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ORIGINAL ARTICLE
Translational basic science

Fluoroquinolones and the risk of aortic aneurysm or aortic dissection: evidence from a nationwide nested case–control study paralleled with matched experimental models

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Aims Fluoroquinolones (FQ) have been associated with aortic aneurysm and aortic dissection (AA/AD) resulting in an official warning. Recently, large-scale epidemiological studies failed to confirm this.

Methods and Results The current study aimed to scrutinize the FQ-AA/AD association through a retrospective nested case-cohort analysis supplemented with animal experimentation. FQ exposure was not associated with increased AA/AD hazard ratios in main and high-risk (elderly ≥65 years, hypertensive, and prevalent aortic disease) populations. Additionally, FQ did not cause increased mortality or aortic interventions in aortic disease patients. In addition, in animal experimentation, ciprofloxacin did not enlarge aortic diameters nor increase arterial stiffness.

Conclusion Conventional use of FQ should not be avoided when clinically indicated.

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Structured Graphical Abstract

Key Question

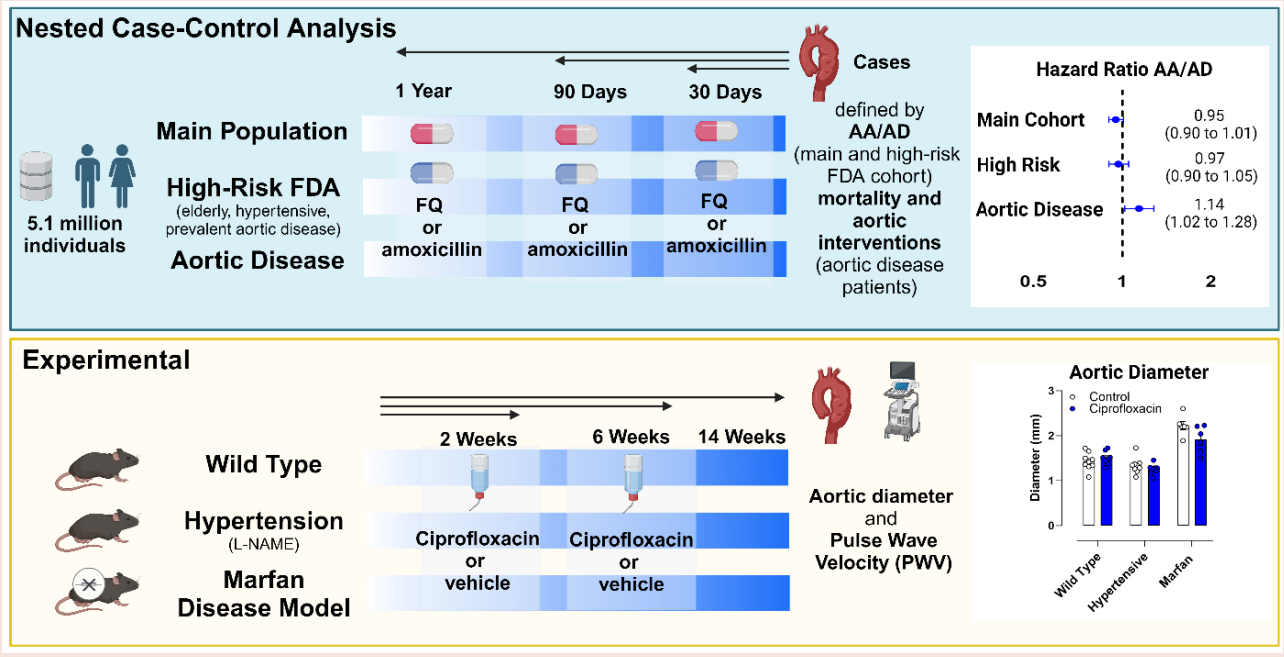
Fluoroquinolones (FQ) have been associated with aortic aneurysm and aortic dissection (AA/AD) resulting in an official warning. Recently, large-scale epidemiological studies failed to confirm this. The current study aimed to scrutinize the FQ-AA/AD association through a retrospective nested case-cohort analysis supplemented with animal experimentation.

Key Findings

FQ exposure was not associated with increased AA/AD hazard ratios in main and high-risk (elderly ≥65 years, hypertensive, and prevalent aortic disease) populations. Additionally, FQ did not cause increased mortality or aortic interventions in aortic disease patients. In addition, in animal experimentation ciprofloxacin did not enlarge aortic diameters nor increase arterial stiffness.

Take Home Message

Conventional use of FQ should not be avoided when clinically indicated.



FDA, U.S. Food and Drug Administration; FQ, fluoroquinolone; AA/AD, aortic aneurysms or aortic dissections; L-NAME, Nω-Nitro-L-arginine methyl ester hydrochloride; mm, millimetre.

Keywords Fluoroquinolones • Aortic aneurysm • Aortic dissection

Introduction

Fluoroquinolones (FQ) are among the most commonly used antibiotic classes worldwide owing to their broad-spectrum antimicrobial activity.[1,2] However, FQ have a black box warning issued by the US Food and Drug Administration (FDA) for increased risk of tendon ruptures and tendonitis through induction of degenerative changes in collagen fibres.[3] Collagen is a main constitute of the arterial wall,[4,5] and a substantial number of epidemiological studies have reported an association between FQ and life-threatening aortic complications such as aortic aneurysms or aortic dissections (AA/AD). As a result, the FDA has issued a warning for FQ use in high-risk patients (e.g. elderly, hypertensive subjects, and patients with prevalent aortic disease).[6] Additionally, FQ have been shown to

exacerbate AA/AD in experimental animal models.[7,8] As a result, clinicians have been discouraged from prescribing FQ even when they provide a substantial benefit for the treatment of indicated infections.[9] However, recently two studies independently reported a lack of association between FQ and AA/AD, yet the controversy is not fully resolved.[9,10]

Hence, we aimed to scrutinize the potential relationship between FQ exposure and aortic disease by combining both large-scale epidemiological data from Danish nationwide clinical databases containing more than 5 million individuals included between 2003 and 2021. In addition, the effect of ciprofloxacin on aortic diameter, as well as on Pulse Wave Velocity (PWV, as a marker of arterial remodelling), was determined in a series of independently designed animal experiments mimicking healthy, hypertensive, and Marfan disease conditions.

