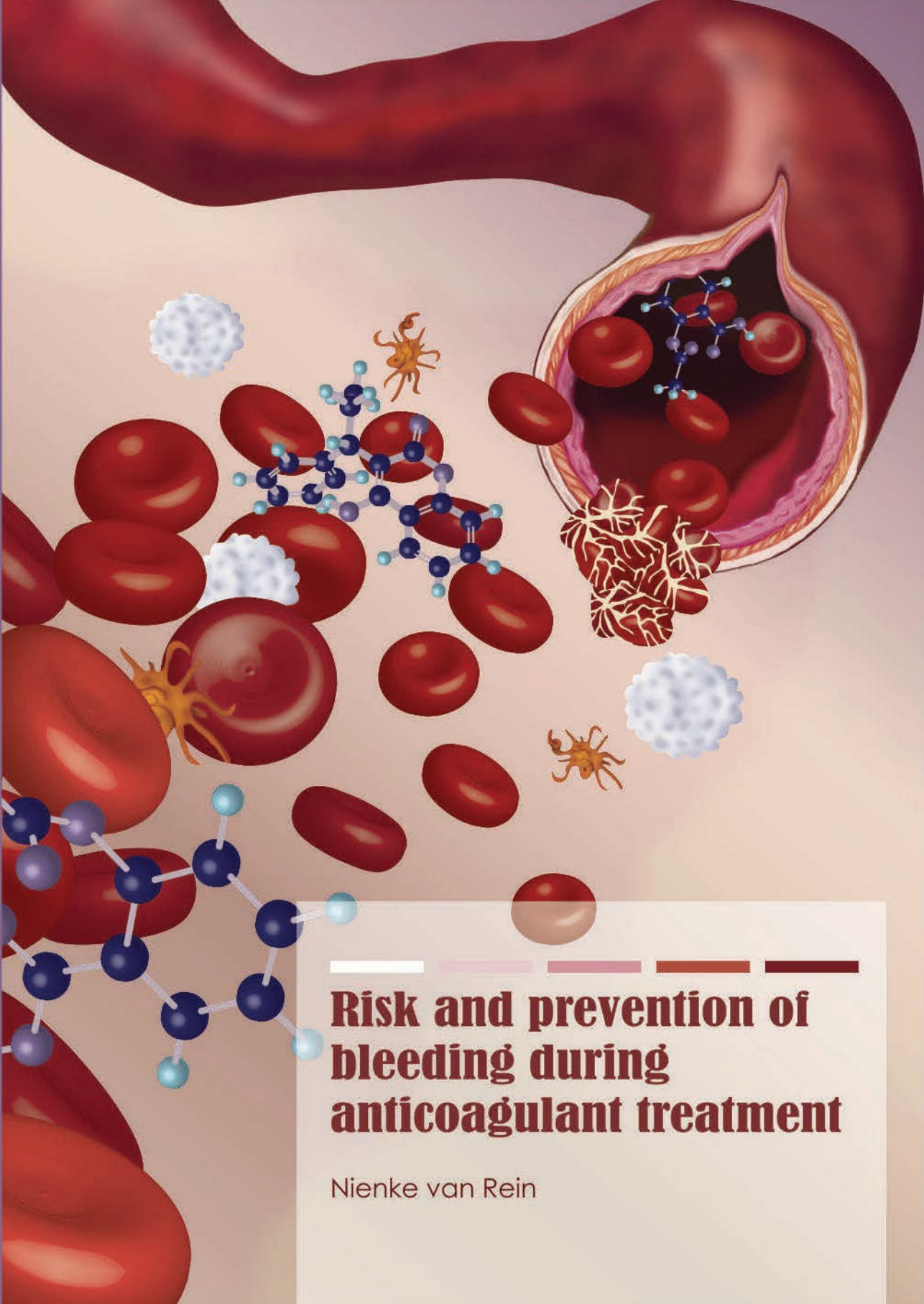

Risk and prevention of bleeding during anticoagulant treatment

Nienke van Rein

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Chapter 1

Introduction and outline

PHARMACOEPIDEMIOLOGY

The term 'epidemiology' is a source of confusion about the nature of this discipline. For the public, 'epidemiology' evokes a medical discipline that deals with large scale outbreaks of infectious diseases. This was the context in which the term was initially used: in the 16th century the Spanish physician Angelerio published a study on the plague entitled 'Epidemiologica'.[1] In 1850, the *London Epidemiologic Society* was created, which assembled scientists, public health practitioners, and physicians to unite their efforts to fight against 'epidemics'.[1] Today, epidemiology remains associated with fight against infectious diseases, but it has expanded and is not restricted to specific diseases anymore.[1] It is currently defined as the study of patterns, causes, and effects of health and disease conditions in defined populations.[2] Epidemiology consists of multiple sub-specialties of which clinical epidemiology, genetic epidemiology, molecular epidemiology and pharmacoepidemiology are examples.

Pharmacoepidemiology is the study of utilization and effects of drugs in large numbers of people. It provides estimates of the probability of beneficial effects and the probability of adverse events.[3] When studying effects of drugs, it is important to combine the knowledge on the epidemiology and pharmacology, but also on prescription behavior of physicians.

The first part of this thesis will look deeper into the methods of observational studies as the methods form the 'foundation' of the 'pharmacoepidemiological framework'.

Methodological rigidity and pharmacoepidemiology

Chapters 2 and 3 describe methodological aspects of case-control- and cohort studies that examine the effects of statins on bleeding. Statins are known to halt or reduce atherosclerosis but are also claimed to have a wide range of unintended and unexplained beneficial effects.[4] In both chapters, methodological aspects of these studies are considered that could explain why the wide and varied claims of unintended effects of statins are the result of bias and do not represent true benefit. In **Chapter 4**, we examine whether previous claims that vitamin preparations decrease the risk for venous thrombosis could be explained by lack of proper adjustment for the lifestyle-related factors. We look further into explanations for discrepancies between study outcomes in **Chapter 5**. In this chapter we describe how and why outcomes may differ when using either of two control groups in a case-control study. The second part of this thesis comprises of pharmacoepidemiological studies which focus on preventing bleeding complications during treatment with anticoagulants and in particular vitamin K antagonists.

