Associations between ticagrelor use and the risk of infections: A Mendelian randomization study

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Aims: We conducted a Mendelian randomization (MR) study to elucidate the anti-infective effects of ticagrelor.

Methods and results: Single-nucleotide polymorphisms (SNPs) associated with serum levels of ticagrelor or its major metabolite AR-C124910XX (ARC) in the PLATElet inhibition and patient Outcomes trial were selected as genetic proxies for ticagrelor exposure. Positive control analyses indicated that genetically surrogated serum ticagrelor levels (six SNPs) but not ARC levels (two SNPs) were significantly associated with lower risks of coronary heart disease. Therefore, the six SNPs were used as genetic instruments for ticagrelor exposure, and the genome-wide association study data for five infection outcomes were derived from the UK Biobank and FinnGen consortium. The two-sample MR analyses based on inverse variance-weighted methods indicated that genetic liability to ticagrelor exposure could reduce the risk of bacterial pneumonia (odds ratio [OR]: 0.82, 95% confidence interval [CI]: 0.71–0.95, P = 8.75E-03) and sepsis (OR: 0.83, 95%...
CI: 0.73–0.94, $P = 3.69 \times 10^{-3}$); however, no causal relationship between ticagrelor exposure and upper respiratory infection, pneumonia, and urinary tract infection was detected. Extensive sensitivity analyses corroborated these findings.

**Conclusion:** Our MR study provides further evidence for the preventive effects of ticagrelor on bacterial pneumonia and sepsis.

**Keywords:** Ticagrelor; Infection; Mendelian randomization; Causality

**BACKGROUND**

Despite advances in prevention and treatment, coronary heart disease (CHD) remains a significant cause of morbidity and mortality worldwide. Ticagrelor, a nonthienopyridine and direct-acting P2Y12 adenosine diphosphate inhibitor, has been approved for lowering cardiovascular events in patients with CHD, especially those with acute manifestations (i.e., acute coronary syndrome [ACS]). Unlike the thienopyridine antagonists of the P2Y12 receptor clopidogrel and prasugrel, ticagrelor suppresses the intracellular uptake of adenosine, an endogenous regulator of inflammatory processes and innate immunity. This finding raises the possibility that ticagrelor may possess anti-infective properties. A post-hoc analysis of the PLATElet inhibition and patient Outcomes (PLATO) trial found that compared with clopidogrel, ticagrelor treatment was associated with a decreased rate of pneumonia and death from pneumonia and sepsis in patients with ACS. A nationwide cohort study involving 24,456 patients demonstrated that ticagrelor prescription might help prevent *Staphylococcus aureus* bacteremia following ACS episodes and percutaneous coronary intervention (PCI).

Nevertheless, other studies failed to achieve significant associations between ticagrelor use and infection outcomes. A recent observational study indicated that ticagrelor treatment did not significantly alter the risk of infections in patients undergoing primary PCI during hospitalization for myocardial infarction. Another propensity-score matched analysis of patients receiving coronary artery bypass grafting demonstrated a similar incidence of infectious complications in the ticagrelor versus clopidogrel groups. So far, the effects of ticagrelor use on infection outcomes are inconsistent and largely driven by observational data. Given the detrimental impacts of infections and the crucial role of ticagrelor in managing CHD, uncovering the association between ticagrelor use and the risk of infectious diseases is vital to public health.

Mendelian randomization (MR) is used to explore causality between exposures and outcomes. This approach employs single-nucleotide polymorphisms (SNPs) as unbiased instrumental variables (IVs) for exposures, which are much less prone to residual confounding and reverse causation inherent to traditional observational study designs. Genetic variants are randomly allocated during gametogenesis and would not be interfered with the environment; thus, MR procedures can somewhat mimic randomized controlled trials (RCTs). Therefore, this study...
performed two-sample MR analyses to examine whether ticagrelor has causal effects on the risks of infections using summary statistics from open genome-wide association studies (GWASs).

METHODS

Study design

We conducted a two-sample MR study to investigate the potential causal effects of ticagrelor on susceptibility to infectious diseases. Figure 1 shows that the MR approach was based on three essential assumptions: 1) IVs must be strongly related to ticagrelor exposure (correlation); 2) IVs should not be associated with any confounders for the ticagrelor-infection association (independence); 3) IVs influence the risk of infections only through ticagrelor exposure (exclusion restriction). Table S1 presents a checklist for Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomization (STROBE-MR) statement.13 All data used in this MR study were publically accessible; therefore, no additional ethical approvals are required.