Methods

Data sources and study population

Danish nationwide registers were used to conduct a nested case–control study including individuals in Denmark aged 30–100 years during 2003–2021. The Danish registers have been used extensively for research and have previously been described in detail.[11–14] In brief, a unique identifying number is assigned to each Danish resident, allowing for comprehensive healthcare tracking of citizens. This identifier facilitates the integration of various nationwide clinical databases, enabling extensive epidemiological research at a national level logging hospital interactions, surgeries performed and all prescriptions filled at Danish pharmacies. For analyses, three separate cohorts were identified: (i) A main cohort of 5.1 million individuals was used to assess whether FQ exposure was associated with incident AA/AD in the main population. Individuals were excluded with a history of AA/AD, bicuspid aortic valve, coarctation of the aorta, and connective tissue disease including rheumatic fever, rheumatoid arthritism, Bechterew's disease, Marfan Syndrome, and Ehlers–Danlos Syndrome. (ii) A high-risk cohort, as defined by the FDA, was used to investigate whether FQ exposure was associated with increased rates of AA/AD. The high-risk cohort included all patients aged ≥65 years or with a medical history of hypertension, ischaemic heart disease, bicuspid aortic valve, coarctation of the aorta, and connective tissue disease including rheumatic fever, rheumatoid arthritism, Bechterew's disease, Marfan Syndrome, and Ehlers–Danlos Syndrome at time of first criteria fulfilment.[6] (iii) A cohort with the known aortic disease was analysed to evaluate whether FQ exposure was associated with poor outcomes in potentially susceptible patients. To this end, a nest of all patients alive 60 days after a first-time hospitalization for AA/AD was identified. In this nest of patients, the risk of all-cause mortality and aortic interventions (defined as aortic surgery and aortic stenting as a proxy for worsening aortic disease) were investigated.

Case definitions and matching

For the main analysis as well as the high-risk cohort, cases were defined as incident AA/AD, whichever came first. As a secondary outcome in these two cohorts, we used a case definition of only ruptured AA/AD to identify severe cases of aortic pathology. For the cohort with known aortic disease, cases were defined as all-cause mortality, and aortic interventions, as well as a composite of the two outcomes. For all analyses, cases were matched on sex, age, and year of index in a 1:30 ratio to controls using exact matching with replacement as reported previously.[15] For example, a 71-year-old male diagnosed with AD in 2016 was matched to a 71-year-old male alive and not diagnosed with AD in 2016. Following matching, summary reports were inspected to ensure a limited number of reused cases/controls. Diagnostic codes used for all variable definitions in the study are listed in Supplementary material online, *Table S1*.

FQ exposure and active comparator

Individuals were assumed exposed in a binary manner if they had claimed a prescription for an oral FQ (ciprofloxacin or moxifloxacin) within the given exposure windows. Generally, 30-day, 90-day, and 1-year exposure windows were considered to evaluate potential short-, intermediate-, and long-term association between FQ exposure and the outcome of interest. A potential dose–response relationship was also evaluated as higher doses of FQ should be associated with increased rates of AA/AD, if a true association exists. All claimed prescriptions of FQ within 1 year prior to the date of interest were identified and cumulative defined daily doses were calculated (cDDD) as done previously.[11] For example, if a patient was diagnosed with AA/AD on 03 January 2017, and had claimed three prescriptions of FQ in the preceding year, all prescriptions were summed up for that patient.

We employed an active comparator design to limit protopathic bias. Amoxicillin was chosen as an active comparator since it shares clinical indications with FQ and amoxicillin is not known to cause AA/AD.[16]

The following comorbidities were included in the analysis: ischaemic heart disease, heart failure, atrial fibrillation, chronic obstructive pulmonary disease (COPD), chronic kidney disease, myocardial infarction, ischaemic stroke, or transient cerebral ischaemia. For concomitant pharmacotherapy, filled prescriptions were identified for the following drugs: beta-blockers, calcium-channel blockers, renin–angiotensin system inhibitors, anticoagulants, antiplatelet drugs, statins, and loop diuretics. A filled prescription for glucose-lowering drugs was used as a proxy for diabetes mellitus.[17] Finally, two filled prescriptions for blood pressure–lowering drugs were used as a proxy for hypertension[18] (see Supplementary material online, *Table S1*).

Animal experimentation

Male wild-type (WT) C57BL/6J mice were purchased from Charles River (Ecully, France), whereas male genetic Marfan model (Fbn1$^{C1039G/+}$) mice originated from internal breeding. At the age of 12 weeks, mice were subjected to the experimental protocol. Mice were housed in the animal facility of the University of Antwerp in standard cages with 12–12 h light–dark cycles, with free access to regular chow and tap water. Cages were stored at constant room temperature (20–24°C) and humidity (45%). The animal procedures conformed to the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes, and all experiments were approved by the ethics committee of the University of Antwerp (File 2021-22 & 2021-83).[19]

Experimental design of mouse study

Ciprofloxacin was evaluated in three different experimental mouse models. (i) WT C57BL/6J mice were used to represent the main population. (ii) Hypertension was induced by adding L-NAME (0.5 mg/mL) to the drinking water of C57BL/6J mice for the entire study duration. (iii) Finally, mice with a mutation in the *Fibrillin 1* gene (Fbn1$^{C1039G/+}$ C57BL/6J mice) were used as an established experimental model of Marfan disease. Mice were randomly assigned to receive either ciprofloxacin (100 mg/kg/day) or vehicle (standard water) via the drinking water. Ciprofloxacin was administered through a two-week on two-week off dosing protocol, repeated twice. The diameter of the ascending aorta, an established marker of AA/AD risk in both human and mouse models, was evaluated longitudinally by echocardiography performed at weeks 0 (baseline), 2, 6, and 14. Additionally, PWV was measured in the abdominal aorta as a marker of arterial remodelling that may associate with AA/AD. After ciprofloxacin exposure, an 8-week follow-up period was included to provide sufficient time for maladaptive arterial wall remodelling to occur. Therefore, ciprofloxacin exposure was confirmed in a parallel cohort of wild-type and hypertensive mice to mitigate washout effects. Limited availability of Marfan mice from internal breeding precluded parallel plasma sample collection for this group. At week 14, mice were humanely killed by sodium pentobarbital (200 mg/kg, i.p.), and the aorta was carefully dissected. A 2-mm segment of the ascending aorta was mounted in the organ bath set-up to measure arterial compliance.[20]

