Appendix 11 (as supplied by the authors): Summary of nine published studies describing cardiovascular events and mortality associated with macrolide use compared with other classes of antibiotics or non-use for the treatment of respiratory tract infections

Author	Study Type/Patient Description	Observations	Putative Biological Mechanism of Action	Potential Risk Factors	Dose/Exposure Time/ Study Limitations	Quality Score*
Randomized Controlled Trials						
Jespersen *et al.*, 2006[1]	-Five Copenhagen University cardiology departments and a coordinating centre -2,172 participants who had a discharge diagnosis of MI or angina pectoris from 1993 to 1999 randomized to clarithromycin (mean age 65.4 years) and 2,200 to placebo (mean age 65.2 years)	-Clarithromycin group: *n* = 2,172; placebo group: *n* = 2,200 -No significant effects of clarithromycin on the primary outcome (344 [15.8%] vs. 307 [13.8%], HR 1.15, 95% CI 0.99-1.34) or secondary outcome (249 [11.5%] vs. 218 [9.9%], HR 1.17, 95% CI 0.98-1.40) -All-cause mortality (212 [9.8%] vs. 172 [7.8%], HR 1.27, 95% CI 1.03-1.54) and CV mortality (111 [5.1%] vs. 78 [3.5%], HR 1.45, 95% CI 1.09-1.92) were significantly higher in the clarithromycin arm	-Macrolide antibiotics are anti-inflammatory and eradicate *Chlamydia pneumoniae* from atherosclerotic plaques	-Age, sex, previous CV disease and risk factors, smoking status, concurrent use of certain drugs	-Two-week treatment with clarithromycin 500 mg/day or matching placebo -Limitation: more smokers were randomized to the clarithromycin arm -Primary outcome: composite of all-cause mortality, MI, or unstable angina pectoris during three years' follow-up; Secondary outcome: composite of CV mortality, MI, or unstable angina pectoris	24
Population-Based Studies						
Asadi *et al.*, 2012[2]	-Population-based prospective cohort study of 2,779 outpatients with	-Macrolide group: *n* = 1,832; fluoroquinolone group: *n* = 947 -30-day	-Immunomodulatory effects mediated by several macrolide properties: decreases in pro-	-Age, sex, clinical radiographic severity of illness at presentati	-10 days of any one of the following: doxycycline 200 mg initially then 100 mg/day,	20

	community-acquired pneumonia assessed at 7 Emergency Departments in Edmonton, Alberta, Canada prescribed macrolide (mean age 46.1 years) or fluoroquinolone (mean age 61.9) enrolled from 2000 to 2002 and followed until 2007	mortality was significantly lower in the macrolide group relative to the fluoroquinolone group (4 [0.2%] vs. 25 [3%], adjusted OR 0.28, 95% CI 0.09-0.86)	inflammatory cytokines (TNF-α, IL-1, IL-6, IL-8 and IFN-γ), increases in anti-inflammatory cytokines, and decreases in neutrophil chemotaxis, leukocyte adhesion, and oxidative metabolism	on, nursing home status	levofloxacin 500 mg/day, azithromycin 500 mg initially then 250 mg/day, clarithromycin 500 mg/day or erythromycin 500 mg/day -Limitations: confounding by indication, physician bias, no post-discharge microbiologic data available, generalizability	
Mortensen *et al.*, 2014[3]	-Retrospective cohort study of VA data comparing 31,863 patients hospitalized with pneumonia from 2002 to 2012 prescribed azithromycin (mean age 77.8 years) to 31,863 patients who received other guideline-concordant antibiotics (mean age 77.8 years)	-Azithromycin users: *n* = 31,863; non-users: *n* = 31,863 -90-day mortality significantly lower in the azithromycin group (17.4% vs. 22.3%, OR 0.73, 95% CI 0.70-0.76) -30-day mortality significantly lower in the azithromycin group (OR 0.76, 95% CI 0.73-0.80) -Significantly increased odds of 90-day MI (5.1% vs. 4.4%, OR 1.17, 95% CI 1.08-1.25) but not any cardiac event (43.0% vs. 42.7%, OR 1.01, 95% CI 0.98-1.05), cardiac arrhythmias	-Plausible anti-inflammatory effects (immunomodulatory agent)	-Tobacco use, alcohol use, liver disease, multiple classes of medications, SES, race	-At least 1 outpatient medication from a VA pharmacy within 90 days prior to admission; at least 1 dose of antimicrobial therapy within the first 48 hours of admission -Limitations: few female patients, only patients 65 and older, reliance on ICD-9 diagnosis of CV events rather than clinical information, ICD-9 codes not validated in VA administration data, undetermined duration of azithromycin therapy	18