1

HISTORY AND PHARMACOEPIDEMIOLOGY OF VITAMIN K ANTAGONISTS

History

The sweet clovers *Melilotus alba* and *Melilotus officinalis* became recognized as valuable farm crops during the first quarter of the twentieth century.[5] In the winter months of 1921-22, veterinarians in Canada found numerous large and small hemorrhages during the postmortems of cattle dying from a mysterious disease. The pathologist Frank W. Schofield found that in every case sweet clover had been consumed by the deceased animals.[6] A few years later, Schofield discovered that moldy sweet clover caused the symptoms while a clean stack did not.[5] It did not take long before it was known that sweet clover disease came with a deficiency of prothrombin.[7] In 1935, Henrik Dam discovered that the fat soluble vitamin K was an anti-hemorrhagic factor.[8] Not much later, Campbell saw the first crystals of dicoumarol under a microscope, who was supervised by Karl Link in 1939.[9,10] Link kept on working on a more potent rodent poison, resulting in warfarin in 1948. In 1951, a US army inductee unsuccessfully attempted suicide with warfarin and fully recovered after administration of vitamin K in the hospital. The incident was a catalyst for the use of warfarin as a therapeutic agent. It was found to be superior to dicoumarol and warfarin was approved for medical use in 1954,[10] after which phenprocoumon and acenocoumarol followed soon.[11,12]

Pharmacology

When warfarin was approved for medical use, it was unknown what the biochemical basis of the anticoagulant effect was. During the beginning of the 1970s, it was discovered that vitamin K undergoes a cyclic conversion. The cyclic conversion was named the vitamin K cycle, and it is this cycle that is inhibited by warfarin.[13,14] Vitamin K has three forms, and in the cycle the quinone-form becomes converted to the hydroquinone then to the epoxide, and back to quinone (see Figure 1).[14] Vitamin K-epoxide reductase complex 1 (VKORC1) converts the epoxide- to the quinone- to the hydroquinone form.[11] Vitamin K hydroquinone supports carboxylation of γ-glutamic acid residues to γ-carboxyglutamic acid (Gla) residues in vitamin K dependent coagulation factors. This latter reaction is catalyzed by Gamma-Glutamyl carboxylase (GGCX).[11] The Gla residues can bind Ca^{2+} with high affinity which is necessary for biological function - in particular binding to negatively charged phospholipid membranes - of vitamin K dependent coagulation factors.[11] As warfarin inhibits the actions of VKORC1, and thereby the vitamin K cycle, lower levels of carboxylated vitamin K dependent coagulation factors (i.e. II, VII, IX, X, protein C, S and Z) will circulate and this impairs coagulation reactions.[15]

The most commonly prescribed vitamin K antagonists are warfarin, phenprocoumon and acenocoumarol, which are all racemic mixtures with one enantiomer being more potent than the other.[16] An important difference between the vitamin K antagonists is

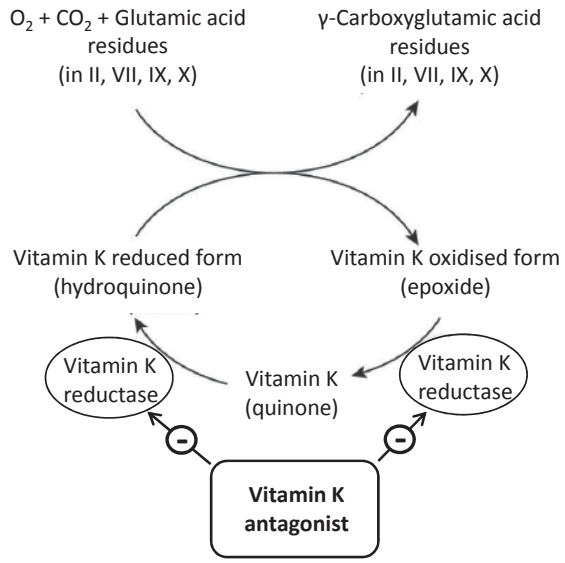


Figure 1. Vitamin K cycle and pharmacology vitamin K antagonists

their half-live. Acenocoumarol has the shortest half-life (7 h), warfarin a longer (40 h), and phenprocoumon the longest half-life (120 h).[15,17] Another difference lies in the pharmacokinetics of the drugs: the more potent enantiomers of phenprocoumon and warfarin are metabolized by CYP2C9, while the more potent enantiomer of acenocoumarol is metabolized by CYP2C19 and CYP2C9.[15,16]

Therapy

Warfarin is the most commonly prescribed vitamin K antagonist in the world, but in the Netherlands only phenprocoumon and acencoumaol are available.[18] Vitamin K antagonists are used to treat and prevent thrombosis and are prescribed for several indications. The most common indication is prevention of thromboembolic stroke in atrial fibrillation patients.[19] Examples of other indications are treatment of acute venous thrombosis and prevention of thrombosis after a prosthetic heart valve implantation or after vascular surgery.[18] The duration of therapy depends on the indication: patients who have atrial fibrillation are usually treated for life, whereas patients with a first venous thrombosis are often treated for three to six months only.

Appropriate dosing is a challenge in therapy with vitamin K antagonists: the required dose of vitamin K antagonists varies between persons, but can also vary over time within a person. The variations within persons are due to changes in for example diet, co-medication or vitamin K intake, which can vary on a day-to-day basis.[15] As the drugs also have a narrow therapeutic window, monitoring of the effect of treatment is required.[15]

In the Netherlands, anticoagulation clinics were set up to maximize the effectiveness and to minimize the bleeding risk of the therapy with vitamin K antagonists.[18,20] In these clinics the international normalized ratio (INR) is measured on a regular basis, on which the dose of the vitamin K antagonist for the next period is based.[18] The target range of the INR depends on the indication of treatment and can be 2.5 to 3.5 or 3.0 to 4.0 in the Netherlands.[18] After determining the INR, patients receive a calendar with their individualized dosing schedule until the next visit, which is generally within six weeks. The frequency of the visits depends on the stability of the INR and the value of the INR.[18]

Despite this monitoring system, the most common side effects of treatment with vitamin K antagonists remain bleeding complications.[15] Whether therapy should be initiated and continued, always depends on the balance between benefit (preventing or treating thrombosis) and harm (bleeding events).[15] Major bleeding events occur in 1-3% of the patients treated every year.[21] The second part of this thesis describes strategies that may result in fewer of these major bleeding events in the future, and thus better safety of anticoagulant treatment.