Echocardiography

Echocardiography was performed under anaesthesia [isoflurane (1.5–2.5% (v/v) (Forene; Abbvie, Belgium)] using a high-frequency ultrasound system (Vevo2100, VisualSonics). Heart rate and body temperature were kept between 550 ± 50 beats/min and 37°C, respectively. M-mode images were obtained to assess vascular parameters, captured with a 24-MHz

transducer. The ascending aortic diameter was derived using Vevo LAB Software (Version 3.2.0, VisualSonics). Pulse wave velocity was measured in the abdominal aorta using a previously described method.[21] In brief, aortic flow velocity (V) with pulse wave Doppler and aortic diameter (D) from 700 frames-per-second B-mode images in electrocardiogram-gated kilohertz visualisation (EKV) mode were used to plot a ln(D)-V loop to calculate PWV using MathLab v2014 software (MathWorks).

Ex vivo aortic diameter and stiffness

Ex vivo arterial stiffness was determined in a Rodent Oscillatory Tension Set-up to study Arterial Compliance (ROTSAC).[20] Briefly, ascending aorta segments were continuously stretched between alternating preloads, corresponding to a 'diastolic' and 'systolic' transmural pressure at a frequency of 10 Hz to mimic the physiological heart rate in mice (600 bpm). The Laplace relationship was used to calculate the transmural pressure. At any given pressure, calibration of the upper hook allowed the calculation of the vessel diameter (both systolic and diastolic diameter) and the Peterson pressure-strain modulus of elasticity (Ep) at pressures of 80–120 mmHg.

Ciprofloxacin plasma concentrations

Blood samples were collected from parallel cohorts ($n = 6$) of WT treated mice in a terminal procedure. The concentration of ciprofloxacin was determined by a high-performance liquid chromatography with diode-array detection (HPLC-DAD) system from Agilent (Infinity 1260) as previously described,[22] sodium sulfadimidine (Sigma-Aldrich, product no. 46560) was administered as an internal standard.

Histology

Aortic segments were fixed in 4% buffered formaldehyde solution (BDH Prolabo, VWR, Belgium) for 24 h, subsequently dehydrated in 60% isopropanol (BDH Prolabo, VWR, Belgium), followed by paraffin-embedding. Aortic wall morphology was assessed using haematoxylin and eosin stains. Total collagen and elastin content was evaluated by Picrosirius red stains (Abcam, ab150681) and orcein stains (Abcam, ab245881), respectively. Mosaic images were acquired at 20× magnification using an Olympus BX40 microscope and quantified using ImageJ software.

Statistical methodology

The statistical methods of the epidemiological analyses have been previously described in detail.[11,23,24] Briefly, characteristics of cases and controls are presented with continuous variables described with medians and interquartile ranges (IQR) and categorical variables with counts and percentages, i.e. binarily as present or not present. The association between FQ exposure and the rates of the outcomes was estimated using time-dependent, multivariable Cox proportional hazards regression models, and all variables included in this model were handled as categorical variables. From these models, conditional logistic regression software was used to obtain hazard ratios (HR) with 95% confidence intervals (95% CI) as done previously.[11] The reported HR should be interpreted as what would have been obtained from a Cox model based on the whole study population fitted with time-varying exposure and adjustment.[23] In the analysis of the dose–response relationship, the cDDD 1–5 group served as the reference group. In all analyses, the models were adjusted for hypertension, ischaemic heart disease, atrial fibrillation, heart failure, COPD, diabetes mellitus, and chronic kidney disease.[25] The level of statistical significance was set at 5%. Analyses were