Selection of instrument variables

We extracted IVs for ticagrelor \textit{invivo} exposure from the GWAS summary statistics of serum steady-state area under the curve (AUCss) of ticagrelor or its major active metabolite AR-C124910XX (ARC), which were measured within 3,753 ACS patients (predominately European ancestry) administered ticagrelor from the PLATO trial.14 After adjusting covariables, the authors identified 23 SNPs strongly associated with ticagrelor AUCss and 56 SNPs with ARC AUCss ($P < 1E-08$, controlling for 5 million independent tests). The two sets of SNPs were pruned using linkage disequilibrium (LD) $r^2 < 0.01$ within 100-kb windows to ensure the independence of IVs. SNPs that directly affected the risk of outcomes ($P < 5E-08$) were also discarded. If any SNPs for ticagrelor use were absent in the outcome GWAS data, we applied the LDlink tool to identify proxy SNPs with LD $r^2 > 0.8$. The SNPs were removed from subsequent analyses if no appropriate surrogates were available. The remaining SNPs were selected as IV for the AUCss of ticagrelor or ARC, and the $F$-statistics were calculated to quantify the strength of each genetic instrument.

Positive control analyses

AUCss, a key pharmacokinetic index, correlates directly with drug absorption and clearance. Theoretically, genetic instruments for AUCss can predict the therapeutic effects of corresponding drugs. To ensure the efficacy of the selected two sets of IVs (IVs for ticagrelor AUCss or ARC AUCss), we evaluated their associations with CHD because this illness is the main indication of ticagrelor. GWAS summary-level statistics for CHD and its subtypes (myocardial infarction and angina pectoris) were acquired from UK Biobank, FinnGen (Release 6), or large-scale GWAS meta-analyses of UK Biobank and CARDIoGRAMplusC4D (Table S2). A two-sided $P$-value of $< 0.017$ (0.05/3 outcomes) was deemed significant in positive control analyses.

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Data sources for outcomes

Based on previously reported data for ticagrelor and disease incidence, we examined the risk of the following infections: upper respiratory infections, pneumonia, bacterial pneumonia, urinary tract infections, and sepsis. We analyzed the GWAS summary-level data from two independent cohorts of European ancestry: UK Biobank\(^\text{17}\) and FinnGen (Release 6)\(^\text{18}\) (Table S2). The GWAS within the UK Biobank was conducted using Scalable and Accurate Implementation of GEneralized mixed model (SAIGE), controlled for age, gender, genetic relatedness, and the first four principal components. The GWAS data within FinnGen were also analyzed using SAIGE with adjustment for age, gender, genotyping batch, and the first 10 principal components.

Statistical analyses

All statistical analyses were performed using the "TwoSampleMR" package in R 4.2.0 software (The R Foundation for Statistical Computing, Vienna, Austria). The random-effects inverse-variance weighted (IVW) algorithm\(^\text{21}\) was employed as the main approach for MR analyses. Estimates of associations were reported as odds ratios (ORs) and 95% confidence intervals (95% CIs) per 1-log unit increment in the AUCs of ticagrelor or ARC. Causal directions for positive IVW estimates were tested using the Steiger test\(^\text{22}\). Potential heterogeneity within the IVW model was detected using Cochran’s Q statistical test and \(I^2\)-statistics. We considered that there was apparent heterogeneity when \(P > 50\%\) and Cochran’s Q-test \(P < 0.05\). MR analyses were conducted separately for the outcomes data from UK Biobank and FinnGen, and we used fixed-effects meta-analytic methods to combine the two cohorts. If not otherwise specified, the combined results were adopted as the final effects of ticagrelor exposure. The statistical significance level of infectious diseases was set at \(P < 0.01\) (0.05/5 outcomes) for correction of multiple testing.

Although the IVW method offers the highest statistical power, it may be biased when horizontal pleiotropy exists\(^\text{23}\), a phenomenon in which genetic variants affect the outcome through mechanisms independent of exposure. Thus, we first assessed whether the IVs were linked to other phenotypes (\(P < 5\times 10^{-8}\)) in the PhenoScanner V2 database\(^\text{24}\). The MR-Egger regression intercept test\(^\text{23}\) and MR-pleiotropy residual sum and outlier (MR-PRESSO) global test\(^\text{25}\) were then applied to investigate the potential of pleiotropic bias. We also introduced MR-Egger regression with bootstrapping\(^\text{23}\) and weighted median\(^\text{26}\) as supplementary methods to generate the MR results. The MR-Egger approach provides estimates adjusted for horizontal pleiotropy, and the weighted median method affords unbiased causality when at least 50% of the IVs are valid. Finally, we repeated the MR analyses by omitting proxy SNPs to evaluate the robustness of the results.
RESULTS