Therapeutic interventions

Atrial fibrillation patients can have co-morbidities that can result in treatment with a combination of anticoagulants.[22,23] Some high risk patients receive combination therapy with a vitamin K antagonist, aspirin and clopidogrel (i.e. triple therapy). Literature shows that patients who receive triple therapy are at an almost four-fold increased risk for major bleeding as compared to those on warfarin monotherapy.[24] Overall absolute risks for safety and effectiveness outcomes were previously reported,[24] but data on safety and effectiveness outcomes in groups of patients with different baseline risks of bleeding complications or ischemic stroke (e.g elderly or those with a history of a bleeding complication) are currently not known. **Chapter 6** shows rates of safety- (major bleeding) and effectiveness- (myocardial infarction and ischemic stroke) outcomes for various forms of anticoagulant treatment in atrial fibrillation patients.

Chapter 7 and 8 examine anticoagulant treatment of patients with acute venous thrombosis. This treatment can routinely consists of administration of rapid-onset low-molecular-weight-heparins (LMWHs) overlapped and followed by vitamin K antagonists.[25] The combination is indicated during the initial period of treatment because the onset of the anticoagulant effect of vitamin K antagonists will take several days. Therefore LMWHs are prescribed until two consecutive INRs are within the target range.[15] It is assumed that all LMWHs are equally effective and have similar rates of side effects.[26] However, pharmacodynamics and pharmacokinetics of these individual LMWHs do differ and therefore this assumption may be erroneous.[26] **Chapter 7 and 8** present the safety (bleeding risk) and efficacy (recurrent thrombosis risk) outcomes of individual LMWHs in concurrent therapy with vitamin K antagonists.

Practical safety interventions for treatment

Interventions to prevent bleeding complications also include ways to increase therapeutic compliance. Multidose drug dispensing (MDD) is a dosing aid that provides patients with disposable bags containing all drugs intended for one dosing moment.[27] This is especially useful when non-compliant polypharmacy patients have complex dosing patterns. Better adherence to treatment with vitamin K antagonists may result in fewer thrombotic- and bleeding events, but whether compliance increases after initiating MDD in anticoagulated patients has not been studied yet. In **Chapter 9**, we describe whether MDD improves adherence in patients using vitamin K antagonists.

Another practical intervention to reduce the risk of bleeding is to make the route of administration of antidotes to anticoagulant drugs easier. Vitamin K is used to antagonize the anticoagulant effect of vitamin K antagonists.[20] A liquid formulation for oral administration of vitamin K has been used for a long time,[28] but recently tablets have become available. Tablets are easier to ingest and taste better. However, it is not known whether the tablets are as effective as the liquid formulation in reversing anticoagulant effects of vitamin K antagonists. As the absorption of vitamin K is stimulated by fat,[29,30] it is possible that an oily solution results in higher bioavailability and is therefore a more effective reversal formulation (i.e. decreasing the INR) than tablets. **Chapter 10** of this thesis describes three trials that relate to this issue. The first crossover trial shows the absorption profiles of the liquid formulation as compared with the tablets. The other two trials show the effects of the solution and tablets on the INR.

Prediction of bleeding complications

Preventing major bleeding complications can also be achieved by ceasing or not initiating anticoagulant treatment with vitamin K antagonists. This could be considered if the harm of the treatment (bleeding) outweighs its benefit (prevention of thrombosis). To establish which patients do not benefit from treatment with vitamin K antagonists, it is necessary to predict who is at high risk for major bleeding complications. Much research has been performed on risk factors for major bleeding complications, which can be divided into risk factors related to patient characteristics, comorbidities, co-medication and the anticoagulant treatment (Table 1).

These risk factors can be combined in risk prediction models, but these all perform relatively poorly.[55] Available data also shows that patients who are at high risk of recurrent ischemic stroke have the highest risk for intracerebral bleeding,[56] which complicates the decision whether to treat or continue anticoagulant treatment or to discontinue. New predictors for major bleeding complications are therefore necessary. **Chapter 11** gives leads to what type of predictors we should be looking for. In addition, **Chapter 12** describes a large prospective cohort study where plasma and DNA is collected from over 13 500 patients who started treatment with vitamin K antagonists

between 2012 and 2014. This study is set-up in order to discover new leads to improve prediction of major bleeding complications in the future, and its methods are discussed. **Chapter 13** provides the first results whether markers of endothelial damage could be considered as predictors for major bleeding.

Table 1. Predictors for major bleeding complications during treatment with vitamin K antagonists

Patient characteristics	Comorbidity	Co-medication	Anticoagulant treatment
Advanced age[31-33]	Uncontrolled hypertension[34-36]	Antiplatelet drugs[24]	High INR[37-41]
Genetic profile[42,43]	Diabetes[44,45]	Antibiotics[46]	Low time in therapeutic range[47-49]
Bleeding history[34,36,50,51]	Cancer[52]	NSAIDs[53,54]	Labile INRs[36]
Drug abuse[45]	Abnormal liver and renal function[35,44,50]		
Alcohol abuse[35,45]			