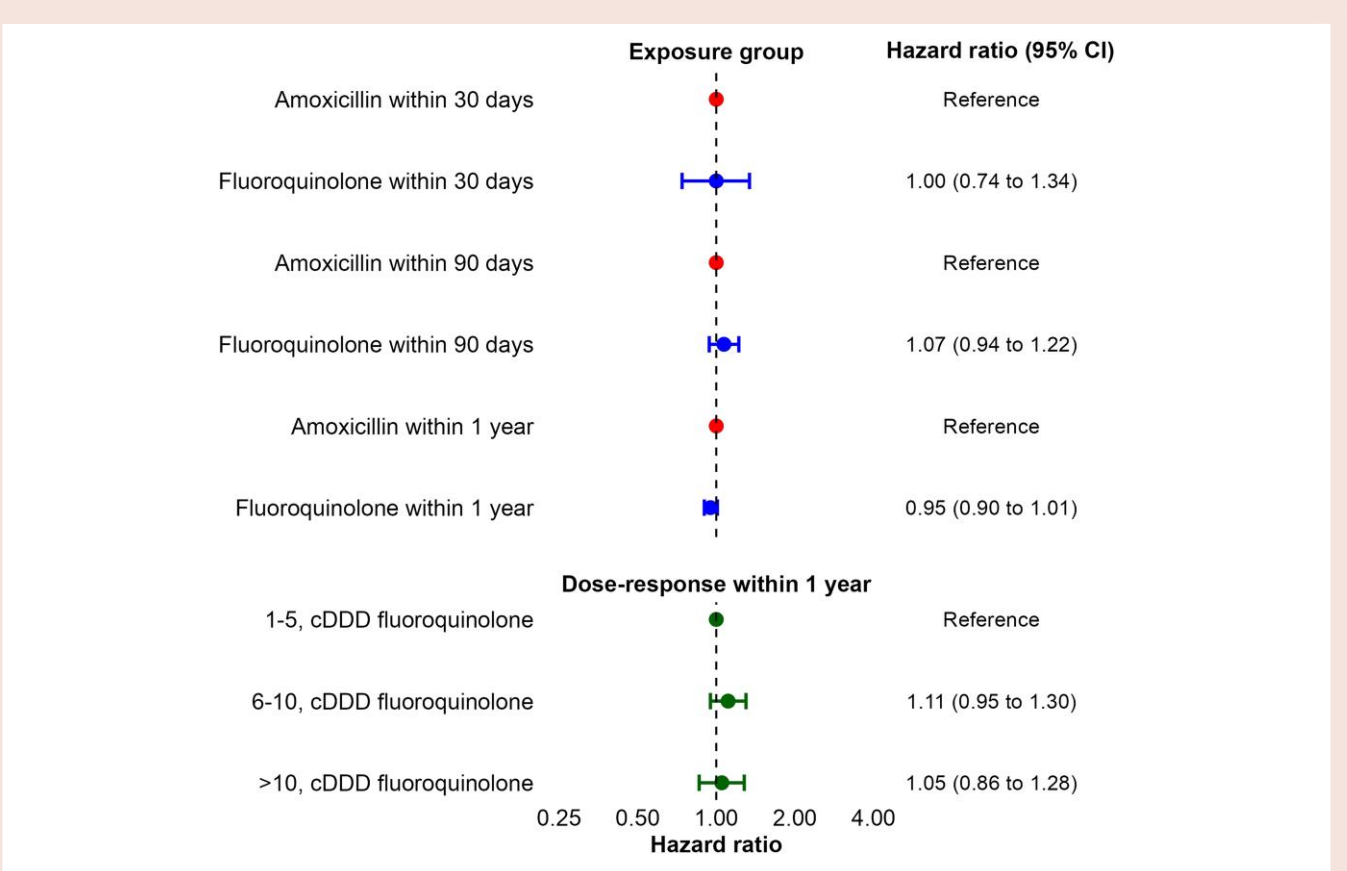


Figure 1 Risk of first-time aortic aneurysm/aortic dissection (AA/AD) by antibiotic use in the main population. Forest plot depicting the association between fluoroquinolone (FQ) use and first-time AA/AD as well as cumulative defined daily doses (cDDD) of FQ and the rates of AA/AD in the main population. The *x*-axis depicting the hazard ratios with 95% confidence intervals and the *y*-axis exposure groups of claimed FQ prescriptions.

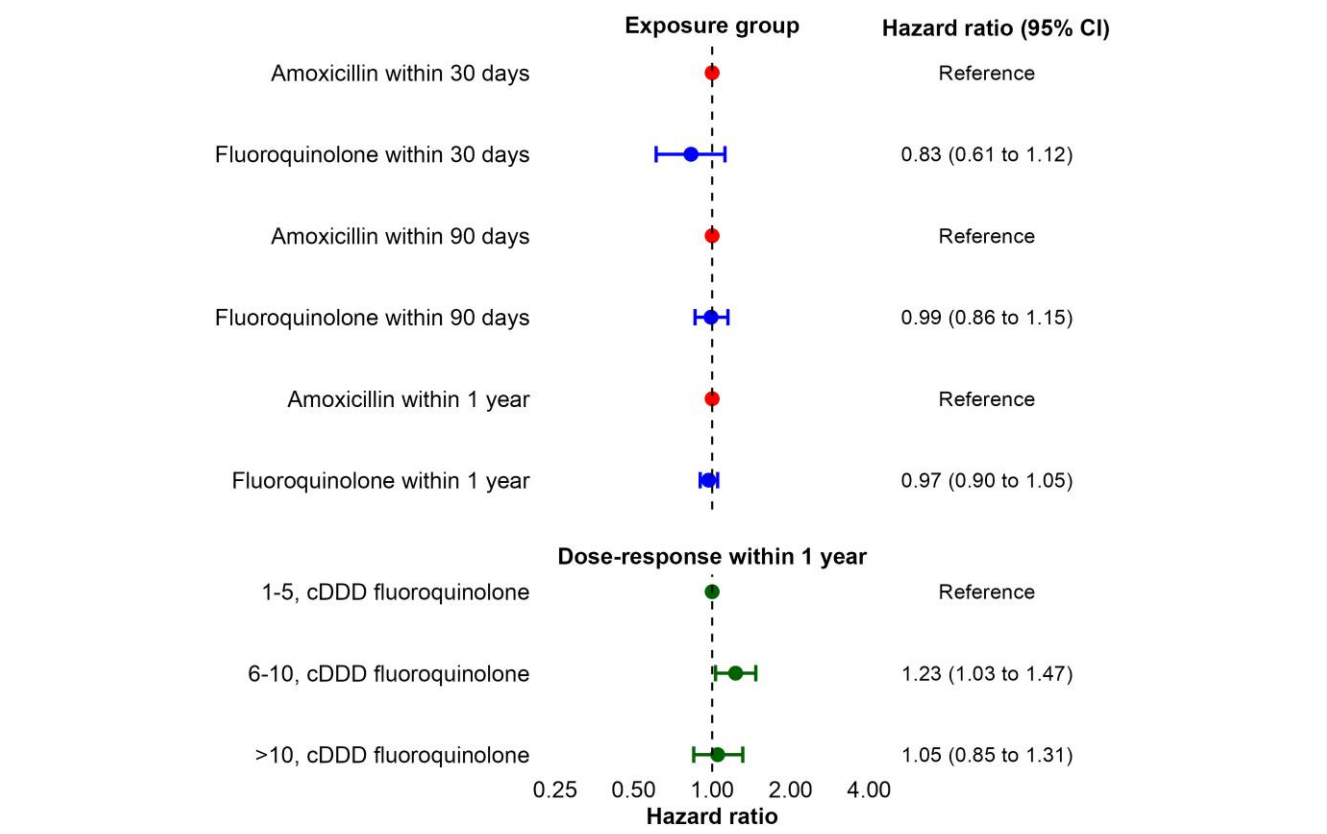


Figure 2 Risk of first-time aortic aneurysm/aortic dissection (AA/AD) by antibiotic use in high-risk nested patients. Forest plot depicting the association between FQ use and first-time AA/AD as well as cumulative defined daily doses (cDDD) of FQ and the rates of AA/AD in a high-risk nested population. The *x*-axis depicting the hazard ratios with 95% confidence intervals and the *y*-axis exposure groups of claimed FQ prescriptions.

conducted in R (version 3.6.1) using the 'clogit' function from the 'survival' package.

