UPPER GASTROINTESTINAL HEMORRHAGE DURING WARFARIN THERAPY ASSOCIATED WITH CO-TRIMOXAZOLE AND OTHER URINARY TRACT ANTI-INFECTIVES

by

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ABSTRACT

Some antibiotics, including co-trimoxazole, inhibit warfarin’s metabolism and may be associated with greater risk of hemorrhage in warfarin-treated patients. This thesis examines the risk of upper gastrointestinal (UGI) hemorrhage in older patients receiving warfarin and antibiotics commonly used to treat urinary tract infections, with a focus on co-trimoxazole. This population-based nested case-control study using Ontario healthcare databases, identified residents aged 66 years or older continuously on warfarin (n=134,637). Cases were those hospitalized with UGI hemorrhage (n=2151). Cases were nearly 4 times more likely than controls to have recently received co-trimoxazole (adjusted odds ratio (OR), 3.84; 95% confidence interval (CI) 2.33 – 6.33). Ciprofloxacin was also associated with an increased risk (adjusted OR 1.94; 95% CI 1.28 – 2.95), but no significant association was observed with amoxicillin, ampicillin, nitrofurantoin or norfloxacin. Among older patients receiving warfarin, co-trimoxazole is associated with a significantly higher risk of UGI hemorrhage than other commonly used antibiotics.
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1.1 Rationale

Warfarin is the oral anticoagulant of choice in North America,\(^1\) with over 30 million outpatient prescriptions in the United States in 2004.\(^3\) Warfarin has a narrow therapeutic index and is prone to multiple drug interactions.\(^4\)–\(^7\) Some drugs, among them antibiotics, can increase the risk of hemorrhage during warfarin therapy by inhibiting cytochrome P450 (CYP) isoenzyme 2C9, which is responsible for metabolizing the (S)-enantiomer of warfarin.\(^5\)–\(^7\) One of these is co-trimoxazole (trimethoprim-sulfamethoxazole), a widely used antibiotic often used to treat urinary tract infections.\(^5\)–\(^9\)

Few observational studies have examined the clinical consequences of co-trimoxazole use in patients receiving warfarin, and these studies did not focus on antibiotics which are indicated for urinary tract infection (UTI).\(^10\)–\(^11\) Because warfarin is commonly co-prescribed with antibiotics used to treat UTIs and UTI is very common among older adults\(^12\)–\(^13\), we sought to characterize the risk of upper gastrointestinal (UGI) hemorrhage associated with the use of these antibiotics in patients receiving warfarin, with a primary focus on co-trimoxazole (TMP/SMX). We hypothesized that TMP/SMX would be associated with a greater risk of UGI hemorrhage than other antibiotics that are also indicated for UTI (amoxicillin, ampicillin, ciprofloxacin, norfloxacin, and nitrofurantoin), which are not considered to have such a strong drug interaction with warfarin.

It is important to evaluate drug-drug interactions in the elderly in a “real world” setting since older adults are generally prone to polypharmacy.\(^6\) In addition, many
warfarin drug interaction studies were performed at a time when the Food and Drug Administration did not require the inclusion of elderly patients in clinical trials and healthy volunteers data is probably not representative of the real world patient population.\textsuperscript{2,6} Randomized controlled trials (RCTs) generally exclude high-risk patients and have better monitoring and management of anticoagulation compared to usual clinical practice.\textsuperscript{2} As well, RCTs often do not have enough power to reliably examine drug-drug interactions.

1.2 Research question

Among Ontario patients 66 years and older who are continuously treated with warfarin, are those with a hospital admission for upper gastrointestinal bleeding more likely to have been exposed to TMP/SMX as compared to other antibiotics commonly used for UTI (amoxicillin, ampicillin, ciprofloxacin, norfloxacin, or nitrofurantoin), than those who did not bleed?

1.3 Hypothesis

We hypothesized that in older adults continuously treated with warfarin, co-prescribing of TMP/SMX would be associated with the greatest increased risk of UGI hemorrhage relative to other commonly used antibiotics used to treat UTI. We hypothesized that amoxicillin/ampicillin, nitrofurantoin and norfloxacin would be associated with the least risk and that ciprofloxacin would be associated with some increased risk of UGI bleeding, but less than TMP/SMX.\textsuperscript{4}
BACKGROUND

2.1 Warfarin – clinical use and challenges

2.1.1 Clinical use of warfarin

After more than half a century, warfarin sodium is still the anticoagulant of choice in North America and worldwide.\(^1,2,14\) Warfarin is commonly used for the prevention and/or treatment of various thromboembolic conditions, such as deep vein thrombosis, pulmonary embolism, and complications associated with atrial fibrillation, mechanical heart valves, and myocardial infarction.\(^2\) Being the mainstream therapy for such a large range of indications, it is not surprising that warfarin is a widely used drug with increasing prescriptions over time.\(^3,15\) A cross-sectional time-series analysis done on Ontario data showed that the prescription rate nearly doubled between 1997 and 2002 and close to 37,000 patients were treated with warfarin in 2002.\(^15\) Similar trends have been observed in the United States where outpatient prescriptions for warfarin have increased 1.45 fold from 1998 to 2004, reaching over 30 million yearly prescriptions in 2004.\(^3\) A more recent nationally representative survey of community-dwelling adults aged 57 – 84 years in the U.S. in 2005-2006, which used in-home interviews and medication logs, estimated that 4.4% (95% confidence interval 3.7 – 5.1) of people used warfarin.\(^16\)

Warfarin’s mechanism of action is through interference with the synthesis of the vitamin K-dependent coagulation factors II (prothrombin), VII, IX, and X.\(^1\) Warfarin anticoagulation is monitored using the international normalized ratio (INR), a standardized test to measure prothrombin time.
2.1.2 Warfarin pharmacokinetics and pharmacodynamics

Warfarin is well absorbed through the gastrointestinal tract and maximum plasma concentrations are reached within 2 hours.\(^1,\)\(^17\) Warfarin is mostly protein bound (approximately 99\%) and has a half-life, \(t_{1/2}\), of about 36 - 42 hours.\(^1,\)\(^2\) Warfarin exists as two enantiomeric forms ((S)-warfarin and (R)-warfarin) and is clinically only available in a racemic mixture of these two optically active isomers.\(^1,\)\(^2\) The two enantiomers are almost entirely metabolised in the liver, by 2 different pathways.\(^2\) (S)-warfarin is approximately 3-5 times more potent than (R)-warfarin and is metabolized principally by CYP 2C9.\(^2,\)\(^18\) (R)-warfarin is metabolized predominantly by CYP 1A2 and 3A4.\(^2\) Elderly patients may require decreased doses of warfarin, however pharmacokinetic changes are negligible and cannot explain this increased sensitivity of older adults to warfarin.\(^17\)

CYP450 enzymes are bound to membranes inside cells and their heme pigment absorbs light at a wavelength of 450nm when in contact with carbon monoxide.\(^19\) There are more than 50 CYP450 enzymes, however fewer than ten, among them CYP 2C9, metabolize 90\% of drugs.\(^19,\)\(^20\) Important drug interactions with warfarin work through CYP 2C9 induction, which increases warfarin clearance or through CYP 2C9 inhibition, which decreases warfarin metabolism.\(^7,\)\(^14\) Drugs that induce the expression of CYP 2C9 reduce the antithrombotic effect of warfarin (e.g. rifampin, phenytoin, phenobarbital).\(^5,\)\(^7\) Drugs that inhibit CYP 2C9 are clinically important because they inhibit the metabolism of the more potent (S)-warfarin and thus increase warfarin’s anticoagulation effect, and concomitantly increase the INR.\(^6,\)\(^20\) Drugs that fall into this category include amiodarone, metronidazole, phenylbutazone, fluvoxamine, TMP/SMX and others.\(^5,\)\(^7,\)\(^20\)
Genetic polymorphism that occurs in CYP 2C9 can impact the required maintenance dose of warfarin since it metabolizes (S)-warfarin.\textsuperscript{19,20} There are two common allelic variants (CYP2C9*2 and CYP2C9*3) that are associated with a significant reduced activity of CYP 2C9.\textsuperscript{20,21} Patients who are heterozygous for the variant enzyme will require a lower maintenance dose of warfarin and those who are homozygous require even smaller doses.\textsuperscript{20,21}

Warfarin inhibits vitamin K-dependent carboxylation of coagulation factors II, VII, IX, and X through the inhibition of vitamin K epoxide reductase (VKOR) enzyme.\textsuperscript{2,21} The gene that encodes this enzyme is vitamin K epoxide reductase complex subunit 1 (VKORC1).\textsuperscript{2,21} Polymorphism in the VKORC1 gene can affect the pharmacodynamics of warfarin since it leads to varying sensitivity of the enzyme to warfarin.\textsuperscript{2,21} Mutations in VKORC1 may be the reason for hereditary resistance to warfarin in some individuals and may account for some of the variable dose requirements in patients.\textsuperscript{2,21,22}

\section*{2.1.3 Clinical challenges of warfarin}

Warfarin has a narrow therapeutic index, and the response to warfarin is influenced by pharmacogenetic and pharmacokinetic polymorphisms, vitamin K status, and multiple drug interactions.\textsuperscript{2,4-7,22} Warfarin’s laboratory monitoring is difficult to standardize and maintenance of therapeutic levels requires an in-depth understanding of warfarin and good clinician-patient communication.\textsuperscript{2} In addition, patients need to understand and comply with the drug regimen and consequently, safe and effective treatment with warfarin poses a challenge in clinical practice.
The main potential adverse effect of warfarin is bleeding. Bleeding complications most often involve the gastrointestinal tract, soft tissue and the urinary tract.\textsuperscript{23} Various factors may contribute to the risk of bleeding in warfarin-treated patients, including the intensity of anticoagulation therapy (generally based on clinical indication), patient characteristics, concomitant use of potentially interacting drugs, and the length of time since the initiation of anticoagulation therapy.\textsuperscript{24} The risk of bleeding is the highest at the outset of warfarin therapy.\textsuperscript{23,24}

There is wide variability in the range of the reported frequency of bleeding events while on warfarin therapy.\textsuperscript{23} One reason for the differences in bleeding statistics is that clinical randomized trials generally report fewer events than observational studies (randomized trials may report up to less than half of the frequency).\textsuperscript{23,24} The rationale being that clinical trials generally include healthier patients and warfarin therapy is monitored more closely.\textsuperscript{23,24} A recent review reported that vitamin K antagonists in clinical studies increased the risk of major bleeding by 0.3-0.5%/year compared to controls.\textsuperscript{24} A literature review of 21 randomized and 4 observational studies of patients on warfarin therapy for various indications summarized that the average frequency of fatal and major bleeding was 0.6% and 3.0%, respectively.\textsuperscript{23} Another review article described an incidence of major bleeding in the range of 0% - 16%.\textsuperscript{3,25} A study focusing on elderly patients with atrial fibrillation during the first year of warfarin therapy found that the cumulative incidence of major hemorrhage for patients aged 80 years and older was 13.1 per 100 person-years and for patients 65 -80 years of age, it was 4.7 per 100 person years.\textsuperscript{26}
Warfarin is still one of the top ten drugs in the FDA’s Adverse Event Reporting System (AERS), with bleeding being the main adverse event. The AERS has received hundreds of reports of warfarin-suspected cases of bleeding annually, with a majority of cases having a serious outcome. Warfarin is one of the leading medications linked to emergency department visits for adverse drug events in older adults. Based on 2004-2005 data from the U.S. National Electronic Injury Surveillance System-Cooperative Adverse Drug Event Surveillance System (Centers for Disease Control and Prevention), it was estimated that warfarin accounts for approximately 17% of emergency department visits for adverse drug events in patients 65 years or older. In this study, more than 70% involved clinically evident bleeding and over 40% required hospitalization.