REFERENCES

1. Morabia A. Introduction; A contextual name. *A History of Epidemiologic Methods and Concepts*2004:3-4.
2. JM L. *Dictionary of epidemiology.* 4th edition ed: New York: Oxford University Press; 2001.
3. http://www.hopkinsmedicine.org/gim/research/content/pharmacoepi.html; visited 28-12-2015.
4. Rosendaal FR. Statins and venous thrombosis: a story too good to be true? *PLoS.Med.* 2012 2012;9(9):e1001311.
5. Schofield F. Damaged sweet clover: the cause of a new disease in cattle simulating hemorrhagic septicemia and blackleg. *American Veterinary Medical Association.* 1923 1923-1924 1923;64:553-575.
6. Schofield F. A brief account of a disease in cattle simulating hemorrhagic septicemia due to feeding sweet clover. *Can.Vet.Record.* 1922 1922;3:74-78.
7. LM R, AF S. Studies on sweet clover disease. *Agrie Exp Sta Bull North Dakota Agricultural College, Fargo.* 1931 1931;250.
8. Griminger P. Vitamin K antagonists: the first 50 years. *J.Nutr.* 7/1987 1987;117(7):1325-1329.
9. Nutrition classics from The Journal of Biological Chemistry 138:21-33, 1941. Studies on the hemorrhagic sweet clover disease. IV. The isolation and crystallization of the hemorrhagic agent by Harold A. Campbell and Karl Paul Link. *Nutr.Rev.* 8/1974 1974;32(8):244-246.
10. LINK KP. The discovery of dicumarol and its sequels. *Circulation.* 1/1959 1959;19(1):97-107.

11. Oldenburg J, Marinova M, Muller-Reible C, Watzka M. The vitamin K cycle. *Vitam.Horm.* 2008 2008;78:35-62.
12. BAER S, YARROW MW, KRAVITZ C, MARKSON V. Clinical experiences with warfarin (coumadin) sodium as an anticoagulant. *J.Am.Med.Assoc.* 6/7/1958 1958;167(6):704-708.
13. Bell RG, Matschiner JT. Vitamin K activity of phylloquinone oxide. *Arch.Biochem.Biophys.* 12/1970 1970;141(2):473-476.
14. Bell RG, Matschiner JT. Warfarin and the inhibition of vitamin K activity by an oxide metabolite. *Nature.* 5/5/1972 1972;237(5349):32-33.
15. Ansell J, Hirsh J, Hylek E, Jacobson A, Crowther M, Palareti G. Pharmacology and management of the vitamin K antagonists: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest.* 6/2008 2008;133(6 Suppl):160S-198S.
16. Beinema M, Brouwers JR, Schalekamp T, Wilffert B. Pharmacogenetic differences between warfarin, acenocoumarol and phenprocoumon. *Thromb.Haemost.* 12/2008 2008;100(6):1052-1057.
17. Ufer M. Comparative pharmacokinetics of vitamin K antagonists: warfarin, phenprocoumon and acenocoumarol. *Clin.Pharmacokinet.* 2005 2005;44(12):1227-1246.
18. van der Meer FJ, Rosendaal FR, Vandenbroucke JP, Briet E. Bleeding complications in oral anticoagulant therapy. An analysis of risk factors. *Arch.Intern.Med.* 7/12/1993 1993;153(13):1557-1562.
19. Bergman M, Ori Y, Blumberger N, Brener ZZ, Salman H. A study of elderly adults taking warfarin admitted with prolonged international normalized ratio: a community hospital's experience. *J.Am.Geriatr.Soc.* 9/2012 2012;60(9):1713-1717.
20. Ageno W, Gallus AS, Wittkowsky A, Crowther M, Hylek EM, Palareti G. Oral anticoagulant therapy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest.* 2/2012 2012;141(2 Suppl):e44S-e88S.
21. Schulman S, Beyth RJ, Kearon C, Levine MN. Hemorrhagic complications of anticoagulant and thrombolytic treatment: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest.* 6/2008 2008;133(6 Suppl):257S-298S.
22. Fuster V, Ryden LE, Cannom DS, et al. ACC/AHA/ESC 2006 guidelines for the management of patients with atrial fibrillation--executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the European Society of Cardiology Committee for Practice Guidelines (Writing Committee to Revise the 2001 Guidelines for the Management of Patients With Atrial Fibrillation). *J.Am.Coll.Cardiol.* 8/15/2006 2006;48(4):854-906.
23. Singer DE, Albers GW, Dalen JE, et al. Antithrombotic therapy in atrial fibrillation: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest.* 6/2008 2008;133(6 Suppl):546S-592S.
24. Hansen ML, Sorensen R, Clausen MT, et al. Risk of bleeding with single, dual, or triple therapy with warfarin, aspirin, and clopidogrel in patients with atrial fibrillation. *Arch.Intern.Med.* 9/13/2010 2010;170(16):1433-1441.