Results from animal experimentations were expressed as mean ± standard error of the mean (SEM) with *n* representing the number of mice. The normality of the distribution of continuous variables was evaluated using the Shapiro–Wilk test, which confirmed a normal distribution. Additionally, an outlier analysis was conducted using the robust regression and outlier removal (ROUT) method at $Q = 1\%$; however, no outliers were identified. Statistical analyses were performed in GraphPad Prism 10.0. Statistical tests are mentioned in the figure legends. Significance was accepted at $P < 0.05$.

Results

Epidemiological analyses

The main cohort comprised 5.10 million individuals with 58 919 cases (incident AA/AD) and 1 767 510 sampled controls. In general, cardiovascular comorbidity was much more prevalent among cases than controls (see Supplementary material online, *Table S2*). For the case definition of incident AA/AD, short-term 30-day, intermediate-term 90-day, and long-term 1-year FQ exposure windows were all not associated with increased HRs (30-day HR, 1.00 [95% CI: 0.74–1.34]; 90-day HR, 1.07 [95% CI 0.94–1.22]; 1-year HR, 0.95 [95% CI 0.90–1.01]) (*Figure 1*). Increasing cumulative dose of FQ did not confer increased rates of AA/AD in a dose–response manner (1–5 cDDD: Reference group; 6–10 cDDD: HR 1.11 [95% CI: 0.95–1.30]; >10 cDDD: HR 1.05 [95% CI: 0.86–1.28]) (*Figure 1*). Using a severe case definition of ruptured AA/AD yielded comparable results with no increased rates of rupture in patients exposed to FQ irrespective of the exposure window as well as increasing cumulative dose (see Supplementary material online, *Figure S1*).

The secondary cohort consisting of high-risk patients comprised 990 170 patients with 28 376 cases (see Supplementary material online, *Table S3*). In this high-risk nest, FQ was not associated with increasing rates of AA/AD (30-day HR 0.83 [95% CI: 0.61–1.12]; 90-day HR 0.99 [95% CI: 0.86–1.15]; 1-year HR 0.97 [95% CI: 0.90–1.05]) (*Figure 2*).

The third cohort comprising individuals at high risk of severe aortic disease (patients with prevalent aortic conditions) included 28 489 patients with 15 363 cases of all-cause mortality and 2357 cases of aortic interventions (see Supplementary material online, *Table S4*). Exposure to FQ was not associated with increased mortality in patients with aortic disease (30-day HR, 0.98 [95% CI: 0.79–1.22]; 90-day HR, 1.06 [95% CI: 0.95–1.19]). In addition, no association between FQ and increased rates of aortic interventions, as a proxy for worsening disease, was found (*Figure 3*).

Overall, being exposed to increasing cumulative doses of FQ did not confer increasing rates of AA/AD in a dose–response manner. Having a cDDD of 6–10 was associated with a signal towards slightly increased rates whereas a cDDD >10 was not (1–5 cDDD: Reference group; 6–10 cDDD: HR, 1.23 [95% CI: 1.03–1.47]; >10 cDDD: HR, 1.05 [95% CI: 0.85–1.31]). Using a case definition of ruptured AA/AD showed overall similar results with no associations (see Supplementary material online, *Figure S2*).