2.1.4 Mechanisms of warfarin drug interactions

Numerous drugs can affect warfarin’s dose response by altering warfarin’s pharmacokinetics, and pharmacodynamics. Warfarin drug interactions that may increase the risk of bleeding can be categorized according to five major mechanisms:

- interference with platelet function (e.g. acetylsalicylic acid, clopidogrel, and selective serotonin reuptake inhibitors);
- injury to the gastrointestinal mucosa (e.g., selective and non-selective non-steroidal anti-inflammatory drugs);
- reduced synthesis of vitamin K by the intestinal flora (many antibiotics);
- interruption of the vitamin K cycle (e.g., acetaminophen); and
interference with warfarin metabolism by inhibiting or inducing the CYP 2C9 isoenzyme.\textsuperscript{2,5,7}

\subsection*{2.2 Warfarin, TMP/SMX and UTI}

\subsection*{2.2.1 Mechanisms of antibiotics and warfarin interactions}

Antibiotics potentially interact with warfarin through two different mechanisms. The more general mechanism is common to most antibiotics and works through changing the gut flora balance, therefore reducing the bacterially synthesized vitamin K\textsubscript{2} (menaquinone), which contributes to Vitamin K balance.\textsuperscript{29} The expression of this kind of interaction is variable.\textsuperscript{5} The second mechanism is through the inhibition of CYP 2C9.

This thesis focuses on the interaction between warfarin and antibiotics indicated for UTI, with a primary focus on TMP/SMX, an antibiotic which inhibits CYP 2C9.\textsuperscript{8,9,30}

\subsection*{2.2.2 Urinary tract infection in older adults}

Urinary tract infection (UTI) is the second most common infection among community-dwelling elderly and accounts for nearly 25\% of all infections.\textsuperscript{12} Data from a population-based laboratory surveillance study on community onset UTI in the Calgary Health Region showed that urinary tract infections are common in the elderly and the annual incidence of urinary tract infection increases substantially with advancing age; rising from approximately 30/1,000 in patients aged 70-79 years to about 100/1,000 in patients aged 80-89.\textsuperscript{13} Drugs commonly indicated for UTI include TMP/SMX, amoxicillin and other penicillins, fluoroquinolones, nitrofurantoin, cephalosporins, etc.\textsuperscript{31}
2.2.3  **Co-trimoxazole (trimethoprim / sulfamethoxazole)**

TMP/SMX is a combination of a medium-long acting sulphonamide (sulfamethoxazole) and a diaminopyrimidine (trimethoprim).\textsuperscript{32} TMP/SMX has been the core therapy for UTI for more than 20 years and is still one of the first line therapies, except when high resistance rates to TMP/SMX in specific communities are known.\textsuperscript{31,33} The most common adverse events of TMP/SMX include gastrointestinal upset and skin rashes, which are well-known side effects of sulphonamides.\textsuperscript{32} Serious adverse effects associated with TMP/SMX include blood and liver disorders, toxic epidermal necrolysis and Stevens-Johnson syndrome, which has led to a “serious warnings and precautions” box in the Canadian Compendium of Pharmaceuticals and Specialties and has led some jurisdictions to discourage its use.\textsuperscript{32,34}

2.2.4  **Warfarin and urinary tract anti-infectives**

UTI in older adults is more complicated since these patients frequently have various comorbidities and are exposed to polypharmacy, thus making them susceptible to drug-drug interactions.\textsuperscript{35} One of these potential drug-drug interactions is the use of antibiotics, and specifically TMP/SMX in patients who are receiving warfarin therapy. Trimethoprim and sulfamethoxazole individually (and in combination as co-trimoxazole), interfere with the metabolism of warfarin by inhibiting (CYP) isoenzyme 2C9.\textsuperscript{8,9,30} This is different from other antibiotics indicated for UTI (amoxicillin/ampicillin, ciprofloxacin, norfloxacin, or nitrofurantoin), The other antibiotics may interact with warfarin by altering the gut flora\textsuperscript{29} and ciprofloxacin
inhibits CYP 1A2 and 3A4, which are involved in the metabolism of (R)-warfarin.\textsuperscript{4,8,9,11,36}

Co-prescribing of warfarin and potentially interacting drugs, particularly antibiotics, is a frequent occurrence.\textsuperscript{37,38} One pharmacy benefits manager, providing benefits to more than 65 million patients in the U.S. reported that, in a cohort of 134,833 warfarin users, 3.81% received a prescription for TMP/SMX.\textsuperscript{37}

TMP/SMX is listed as a drug that may potentially increase the INR or the risk of bleeding in patients on warfarin.\textsuperscript{39} Holbrook et al.’s\textsuperscript{4} review of the literature on warfarin and its drug and food interactions, summarizes the interactions’ direction, severity and quality of evidence. TMP/SMX is categorized as “highly probable” to potentiate the effect of warfarin, based on evidence from healthy volunteers as well as patient-based reports that described the increase of warfarin’s anticoagulant effect in the presence of TMP/SMX.\textsuperscript{4,30,40-42}

2.3 Previous observational studies of TMP/SMX and coumarin anticoagulants

2.3.1 TMP/SMX and non-warfarin coumarin anticoagulants

Several Dutch studies have investigated overanticoagulation and the risk of hemorrhage when antibiotics were co-prescribed with non-warfarin coumarin anticoagulants (acenocoumarol and phenprocoumon).\textsuperscript{43-46} Table 1 summarizes the main study details and results. Penning-van Beest et al.\textsuperscript{43} performed a nested case-control study using a cohort of all patients treated with anticoagulants in one anticoagulation clinic in the Netherlands. They identified 300 cases of overanticoagulation (defined as an INR value $\geq 6$) and 302 matched controls.\textsuperscript{43} To address increased risk of
overanticoagulation due to coumarin initiation or unstable anticoagulation, eligible cases and controls had to have stable anticoagulation levels in the 3 months prior to the index date. Data was collected from medical records as well as interviews with patients. They predefined 69 drugs that may potentially interact with coumarins and collected information on changes in drug use during the risk period, which was defined as 4 weeks prior to the index date. They found that the risk of overanticoagulation was increased when TMP/SMX was co-prescribed with acenocoumarol or phenprocoumon (adjusted odds ratio 24.2; 95% confidence interval (CI), 2.8-209.1) and the stratified analysis showed that the risk of overanticoagulation was more present in patients on acenocoumarol.

Another study, using a sample from the Rotterdam population-based cohort, examined which antibacterial drugs were associated with increased overanticoagulation when used concomitantly with coumarins. The study cohort consisted of 1124 patients of whom 351 developed an INR ≥ 6. A Cox proportional hazards regression model for time-dependent variables was used to calculate the relative risk of an INR ≥ 6 associated with exposure to antibacterial drugs. The model examined exposure to the combination of coumarins with antibacterial drugs on the index date of a case patient compared with that of all other non-censored patients in the cohort at the same time as the case patient. The study showed that the greatest risk was associated with the co-administration of TMP/SMX (adjusted relative risk (RR) 20.1; 95% CI, 10.7-37.9). As well, norfloxacin was associated with an increased risk (adjusted RR 9.8, 95% CI 3.0 – 31.6), and contrary to the previous study, amoxicillin was also associated with an increased risk (adjusted RR 10.5, 95% CI 5.1 – 21.7).
Both of the abovementioned studies\textsuperscript{43,44} did not include patients treated with warfarin and they focused on overanticoagulation (as measured by increased INR values) and not on bleeding events. The low exposure rates in these studies probably resulted in imprecise relative risks, unstable point estimates and wide confidence intervals.

Two additional Dutch studies\textsuperscript{45,46} used the PHARMO Record Linkage System which includes drug-dispensing information from community pharmacies and hospital discharge information of 42 demographically defined areas in the Netherlands.\textsuperscript{46} Both studies looked at the risk of major hemorrhage requiring hospitalization in patients treated with acenocoumarol or phenprocoumon and the concomitant use of potentially interacting drugs and antibiotic therapy.\textsuperscript{45,46} The unadjusted relative risk of bleeding was calculated by dividing the incidence rates of bleeding during the coumarin and antibiotic-exposed period by the incidence during the non-exposed (exposed to coumarin alone) period.\textsuperscript{45,46} The cohort in the first study included 19,935 new users of acenocoumarol (95\% of the cohort) or phenprocoumon, of whom 552 patients were hospitalized for bleeding.\textsuperscript{45} The more recent study examined prevalent coumarin users and included 59,987 patients in the cohort (87\% used acenocoumarol, the rest used phenprocoumon), of whom 1850 patients were hospitalized for bleeding.\textsuperscript{46}

These studies found that several antibiotics were associated with a statistically significant increased risk of bleeding, and in the more recently published study, the adjusted relative risk (RR) associated with the use of TMP/SMX was 5.1 (95\% CI, 2.1-12.3).\textsuperscript{45,46} This study also reported an increased adjusted RR for all bleeding requiring hospitalization with the use of amoxicillin (RR 3.1; 95\% CI, 1.6-6.3), and ciprofloxacin
(RR 3.2; 95% CI, 1.3-7.7) whereas the RR associated with norfloxacin and nitrofurantoin was not statistically significant.\textsuperscript{46} Although the results of the two studies were similar for TMP/SMX, the smaller study also showed an increased association of bleeding when norfloxacin was co-prescribed (adjusted RR 5.9, 95% CI 1.9 - 18.6).\textsuperscript{45}

The wide confidence intervals may be partially explained by the relative small number of bleeding events per potentially interacting drug.\textsuperscript{45,46} In the most recent study,\textsuperscript{46} the authors excluded bleeding that required hospitalization as an outcome if the patient was exposed to more than one antibiotic at the time of hemorrhage. Their analysis was adjusted for age, gender, and concomitant non-steroidal anti-inflammatory drugs (NSAIDs). Also, they did not focus on the co-administration of antibiotics with specific indications, but examined all antibiotics and thus most probably included patients with a variety of infections in their cohort.\textsuperscript{46}

\subsection*{2.3.2 Limitation of non-warfarin coumarin anticoagulants}

\textbf{Pharmacoepidemiological studies}

All the studies mentioned above did not focus on specific drugs or antibiotics, but examined a wider range of medications that may potentially interact with warfarin.\textsuperscript{43-46} Although these studies\textsuperscript{43-46} have shown an interaction between non-warfarin coumarin anticoagulants and antibiotics, some examining overanticoagulation and some hemorrhage, we believe that it is important to examine the risk of upper gastrointestinal (UGI) bleeding when antibiotics are prescribed in patients on warfarin as opposed to acenocoumarol and phenprocoumon. Although warfarin, acenocoumarol, and phenprocoumon have similar chemical structure, their pharmacokinetics are
substantially different.\textsuperscript{47} Although the active (S)-form of acenocoumarol, which the majority of the Dutch study population received, and (S)-warfarin are both primarily metabolized by CYP 2C9, they are different drugs and CYP 2C9 is most important for the clearance of warfarin and least important for the clearance of phenprocoumon.\textsuperscript{47} In addition, it seems that the Netherlands, where the studies on acenocoumarol and phenprocoumon were conducted, has a strict anticoagulation monitoring system with anticoagulation clinics, which may lead to a lower risk of bleeding than other countries.\textsuperscript{46}

