of Cohort Studies in Surgery (STROCSS) 2019 guideline [22].

2.4. Measurement of CRP levels

Venous blood samples from each participant were collected into heparin tubes. Serum was separated by centrifugation at 3000 rpm for 5 min and stored at 4 °C for 24 h for the analysis [11]. CRP levels were determined by an immunoturbidimetric assay using SIEMENS Dimension clinical chemistry system for the quantitative determination of CRP in serum and plasma. This instrument automatically calculates and prints the concentration of CRP in mg/L or mg/dL. Analytical measurement range was 0.5–250.0 mg/L or 0.05–25.00 mg/dL.

2.5. Measurement of QTc prolongation

Electrocardiography (ECG) was defined as 12-lead surface heart recording using an ECG machine. The QTc referred to the corrected QT interval using the Fredericia formula. QTc prolongation was defined as QTc interval ≥450 ms in men and ≥470 in women [3,9]. The QT interval and R-R interval were automatically measured using ECG machine merc BLT E30 (Guangdong Biolight Meditech, China). The means of the QT intervals and R-R intervals were obtained by measuring the QT intervals and R-R intervals of three consecutive beats in each lead. The QT interval is dependent on the heart rate: the faster the heart rates the shorter the QT interval. The heart rate-corrected QT (QTc) interval was calculated using the Fredericia formula [9,15].

2.6. Statistical analysis

Levels of CRP and QTc interval in two groups of RR-TB patients were analyzed statistically using SPSS 23.0 software (IBM Corp., Armonk, NY, USA). $p < 0.05$ was considered as significant statistically. Statistical analysis was according to normality test, using Chi-Square, Independence T-test, Mann-Whitney test, or Spearman Rank test.

3. Results

3.1. Characteristics of participant

29 RR-TB patients on intensive phase (64%) and 16 RR-TB patients on continuation phase (36%) were included in this study. Most of the male participants were 23 participants (51.1%; 55.2 vs 43.8%; $p = 0.463$). In addition, there were 19 participants who had diabetes mellitus (42.2%; 48.30 vs 31.30%; $p = 0.268$). The average age of the participants was 37.71 ± 13.55 years with a median value of 41.00 (18.00–62.00) years, which in intensive phase was 37.03 ± 13.76 years and continuation phase was 38.94 ± 13.51 years ($p = 0.569$). Meanwhile, the participant's BMI value obtained a value of 21.61 ± 3.57 m/kg^2 with a median value of 20.28 (18.03–28.65) m/kg^2, which intensive phase was 21.90 ± 3.55 m/kg^2 and continuation phase was 21.08 ± 3.66 m/kg^2 (p = 0.530; Table 1).

Significant differences between the intensive and continuation phases were found in potassium (4.30 ± 0.45 vs 3.97 ± 0.41 mmol/l; CI 0.056–0.606; $p = 0.019$), chloride (98.76 ± 2.78 vs 103.50 ± 4.79 mmol/l; CI 0.109–0.603; $p < 0.001$), calcium (9.03 ± 0.46 vs 8.68 ± 0.20 mg/dl; CI 0.109–0.602; $p = 0.006$), and magnesium levels (2.32 ± 0.16 vs 1.91 ± 0.12 mg/dl; $p = 0.003$). Meanwhile, there was no significant difference in sodium values between the invesive and continuation phases (139.07 ± 4.09 vs 140.75 ± 5.87 mmol/l; CI -4.689 – 1.328; $p = 0.266$; Table 1).

Table 1
Characteristics of participants.

Characteristics	RR-TB patients		*p*
	Intensive phase (n = 29)	Continuation phase (n = 16)	
Age (years)	37.03 ± 13.76	38.94 ± 13.51	0.569
BMI (m/kg^2)	21.90 ± 3.55	21.08 ± 3.66	0.530
Na (mmol/l)	139.07 ± 4.09	140.75 ± 5.87	0.266
K (mmol/l)	4.30 ± 0.45	3.97 ± 0.41	0.019*
Cl (mmol/l)	98.76 ± 2.78	103.50 ± 4.79	<0.001**
Ca (mg/dl)	9.03 ± 0.46	8.68 ± 0.20	0.006*
Mg (mg/dl)	2.06 ± 0.16	1.91 ± 0.12	0.003*
CRP (mg/dl)	2.32 ± 2.67	0.19 ± 0.14	<0.001**
QTc interval (ms)	417.28 ± 31.22	455.94 ± 16.64	<0.001**

Note: BMI = Body mass index; Na = sodium; K = potassium; Cl = chloride; Ca = Calcium; Mg = Magnesium; CRP = C-reactive protein; *significant $p < 0.05$; **significant $p < 0.001$.