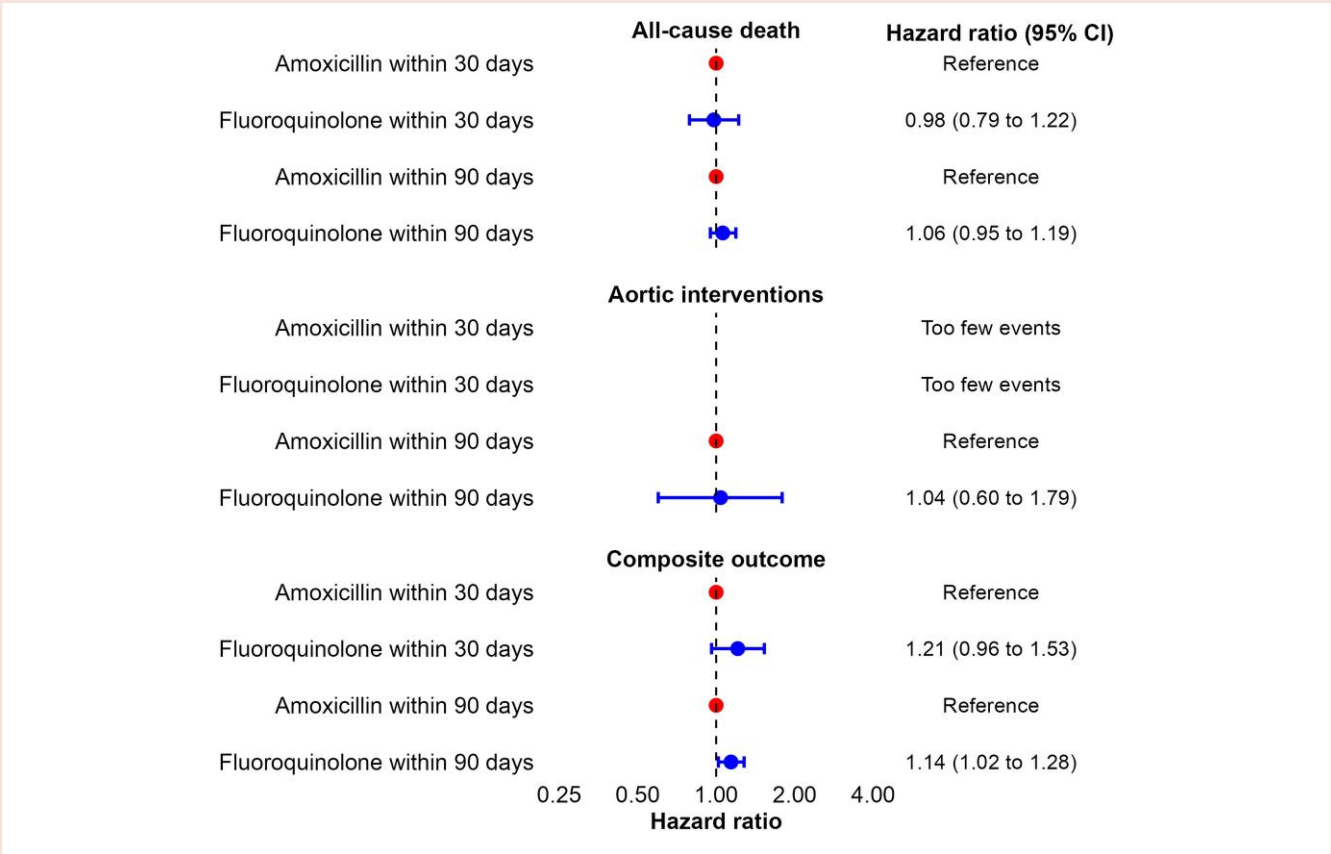


Figure 3 Risk of increased mortality and aortic interventions by antibiotic use in patients with known aortic disease. Forest plot depicting the association between FQ use all-cause death, aortic interventions as well as composite outcome. The *x*-axis depicting the hazard ratios with 95% confidence intervals and the *y*-axis exposure groups of claimed FQ prescriptions.

Animal experimentation

In vivo studies were conducted in mice to supplement the clinical findings (*Figure 4A*). Notably, clinically relevant ciprofloxacin plasma concentrations were confirmed by HPLC-DAD in satellite groups of Wild-Type (1.7 ± 0.2 µg/mL) and Hypertensive groups (2.8 ± 0.6 µg/mL). Ciprofloxacin concentration was not different between both groups ($P = 0.103$). Plasma concentrations were not evaluated in the Marfan cohort due to the limited availability of these mice. Administration of ciprofloxacin did not result in any significant changes in *in vivo* aortic diameter, a parameter of aortic dilation/aneurysm development in WT (two-way ANOVA: ciprofloxacin treatment $P = 0.63$; time value $P < 0.0001$), L-NAME-induced hypertensive (two-way ANOVA: ciprofloxacin treatment $P = 0.09$; time value $P < 0.0001$), as well as Marfan (two-way ANOVA: ciprofloxacin treatment $P = 0.48$; time value $P = 0.0023$) mice (*Figure 4B*, Supplementary material online, *Figure S3*). Arterial stiffness measured by *in vivo* PWV (*Figure 5A*) as well as *ex vivo* arterial stiffness (*Figure 5B*) was not affected by a combined period of 4 weeks of ciprofloxacin treatment in WT, L-NAME-induced hypertensive, as well as Marfan mice. Nevertheless, the diameter of the ascending aorta exhibited significant (one-way ANOVA: animal model $P < 0.0001$) differences between animal models. This observation validates our experimental set-up, as hypertensive mice showed smaller diameters while Marfan mice exhibited larger diameters compared to WT mice. Additionally, PWV differed between models (two-way ANOVA: animal model $P = 0.0011$; ciprofloxacin treatment $P = 0.87$) as well as Ep (two-way ANOVA: animal model $P < 0.0001$; ciprofloxacin treatment $P = 0.87$). Furthermore, histological evaluation showed no ciprofloxacin effect on wall thickness or lumen diameter (H&E stain; Supplementary material online, *Figure S4*), collagen content (Picrosirius Red stain; Supplementary material online, *Figure S5*), and elastin breaks (Orcein stain; Supplementary material online, *Figure S6*).

Discussion

This study using nationwide Danish register-data did not show any convincingly persistent associations between FQ and risk of AA/AD, in neither the main or high-risk (elderly or hypertensive patients) population. Further, a sub-analysis did not discern a consistent dose–response relationship. Finally, no signs of mortality or higher aortic intervention rate were observed in FQ-treated patients with prevalent aortic disease. As such, our results contradict both older studies on this debateable topic as well as an FDA warning, yet independently support recent (2023) large-scale studies reporting a lack of association between FQ use and AA/AD risk. Recently, Huh *et al.*[9] analysed >700.000 FQ prescriptions from the Taiwanese healthcare register and reported no increased risk of AA/AD (HR:0.52–1.10) after adjusting for confounders. Similarly, Brown *et al.*[10] analysed >1.000.000 FQ prescriptions from the UK primary care records and did not observe an association of FQ-induced hospitalization with AA/AD (vs. cephalosporin odds ratio (OR): 0.87–1.27; vs. trimethoprim, 0.75–1.06; vs. co-amoxiclav, 0.82–1.18). In contrast, previous epidemiological studies did report an FQ-AA/AD association. However, methodological details should be