2.3.3 TMP/SMX and warfarin

We identified two observational studies examining overanticoagulation and hemorrhage when anti-infectives, including TMP/SMX, were co-prescribed with warfarin.\textsuperscript{10,11} A retrospective cohort study by Glasheen et al.\textsuperscript{10} assessed the risk of overanticoagulation associated with the prescription of three commonly prescribed antibiotics in patients with stable warfarin and INR levels. Data was obtained from electronic medical records. The study population consisted of patients treated at a university-affiliated Veteran’s Affairs Medical Center, and was mainly male (average age of 70 years). After inclusion and exclusion criteria were considered, the study included 32 patients who were prescribed azithromycin, 27 patients on levofloxacin, and 16 patients on TMP/SMX.\textsuperscript{10} Terazosin was used as a control since it is not known to interact with warfarin.\textsuperscript{10} The antibiotics were prescribed for various indications. In warfarin-treated patients, who were prescribed azithromycin, levofloxacin, or TMP/SMX, the mean INR increased by 0.51, 0.85, and 1.76, respectively.\textsuperscript{10} The
incidence of a supratherapeutic INR level \( \geq 4 \) was 16% for azithromycin, 19% for levofloxacin, and 44% for TMP/SMX. In the TMP/SMX group, 31% of patients had an INR \( \geq 5 \) and two patients (13%) had a documented bleeding episode.\(^{10}\) The increased risk for overanticoagulation (incidence as well as degree), when warfarin-treated patients were concurrently on TMP/SMX, was statistically significant compared to the control drug, terazosin.\(^{10}\) The study by Glasheen et al. is small, focuses on overanticoagulation and lacked sufficient power to evaluate bleeding events.

The second observational study used administrative healthcare data from the Medicaid program to examine drug interactions between warfarin and fluoroquinolones, sulfonamides and azoles.\(^{11}\) They used a nested case-control and case-crossover design to study the association between these anti-infectives and gastrointestinal hemorrhage in warfarin-treated patients. The study included 11,444 cases of hospitalization for gastrointestinal bleeding and 586,744 matched controls.\(^{11}\) The main exposure period of interest was defined as 6-10 days prior to the index date (the index date was the hospitalization date for the cases and the same for their matched controls).\(^{11}\) In comparison to no antibiotic exposure, all anti-infectives were associated with an increased risk of GI hemorrhage. In regards to the antibiotics we have included in the current project, Schelleman et al.\(^{11}\) found in their main analysis that TMP/SMX was associated with a two and a half-fold increased risk of GI hemorrhage (adjusted OR 2.54, 95% CI 2.08 – 3.10), ciprofloxacin had an almost two-fold increased risk (adjusted OR 1.62, 95% CI 1.31 – 1.99) and amoxicillin was also significantly associated with a small increased risk of GI bleeding (adjusted OR 1.28, 95% CI 1.03 – 1.58).\(^{11}\) Various analyses, such as the exclusion of patients with prior GI hemorrhage, resulted in
consistent results. However, after adjusting for the indication of the anti-infective and choosing the reference group as patients exposed to amoxicillin or cephalexin, only TMP/SMX and fluconazole remained statistically significantly associated with an increased risk of hemorrhage (Table 1).\textsuperscript{11}

The authors concluded that warfarin users who took anti-infectives were prone to an increased risk of GI hemorrhage and they suggested that infection or its related sequelae (e.g. fever, change in vitamin K status, antipyretic medication, etc.) may contribute to this increased risk of bleeding, while TMP/SMX adds to the risk further.\textsuperscript{11} This study examined a number of anti-infectives with very different indications.\textsuperscript{11} As this study used administrative healthcare data, its limitations included unmeasured confounders (diet, over-the-counter medications, presence of \textit{helicobacter pylori}, etc.) and lack of information about the level of anticoagulation at the time of hospitalization (as measured by INR).\textsuperscript{11}

The literature on drug interactions is generally not of high quality and the majority of the studies included in the review by Holbrook et al.\textsuperscript{4} were case reports or studies on healthy volunteers. Although there have been European population-based studies examining non-warfarin coumarin anticoagulants and the risk of bleeding when antibiotics are co-prescribed, to date, there is only one large epidemiological study that used large administrative databases to evaluate the risk of hemorrhage associated with TMP/SMX in warfarin-treated patients.\textsuperscript{11,45,46} Both the European studies\textsuperscript{45,46} as well as Schelleman et al.\textsuperscript{11} used large administrative databases to examine various anti-infectives. However, they did not focus on antibiotics indicated for urinary tract infection. It is important to quantify the increased clinical risk of hemorrhage when
TMP/SMX is prescribed to patients treated with warfarin, and to compare this risk with alternative antibiotics indicated for UTI. Examining alternative oral anti-infectives used for UTI provides information about TMP/SMX, but also about the alternative antibiotics that could be used for UTI. To date, there are no large epidemiological studies that have evaluated this issue with a focus on anti-infectives indicated for UTI and therefore this population-based nested case-control study will add to existing knowledge.
METHODS

3.1 Study design and setting

We conducted a population-based, retrospective, nested case-control analysis of multiple linked administrative healthcare databases in Ontario, Canada, between April 1, 1997 and March 31, 2007. This methodology has been previously used to examine various drug-drug interactions. Ontario is the most populous province in Canada, with over 1.4 million individuals aged 66 years or older at the middle of the study period and provides universal access to physician services, hospital care and selected prescription drugs for patients over 65 years of age.

3.2 Data sources

We used four linkable Ontario administrative healthcare databases. The Ontario Drug Benefit (ODB) database includes data on prescription drugs reimbursed by the Ontario government for all Ontario residents aged 65 and older. The pharmacist submits a claim for each prescribed drug that is covered under the ODB formulary and these claims form the basis of the ODB database. Information in the database includes: drug identification number (DIN), dispensing date, quantity of drug dispensed, number of days supplied (from 1997 onward), as well as encrypted patient and physician identifiers. Levy et al. have shown that these data are reliable (with an overall error rate of 0.7%). The ODB data do not include information on prescriptions that are reimbursed by private insurers or those paid out-of-pocket by patients.
Information regarding all hospital admissions was obtained from the Canadian Institute for Health Information Discharge Abstract Database (CIHI DAD). All hospitals in Ontario are required to submit demographic and clinical information about all hospital admissions and discharges, including transfers and deaths, to CIHI which collates these data. Trained hospital medical records staff transcribe information from each patient’s medical chart using standard diagnosis and procedure codes. We used these data for information on each patient’s hospital-based diagnoses. In April 2002, diagnosis coding for hospital admissions changed from the *International Classification of Diseases, Ninth Revision Clinical Modification (ICD-9-CM)* to the *International Statistical Classification of Diseases and Related Health Problems, Tenth Revision, Canada (ICD-10-CA)*. Therefore we used both ICD-9 as well as ICD-10 to define discharge diagnoses. A recent reabstraction study\(^55\) showed that the degree of agreement for demographic information in the CIHI DAD was excellent (over 98%) and previous reabstraction studies\(^56\) also showed that agreement on demographic data was 95% or higher. The most responsible diagnosis (the one diagnosis responsible for the greatest contribution to the length of stay) tends to be well coded overall, although there is variability in the quality of various diagnoses.\(^55,56\) In contrast, coding of secondary codes, (Type 1 pre-admission diagnoses or Type 2 in-hospital diagnoses) is frequently poor.\(^55\)

The Ontario Health Insurance Plan (OHIP) database records all physician claims, including procedures. In Ontario, physicians are reimbursed after submitting claims to OHIP for each service provided. These services include: physician consults or assessments in private offices, acute care, and long-term care facilities; technical and
professional components of diagnostic and therapeutic procedures; surgical procedures; and laboratory services (ICES intranet). These service data are relatively accurate because the information submitted is associated with a reimbursement fee. These data only capture information for those physicians who work on a fee-for-service basis (it is estimated that approximately 95% of all physicians in Canada are remunerated on a fee-for-service basis). In Ontario, approximately 90% of physicians have the majority of their practice within the OHIP fee for service billing system. (Internal communication, Sue Schultz, ICES) The rest of the physicians are reimbursed by Alternate Funding Plans or salary, such as pathologists, etc. (ICES intranet). Finally, we used the Registered Persons Database (RPDB) for basic demographic information and vital status information. The Registered Persons Database is developed and maintained by the Ministry of Health and Long-Term Care. It consists of the health card number, date of birth, sex, postal code and death date (where applicable) for individuals with a valid health card (ICES intranet).

These administrative health care databases were anonymously linked through individual, encrypted health card numbers. The linkage among these databases is based on deterministic matching and therefore its rate is 100%. These linkable administrative databases are available through the Institute for Clinical Evaluative Sciences.

3.3 Individual observation period & cohort definition

3.3.1 Inclusion criteria

Our cohort consisted of all elderly Ontario residents who had been prescribed warfarin continuously for at least 180 days (6 months), starting from the first warfarin
prescription after the patient’s 66th birthday (i.e., cohort entry was defined as day 180 of warfarin treatment). Continuous warfarin use was defined by using a grace period of 50% (i.e., a patient who was dispensed a prescription for warfarin for 30 days would need to fill another prescription within 45 days of the previous dispensing date to be considered a continuous warfarin user).

3.3.2 Exclusion criteria

We excluded patients younger than 66 years of age to avoid incomplete medication records, since we would not have a one year look-back for previous drug use. Patients who had been hospitalized with an admission diagnosis of any bleeding (see Table 2) within the initial 180 days of continuous warfarin use prior to cohort entry were also excluded.

We defined the inclusion and exclusion criteria of continuous warfarin use and no history of bleeding in the 180 days prior to cohort entry based on reports stating that there is an increased risk of bleeding at the start of warfarin therapy. In addition, we did not have laboratory data to confirm “stable” therapeutic INR levels. The inclusion criterion requiring continuous use for at least 180 days also restricted the cohort to those patients who were chronically treated with warfarin.

The observation period after cohort entry ended when one of the following events occurred (whichever occurred first): first hospitalization for UGI hemorrhage, discontinuation of warfarin, death, or end of study period (March 31, 2007). Discontinuation of warfarin treatment followed the same definition as that of the inclusion criterion (i.e., discontinuation of warfarin meant that more than 50% days
supplied had passed since the end date of the previous prescription of warfarin). When warfarin was discontinued, we extended the follow-up period by 50% of the days supplied to capture admissions for UGI bleeds, which may have triggered the cessation of warfarin treatment (i.e., a patient whose last warfarin prescription prior to discontinuation was for 30 days, was followed up for 45 days from the last dispensing date).