		(25.8% vs. 26.0%, OR 0.99, 95% CI 0.95-1.02) or heart failure (26.3% vs. 26.2%, OR 1.01, 95% CI 0.97-1.04)				
Rao *et al.*, 2014[4]	-Retrospective cohort study of US veterans (mean age 56.5 years) who received an outpatient prescription of either amoxicillin (*n* = 979,380), azithromycin (*n* = 594,792) or levofloxacin (*n* = 201,798) between September 1999 and April 2012	-During treatment days 1 to 5, patients receiving azithromycin had significantly increased risk of death (HR 1.48, 95% CI 1.05-2.09) and serious arrhythmia (HR 1.77, 95% CI 1.20-2.62) compared with patients receiving amoxicillin -On treatment days 6 to 10, when comparing azithromycin with amoxicillin, risks were not statistically different for death from any cause (HR 1.14, 95% CI 0.81-1.62) and arrhythmia (HR 1.37, 95% CI 0.91-2.05)	-Increases cardiac arrhythmogenic risks, including QT interval prolongation, torsades de pointes, and polymorphic ventricular tachycardia	-Race, age, sex, indication for antibiotics cardiac morbiditie s, laboratory findings, medicatio n	-Follow-up times were separated into the first 5 days and days 6 through 10 after antibiotics were dispensed, with day 1 being the first day the drug was dispensed -Azithromycin, levofloxacin, or amoxicillin (including amoxicillin with clavulanate potassium) within 30 days after a VA outpatient visit -Limitations: residual confounding, baseline differences between antibiotic groups, exclusion criteria	18
Ray *et al.*, 2004[5]	-Retrospective cohort study of Tennessee Medicaid patients between January 1988 and December 1993 -5,305 person-	-Rate of sudden death from cardiac causes was twice as high among current users of erythromycin compared to non-users (10	-Prolongs QT interval and increases the risk of TdP	-Smoking, higher BMI, high consumpti on of saturated fats, lack of physical activity,	-Current use: days of supply from the day the prescription was filled; non-use: no use within the previous 365 days; former	19

	years of current use of erythromycin (mean age 41.4 years) and 111,779 person-years of former use (mean age 42.2 years) compared to 1,126,013 person-years of non-use (mean age 45.0 years) and 6,846 person-years of current use of amoxicillin (mean age 41.7 years)	vs. 1,358 deaths, adjusted incidence-rate ratio 2.01, 95% CI 1.08-3.75) -No significant increase for former users of erythromycin (100 vs. 1,358 deaths, adjusted incidence-rate ratio 0.89, 95% CI 0.72-1.09) or current users of amoxicillin (8 vs. 1,358 deaths, adjusted incidence-rate ratio 1.18, 95% CI 0.59-2.36)		pre-existing CV disease (heart failure, angina and MI), concurrent use of CYP314 inhibitors	use: some use of a study medication that was not current but had occurred within the previous 365 days. -Limitations: drug compliance, no information on a number of behavioural risk factors	
Ray *et al.*, 2012[6]	-Retrospective cohort study of Tennessee Medicaid patients between 1992 and 2006 comparing patients who took azithromycin (347,795 prescriptions, mean age 48.6 years), no antibiotics (1,391,180 prescriptions, mean age 48.6 years), amoxicillin (1,348,672 prescriptions, mean age 47.7 years), ciprofloxacin (264,626 prescriptions, mean age 50.5 years) or levofloxacin	-Increased risk of 5-day CV death (HR 2.88, 95% CI 1.79-4.63) and death from any cause (HR 1.85, 95% CI 1.25-2.75) from azithromycin (adjusted) relative to no antibiotics (adjusted) -Relative to amoxicillin (unadjusted), azithromycin (adjusted) was associated with an increased risk of CV death (HR 2.49, 95% CI 1.38-4.50) and death from any cause (HR 2.02, 95% CI 1.24-3.30) -Among	-Proarrhythmic effects	-CV disease and other behavioural risk factors associated with CV disease, indication for antibiotic therapy	-Duration of treatment: 5-day period generally recommended for azithromycin and 10-day period most commonly suggested for other study antibiotics -Limitation: misclassification	19

	(193,906 prescriptions, mean age 51.5 years)	patients who took azithromycin, there were 29 CV deaths during the 5-day course of treatment (85.2 per 1 million courses); for those not taking antibiotics, there were 41 CV deaths (29.8 per 1 million periods); for the amoxicillin group, there were 42 CV deaths (31.5 per 1 million courses) -Risk of CV death significantly greater with a 5-day course of azithromycin (adjusted) than with the first 5 days of a course of ciprofloxacin (unadjusted HR 3.49, 95% CI 1.32-9.26) but not significantly different from levofloxacin (unadjusted HR 1.27, 95% CI 0.66-2.47)				
Schembri *et al.*, 2013[7]	-Secondary analysis of a prospectively collected dataset of 1,631 patients admitted to NHS Lothian Hospitals in Edinburgh, UK	-Clarithromycin users: $n = 980$; non-macrolide users: $n = 651$ -Significant association between clarithromycin use and CV	-Short-term events may be associated with clarithromycin's pro-arrhythmic effects mediated through prolongation of the QT interval -Long-term events	-Age, history of CV events	-Macrolide users included all patients who received at least one dose of clarithromycin during their hospital admission -Duration of	16