Genetic instruments for ticagrelor use

After LD clumping, we obtained six and two SNPs as genetic instruments for the AUCs of ticagrelor and ARC, respectively (Table S3). The variance explained by these IVs was 7.23% for ticagrelor AUCs and 2.41% for ARC AUCs. The F-statistics for the IVs were all beyond 30, suggesting that weak instruments were unlikely to influence our MR analyses. Positive control analyses demonstrated that genetically predicted ticagrelor AUCs was associated with reduced risks of CHD (OR: 0.81, 95% CI: 0.75–0.87, \( P = 1.34 \times 10^{-8} \)), myocardial infarction (OR: 0.89, 95% CI: 0.82–0.97, \( P = 7.75 \times 10^{-3} \)), and angina pectoris (OR: 0.86, 95% CI: 0.77–0.97, \( P = 1.13 \times 10^{-2} \)) (Table S4); however, we found no evidence of protective effects on CHD or its subtypes for the genetically determined ARC AUCs (Table S5). Therefore, the subsequent analysis used the six IVs for serum ticagrelor AUCs as the proxy of exposure to ticagrelor. Of these, rs140607780 (G/A) was not present in the GWAS data of most infectious outcomes; thus, we selected rs7350033 (C/G) instead (\( r^2 = 0.872 \)) where necessary. In the exposure dataset, rs7350033 was also significantly associated with serum AUCs of ticagrelor (\( \beta = 0.273, P = 3.47 \times 10^{-11} \)).

Ticagrelor use and infectious diseases

Figure 2 presents the main MR results from the IVW method for infection outcomes, showing that genetic liability to ticagrelor exposure was correlated with decreased risks of bacterial pneumonia (OR: 0.82, 95% CI: 0.71–0.95, \( P = 8.75 \times 10^{-3} \)) and sepsis (OR: 0.83, 95% CI: 0.73–0.94, \( P = 3.69 \times 10^{-3} \)). Further analysis in the UK Biobank cohort indicated that genetically determined ticagrelor AUCs could also lower the risk of sepsis in critical care (OR: 0.59, 95% CI: 0.40–0.88, \( P = 9.21 \times 10^{-3} \)). However, there was no evidence for causal linkages between ticagrelor exposure and upper respiratory infection (\( P = 1.04 \times 10^{-1} \)), all pneumonia (\( P = 9.22 \times 10^{-2} \)), and urinary tract infection (\( P = 9.18 \times 10^{-1} \)). The associations based on the MR-Egger and weighted median methods were directionally consistent with the IVW estimates (all OR <1; Table S6), albeit with noticeably wider CIs.

Sensitivity analyses

No heterogeneity was observed from the Cochran's Q-test for the MR results (Table S7). The MR Steiger test confirmed no reverse causality from ticagrelor to bacterial pneumonia or sepsis (Table S7). In the PhenoScanner V2 database, we found that the IVs used for ticagrelor exposure were not associated with other phenotypes. Accordingly, the MR-Egger regression intercept or the MR-PRESSO global tests (Table S7) showed no horizontal pleiotropy for all infectious outcomes. Omitting the proxy SNP rs7350033 had no significant impact on the risk of bacterial pneumonia (OR: 0.78, 95% CI: 0.67–0.91, \( P = 1.74 \times 10^{-3} \)) or sepsis (OR: 0.83, 95% CI: 0.73–0.94, \( P = 3.03 \times 10^{-3} \); Figure S1).
DISCUSSION

This investigation of common infectious diseases found that patients with ticagrelor exposure had a lower incidence of bacterial pneumonia and sepsis; however, no causal effects of ticagrelor were observed on upper respiratory infection, all pneumonia, and urinary tract infection. To our knowledge, this is the first comprehensive MR study to address this research topic.

P2Y12 receptor antagonists are generally considered to increase the risk of infections because of their blocking effects on platelet activation, a first-line modulator of the immune defensive responses against pathogen invasion. Previous cohort studies of P2Y12 inhibitors support this perspective, where clopidogrel increased the risk of infections by 48%–51% compared to placebo. Therefore, as a more potent P2Y12 inhibitor than clopidogrel, ticagrelor may not realize its anti-infective function through platelet inhibition. In contrast to other P2Y12 inhibitors, ticagrelor also suppresses cellular absorption of adenosine via inhibition of the equilibrative nucleoside transporter 1, increasing blood adenosine concentrations. Such effects of ticagrelor could enhance the effects of adenosine on neutrophil chemotaxis and phagocytosis at nanomolar concentrations, thereby boosting the host's defense against infections. Ticagrelor also blocks α-toxin-induced platelet injury through the hepatic Ashwell-Morell receptor, thereby promoting platelet-mediated bacterial clearance. Moreover, ticagrelor exerts direct *in vitro* bactericidal activity against a spectrum of gram-positive bacterial strains, which has not been reported for other P2Y12 inhibitors. In *Staphylococcus aureus*-infected mice, a conventional dosage of ticagrelor suppressed biofilm growth and bacterial dissemination to surrounding tissues. These mechanisms, in combination, may account for the anti-infective properties of ticagrelor, but further research is required.