3.4 Cases and controls

3.4.1 Case patients

Within the cohort of continuous warfarin users, we defined case patients as those who had been admitted to hospital with an “admission diagnosis” of UGI bleeding between October 1, 1997 and March 31, 2007. Only the first hospitalization for UGI bleeding was considered a case and the patient was censored thereafter. Admissions for UGI bleeds were obtained from the CIHI DAD. We defined UGI bleeding as ICD-9 diagnostic codes 531.0, 531.2, 531.4, 531.6, 532.0, 532.2, 532.4, 532.6, 533.0, 533.2, 533.4, 533.6, 534.0, 534.2, 534.4, 534.6, 578.0, 578.1, and 578.9; and ICD-10 diagnostic codes K25.0, K25.2, K25.4, K25.6, K26.0, K26.2, K26.4, K26.6, K27.0, K27.2, K27.4, K27.6, K28.0, K28.2, K28.4, K28.6, K92.0, K92.1, and K92.2 (see Appendix 1 for description). An internal review at the Institute for Clinical Evaluative Sciences of these UGI hemorrhage ICD-9 and ICD-10 codes has shown that the rates have been stable over time and that the abovementioned codes capture the same clinical conditions before and after the switch from ICD-9 to ICD-10 codes (verbal communication Alex Kopp, ICES). The same ICD-9 codes were used in a previous
study examining the risk of UGI hemorrhage in patients prescribed warfarin and cyclooxygenase-2 inhibitors.\textsuperscript{50} The ICD-9 codes were found to have a positive predictive value of well above 80\% for UGI bleeding based on a Saskatchewan hospital database.\textsuperscript{57} The hospital admission date served as the index date for all analyses. See Figure 1 for a schematic presentation of the study design.

We limited the case definition to upper GI hemorrhage and did not include lower GI hemorrhage due to data accuracy. The CIHI DAD discharge ICD-9 diagnoses available at ICES only include the 4-digit level ICD-9 information. However, most lower GI bleeds are specified at the 5-digit level of the ICD-9 diagnoses. For example, ICD-9 code 5621 is diverticulosis of colon, which includes 56210 (diverticulosis of colon without hemorrhage), 56211 (diverticulitis of colon without hemorrhage), as well as 56212 (diverticulosis of colon with hemorrhage), and 56213 (diverticulitis of colon with hemorrhage). Thus, the 4-digit ICD-9 code information is not specific enough and would have included patients admitted for lower GI conditions that were not lower GI bleeds. The issue of added specificity of the ICD-9 5-digit level information does not apply for upper GI bleeding codes since hemorrhage is identified at the level of the 4\textsuperscript{th} digit. For the upper GI hemorrhage codes, the 5-digit level information adds information if the event included obstruction or not. In addition, we did not look at other hemorrhagic outcomes, such as intracranial hemorrhage or genitourinary tract hemorrhage, which are less reliably coded in the administrative database. We also decided not to define the outcome in a broader way since this may have introduced more potential confounders (i.e., different conditions are associated with upper GI
hemorrhage compared to intracranial hemorrhage and thus the covariates would have had to be defined more broadly).

3.4.2 Control patients

From the cohort of patients continuously using warfarin who were alive at the time of a case event and who had not yet been admitted with an admission diagnosis of UGI bleeding, we randomly matched a case to up to 10 controls. We matched controls based on date of birth (within 100 days of case patient), sex, and continuous warfarin use on the date of the case event. Controls were assigned the same index date as the corresponding case. If fewer than 10 patients could be matched to a case patient, we used the available controls and did not alter the matching algorithm. If more than 10 controls were available, ten controls were chosen randomly. Each control may have been used more than once (for different case patients) and a case could serve as a control for a different case patient prior to their hospital admission for UGI bleeding.\textsuperscript{58,59} Controls were not matched on the length of continuous warfarin prescription since the cohort included patients who had to have been prescribed warfarin for at least 180 days and were all considered stable warfarin users.

We excluded cases and controls who had been discharged from hospital (for any admission diagnosis, except UGI bleeding) within 30 days prior to the index date. This was done for two reasons; first, acute illness and hospitalization may predispose the patients to bleeding and second, the ODB does not capture drugs prescribed to patients in hospital and thus we may have missed antibiotic exposures. We also excluded cases and controls who had been prescribed the combination kit amoxicillin & lansoprazole &
clarithromycin within 14 days of the index date. This treatment is indicated for *H. pylori* eradication and thus serves as a marker for a population associated with a higher risk of peptic ulcer disease. In addition, we excluded patients who had been exposed to more than one antibiotic of interest (i.e., TMP/SMX, amoxicillin/ampicillin, ciprofloxacin, norfloxacin, and nitrofurantoin).

3.5 **Definition of exposure to interacting drugs**

The main exposure of interest in the study was oral TMP/SMX compared to other oral antibiotics indicated for urinary tract infection but which are not considered to have such a strong drug interaction with warfarin: amoxicillin or ampicillin (included in one group), ciprofloxacin, norfloxacin, and nitrofurantoin. We defined the exposure as the oral form of the antibiotics of interest to create a relative homogeneous group of patients. All oral preparations of TMP/SMX, amoxicillin/ampicillin, ciprofloxacin, norfloxacin, and nitrofurantoin were defined based on their Drug Identification Numbers (DINs). The cases and controls were linked with the ODB database and all prescriptions were identified. Our primary exposure of interest was the dispensation of at least one oral prescription of the above-mentioned antibiotics within 0-14 days of the index date. We performed sensitivity analyses examining antibiotic exposure within 7 and 21 days prior to the index date. To assess the robustness and specificity of our results we repeated the analyses examining the exposure to prescriptions of ocular antibiotics. Ocular antibiotics are not considered to have any interaction with warfarin and an increased risk of UGI bleeding was not expected.
3.6 Covariates

We defined various covariates to adjust for potential confounders. The covariates also included conditions and medications that may influence the outcome of UGI bleeding. Table 2 summarizes the covariates and the databases used to obtain the information.

3.6.1 History of UGI hemorrhage

Patients who have a history of UGI bleeding may be pre-disposed to bleeding while on warfarin and therefore we adjusted for a history of UGI bleeding.\textsuperscript{2} We used the CIHI DAD to identify hospitalizations with admission diagnoses of UGI hemorrhage, using the same diagnostic codes as the case definition, in the five years prior to the cohort entry date. In addition we adjusted for upper gastrointestinal endoscopic and radiologic studies during the 1 year prior to the index date (not including the index date). These data were obtained from the OHIP physician billing information (fee codes X103, X104, X109, Z399, Z400, Z515, Z527, Z528, Z560, Z749). We used the ODB data to include a category of gastroprotective agents in the model, since these drugs may reduce the risk of bleeding and also serve as a marker of a pre-existing gastrointestinal disease.

3.6.2 Other potentially interacting medications

We used the ODB database to adjust for other concomitant medications that may be associated with an increased risk of UGI bleeding or may interfere with warfarin metabolism. All the drug exposures were defined using the index date as the reference date. The index date was defined as the hospital admission date for UGI bleeding for the cases and the same date was assigned to the matched controls. We examined drug
dispensation in the 120 days prior to the index date. We categorized the potentially confounding drugs based on their mechanism of action or drug groups. The following mutually exclusive drug categories were included in the adjusted model (Table 2): antiplatelet agents including acetylsalicylic acid (ASA), clopidogrel, ticlopidine and dipyridamole, all of which interfere with primary hemostasis. It has been shown that drugs, such as ASA increase the risk of major hemorrhage when combined with warfarin. We also included a category of “other anticoagulants” to include medications such as low molecular weight heparins, nicoumalone, lepirudin, and fondaparinux. Selective cyclooxygenase (COX)-2 inhibitors and nonselective nonsteroidal anti-inflammatory drugs (NSAIDs) can cause gastrointestinal erosions and have been shown to increase the risk of UGI bleeding in patients who are concomitantly prescribed warfarin. Another group of drugs that may have the potential to increase the risk of UGI bleeding are corticosteroids. Another category of medications are acetaminophen and its combination drugs. Some studies suggest that selective serotonin reuptake inhibitors (SSRIs) deplete platelet serotonin levels and therefore they may affect primary hemostasis by inhibiting platelet aggregation and be associated with increased gastrointestinal bleeding. In addition, some of the SSRIs (fluvoxamine, fluoxetine, sertraline) interfere with warfarin metabolism by inhibiting CYP 2C9, which may increase the risk of bleeding. We decided to group all SSRIs into one category, irrespective of their metabolic effect on warfarin. The model also included a term encompassing all other systemic antibiotics, excluding our antibiotics of interest, since many antibiotics have the potential to impact the balance of the gut flora and thus may increase the effect of warfarin. Medications other than TMP/SMX that interfere
with warfarin metabolism and were not included in any of the other above mentioned covariate drug categories, are another important group. The following systemic medications, which inhibit CYP 2C9, were grouped together in one category: amiodarone, anastrozole, disulfiram, fenofibrate, fluconazole, fluvastatin, imatinib, isoniazid, ketoconazole, leflunomide, lovastatin, metronidazole, miconazole, modafinil, nateglinide, probenecid, sildenafil, teniposide, valproic acid, voriconazole, and zafirlukast. Due to the nature of our older population, we did not include HIV medications that are inhibiting CYP 2C9. Bosentan, carbamazepine, phenobarbital, phenytoin, primidone, rifampin, and secobarbital were categorized under CYP 2C9 inducers which may decrease warfarin’s effectiveness.

Some of the medications included in the categories above (e.g., ASA, acetaminophen, some NSAIDs) are non-prescription drugs which are available “over-the-counter” in Ontario. Although we were not able to completely and reliably capture all of these drugs, we included them in the covariate categories since some preparations are available by prescription and patients over 66 years have a financial incentive to obtain these drugs by prescription.

3.6.3 Comorbidities
Since warfarin is metabolized by the liver, we adjusted for cirrhosis, which we identified by using the main and comorbidity diagnoses listed in hospital admissions and the OHIP diagnosis codes in the 5 years prior to the index date (see Table 2 for specific codes). We also adjusted for previous and/or current alcohol abuse using hospital admission and physician claims from the 5 years prior to the index date (See Table 2).
Other comorbidities, such as renal insufficiency, anemia, hypertension, serious heart
disease or a history of stroke have been reported to be associated with an increased risk
of bleeding while on warfarin.\textsuperscript{2,24} In order to capture the patients’ comorbidities, we
included in the adjusted model a frequently used validated measure of comorbidity,
which is the number of different Drug Identification Numbers (DINs) used in the year
prior to the index date.\textsuperscript{69} We also adjusted for any bleeding (except UGI hemorrhage) in
the year prior to the index date). We used prescriptions with a long-term care (LTC) flag
in the ODB database within the 180 days prior to the index date to identify patients who
were residing in LTC facilities. Age has been reported to be an important independent
risk factor for bleeding while on warfarin.\textsuperscript{24} Since we matched the cases and controls on
age, adjusting for it was not required.

3.7 Statistical analysis

3.7.1 Descriptive analysis

We described the characteristics of the cases and their matched controls, using the
number of patients and percentage (for binary variables) or the mean and standard
deviation (for continuous variables). Most variables were described at the time of the
index case (except for the history of UGI bleeds).

3.7.2 Primary analyses

We used conditional logistic regression to estimate the odds ratio (OR) and respective
95\% confidence interval (CI) for the association between hospital admission for UGI
hemorrhage and the prescription of TMP/SMX in patients continuously on warfarin. We
also estimated the OR and 95% CI for the association between hospital admission for UGI hemorrhage and the prescription of each of the other four alternative antibiotics (amoxicillin/ampicillin, ciprofloxacin, norfloxacin, and nitrofurantoin). Each of the five analyses used the group who had “no-exposure” to the antibiotic of interest as the reference group. Multivariate analyses were performed, adjusting for the covariates listed in Table 2. We also included in each of the adjusted models, the other four antibiotics that were not the exposure in question for that analysis. For example, when ciprofloxacin was the analysis’ exposure of interest, we adjusted for TMP/SMX, norfloxacin, amoxicillin/ampicillin, and nitrofurantoin as four individual terms in the model. The primary analysis examined exposure to the antibiotic of interest in the 14 days prior to the index date.