25. Kearon C, Akl EA, Comerota AJ, et al. Antithrombotic therapy for VTE disease: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest.* 2/2012 2012;141(2 Suppl):e419S-e494S.
26. White RH, Ginsberg JS. Low-molecular-weight heparins: are they all the same? *Br.J.Haematol.* 4/2003 2003;121(1):12-20.
27. Kwint HF, Stolk G, Faber A, Gussekloo J, Bouvy ML. Medication adherence and knowledge of older patients with and without multidose drug dispensing. *Age Ageing.* 9/2013 2013;42(5):620-626.
28. Fytomenadiondrank 10 mg/ml. *Formularium Nederlandse Apothekers*. 2013 ed2009.
29. Gijsbers BL, Jie KS, Vermeer C. Effect of food composition on vitamin K absorption in human volunteers. *Br.J.Nutr.* 8/1996 1996;76(2):223-229.
30. Shearer MJ, McBurney A, Barkhan P. Studies on the absorption and metabolism of phylloquinone (vitamin K1) in man. *Vitam.Horm.* 1974 1974;32:513-542.
31. Fang MC, Go AS, Hylek EM, et al. Age and the risk of warfarin-associated hemorrhage: the anticoagulation and risk factors in atrial fibrillation study. *J.Am.Geriatr.Soc.* 8/2006 2006;54(8):1231-1236.
32. Palareti G, Hirsh J, Legnani C, et al. Oral anticoagulation treatment in the elderly: a nested, prospective, case-control study. *Arch.Intern.Med.* 2/28/2000 2000;160(4):470-478.
33. Tincani E, Baldini P, Crowther MA, et al. Bleeding rates in patients older than 90 years of age on vitamin K antagonist therapy for nonvalvular atrial fibrillation. *Blood Coagul.Fibrinolysis.* 1/2009 2009;20(1):47-51.
34. Fang MC, Go AS, Chang Y, et al. A new risk scheme to predict warfarin-associated hemorrhage: The ATRIA (Anticoagulation and Risk Factors in Atrial Fibrillation) Study. *J.Am.Coll.Cardiol.* 7/19/2011 2011;58(4):395-401.
35. Gage BF, Yan Y, Milligan PE, et al. Clinical classification schemes for predicting hemorrhage: results from the National Registry of Atrial Fibrillation (NRAF). *Am.Heart J.* 3/2006 2006;151(3):713-719.
36. Pisters R, Lane DA, Nieuwlaat R, de Vos CB, Crijns HJ, Lip GY. A novel user-friendly score (HAS-BLED) to assess 1-year risk of major bleeding in patients with atrial fibrillation: the Euro Heart Survey. *Chest.* 11/2010 2010;138(5):1093-1100.
37. Optimal oral anticoagulant therapy in patients with nonrheumatic atrial fibrillation and recent cerebral ischemia. The European Atrial Fibrillation Trial Study Group. *N.Engl.J.Med.* 7/6/1995 1995;333(1):5-10.
38. Cannegieter SC, Rosendaal FR, Wintzen AR, van der Meer FJ, Vandenbroucke JP, Briet E. Optimal oral anticoagulant therapy in patients with mechanical heart valves. *N.Engl.J.Med.* 7/6/1995 1995;333(1):11-17.
39. Hull R, Hirsh J, Jay R, et al. Different intensities of oral anticoagulant therapy in the treatment of proximal-vein thrombosis. *N.Engl.J.Med.* 12/30/1982 1982;307(27):1676-1681.
40. Saour JN, Sieck JO, Mamo LA, Gallus AS. Trial of different intensities of anticoagulation in patients with prosthetic heart valves. *N.Engl.J.Med.* 2/15/1990 1990;322(7):428-432.

41. van der Meer FJ, Rosendaal FR, Vandenbroucke JP, Briet E. Assessment of a bleeding risk index in two cohorts of patients treated with oral anticoagulants. *Thromb.Haemost.* 7/1996 1996;76(1):12-16.

42. Aithal GP, Day CP, Kesteven PJ, Daly AK. Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. *Lancet.* 2/27/1999 1999;353(9154):717-719.

43. Reitsma PH, van der Heijden JF, Groot AP, Rosendaal FR, Buller HR. A C1173T dimorphism in the VKORC1 gene determines coumarin sensitivity and bleeding risk. *PLoS.Med.* 10/2005 2005;2(10):e312.

44. Beyth RJ, Quinn LM, Landefeld CS. Prospective evaluation of an index for predicting the risk of major bleeding in outpatients treated with warfarin. *Am.J.Med.* 8/1998 1998;105(2):91-99.

45. Shireman TI, Mahnken JD, Howard PA, Kresowik TF, Hou Q, Ellerbeck EF. Development of a contemporary bleeding risk model for elderly warfarin recipients. *Chest.* 11/2006 2006;130(5):1390-1396.

46. Glasheen JJ, Fugit RV, Prochazka AV. The risk of overanticoagulation with antibiotic use in outpatients on stable warfarin regimens. *J.Gen.Intern.Med.* 7/2005 2005;20(7):653-656.

47. Connolly SJ, Pogue J, Eikelboom J, et al. Benefit of oral anticoagulant over antiplatelet therapy in atrial fibrillation depends on the quality of international normalized ratio control achieved by centers and countries as measured by time in therapeutic range. *Circulation.* 11/11/2008 2008;118(20):2029-2037.

48. Veeger NJ, Piersma-Wichers M, Tijssen JG, Hillege HL, van der Meer J. Individual time within target range in patients treated with vitamin K antagonists: main determinant of quality of anticoagulation and predictor of clinical outcome. A retrospective study of 2300 consecutive patients with venous thromboembolism. *Br.J.Haematol.* 2/2005 2005;128(4):513-519.

49. White HD, Gruber M, Feyzi J, et al. Comparison of outcomes among patients randomized to warfarin therapy according to anticoagulant control: results from SPORTIF III and V. *Arch. Intern.Med.* 2/12/2007 2007;167(3):239-245.

50. Landefeld CS, Goldman L. Major bleeding in outpatients treated with warfarin: incidence and prediction by factors known at the start of outpatient therapy. *Am.J.Med.* 8/1989 1989;87(2):144-152.

51. Ruiz-Gimenez N, Suarez C, Gonzalez R, et al. Predictive variables for major bleeding events in patients presenting with documented acute venous thromboembolism. Findings from the RIETE Registry. *Thromb.Haemost.* 7/2008 2008;100(1):26-31.