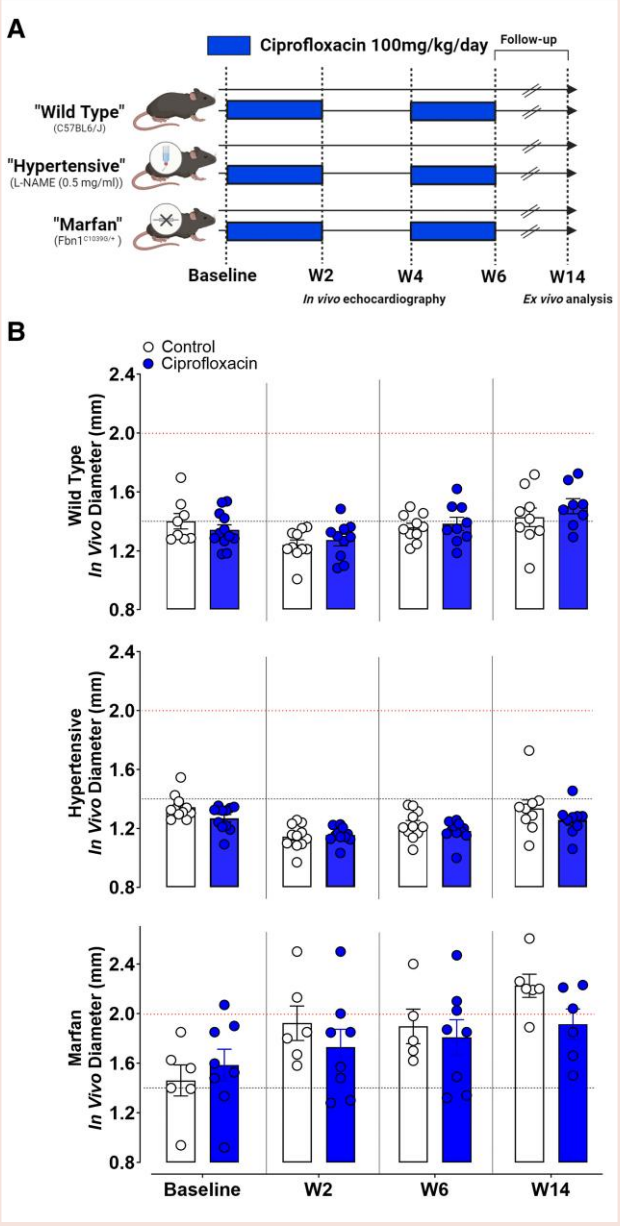


Figure 4 Aortic dilation or aneurysm formation following ciprofloxacin treatment in Wild-Type (WT), hypertensive, and genetic ($Fbn1^{C1039G/+}$) Marfan model mice. (*A*) Experimental overview. Ciprofloxacin (100 mg/kg/day) was administered for two periods of 2 weeks. The diameter of the ascending aorta was determined longitudinally by echocardiography at baseline, week 2, week 6, and week 14. Hypertension was induced by adding L-NAME (0.5 mg/mL) to the drinking water for the entire study duration. (*B*) Ascending aorta diameters were not affected by ciprofloxacin treatment *in vivo* in WT, L-NAME-induced hypertensive, as well as Marfan mice. Statistical analyses: Two-way ANOVA with Sidak *post hoc* test for multiple comparisons. $n = 10$ per group for WT and hypertensive mice, $n = 8$ per group for Marfan mice. L-NAME , Nω-Nitro-L-arginine methyl ester hydrochloride; lower dotted line, mean WT aortic diameter *in vivo*; upper dotted line, aortic aneurysm identification point, 150% aortic diameter increase from baseline WT value.

carefully considered and are summarized in Supplementary material online, *Table S5*.

It is noteworthy that a number of studies have insufficiently suitable comparators.[16] Control groups lacking antibiotic administration[26–29] differ fundamentally due to lack of infection. Infection has been suspected as a risk factor for aortic aneurysms (AA) because of bacterial invasion of the arterial wall, haemodynamic instability, and systemic inflammation, underscoring the significance of considering co-existing infections.[30–32] Moreover, patients with severe infections end up in a hospital setting and are more likely to be subjected to advanced imaging procedures which may incidentally reveal asymptomatic AA as a result of surveillance bias. Dong *et al.*[30] reported no association of FQ with the risk of AA/AD when compared to other antibiotics. In contrast, other studies using active comparators did report an association.[28,31,33–35] The choice of comparators (i.e. amoxicillin, azithromycin, and trimethoprim-sulfamethoxazole) is based on shared FDA-approved indications.[28,31,33–35] However, the heterogeneous use of antibiotics across calendar time and countries makes it difficult to choose a 'one size fits all' active comparator for cross-country/cross-study comparisons. For instance, in Denmark, FQ usage is limited to severe infections.[11] Along this line, Gopalakrishnan *et al.*[31] reported an FQ-AA/AD association when indicated for pneumonia, but not in urinary tract infections indicating possible bias due to severity or type of infection. Unfortunately, the Danish nationwide registry does not include information on the clinical nature of the infection. Information on the indication of FQ use would be available from discharge codes during hospitalizations, but this approach would introduce a bias towards patients with severe infections requiring hospital care. Consequently, patients prescribed oral antibiotics for less severe infections in primary care would not be represented. Another methodological aspect involves different study designs amongst epidemiological studies. Various study designs are employed, each exhibiting unique strengths and limitations posing a challenge for comparison of outcomes.

Interestingly, most previous studies reported increased AA/AD risk within a short period, i.e. 60–120 days. The biological feasibility of vascular events transpiring within such a short time has risen controversy as AA progresses slowly.[36] While literature commonly reports ≤90 days exposure periods, the current study incorporated claimed FQ prescriptions of up to 1 year prior to the event date, with no increased signal being reported at 1 year. Further, the current work investigated AA/AD risk with prolonged or repetitive FQ exposure by calculating cDDD. Consistently, no dose–response relationship was detected, although cDDD may not precisely reflect individual dosages and adherence.

Another strength of the current analyses is the sub-analyses of a high-risk (cf. FDA warning) and prevalent aortic disease cohort.[6] The lack of association between FQ and AA/AD in high-risk cohorts provide assurance on the safety of FQ. Similarly, our series of experiments in mouse models failed to demonstrate a direct link of ciprofloxacin exposure to aortic dilation or aneurysms. Mice were treated with ciprofloxacin, the most commonly prescribed FQ, accounting for 85% of all prescriptions and 88% of all FQ case-reported AA episodes.[35] Considering the higher incidence of AA formation in male patients and mouse models,[37,38] male mice were exclusively used to enhance the probability of detecting an effect. While independently designed, in retrospect the study designs largely paralleled the clinical cohorts. Despite reaching clinically relevant exposures (2.3 ± 0.4 µg/mL), no aortic dilation by ciprofloxacin was detected. Measurement of aortic diameter *in vivo* by echocardiography presents an established marker of AA/AD, both in patients as well as mouse models.[8,39] Additionally, arterial stiffness, as determined by PWV, is an early indicator of arterial remodelling and is known to correlate with the development of AA,