In order to evaluate the robustness and specificity of the results we repeated the primary conditional logistic regression analysis while examining exposure to ocular antibiotics. We used ocular antibiotics as a neutral tracer exposure since they are not known to have a drug interaction with warfarin and therefore we expected no association with UGI bleeding.

We applied a test of interaction\textsuperscript{70} comparing the adjusted OR for UGI bleeding of TMP/SMX with the adjusted OR of amoxicillin/ampicillin. We pre-defined this comparison since our hypothesis was that the risk of UGI bleeding while on amoxicillin or ampicillin would be minimal and these drugs are frequently used for urinary tract infection.

In addition, we estimated the proportions of admissions that could have been averted by avoiding the interacting antibiotic TMP/SMX and using an alternative
antibiotic instead. Attributable fractions were calculated based on the main exposure analysis (14 days prior to index date), using standard methods where attributable fraction = [((OR-1)/OR)*[proportion of cases exposed to the risk factor (i.e., interacting antibiotic)]. We used the ratio of ORs obtained from the test of interaction comparing co-trimoxazole with amoxicillin/ampicillin, since in a patient with a UTI the alternative to TMP/SMX is to prescribe another “safer” antibiotic, not to avoid antibiotics altogether.

3.7.3 Sensitivity analyses
We performed sensitivity analyses examining exposure windows of 7 days and 21 days prior to the index date. All analyses were performed using SAS version 9.13 (SAS Institute, Cary, NC).

3.8 Ethics
This study was approved by the Ethics Review Board of Sunnybrook Health Sciences Centre, Toronto, Ontario.
RESULTS

4.1 Description of cohort and cases & controls

We identified 134,637 patients aged 66 years and older who were continuously treated with warfarin for at least 180 days and thus were included in our cohort. The median (interquartile range (IQR)) age at cohort entry was 76.3 (IQR = 70.8 – 81.9) and 49.9% were women. The cohort included a total of 198,910 person-years of continuous warfarin treatment (median 291 days, IQR = 98 – 732 days). During the study period there were 16,986 TMP/SMX prescriptions dispensed to patients on chronic warfarin treatment, 53,273 prescriptions for amoxicillin or ampicillin, 34,691 for ciprofloxacin, 15,701 for norfloxacin and 20,809 for nitrofurantoin. TMP/SMX was dispensed to 9,751 (7.24%) patients, amoxicillin/ampicillin to 24,135 (17.93%) patients, ciprofloxacin to 17,815 (13.23%) patients, norfloxacin to 8,231 (6.11%) patients and nitrofurantoin to 7,617 (5.66%) patients. Collectively, 34% (45,972) of patients on continuous warfarin therapy received at least one prescription for an antibiotic of interest during the study period.

Of the 134,637 patients in the cohort, 14,850 (11.03%) died during the observation period and 98,896 (73.45%) discontinued warfarin before the end of the study period. After excluding 49 patients who had a bleeding associated admission in the initial 180 days of warfarin therapy, we identified 2,441 patients who were admitted to a hospital with an admission diagnosis of UGI hemorrhage. After excluding patients who had been on more than one antibiotic of interest (n=7), or the combination kit amoxicillin & lansoprazole & clarithromycin within 14 days of the index date (n≤5), those who had a hospital admission within 30 days of the index date (n=270), including
those who fit more than one exclusion criteria (n=12), there were 2,151 patients hospitalized for UGI bleeding who met our definition of a case. Almost all cases (2135; 99.3%) were matched to 10 controls.

After cohort entry, controls had been continuously on warfarin for a mean duration of 1194 days (SD = 930 days; median = 971 days; IQR = 431 – 1774). Cases had been continuously on warfarin for a mean duration of 858 days (SD = 764 days; median = 621 days; IQR = 273 – 1230 days). The median length of the initial hospital stay for the cases hospitalized for UGI bleeds was 6 days (IQR = 3 - 11 days) and 224 died while in hospital. The characteristics of the cases and controls are described in Table 3. Their median age at the index date was 80 years (IQR = 74 – 85 years) and 52% were men. Cases were more likely than controls to have had a history of UGI bleeds or other bleeds, had undergone more UGI examinations, and more of them were prescribed gastroprotective medications. Case patients were, as expected, sicker and more likely to be given other medications that have the potential to cause UGI bleeds (Table 3).

Among the 2151 cases, 25 (1.2%) were prescribed TMP/SMX in the 14 days prior to hospitalization, 30 (1.4%) were prescribed amoxicillin or ampicillin, 31 (1.4%) received ciprofloxacin, 11 (0.5%) received nitrofurantoin and less than 6 cases received a prescription for norfloxacin (Table 4). Among the 21,434 controls, 56 (0.3%) were prescribed TMP/SMX in the 14 days prior to hospitalization, 209 (1.0%) were prescribed amoxicillin or ampicillin, 124 (0.6%) received ciprofloxacin, 64 (0.3%) were dispensed nitrofurantoin and 61 (0.3%) received norfloxacin (Table 4).
4.2 Primary analyses

We compared the exposure to TMP/SMX in cases (patients hospitalised with UGI bleeding) versus controls. The univariate and multivariate odds ratios (ORs) are provided in Table 4. The multivariate analyses adjusted for confounders and other variables that could affect the risk of UGI bleeding (Table 2). The multivariate results were similar to the univariate ones but with more attenuated odds ratios (Table 4). We found that cases were nearly 4 times more likely to have received a TMP/SMX prescription in the 14 days prior to hospitalization (adjusted odds ratio (adjusted OR) was 3.84 (95% confidence interval (CI) 2.33 – 6.33) (Table 4).

Hospitalization for UGI bleeding in patients on warfarin and co-administration of ciprofloxacin in the 2 weeks prior to the index date was also associated with a significant, close to two fold, increased risk (adjusted OR 1.94, 95% CI 1.28 – 2.95). There was no significant association between hospitalization for UGI bleeding and the prescription of amoxicillin / ampicillin (adjusted OR 1.37, 95% CI 0.92–2.05), nitrofurantoin (adjusted OR 1.40, 95% CI 0.71–2.75), or norfloxacin (adjusted OR 0.38, 95% CI 0.12–1.26). (Table 4).

As expected, there was no significant association between UGI bleeding risk and ocular antibiotics (AOR 0.99, 95% CI 0.5 – 1.93).

We compared the risk estimate associated with co-prescription of TMP/SMX in the 14 days prior to the index date to that of co-prescription of amoxicillin/ampicillin using the test of interaction. The increased risk of UGI bleeding with TMP/SMX was found to be statistically significantly different from that with amoxicillin/ampicillin (Ratio of odds ratios 2.80, 95% CI 1.48 – 5.32).
Using the ratio of odds ratios comparing TMP/SMX and amoxicillin / ampicillin, we estimate that approximately 0.75%, or 1 out of every 134 of the hospital admissions for UGI bleeding in older adults continuously on warfarin could have been averted if simultaneous TMP/SMX use would have been avoided and amoxicillin or ampicillin would have been used instead.

4.3 Sensitivity analyses

Sensitivity analyses examined the exposure to TMP/SMX and the other antibiotics indicated for urinary tract infection in the 7 and 21 days prior to index date (Table 4). The results were consistent with the primary analyses. However, the magnitude of risk was attenuated when we examined the risk associated with exposure to TMP/SMX and the other antibiotics within the extended window of 21 days. The analysis examining the risk of UGI bleeds within 7 days of drug exposure showed that amoxicillin/ampicillin exposure was associated with an increased risk of UGI bleeds (adjusted OR 1.88, 95% CI (1.18 – 2.99)).
DISCUSSION

5.1 Major findings

We found that the concomitant use of TMP/SMX in patients on chronic warfarin therapy was associated with a four-fold increase in the risk of hospitalization for UGI bleeding (adjusted OR 3.84; 95% CI 2.33 – 6.33), which was considerably higher than that with other antibiotics. This association was consistent in sensitivity analyses that varied the exposure windows. Ciprofloxacin was also, but less strongly, associated with an increase in UGI hemorrhage (adjusted OR 1.94, 95% CI 1.28–2.95). The other antibiotics were not associated with a significant increase in risk. When comparing TMP/SMX to amoxicillin/ampicillin by using the test of interaction\textsuperscript{70}, the risk of UGI hemorrhage was statistically significantly higher with TMP/SMX (ratio of odds ratios 2.80, 95% CI 1.48 – 5.32). We estimate that 0.75% of all hospitalization for UGI hemorrhage in older patients treated with warfarin could have been avoided by not co-prescribing TMP/SMX but using alternative antibiotics with a lower propensity to interact with warfarin, such as amoxicillin or ampicillin.

5.2 Limitations

Our study has several limitations. First, we used administrative data and did not have access to laboratory data. Therefore we were unable to assess whether patients’ anticoagulation levels were stable when antibiotics were started, nor could we assess changes in INR levels after the start of antibiotics. To address the former, we included only patients who had been continuously receiving warfarin for six months. Lack of laboratory data also prevented us from assessing the impact of TMP/SMX on increased
INR levels which did not result in admission for UGI bleeding. However, although more frequent than UGI bleeding, asymptomatic increases in INR are less clinically important than an UGI bleed.

Second, we have no information about patients’ compliance with their medication regimen, or whether there was a change in INR monitoring and warfarin dose when antibiotics were prescribed. However, this is not a major problem because our study’s objective was to determine the risk of clinically important adverse outcomes in regular practice.

Third, as expected, cases tended to suffer from more comorbidities and have more risk factors for UGI hemorrhage than controls. However, we adjusted for an extensive array of potential confounders, and this limitation applies regardless of antibiotic therapy and is highly unlikely to explain the differential risk of UGI hemorrhage observed in our study.

Although we adjusted for many potential confounders in the multivariate model, we could not adjust for unmeasured confounders, such as over-the-counter medications (e.g., ASA, acetaminophen, ibuprofen, etc.), particular foods, herbal supplements, and dietary intake of vitamin K. For example, mango potentiates warfarin, while ginseng and avocado inhibit it. However, there is no reason to believe that the frequency of confounders would systematically differ among the patients exposed to the different antibiotics. Another unmeasured confounder is the genetic variability among patients, which can contribute to hyper-responsiveness to warfarin. This effect may be due to common mutations in the gene for CYP 2C9 and the association of some variants with decreased metabolism capability of the CYP 2C9 enzyme and therefore reduced (S)-
warfarin clearance.\textsuperscript{20-22} Polymorphism in the vitamin K epoxide reductase complex subunit 1 (VKORC1) is another potential factor in warfarin’s genetically influenced dose requirements.\textsuperscript{21,22} Including patients in the cohort who have been on warfarin for at least 180 days tries to address this, since patients would have been monitored at initiation of therapy and stabilized on an appropriate warfarin dose. In addition, genetic variability is also not expected to systematically differ among antibiotic exposure groups.

Fifth, hypermetabolic states such as fever and infection may increase the response to warfarin and we may be unable to distinguish between the effect of the acute illness and the interacting antibiotics.\textsuperscript{2,10,11,72,73} This, in addition, to potentially interacting analgesics (e.g., ASA, acetaminophen, NSAIDs) given over-the-counter, could partially explain the increased risk with amoxicillin/ampicillin in the sensitivity analyses looking at a 7 days exposure window and which has also been shown in another study.\textsuperscript{11} Nevertheless, all our patients exposed to antibiotics had acute illnesses requiring treatment with antibiotics and the magnitude of the increase in INR in outpatients due to the illness is likely to apply to all antibiotics.