	with radiologically confirmed community acquired pneumonia and prescribed clarithromycin (median age 65 years) compared with patients who received non-macrolide antibiotics (median age 68 years) during their admission between 2005 and 2009	events compared with non-macrolide users after propensity matching (123 [12.6%] vs. 48 [7.4%], HR 1.58, 95% CI 1.08-2.30) -Clarithromycin use was not associated with a significant difference in all-cause mortality (adjusted HR 1.13, 95% CI 0.85-1.51) or CV mortality (adjusted HR 1.58, 95% CI 0.93-2.71)	after cessation of clarithromycin support an ischemic mechanism (e.g. clarithromycin may activate macrophages leading to an inflammatory cascade resulting in more vulnerable plaques that over time may lead to acute coronary syndrome or sudden cardiac death by plaque rupture)		use: < 3 days, 3-6 days, 7 days, > 7 days -1-year follow-up -Limitations: bias due to unrecorded factors, patients with more severe illness were more likely to be prescribed clarithromycin and thus clarithromycin may be a marker for more severe infection and hence increased CV events	
Svanström *et al.*, 2013[8]	-Retrospective cohort study of Danish adults comparing 1,102,050 episodes of azithromycin use (mean age 39.7 years) with no use of antibiotic (mean age 39.5 years) matched in a 1:1 ratio and comparing 1,102,419 episodes of azithromycin use (mean age 39.7 years) with 7,364,292 episodes of penicillin V use (mean age 42.0 years) between 1997 and 2010	-With propensity score matched analysis, risk of death from CV causes significantly increased with current use of azithromycin as compared with no use of antibiotics (rate ratio 2.85, 95% CI 1.13-7.24) -With adjustment for propensity scores, current azithromycin use was not associated with an increased risk of CV death when compared with penicillin V (rate ratio 0.93, 95% CI 0.56-1.55)	-Proarrhythmic effect as evidenced by other macrolides known to prolong QT interval	-Age, sex, history of CV disease	-Current use (1-5 days), recent use (6-10 days) and past use (11-35 days) -Limitations: no information on the indication for treatment and several known risk factors (e.g. smoking and BMI), primary definition, including all CV causes of death, was broad	20

Svanström *et al.*, 2014[9]	-Retrospective cohort study of Danish adults who received prescriptions for clarithromycin (*n* = 108,767, mean age 57.2 years), roxithromycin (*n* = 350,575, mean age 56.6 years) or penicillin V (*n* = 1,519,324, mean age 55.7 years)	-Compared with use of penicillin V (235 deaths, incidence rate 2.5 per 1,000 person years), use of clarithromycin was associated with a significantly increased risk of cardiac death (18 deaths, incidence rate 5.3 per 1,000 person years, adjusted rate ratio 1.76, 95% CI 1.08-2.85) but use of roxithromycin was not (32 deaths, incidence rate 2.5 per 1,000 person years, adjusted rate ratio 1.04, 95% CI 0.72-1.51)	-Interference with the delayed rectifier potassium current (I_{Kr}), which results in accumulation of potassium ions in cardiac myocytes and thereby delays cardiac repolarisation	-Sex, age, cardiac risk score, concomitant use of cytochrome P450 3A inhibiting drugs	-Treatment: 7-day course of antibiotic -Limitations: lack of information on lifestyle and health factors known to influence the risk of cardiac death (e.g. smoking and BMI), missing infection information, limited power to detect difference in subgroup analyses	16

Abbreviations: BMI = body mass index, CI = confidence interval, CV = cardiovascular, HR = hazard ratio, ICD-9 = International Classification of Diseases, Ninth Revision, IL = interleukin, MI = myocardial infarction, NHS = National Health Service, OR = odds ratio, SES = socioeconomic status, TdP = torsades de pointes, TNF = tumor necrosis factor, VA = Department of Veterans Affairs

*We evaluated the quality of individual studies using the Downs and Black quality assessment method, which is a list of 27 criteria to evaluate both randomized and non-randomized trials (Appendix 12)[10]. This scale assesses the completeness and clarity of study reporting, external validity, internal validity (e.g. bias and confounding) and power. The tool was modified slightly for use in our review. Specifically, the scoring for question 27 dealing with statistical power was simplified to a choice of awarding either 1 or 0 points depending on whether there was sufficient power to detect a clinically important effect. On the modified scale, we gave all included studies a score from 0 to 28, grouped into the following four quality levels: excellent (26 to 28), good (20 to 25), fair (15 to 19), and poor (less than 14).

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