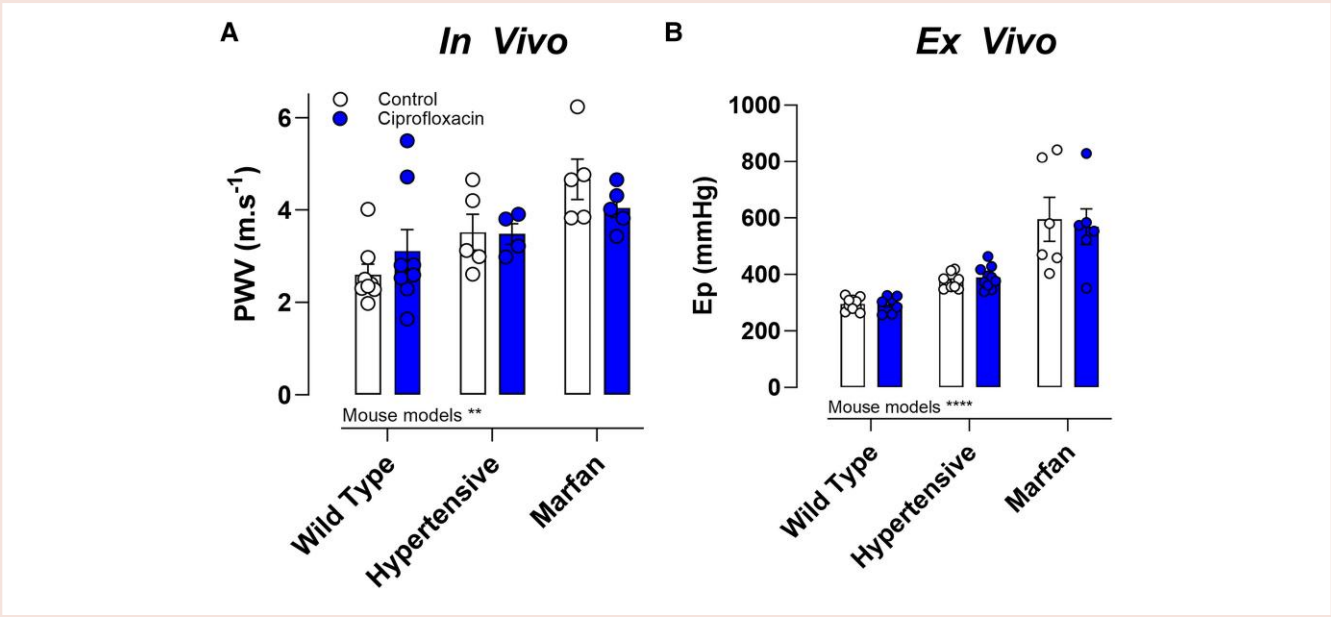


Figure 5 Arterial stiffness *in vivo* and *ex vivo* following ciprofloxacin treatment in wild-type (WT), hypertensive, and Marfan model mice. No changes in (*A*) *in vivo* or (*B*) *ex vivo* arterial stiffness, Ep (80–120 mmHg) were reported after a combined period of 4 weeks of ciprofloxacin treatment in WT, hypertensive, and Marfan mice. Significant differences between mouse models were detected. Statistical analyses: Two-way ANOVA with Sidak *post hoc* test for multiple comparisons. $n = 10$ per group for WT and L-NAME mice, $n = 8$ per group for Marfan mice, ** $P < 0.01$; **** $P < 0.0001$. PWV, pulse wave velocity; Ep , Petersons elastin modulus.

both in patients and in mouse models.[40–42] Furthermore, arterial stiffness is associated with a higher incidence of AA in patient populations.[43–45] Interestingly, the Marfan model did show considerably higher PWV values illustrating the validity of the model, as well as the sensitivity of PWV as a proxy of arterial remodelling. Our experimental results contrast with previous literature by Le Maire *et al*. reporting ciprofloxacin-induced aortic dilation and aneurysms in genetic ($Fbn1^{C1039G/+}$) Marfan mice, as well as in an experimental chronic angiotensin-II induced aneurysm model.[7,8] Importantly, both studies used a 4-week continuous ciprofloxacin dosing protocol,[7,8] while the current study used a 2-week on, 2-week off protocol, repeated twice. The latter mimics clinical regimens, as ciprofloxacin is typically administered orally for 7 days, with a prolonged course of 14 days if bacterial infection persists.[46,47] The importance of treatment duration was also demonstrated by Çulpan *et al*.[48] who reported that a 4-week course of ciprofloxacin was associated with AA/AD in a $CaCl_2$-induced rat model, while 2-week treatment did not lead to aortic dilation.

Limitations

Both epidemiological and experimental approaches have a number of shortcomings. Ideally, the association between FQ use and AA/AD is investigated in a randomized controlled trial though the low incidence of AA/AD would require an unrealistic large sample size.[49] Prospective studies employing advanced imaging techniques, such as echocardiography for aortic diameter, PWV, or vascular deformation mapping, may offer more practical and sensitive alternatives.[42] Such studies could address key limitations of observational data, including biases from heterogeneity in FQ indications, and challenges of accurately estimating FQ exposure and infection severity.[31] Remarkably, cases showed higher cardiovascular comorbidity despite a balanced matching procedure. However, this would likely draw the results towards an association between FQ and aortic disease, which is the opposite of our observed lack of association.

Conclusion

The current study failed to identify an association between FQ use and the risk of AA/AD, even among high-risk populations or individuals with pre-existing aortic disease. Further, no aortic dilation was noticed upon 2-week ciprofloxacin treatment of different mouse models. Overall, our results contradict previous reports as well as an FDA warning on this topic, yet we identified important experimental variables, such as the challenge of selecting an appropriate active comparator group (epidemiological studies) or clinically relevant treatment protocol (animal experimentation). Overall, our study corroborates two recent large-scale studies[9,10] that also reported conventional FQ use should be considered safe from an AA/AD risk perspective.

Lead author biography

Callan Wesley earned a PhD in Medical Science at the University of Antwerp, specializing in arterial stiffness and its association with cardiovascular disease. His research focuses on understanding the mechanisms behind arterial biomechanic changes and their role in cardiovascular health, with a specific investigation into arterial stiffness within the field of safety pharmacology.

Data availability

Danish data protection laws prohibit sharing of the data used for this study.

Supplementary material

Supplementary material is available at *European Heart Journal Open* online.

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