Sixth, while the antibiotics on which we have focused are all indicated for UTI, they also have additional indications. Since diagnosis codes based on OHIP data are not reliable on an individual level (ICES intranet), we had no information regarding the reason for the antibiotic therapy and cannot exclude the possibility that patients who were prescribed various antibiotics were systematically different from one another. However, we focused on an outpatient population who received oral antibiotics, thus excluding patients with more serious infection requiring hospitalization and intravenous
antibiotic therapy. We considered using the performance of a urinalysis test recorded in billing information (unfortunately test results are not available) as an indicator for UTI and limiting the study to only patients who were exposed to an antibiotic of interest while using the amoxicillin/ampicillin exposure group as the reference. However, we did not perform this analysis since this would have restricted the analysis to an extremely small number of exposed patients.

Seventh, we limited our outcome definition to upper gastrointestinal hemorrhage. We excluded lower GI hemorrhage due to data inaccuracies, and intracranial and genitourinary hemorrhages were excluded to have a more homogenous study outcome.

Finally, our study includes the elderly, age 66 years and older, and may not be generalizable to a younger population.

5.3 **Strength of the study**

Why did we choose this study design? It was possible to use either a nested case-control or cohort design to address this question. We chose a nested case-control study because we believe this is a simpler approach to address the question in a meaningful manner. Since this study examines a drug-drug interaction and relies on patients being on both drugs at the same time to assess this interaction, rules for concomitant use would be more complicated in a cohort design for those individuals who are repeatedly exposed to antibiotics and time-dependent covariates would need to be used, which would add a layer of complexity that we avoided through the nested case-control design. There is likely also a more comprehensive capture of events (i.e., cases) using a nested
case-control design and thus more power. In addition, in comparison with the analysis of a cohort with time-dependent covariates, a nested case control approach produces similar risk estimates with computational efficiency.\textsuperscript{74} Also, the nested case-control design has been successfully used previously to investigate drug-drug interactions and is intuitively easily explained.\textsuperscript{48-52,75,75}

The strength of our study lies in its population-based design, using data from all elderly people aged 66 years and older residing in Ontario. This population enabled us to have a large sample size of more than 130,000 patients on chronic warfarin therapy, includes everyone in the province of Ontario, and does not only focus on those with health insurance or the very poor who are eligible for government assistance.

The initiation of warfarin therapy is a time that is prone to an increased risk of bleeding.\textsuperscript{23,24} Therefore we chose to use a cohort consisting of patients who had been on warfarin for at least 180 days and excluded patients who had been hospitalized with any bleeding in the initial 180 days of warfarin therapy. Focusing on patients with chronic warfarin use also allowed us to avoid restricting the cohort to a population with more specific indications for warfarin treatment and increased the study’s generalizability. The inclusion of patients who received warfarin for various indications may have created a less homogenous cohort, however these patients were all exposed to warfarin and thus at risk for drug-drug interactions, and therefore we believe they should be included in the study.

This study examines the increased risk of an outcome of hospitalization for UGI hemorrhage, which is an accurately measured and clinically important outcome.\textsuperscript{57} Using
the linkable databases, numerous covariates that could potentially confound or affect the risk of UGI hemorrhage were defined and adjusted for in the multivariate models.

The ex ante specification of the antibiotics most likely to be associated with hemorrhage is a strength of this study. This was based upon each antibiotic’s degree of inhibition of CYP 2C9. Our pre-study hypothesis was that TMP/SMX would be associated with the highest risk, followed by ciprofloxacin and that the other alternative antibiotics would have a minimal risk.\textsuperscript{4,5,8,9} We also repeated the primary analysis using ocular antibiotics as a neutral tracer to examine specificity and robustness of our results.

5.4 Consistency with other literature

Our findings regarding TMP/SMX are consistent with its inhibition of CYP 2C9 and the resulting stereoselective increase of the potent enatiomer (S)-warfarin.\textsuperscript{5,6,30} These findings are in keeping with other research involving non-warfarin coumarin anticoagulants (acenocoumarol and phenprocoumon) not widely used in North America, although these showed relative risks of more than five-six fold.\textsuperscript{45,46} The studies examined a broader outcome of “bleeding requiring hospitalization”, which included gastrointestinal, cerebral, uterine, and other types of hemorrhage.\textsuperscript{45,46} The magnitude of the increased risks may differ between this study and these other two studies for various reasons, including the smaller number of patients with outcomes and less extensive adjusting for potential covariates. Our results were also consistent with previous studies examining warfarin and TMP/SMX, although the magnitude of the association between UGI hemorrhage and TMP/SMX was somewhat higher in our study.\textsuperscript{10,11}
Our study adds to the existing literature by being the first to focus on the specific risks associated with TMP/SMX and other antibiotics indicated for urinary tract infections, which is among the leading reasons for antibiotic therapy in older adults. Schelleman et al. included TMP/SMX, ciprofloxacin and amoxicillin in their study; however they did not only focus on the specific interaction of TMP/SMX with warfarin and their findings should be interpreted with the recognition that they conducted multiple analyses. In addition, we focused our study on patients chronically treated with warfarin, as represented by continuous use of at least 180 days. Our results are also complementary with other observational studies involving warfarin and TMP/SMX since we calculated the attributable fraction to estimate the proportions of admissions that could have been averted by avoiding the interacting antibiotic TMP/SMX and using an alternative “safer” antibiotic instead.

We observed a small increase in the risk of hemorrhage with ciprofloxacin. There are various hypotheses for the mechanism underlying the ciprofloxacin-warfarin interaction, including that ciprofloxacin may reduce the metabolism of (R)-warfarin, the less biologically active enantiomer, by inhibiting CYP 1A2 and 3A4. A clinical impact of this interaction, such as the increased risk of UGI hemorrhage we found in patients who received ciprofloxacin while on warfarin therapy, has not been consistently described in the research literature. Holbrook et al. categorized ciprofloxacin as "highly probable" to interact with warfarin, however it is noteworthy that the cited study in the review (by Israel et al.) found an increase in (R)-warfarin concentration, but the effect on PT was not clinically significant. A study of 18 healthy volunteers, which focused on coadministration of warfarin and the prolonged–release (PR) formulation of
ciprofloxacin, found no significant interaction (except a slightly elevated $t_{1/2}$ for (R)-warfarin).\textsuperscript{78} Although Schelleman et al.\textsuperscript{11} found an increased risk of GI bleeding in patients on warfarin who received ciprofloxacin compared to “no exposure” to an antibiotic, after using amoxicillin or cephalexin as the reference drug and adjusting for various anti-infective indications, the increased risk was not statistically significant.\textsuperscript{11} Several case reports and case series have shown an increased anticoagulation and hemorrhagic complications with ciprofloxacin, however consistent experimental data linking these data to an increased risk of anticoagulation or hemorrhage are lacking.\textsuperscript{36,77,79}

Our findings that ciprofloxacin was associated with an almost two-fold increased risk of UGI bleeding may also partially be contributed to by unmeasured confounding or selection bias. We speculate that ciprofloxacin may be prescribed to patients with infections that were not UTIs (such as respiratory tract infections and intra-abdominal infections), and that patients who received ciprofloxacin may have had systematically more serious infections, which by itself may contribute to an increased risk of hemorrhage in patients on warfarin.\textsuperscript{2,10,11,72,73}

Like ciprofloxacin, norfloxacin has also been reported to inhibit CYP 1A2 and CYP 3A4.\textsuperscript{8} Norfloxacin inhibits CYP 3A4 \textit{in vitro} in rat and human microsomes, however it was concluded that most probably, a drug interaction would only occur when these antibiotics’ concentrations are unusually high.\textsuperscript{8,76} In a study in ten healthy volunteers, no clinically significant interaction between norfloxacin and warfarin was found.\textsuperscript{80} Holbrook et al.\textsuperscript{4} categorized norfloxacin as possibly interacting with warfarin. Unlike ciprofloxacin, norfloxacin is only indicated for UTI and our results did not show
an increased association of UGI hemorrhage with norfloxacin. As well, Holbrook et al.\textsuperscript{4} categorized amoxicillin as possibly interacting with warfarin.

The small, statistically non-significant elevated odds ratios associated with amoxicillin/ampicillin may indicate residual confounding or may indicate that amoxicillin/ampicillin carries a small increased risk. However, the much higher odds ratio associated with TMP/SMX strongly indicates it has much greater impact upon the risk of UGI bleeding than the other antibiotics.

5.5 **Implications for clinical practice**

With the expansion of indications of warfarin in the elderly, it is important for clinicians to be aware of the factors that may increase the risk of major bleeding in patients on warfarin.\textsuperscript{17} Factors such as the targeted intensity of the anticoagulant therapy, patient’s age and other characteristics, concomitant use of interacting drugs, especially those that interfere with the metabolism of (S)-warfarin, and length of therapy are known determinants of bleeding in patients on warfarin.\textsuperscript{17,24} Of these predictors, avoiding concomitant use of interacting drugs should be among the easier to control for since factors such as patients’ age and comorbidities are not modifiable.

Based on our data, a total of 34% of patients 66 years of age and older received at least one of the antibiotics of interest, and over 7% of patients, continuously on warfarin, were exposed to at least one prescription of TMP/SMX, which is higher than has been previously reported based on data from a large pharmacy benefits manager in the United States (close to 4%).\textsuperscript{37} Taken collectively, the results of our and previous observational studies\textsuperscript{11} provide compelling evidence that co-prescribing of TMP/SMX
in patients on warfarin is associated with an important increased risk of UGI hemorrhage, and that this risk is considerable higher than with other commonly used antibiotics for UTI. UGI bleeds that require hospitalization are not trivial and there is morbidity associated with endoscopy, blood transfusion and nosocomial infections. In our study, of the 2,151 cases hospitalized for UGI hemorrhage, 224 (10.4%) patients died before discharge. We estimate that 0.75% of all hospitalization for UGI hemorrhage in older patients treated with warfarin could have been avoided by using alternative antibiotics with a lower propensity to interact with warfarin, such as amoxicillin or ampicillin. These findings are especially important in view of the tens of millions of scripts of warfarin that are prescribed yearly in the United States and other countries.3

Our observations suggest that clinicians should consider alternate antibiotics to TMP/SMX in patients receiving warfarin. In the rare circumstance where TMP/SMX therapy is essential, close monitoring of anticoagulation control is necessary, and temporary reductions in the warfarin dose may be required.

5.6 Future directions

The results of our study are consistent with other observational studies.10,11 Worthwhile future research about the interaction between these antibiotics and warfarin include the use of richer clinical data (such as INR results and indications for the antibiotic) and studies that examine other bleeding outcomes.