3.2. Correlation of biomarker on QTc interval

Participants' biomarker values were such as sodium (139.67 ± 4.80 mmol/l), potassium (4.18 ± 0.46 mmol/l), chloride (100.44 ± 4.24 mmol/l), Calcium (8.90 ± 0.42 mg/dl), magnesium (2.01 ± 0.16 mg/dl), and CRP (1.56 ± 2.37 mg/dl). Meanwhile, the mean value of QTc intervals prolonging participants was 431.02 ± 32.64 ms with a median value of 433.00 (352.00–476.00) ms. The mean of QTc interval in the intensive phase was lower than the continuation phase (417.28 ± 31.22 vs 455.94 ± 16.64 ms; CI -55.65 to −21.68; $p < 0.001$). The CRP was significantly different in these two groups (2.32 ± 2.67 vs 0.19 ± 0.14 mg/dl; Table 1).

Correlation analysis did not establish a correlation between CRP levels and QTc interval in both intensive ($r = -0.232$; $p = 0.226$) and continuation phase ($r = 0.067$; $p = 0.805$). Other variables, including sodium, potassium, chloride, calcium and magnesium, also demonstrated no correlation with QTc interval in the intensive and continuation phase. In the intensive phase there was no correlation between sodium, potassium, chloride, calcium and magnesium with the QTc interval which values of r and p were as follows: sodium ($r = 0.130$; $p = 0.503$), potassium ($r = -0.142$; $p = 0.461$), chloride ($r = 0.229$; $p = 0.232$), calcium ($r = 0.127$; $p = 0.510$), and magnesium ($r = -0.105$; $p = 0.598$). While in the continuation phase as follows sodium ($r = -0.016$; $p = 0.954$), potassium ($r = 0.323$; $p = 0.223$), chloride ($r = -0.080$; $p = 0.769$), calcium ($r = 0.312$; $p = 0.239$), and magnesium ($r = 0.059$; $p = 0.828$; Table 2). A Scatter plot of QTc interval dependent on CRP levels was presented in Fig. 1. In the intensive phase, CRP levels were found high with various of QTc intervals. While on continuation phase, CRP levels were found lower with only slightly greater varies of QTc intervals.

4. Discussions

The difference of CRP levels between the two groups showed that inflammation in the intensive phase was high and reduced after sputum conversion in the continuation phase, showed the role of CRP levels as a marker in treatment monitoring. In clinical practice, CRP is commonly used to assess, diagnose, and prognosis inflammation as its concentration will increase with the presence of injury, inflammation, or tissue death [16].

Table 2
Correlation of biomarker on prolonging QTc intervals in RR-TB patients.

QTc Interval	Intensive phase		Continuation phase	
	r	p	r	p
Na (mmol/l)	0.130	0.503	−0.016	0.954
K (mmol/l)	−0.142	0.461	0.323	0.223
Cl (mmol/l)	0.229	0.232	−0.080	0.769
Ca (mg/dl)	0.127	0.510	0.312	0.239
Mg (mg/dl)	−0.105	0.598	0.059	0.828
CRP (mg/dl)	−0.232	0.226	0.067	0.805

Note: Na = sodium; K = potassium; Cl = chloride; Ca = Calcium; Mg = Magnesium; CRP = C-reactive protein; *significant $p < 0.05$; **significant $p < 0.001$.

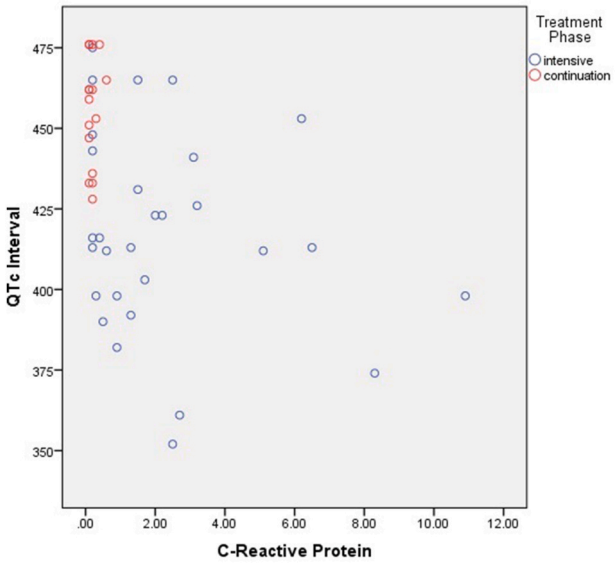

Fig. 1. Scatter plot of CRP levels (mg/dl) and QTc interval (ms).

Effective treatment after the intensive phase of treatment according to acid-fast bacilli (AFB) sputum conversion was correlated with reduced inflammation in the lung which indicated decreasing levels of CRP to normal value. AFB sputum conversion and improvement of the disease correlated with decreased CRP and increased BMI [23], while BMI of patients in intensive and continuation phase in our study showed no significant difference. Levels of CRP 0.2–10.9 mg/dl in the intensive phase showed higher inflammation and reduced CRP 0.2 tp 0.6 mg/dl after being treated in the continuation phase (Table 1) showed the decrease of inflammation caused by *Mycobacterium tuberculosis* infection. CRP is an important factor to determine the etiology of infection. CRP level >100 mg/L strongly indicated the presence of bacterial infection, while CRP <10 mg/L indicated viral infection. CRP 10–100 mg/L was commonly found in TB [16]. CRP is one of the biomarkers in the blood which demonstrated activated macrophages in pulmonary TB. Increasing levels of CRP were found in the early development of disease. CRP levels increased significantly in pulmonary TB patients with severe lung lesion and progressively decrease to normal range in patients received ATD and have acid-fast bacilli (AFB) sputum conversion. CRP was mainly used as a biomarker to evaluate TB treatment success and decrease CRP level indicated the effective treatment response [23].