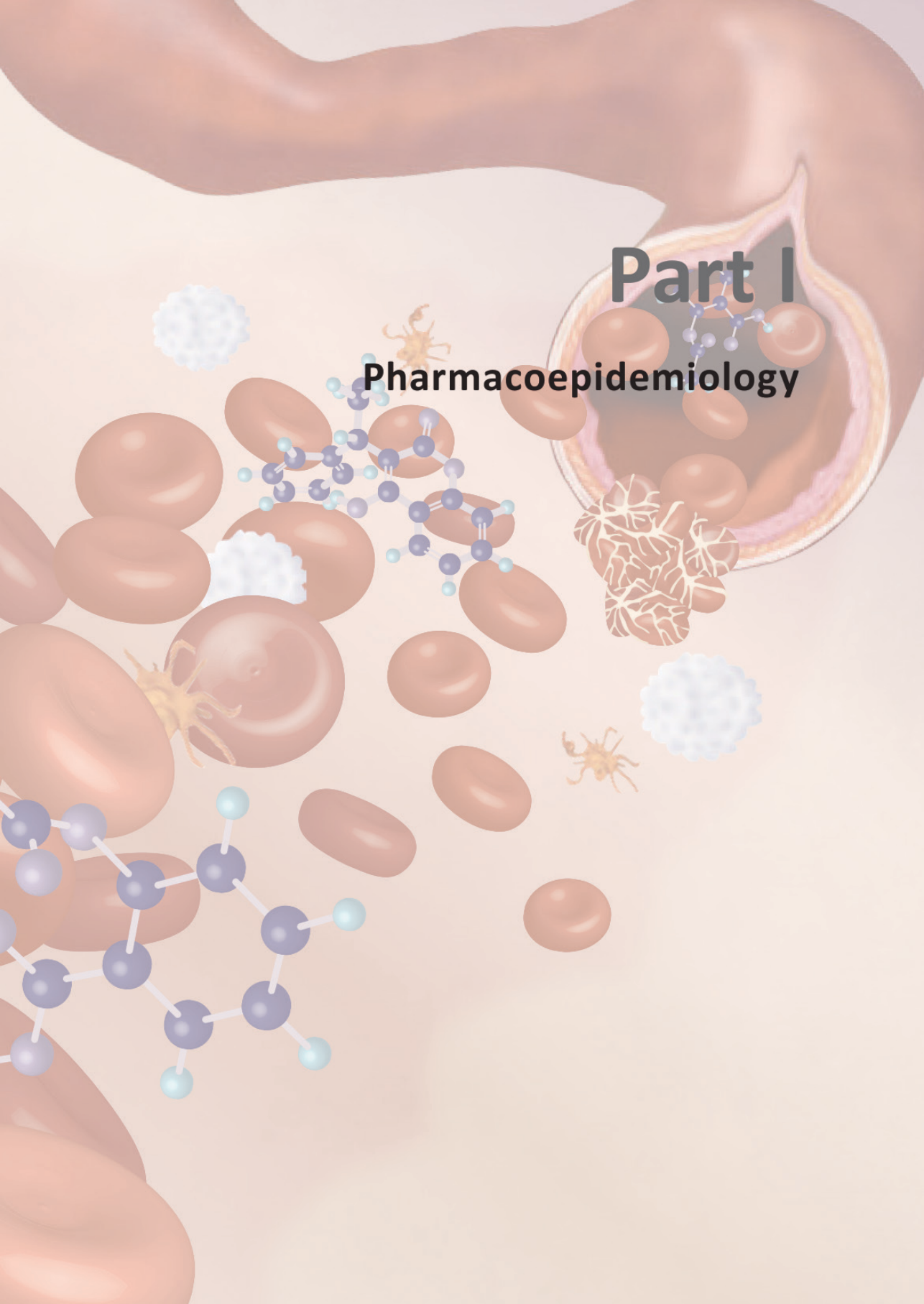
52. Kuijer PM, Hutten BA, Prins MH, Buller HR. Prediction of the risk of bleeding during anticoagulant treatment for venous thromboembolism. *Arch.Intern.Med.* 3/8/1999 1999;159(5):457-460.

53. Mellemkjaer L, Blot WJ, Sorensen HT, et al. Upper gastrointestinal bleeding among users of NSAIDs: a population-based cohort study in Denmark. *Br.J.Clin.Pharmacol.* 2/2002 2002;53(2):173-181.

54. Shorr RI, Ray WA, Daugherty JR, Griffin MR. Concurrent use of nonsteroidal anti-inflammatory drugs and oral anticoagulants places elderly persons at high risk for hemorrhagic peptic ulcer disease. *Arch.Intern.Med.* 7/26/1993 1993;153(14):1665-1670.

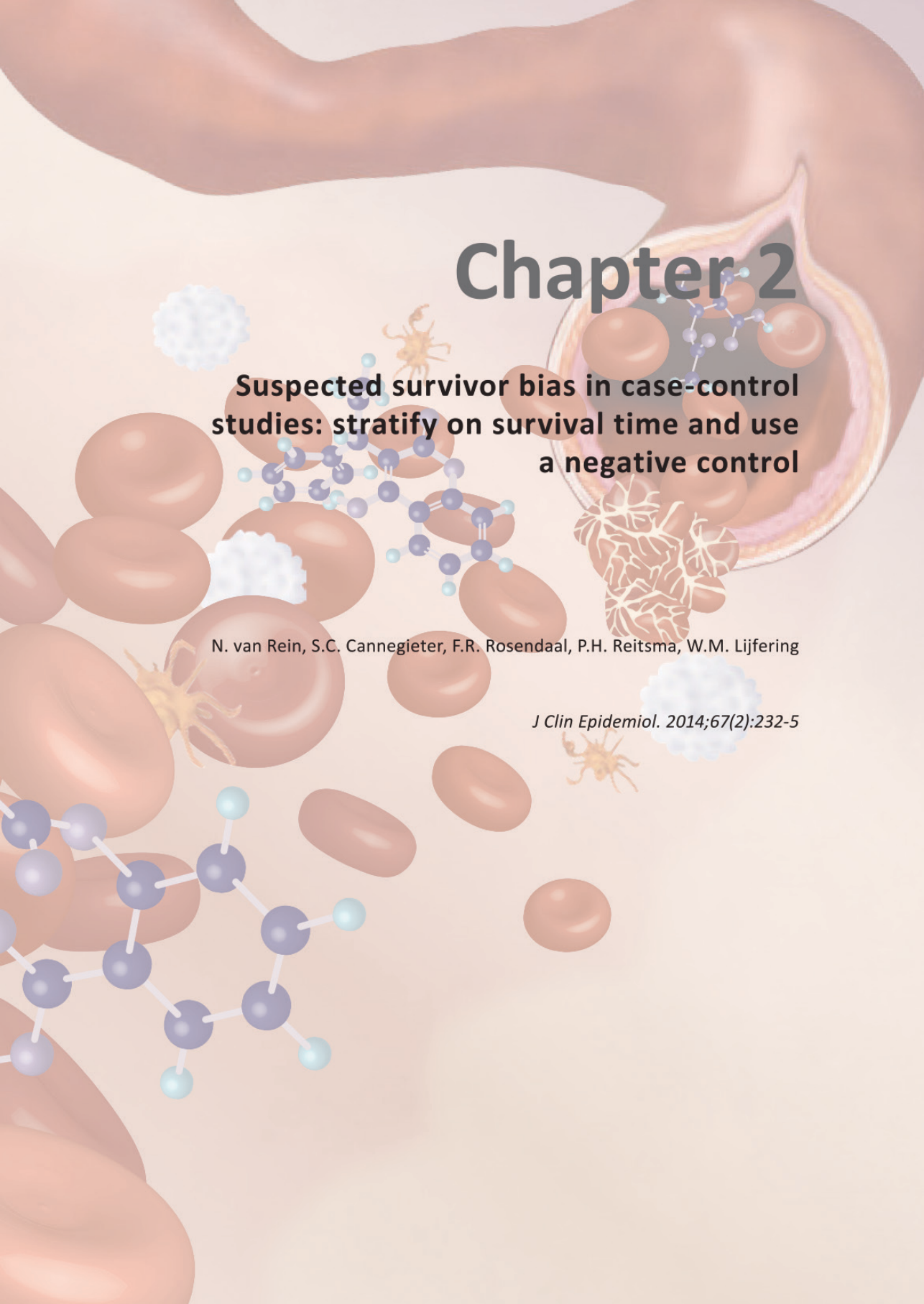
55. Zhu W, He W, Guo L, Wang X, Hong K. The HAS-BLED Score for Predicting Major Bleeding Risk in Anticoagulated Patients With Atrial Fibrillation: A Systematic Review and Meta-analysis. *Clin.Cardiol.* 9/2015 2015;38(9):555-561.