Given the availability of alternative antibiotics for UTI, it is now time to institute a policy change and/or a knowledge translation (KT) strategy.81 Due to TMP/SMX’s
potential for serious adverse effects and various possible drug interactions, it may be prudent to consider the prescription of TMP/SMX only in cases when there are no suitable alternatives (for example, patients with multidrug resistant organisms or in the rare case of *Pneumocystis carinii* pneumonia).\textsuperscript{32,34} KT initiatives could consist of discussing TMP/SMX and its challenges in clinical practice at rounds and continuing education sessions. In addition, there may be opportunities to work with prescribing or infectious disease guideline groups to include this evidence in their recommendations. Treatment of UTI in the elderly is complex. For example, nitrofurantoin should be avoided in older adults due to potential renal impairment and asymptomatic bacteriuria should not unnecessarily be treated.\textsuperscript{35,82} Therefore, it may be useful to incorporate and disseminate the findings of this thesis also as part of comprehensive guidelines for the management of UTI in the elderly. Also, automated prescribing systems could more strongly highlight this interaction when the drugs are co-prescribed.\textsuperscript{83} Policy changes would most probably be more difficult to implement, however this study could be used for potential policy decisions around the listing status of TMP/SMX so it may lead physicians to consider other alternative antibiotics. Of the contributors to the bleeding risk in warfarin-treated patients, drug-drug interactions, and especially antibiotics, are likely one of the few “modifiable” component. Raising awareness and quantification of the risk are crucial in reducing these potentially avoidable hemorrhagic adverse events of warfarin and addressing a clinically important issue.

5.7 Conclusions
In summary, UTI is a common problem in the elderly\textsuperscript{12,13} and TMP/SMX is still regularly prescribed for UTI, including in patients who are continuously receiving warfarin. There is compelling evidence that TMP/SMX is associated with an important increased risk of UGI hemorrhage in older patients receiving warfarin and that there are alternative antibiotics. Clinicians should consider alternate antibiotics to TMP/SMX in patients receiving warfarin. In the rare circumstance where TMP/SMX therapy is essential, close monitoring of anticoagulation control is necessary, and temporary reductions in the warfarin dose may be required. Increased awareness of the risk of bleeding in patients taking warfarin and TMP/SMX is crucial if this preventable cause of bleeding is to be avoided.
REFERENCES


(65) Thijssen HH, Soute BA, Ver voort LM, Claessens JG. Paracetamol (acetaminophen) warfarin interaction: NAPQI, the toxic metabolite of paracetamol, is an inhibitor of enzymes in the vitamin K cycle. Thromb Haemost 2004; 92(4):797-802.


### Table 1 Previous studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Anticoagulant</th>
<th>Design/Outcome/Study period</th>
<th>Sample Size</th>
<th>Relevant Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penning-van Beest FJ et al., 2001&lt;sup&gt;43&lt;/sup&gt;</td>
<td>Acenocoumarol &amp; Phenprocoumon</td>
<td>Nested case-control study</td>
<td>300 cases</td>
<td>TMP/SMX: aOR= 24.2 (2.8-209.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>INR ≥ 6</td>
<td>302 matched</td>
<td>Amoxicillin, Norfloxacin: NS</td>
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<tr>
<td></td>
<td></td>
<td>1997 - 1999</td>
<td>controls</td>
<td></td>
</tr>
<tr>
<td>Visser LE et al., 2002&lt;sup&gt;44&lt;/sup&gt;</td>
<td>Acenocoumarol &amp; Phenprocoumon</td>
<td>Population-based cohort study</td>
<td>N = 1124</td>
<td>TMX/SMX: aRR= 20.1 (10.7-37.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>INR ≥ 6</td>
<td>(sample from Rotterdam study)</td>
<td>Amoxicillin: aRR= 10.5 (5.1-21.7)</td>
</tr>
<tr>
<td>Penning-van Beest FJ et al., 2005&lt;sup&gt;45&lt;/sup&gt;</td>
<td>Acenocoumarol &amp; Phenprocoumon</td>
<td>Population-based retrospective cohort</td>
<td>N = 19,935 New users</td>
<td>TMP/SMX: aRR= 6.2 (2.0-19.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bleeding</td>
<td></td>
<td>Norfloxacin: aRR 5.9 (1.9 – 18.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1992 - 2000</td>
<td></td>
<td></td>
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<tr>
<td>Penning-van Beest FJ et al., 2007&lt;sup&gt;46&lt;/sup&gt;</td>
<td>Acenocoumarol &amp; Phenprocoumon</td>
<td>Population-based retrospective cohort</td>
<td>N=59,987 Coumarin users</td>
<td>TMP/SMX: aRR = 5.1 (2.1 – 12.3);</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bleeding</td>
<td></td>
<td>Amoxicillin: aRR = 3.1 (1.6 – 6.3);</td>
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<tr>
<td></td>
<td></td>
<td>1996 - 2004</td>
<td></td>
<td>Ciprofloxacin: aRR = 3.2 (1.3 – 7.7);</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nitrofurantoin &amp; Norfloxacin: NS</td>
</tr>
<tr>
<td>Glasheen JJ et al., 2005&lt;sup&gt;10&lt;/sup&gt;</td>
<td>Warfarin</td>
<td>Retrospective cohort study</td>
<td>N = 95</td>
<td>TMP/SMX:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>INR ≥ 4 – 44% of patients (p&lt;0.01);</td>
<td>(TMP/SMX = 16)</td>
<td>INR ≥ 5 – 31% of patients (p&lt;0.05);</td>
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<tr>
<td></td>
<td></td>
<td>bleeding</td>
<td></td>
<td>Bleeding – 13%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1998 - 2002</td>
<td></td>
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</tr>
<tr>
<td>Schelleman H et al., 2008&lt;sup&gt;11&lt;/sup&gt;</td>
<td>Warfarin</td>
<td>Nested case-control &amp; Case crossover</td>
<td>N = 308,100 (cases = 11,444, controls = 568,744)</td>
<td>Reference group - no exposure:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gastrointestinal Bleeding</td>
<td></td>
<td>TMP/SMX: aOR = 2.54 (2.08 - 3.10);</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1999 - 2002</td>
<td></td>
<td>Ciprofloxacin: aOR= 1.62 (1.31-1.99);</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Amoxicillin: aOR = 1.28 (1.03-1.58)</td>
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<td></td>
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<td></td>
<td>Reference group – cephalaxin:</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>TMP/SMX: aOR = 2.04 (1.38 - 3.01);</td>
</tr>
</tbody>
</table>

aOR – adjusted odds ratio; aRR – adjusted relative risk, NS – not significant
Table 2 Covariates Included in the Multivariate Analyses (Codes and Databases Used)

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Administrative Database &amp; Codes</th>
<th>Time Frame*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper gastrointestinal hemorrhage</td>
<td><strong>CIHI-DAD Admission</strong> diagnosis <strong>ICD-9</strong>: 5310, 5312, 5314, 5316, 5320, 5322, 5324, 5326, 5330, 5332, 5334, 5336, 5340, 5342, 5344, 5346, 5780, 5781, 5789 or <strong>ICD-10</strong>: K250, K252, K254, K256, K260, K262, K264, K266, K270, K272, K274, K276, K280, K282, K284, K286, K920, K921, K922</td>
<td>5 years prior to cohort entry</td>
</tr>
<tr>
<td>Upper gastrointestinal endoscopic and radiologic studies</td>
<td><strong>OHIP fee codes</strong>: X103, X104, X109, Z399, Z400, Z515, Z527, Z528, Z560, Z749</td>
<td>Within 1 year</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td><strong>CIHI-DAD All diagnoses</strong>: <strong>ICD-9</strong>: 5712, 5715, 5716 <strong>ICD-10</strong>: K702, K703, K740, K746, K742, K744, K743, K745 <strong>OHIP</strong>: 571</td>
<td>Within 5 years</td>
</tr>
<tr>
<td>Alcoholism</td>
<td><strong>CIHI-DAD All diagnoses</strong>:</td>
<td>Within 5 years</td>
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<tr>
<td></td>
<td><strong>ICD-9</strong>: V113, 2910, 2911, 2912, 2913, 2914, 2915, 2918, 2919, 3030, 3039, 3050, 3575, 4255, 5353, 5710, 5711, 5713, 7903, 9800</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>OHIP</strong>: 291, 303</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of DINs</th>
<th>ODB</th>
<th>Within 1 year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long-term care status</td>
<td>ODB flag</td>
<td>Within 180 days</td>
</tr>
</tbody>
</table>

**Concomitant drug use**

<table>
<thead>
<tr>
<th><strong>Concomitant drug use</strong></th>
<th><strong>ODB</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anti-platelet agents</strong></td>
<td>Acetylsalicylic acid (ASA) &amp; combinations, clopidogrel, dipyridamole, ticlopidine</td>
<td>Within 120 days</td>
</tr>
<tr>
<td><strong>Anti-inflammatory</strong></td>
<td>Non-steroidal anti-inflammatory drugs (NSAIDs), including cyclooxygenase-2 inhibitors: Celecoxib, diclofenac, diclofenac &amp; misoprostol, diflunisal, etodolac, fenoprofen, floctafenine, flurbiprofen, ibuprofen, indomethacin, ketoprofen, ketorolac tromethamine, mefenamic acid, meloxicam, nabumetone, naproxen, oxaprozin, phenylbutazone, piroxicam, rofecoxib, sulindac, tenoxicam, tiaprofenic acid, tolmefin, valdecoxib</td>
<td>Within 120 days</td>
</tr>
<tr>
<td><strong>Other anticoagulants</strong></td>
<td>Vitamin K antagonists (nicoumalone), synthetic antithrombotic agents (fondaparinux), lepirudin, low molecular weight heparins</td>
<td>Within 120 days</td>
</tr>
<tr>
<td><strong>Acetaminophen &amp; combinations</strong></td>
<td>Acetaminophen &amp; combinations</td>
<td>Within 120 days</td>
</tr>
<tr>
<td><strong>Systemic Corticosteroids</strong></td>
<td>Betamethasone, budesonide, corticotropin, cortisone, dexamethasone, fludrocortisone, hydrocortisone, methylprednisolone, prednisolone, prednisone, triamcinolone,</td>
<td>Within 120 days</td>
</tr>
<tr>
<td><strong>Gastroprotective medications</strong></td>
<td>H₂ antagonists, misoprostol, proton pump inhibitors, sucralfate</td>
<td>Within 120 days</td>
</tr>
<tr>
<td>All other systemic antibiotics</td>
<td>All other systemic antibiotics that are not included in the study antibiotics</td>
<td>Within 120 days</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>Selective serotonin reuptake inhibitors (SSRIs)</td>
<td>Citalopram, escitalopram, fluoxetine, fluvoxamine, nefazodone, paroxetine, sertraline, venlafaxine</td>
<td>Within 120 days</td>
</tr>
<tr>
<td>Cytochrome P450 isoenzyme 2C9 (CYP 2C9) Inhibitors</td>
<td>Amiodarone, disulfiram, divalproex, fenofibrate, fluconazole, fluvastatin, imatinib, isoniazid, ketoconazole, leflunomide, lovastatin, metronidazole, modafinil, nateglinide, probenecid, sildenafil, valproic acid, voriconazole, zafirlukast</td>
<td>Within 120 days</td>
</tr>
<tr>
<td>CYP 2C9 Inducers</td>
<td>Bosentan, carbamazepine, phenobarbital, phenytoin, primidone, rifampin, secobarbital</td>
<td>Within 120 days</td>
</tr>
<tr>
<td>Antibiotics of interest</td>
<td>TMP/SMX, amoxicillin/ampicillin, ciprofloxacin, nitrofurantoin, norfloxacin</td>
<td>Within 120 days</td>
</tr>
<tr>
<td>Ocular antibiotics</td>
<td>Bacitracin, chloramphenicol, ciprofloxacin, erythromycin, framycetin, fusidic acid, gentamicin, ofloxacin, polymyxin B sulfate &amp; combinations, sulfacetamide, tobramycin</td>
<td>Within 120 days</td>
</tr>
</tbody>
</table>

* Time frames are defined relative to the index date unless otherwise specified.
** "Admission diagnosis" includes CIHI discharge diagnosis type M,1,W,X,Y; and, for ICD10 only, also 9. In addition, if a code appears as M and also as a 2 on the same record, we disregarded it, since having the same diagnosis recorded with an “M” and a “2” indicates that this post-admission diagnosis became the reason for the longest length of stay. “All diagnoses” refers to the inclusion of all diagnosis codes regardless of diagnosis type.
† Same definition was used for the exclusion criteria of any bleeding within the initial 180 days of continuous warfarin use prior to cohort entry, but it also included UGI hemorrhage.