Intervals of QTc between the 2 groups also showed significant differences with $p < 0.001$, a higher value of QTc interval in the continuation phase was possibly affected by drugs administration after received treatment with the shorter regimen. As has been known that moxifloxacin is one of the core components of the shorter regimen which potential to carry a risk of QTc prolongation, although it is also likely to be the most effective against DR-TB [5,24]. QTc interval in the continuation phase was only a little higher than the intensive phase (Table 1). A study by Hong et al. also reported that QTc interval after moxifloxacin dose of 800 mg was not significantly higher than 400 mg [25]. CRP is an inflammation marker in much clinical practice, in addition, to predict risk for cardiovascular disease [26]. Elevated CRP levels were a strong independent predictor of cardiovascular disease in asymptomatic individuals. CRP levels have correlated with the prognosis of atherosclerotic disease, congestive heart failure, atrial fibrillation, myocarditis,

aortic valve disease, and heart transplantation, suggesting its role in the pathophysiology of cardiovascular disease [27].

The correlation between CRP and cardiovascular risk is through systemic inflammation. CRP is unlikely to contribute directly to cardiovascular disease as a pathogenic factor. CRP levels of 1, 1 to 3, and 3 mg/L have been classified as low, moderate, and high-risk groups for future cardiovascular events [16]. CRP is a stronger predictor of cardiovascular risk compared to other inflammatory biomarkers [26].

Potassium and calcium were found significantly different between the 2 groups in this study, but no correlation of potassium and calcium levels with QTc intervals in our study. Potassium and calcium have been known to contribute to ventricles depolarization and repolarization phase and correlated with QTc intervals [28]. Significant differences in K, Cl, Ca, and Mg between 2 groups of study participants may also be caused by drug administering during treatment. Although patients with hypokalemia, hypocalcemia, and hypomagnesemia were not included in this study, it was known that potassium levels decreased in the continuation phase along with decreased calcium and magnesium. Aminy and Mardiana declare that hypokalemia is often associated with an imbalance of other electrolytes, such as hypomagnesemia and hypocalcemia in patients who received aminoglycoside drugs [29]. Treatment for RR-TB patients using a shorter regimen, including aminoglycosides in this study perhaps affected reduced K, Ca, and Mg in the continuation phase. Other studies also reported a significant decrease in serum potassium, calcium, and magnesium after being treated using aminoglycoside in their regimen [30].

Higher CRP levels with shorter QTc intervals were dominantly found in the intensive phase, while lower CRP levels with longer QTc intervals were commonly found in the continuation phase. In contrast to our results, another study did not find a difference in hypertensive patients with regard to CRP as an independent predictors for QT prolongation [15]. Elevated CRP levels occurred during inflammatory conditions such as rheumatoid arthritis, some cardiovascular diseases, and infection. CRP was suggested as an important regulator of inflammatory processes and not just a marker of inflammation or infection [27]. Other studies also reported the correlation of CRP levels and QTc prolongation in RA patients [13,14]. Those studies reported the correlation of CRP and QTc prolongation in patients with RA, hypertensive, and cardiac disease in previous studies, but our study revealed that CRP level was not correlated with QTc interval in RR-TB patients.

As the combination second-line, ATD used in DR-TB treatment is more toxic and leads to severe adverse effects such as QTc prolongation which can be life-threatening, the prevention of developing serious adverse effects is important [31]. Therefore, further study was needed to examine other biomarkers which potential be used to predict QTc prolongation in RR-TB patients.

5. Conclusion

Our study concluded no correlation of CRP levels and QTc interval in RR-TB patients. CRP levels in RR-TB patients seem better to be used as a marker for diagnosis and treatment monitoring, but not to predict the incidence of QTc prolongation in RR-TB patients.

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Ethical approval

We have conducted an ethical approval base on Declaration of Helsinki with registration of research at the Health Research Ethics Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

Consent

Written informed consent was obtained from the patient.

Author contribution

All authors contributed toward data analysis, drafting and revising the paper, gave final approval of the version to be published and agree to be accountable for all aspects of the work.

Registration of research studies

1. Name of the registry: Health Research Ethics Committee in the Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.
2. Unique identifying number or registration ID: 1444/KEPK/VIII/2019.
3. Hyperlink to your specific registration (must be publicly accessible and will be checked): -.

Guarantor

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Declaration of competing interest

The authors declare that they have no conflict of interest.

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