56. The International Stroke Trial (IST): a randomised trial of aspirin, subcutaneous heparin, both, or neither among 19435 patients with acute ischaemic stroke. International Stroke Trial Collaborative Group. *Lancet.* 5/31/1997 1997;349(9065):1569-1581.



Part I

Pharmacoepidemiology



Chapter 2

Suspected survivor bias in case-control studies: stratify on survival time and use a negative control

N. van Rein, S.C. Cannegieter, F.R. Rosendaal, P.H. Reitsma, W.M. Lijfering

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2

ABSTRACT

Objectives: Selection bias in case-control studies occurs when control selection is inappropriate. However, selection bias due to improper case sampling is less well recognized. We describe how to recognize survivor bias (i.e., selection on exposed cases) and illustrate this with an example study.

Study Design and Setting: A case-control study was used to analyze the effect of statins on major bleedings during treatment with vitamin K antagonists. A total of 110 patients who experienced such bleedings were included 18-1,018 days after the bleeding complication and matched to 220 controls.

Results: A protective association of major bleeding for exposure to statins (odds ratio [OR]: 0.56; 95% confidence interval: 0.29-1.08) was found, which did not become stronger after adjustment for confounding factors. These observations lead us to suspect survivor bias. To identify this bias, results were stratified on time between bleeding event and inclusion, and repeated for a negative control (an exposure not related to survival): blood group non-O. The ORs for exposure to statins increased gradually to 1.37 with shorter time between outcome and inclusion, whereas ORs for the negative control remained constant, confirming our hypothesis.

Conclusion: We recommend the presented method to check for overoptimistic results, that is, survivor bias in case-control studies.

INTRODUCTION

Case-control studies are commonly used because it is an efficient way to study rare outcomes. They can be as credible as randomized studies, when correctly designed and performed.[1] Cases are those who experience the event of interest, and controls are a random sample from the source population from which the cases arose.[2] Selection bias in case-control studies is well known to occur when control selection is inappropriate.[2] However, selection of cases can result in bias as well, which is less well recognized. This selection bias can occur when cases are selected a long period after the event, and exposed cases have an increased risk of severe illness or death compared with nonexposed cases.[3] In this article, we provide procedures to check for possible selection bias of cases and illustrate this with an example of a case-control study on the association of statin use and bleeding risk during treatment with vitamin K antagonists.

METHODS

The study used to illustrate this bias is the "factors in oral anticoagulation safety (FACTORS)" case-control study, which has been described before.[4] Briefly, cases reported a nontraumatic (nonfatal) major bleeding complication, during treatment with vitamin K antagonists (oral anticoagulants). Major bleeding was defined as a bleeding leading to hospitalization, a sudden hemoglobin decrease of higher than 1.25 mmol/L, or an intracranial, intra-abdominal, muscle, joint, or intraocular bleeding. These bleedings occurred between 1999 and 2001, and because vitamin K antagonists are characterized by a narrow therapeutic index, careful monitoring is necessary. In the Netherlands, this is performed by anticoagulation clinics.[5]

For every case, one to four controls without major bleeding event were matched on anticoagulation clinic, age, indication of anticoagulation, sex, vitamin K antagonist type (acenocoumarol or phenprocoumon), and whether treatment with vitamin K antagonists stopped before blood collection. Cases and controls were interviewed, and blood was drawn for testing on genetic variants.[4] Inclusion of cases took place 18-1,018 days after the major bleeding event (on average 425 days).

Cases and controls were considered statin users when they reported using this medication at time of the bleeding event (for cases) and during the interview (for controls). Odds ratios (ORs) with 95% confidence intervals (95% CIs) were calculated by means of conditional logistic regression, and were adjusted for comorbidity (diabetes and hypertension) and use of antiplatelet drugs. Written informed consent was obtained from all subjects, and the study was approved by the institutional reviewboards of the Leiden University Medical Center and the Academic Medical Center in Amsterdam. All statistical analyses were performed in SPSS 17.0 for windows (SPSS, Inc., Chicago, IL).

RESULTS

Complete data of the 110 cases and 220 controls were available, except for data on blood group (unavailable in 10 subjects). Clinical characteristics are shown in Table 1. Among both cases and controls, statin users suffered more frequently from comorbid conditions and used antiplatelet drugs more frequently.

The OR of developing a major bleeding event in statin vs. nonstatin users was 0.56 (95% CI: 0.29-1.08; Table 2). We expected that adjustment for comorbidity and use of antiplatelet drugs would lead to an even stronger protective risk estimate, as these confounding factors increase the risk for bleeding complications[6] and are related to statin treatment. However, after adjustment for comorbidity and use of antiplatelet drugs, no stronger protective risk estimate was observed (OR: 0.53, 95% CI: 0.27-1.03).