**Abbreviations:**
DIN – Drug Identification Number; ODB – Ontario
Table 3 Characteristics of Cases and Controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=2,151</td>
<td>N=21,434</td>
</tr>
<tr>
<td>Female</td>
<td>1,023 (47.6%)</td>
<td>10,201 (47.6%)</td>
</tr>
<tr>
<td>Median age (IQR)</td>
<td>80 (74-85)</td>
<td>80 (74-85)</td>
</tr>
<tr>
<td>Upper GI hemorrhage (within 5 yrs of cohort entry)</td>
<td>106 (4.9%)</td>
<td>395 (1.8%)</td>
</tr>
<tr>
<td>Upper GI diagnostic examination within 1 year of index date</td>
<td>469 (21.8%)</td>
<td>1,217 (5.7%)</td>
</tr>
<tr>
<td>Any bleeding within 1 year of index date</td>
<td>72 (3.3%)</td>
<td>331 (1.5%)</td>
</tr>
<tr>
<td>Alcoholism (within 5 yrs of index date)</td>
<td>55 (2.6%)</td>
<td>466 (2.2%)</td>
</tr>
<tr>
<td>Cirrhosis (within 5 yrs of index date)</td>
<td>34 (1.6%)</td>
<td>144 (0.7%)</td>
</tr>
<tr>
<td>Number of DINs in the 1 year prior to index</td>
<td>15.36 ± 7.36</td>
<td>12.69 ± 6.66</td>
</tr>
<tr>
<td>Residence in long-term care</td>
<td>320 (14.9%)</td>
<td>2,332 (10.9%)</td>
</tr>
</tbody>
</table>

Other potentially interacting medications

<table>
<thead>
<tr>
<th>Medication</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-platelets agents</td>
<td>123 (5.7%)</td>
<td>910 (4.2%)</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td>380 (17.7%)</td>
<td>2,035 (9.5%)</td>
</tr>
<tr>
<td>Other anticoagulants</td>
<td>8 (0.4%)</td>
<td>181 (0.8%)</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>174 (8.1%)</td>
<td>1,005 (4.7%)</td>
</tr>
<tr>
<td>Gastroprotective agents</td>
<td>670 (31.1%)</td>
<td>5,128 (23.9%)</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>619 (28.8%)</td>
<td>4,186 (19.5%)</td>
</tr>
<tr>
<td>Selective serotonin reuptake inhibitors</td>
<td>270 (12.6%)</td>
<td>1,937 (9.0%)</td>
</tr>
<tr>
<td>CYP 2C9 inducers</td>
<td>79 (3.7%)</td>
<td>565 (2.6%)</td>
</tr>
<tr>
<td>CYP 2C9 inhibitors</td>
<td>263 (12.2%)</td>
<td>2,128 (9.9%)</td>
</tr>
<tr>
<td>Other systemic antibiotics</td>
<td>449 (20.9%)</td>
<td>3,327 (15.5%)</td>
</tr>
<tr>
<td>Ocular antibiotics</td>
<td>56 (2.6%)</td>
<td>511 (2.4%)</td>
</tr>
</tbody>
</table>

* all medications are prescriptions within 120 days prior to index
Table 4 Association Between Hospital Admission for Upper Gastrointestinal Bleeding and Use of Co-trimoxazole Compared to Other Antibiotics Indicated for UTI in Patients Receiving Warfarin

<table>
<thead>
<tr>
<th>PRIMARY ANALYSIS</th>
<th>No. (%) Exposed</th>
<th>Uncorrected Odds Ratio (95% CI)</th>
<th>Multivariate Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases N=2,151</td>
<td>Controls N=21,434</td>
<td></td>
</tr>
<tr>
<td>Exposure within 14 days of Index date</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMP/SMX</td>
<td>25 (1.2%)</td>
<td>56 (0.3%)</td>
<td>4.53 (2.81 - 7.30)</td>
</tr>
<tr>
<td>Amoxicillin/Ampicillin</td>
<td>30 (1.4%)</td>
<td>209 (1.0%)</td>
<td>1.44 (0.98 - 2.12)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>31 (1.4%)</td>
<td>124 (0.6%)</td>
<td>2.50 (1.68 - 3.73)</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>11 (0.5%)</td>
<td>64 (0.3%)</td>
<td>1.71 (0.90 - 3.24)</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>≤5 (≤0.2%)</td>
<td>61 (0.3%)</td>
<td>0.49 (0.15 - 1.57)</td>
</tr>
<tr>
<td>Ocular antibiotics</td>
<td>10 (0.5%)</td>
<td>81 (0.4%)</td>
<td>1.23 (0.64 - 2.38)</td>
</tr>
</tbody>
</table>

| SENSITIVITY ANALYSES | | Exposure within 7 days of Index date | |                                 |
|----------------------|----------------|------------------------------------|---------------------------------|
| Exposure within 14 days of Index date | | |                                 |
| TMP/SMX              | 12 (0.6%)      | 33 (0.2%)                          | 3.61 (1.87 - 7.00)              | 3.36 (1.69 - 6.67)              |
| Amoxicillin/Ampicillin | 23 (1.1%)     | 127 (0.6%)                        | 1.82 (1.16 - 2.84)              | 1.88 (1.18 - 2.99)              |
| Ciprofloxacin        | 20 (0.9%)      | 68 (0.3%)                         | 2.87 (1.74 - 4.75)              | 2.17 (1.28 - 3.67)              |
| Nitrofurantoin       | 7 (0.3%)       | 35 (0.2%)                         | 1.97 (0.88 - 4.44)              | 1.66 (0.71 - 3.91)              |
| Norfloxacin          | ≤5 (≤0.2%)     | 30 (0.1%)                         | 0.33 (0.05 - 2.45)              | 0.33 (0.05 - 2.46)              |
| Ocular antibiotics   | ≤5 (≤0.2%)     | 37 (0.2%)                         | 1.35 (0.53 - 3.44)              | 1.16 (0.45 - 3.00)              |

<table>
<thead>
<tr>
<th>Exposure within 21 days of Index date</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TMP/SMX</td>
<td>29 (1.3%)</td>
<td>91 (0.4%)</td>
<td>3.22 (2.11 - 4.92)</td>
</tr>
<tr>
<td>Amoxicillin/Ampicillin</td>
<td>42 (2%)</td>
<td>323 (1.5%)</td>
<td>1.30 (0.94 - 1.80)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>36 (1.7%)</td>
<td>172 (0.8%)</td>
<td>2.09 (1.45 - 3.01)</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>14 (0.7%)</td>
<td>91 (0.4%)</td>
<td>1.53 (0.87 - 2.69)</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>9 (0.4%)</td>
<td>97 (0.5%)</td>
<td>0.93 (0.47 - 1.84)</td>
</tr>
<tr>
<td>Ocular antibiotics</td>
<td>11 (0.5%)</td>
<td>126 (0.6%)</td>
<td>0.87 (0.47 - 1.62)</td>
</tr>
</tbody>
</table>

Multivariate analysis adjusts for history of UGI hemorrhage, upper gastrointestinal endoscopic and radiologic studies, and any bleeding (except UGI hemorrhage), history of cirrhosis, history of alcoholism, number of drugs in the year prior to index date, long-term care status, other antibiotics of interest and other concomitant drug use (see Table 2).
FIGURE 1: STUDY DESIGN DESCRIPTION

Index date*  
14 days
Look back for exposure

180 days of continuous warfarin use

Exclude if hospitalized for bleeding

Warfarin start date

Cohort entry (day 180)

First potential cohort entry date: Oct 1, 1997

Max accrual date: Mar 31, 2007

Continuous warfarin use

Index date = 
Case - admission to hospital with an admission diagnosis of UGI hemorrhage.
Control – eligible continuous warfarin users on the case event date, who have not yet been hospitalized with UGI hemorrhage.
Appendix 1 Description of the case definition (ICD-9 & ICD10 codes)

<table>
<thead>
<tr>
<th>ICD-9/ICD-10 Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>531.0 / K25.0</td>
<td>Gastric ulcer – acute with hemorrhage</td>
</tr>
<tr>
<td>531.2 / K25.2</td>
<td>Gastric ulcer – acute with hemorrhage and perforation</td>
</tr>
<tr>
<td>531.4 / K25.4</td>
<td>Gastric ulcer – chronic or unspecified with hemorrhage</td>
</tr>
<tr>
<td>531.6 / K25.6</td>
<td>Gastric ulcer – chronic or unspecified with hemorrhage and perforation</td>
</tr>
<tr>
<td>532.0 / K26.0</td>
<td>Duodenal ulcer - acute with hemorrhage</td>
</tr>
<tr>
<td>532.2 / K26.2</td>
<td>Duodenal ulcer - acute with hemorrhage and perforation</td>
</tr>
<tr>
<td>532.4 / K26.4</td>
<td>Duodenal ulcer - chronic or unspecified with hemorrhage</td>
</tr>
<tr>
<td>532.6 / K26.6</td>
<td>Duodenal ulcer - chronic or unspecified with hemorrhage and perforation</td>
</tr>
<tr>
<td>533.0 / K27.0</td>
<td>Peptic ulcer, site unspecified - acute with hemorrhage</td>
</tr>
<tr>
<td>533.2 / K27.2</td>
<td>Peptic ulcer, site unspecified - acute with hemorrhage and perforation</td>
</tr>
<tr>
<td>533.4 / K27.4</td>
<td>Peptic ulcer, site unspecified - chronic or unspecified with hemorrhage</td>
</tr>
<tr>
<td>533.6 / K27.6</td>
<td>Peptic ulcer, site unspecified - chronic or unspecified with hemorrhage and perforation</td>
</tr>
<tr>
<td>534.0 / K28.0</td>
<td>Gastrojejunal ulcer - acute with hemorrhage</td>
</tr>
<tr>
<td>534.2 / K28.2</td>
<td>Gastrojejunal ulcer - acute with hemorrhage and perforation</td>
</tr>
<tr>
<td>534.4 / K28.4</td>
<td>Gastrojejunal ulcer - chronic or unspecified with hemorrhage</td>
</tr>
<tr>
<td>534.6 / K28.6</td>
<td>Gastrojejunal ulcer - chronic or unspecified with hemorrhage and perforation</td>
</tr>
<tr>
<td>578.0 / K92.0</td>
<td>Hematemesis</td>
</tr>
<tr>
<td>578.1 / K92.1</td>
<td>Blood in Stool / melena</td>
</tr>
<tr>
<td>578.9 / K92.2</td>
<td>Hemorrhage of gastrointestinal tract, unspecified</td>
</tr>
</tbody>
</table>

ICD-9 = *International Classification of Diseases, Ninth Revision Clinical Modification*; ICD-10 = *International Statistical Classification of Diseases and Related Health Problems, Tenth Revision, Canada*