A recent meta-analysis of P2Y12 inhibitors in cardiovascular trials showed that compared with clopidogrel, ticagrelor significantly reduced the risk of pneumonia by 20% but had no effects on other infections, such as sepsis. Notably, infection events were not the pre-specified outcomes of the included RCTs, which may introduce ascertainment bias due to the retrospective nature and lead to underpowered conclusions on infection risks. A nationwide population-based cohort study demonstrated that ticagrelor was associated with a significantly lower one-year risk of pneumonia and sepsis. To date, there is no evidence for the protection of ticagrelor against upper respiratory or urinary tract infections. Pneumonia is commonly caused by gram-positive coccus, whereas upper respiratory tract infection is usually caused by virus attack, and gram-negative organisms cause most urinary tract infections. For sepsis, gram-positive bacteria as a pathogenic cause have increased in frequency over time and are now almost as common as gram-negative infections. Therefore, our MR findings generally agree with previous reports that ticagrelor has antimicrobial properties against gram-positive bacteria, rather than other bacterial species or viruses. Because of no access to the individual-level data of UK Biobank and FinnGen projects, we cannot figure out the microbiological causes of infectious diseases in this study. Although bacterial pneumonia and sepsis can be caused by gram-positive coccus, there are other bacteria to consider such as gram-positive bacteria or atypical bacteria (e.g., mycoplasma or chlamydia), whereas
ticagrelor does not have activity against many of these organisms. Additional researches are warranted to further clarify the antibacterial spectrum of ticagrelor.

The major strength of our work is applying the MR approach based on large-scale GWAS data, which could minimize potential confounding and other biases to strengthen the causal inference. Furthermore, we integrated pharmacogenomics with MR methods using independent SNPs associated with ticagrelor serum AUCss as a genetic proxy for ticagrelor exposure. This approach has important implications for repurposing this antiplatelet drug\textsuperscript{38}. Because ticagrelor has pleiotropic effects other than P2Y12 inhibition, selecting IVs based on \textit{in vivo} pharmacokinetic data may facilitate the discovery of causation between ticagrelor and infections. Finally, we confirmed the efficacy of the selected IVs for ticagrelor exposure using positive control analyses, thus enhancing the reliability of subsequent MR findings.

Several limitations should be recognized. First, horizontal pleiotropy cannot be eliminated, although various sensitivity analyses have been performed to test the MR assumptions. Second, we only provided evidence from a genetic perspective. Because of the lack of relative GWAS data, it is infeasible to conduct additional mediation analysis to clarify the specific regulatory mechanisms involved in the causation between ticagrelor exposure and the risks of infections. Thus, future research is required to confirm our findings. Third, the coefficient of variation (CV\%) for ticagrelor AUCss was reported to be up to 56\% in previous studies\textsuperscript{39}. With a large AUCss CV\%, estimating the correlation between pharmacokinetics and clinical outcomes might be challenging. Last but not the least, our MR study was restricted to individuals of predominantly European ancestry; thus, caution should be considered when extrapolating the conclusions to other populations.

CONCLUSION

This MR study demonstrated that ticagrelor exposure was causally associated with reduced bacterial pneumonia and sepsis risks. Therefore, in patients with indications for P2Y12 inhibitors, ticagrelor may be preferentially considered for those at high risk of bacterial pneumonia and sepsis. These findings may also provide new insights for antibiotic discovery based on the pharmacodynamics of ticagrelor, but require verification in future dedicated RCTs.

DISCLOSURE

The authors declare no conflict of interest.

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**Author Contribution:** C.Q. conceived and designed the MR study; M.X. and Q.W. collected and analyzed the study data; Y.W. and Y.P. contributed to materials and analysis tools; M.X. wrote the paper, with key intellectual contents revised by Q.W. and C.Q. All authors approved the final version of manuscript.

**Data availability:** All data used in this study are acquired from publicly available sources. Details for downloading the GWAS summary-level statistics were released in Table S2.

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**Abbreviations**

ACS: Acute coronary syndrome  
ARC: AR-C124910XX  
AUCss: steady-state area under the curve  
CHD: Coronary heart disease  
CI: Confidence interval  
CV%: coefficient of variation  
GWAS: Genome-wide association study  
IV: Instrumental variable  
IVW: Inverse-variance weighted  
LD: Linkage disequilibrium  
MR: Mendelian randomization  
OR: Odds ratio  
PLATO: PLATElet inhibition and patient Outcomes  
PCI: Percutaneous coronary intervention

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RCT: Randomized controlled trial

SAIGE: Scalable and Accurate Implementation of GEneralized mixed model

SNP: Single-nucleotide polymorphism

References


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Figure 1. Design flow chart for the Mendelian randomization study
Figure 2. Inverse-variance weighted (IVW) Mendelian randomization association between ticagrelor exposure and the risks of infectious diseases. CI, confidence interval; OR, odds ratio

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