These findings were somewhat counterintuitive: first of all, statins gave a nearly 50% risk reduction of major bleeding, even if the indications for which statins are prescribed give an increased risk of major bleeding events. Second, adjustment for these confounders did not lead to a stronger protective risk estimate. We could have concluded that statins are powerful drugs, but instead hypothesized that this result might be biased.

Survivor bias occurs when exposed cases are less likely to take part in a study (e.g., because they died or became severely ill) than unexposed cases. This could mean that exposed cases in this study (i.e., patients who experienced a bleeding event and used statins) were less likely to participate (because of death or severe illness) when time between the event and inclusion in the study increased. Therefore, time between bleeding event and inclusion was taken into account because with more time between

Table 1. Clincal characteristics by cases or controls and statin use

	Cases (*n* = 110)				**Controls (*n* = 220)**			
	Statin users (*n* = 18)		**Non-statin users (*n* = 92)**		**Statin users (*n* = 56)**		**Non-statin users (*n* = 164)**	
	%	**Mean (SD)**	**%**	**Mean (SD)**	**%**	**Mean (SD)**	**%**	**Mean (SD)**
Age, years		69 (10)		67 (12)		67 (10)		70 (11)
Men	94		53		71		55	
Body mass index, kg/m^2		26.6 (3.4)		25.2 (4.0)		27.2 (4.2)		25.8 (3.8)
Positive smoking history	83		78		88		66	
Hypertension	28		30		34		35	
Diabetes	33		9		21		14	
Antiplatelet drugs	6		4		4		1	
Cancer	28		14		13		10	
Years of treatment		6.6 (5.9)		5.1 (6.2)		2.9 (4.3)		2.9 (4.4)

Table 2. Association of major bleeding complications by statin use or blood group non-O, stratified on time between bleeding complication and enrollment

Time between bleeding event and enrollment	Statin use			Blood group non-O		
	exp./not exp. cases, controls	odds ratio* (95% CI)	odds ratio† (95% CI)	exp./not exp. cases, controls	odds ratio* (95% CI)	
Any time	18/92, 56/164	0.56 (0.29-1.08)	0.53 (0.27-1.03)	59/50, 133/78	0.70	(0.43-1.14)
< 2.00 years	18/89, 56/158	0.56 (0.29-1.08)	0.55 (0.28-1.08)	56/50, 129/76	0.68	(0.42-1.11)
< 1.75 years	18/79, 53/140	0.60 (0.31-1.17)	0.59 (0.30-1.20)	51/45, 115/69	0.71	(0.43-1.17)
< 1.50 years	17/69, 45/130	0.74 (0.37-1.51)	0.71 (0.34-1.48)	44/41, 106/63	0.68	(0.40-1.17)
< 1.25 years	13/46, 30/89	1.01 (0.46-2.23)	1.00 (0.42-2.36)	29/29, 73/41	0.58	(0.30-1.12)
< 1.00 year	10/25, 18/54	1.34 (0.52-3.42)	1.37 (0.50-3.81)	17/18, 44/25	0.48	(0.19-1.24)

* Based on conditional logistic regression. † Based on conditional logistic regression and adjusted for diabetes, hypertension and antiplatelet drugs.

a bleeding event and inclusion the higher the possibility that a potential (exposed) case was not able to participate in our study. Therefore, selected cases (with their matched controls) were stratified on time between the bleeding event and inclusion (less than 2.00, 1.75, 1.50, 1.25, and less than 1.00 year). We saw that ORs increased gradually from 0.56 (95% CI: 0.29-1.08) to 1.34 (95% CI: 0.52-3.42) when cases were included within 3 and 1 year(s) after the bleeding event, respectively. After adjustment for comorbidity, this pattern remained the same (Table 2).

Although this stratified analysis suggests that our results were due to survivor bias, numbers were small, which may have led to this finding by chance. We therefore decided to explore this potential bias further, and repeated the analysis, only this time using exposure to blood group non-O as a "negative control", meaning an exposure that, although related to the outcome, is not related to increased risk of death or severe illness.[7] Risk estimates should remain stable with increasing time between the event and inclusion in the study to confirm our hypothesis of survivor bias. Indeed, the OR for major bleeding complications in patients with blood group non-O as compared with blood group O was 0.70 (95% CI: 0.43-1.14), and remained stable after stratifying on time between the major bleeding event and inclusion (Table 2).

DISCUSSION

We showed that an association found in a straightforward analysis of a case-control study can be biased due to selective survival of the cases. In our example, cases with major bleeding who were statin users were less likely to participate in our study in the period after the event than cases who did not use statins, resulting in finding a 43% risk reduction of major bleeding during treatment with vitamin K antagonists.

This could be an important phenomenon that could occur more frequently in case-control studies. We confirmed this phenomenon by performing our analysis for two exposures, but a caveat remains that our study is small numbered. We therefore tried to compare this finding with other studies; but to our knowledge, this is the first time of reporting on the association of statin use and major bleeding during treatment with vitamin K antagonists. However, there are many claims that statins have protective effects for several (non)vascular diseases, including multiples sclerosis, depression, Alzheimer's dementia, atherosclerosis, osteoporosis, AIDS, cancer, and venous thrombosis.[8] For venous thrombosis, a recent meta-analysis of observational studies compares statin users with nonstatin users, and shows a relative risk for venous thrombosis of 0.62 (95% CI: 0.45-0.86).[9] The lowest relative risks (0.20-0.60) were found in studies that had enrolled patients who had survived their event until inclusion. All other studies reported relative risk estimates that were closer to unity (0.74-1.02).[9] The studies with the lowest relative risks may therefore have suffered from the same problem, supporting the hypothesis that survivor bias may be more common and not always recognized as such. This is a problem, as causality is often inferred from these studies, which may lead to advice to prevent venous thrombosis with statins.[10]

Although we showed how to detect survivor bias, a possible limitation of our method (stratification on time between outcome and inclusion in the study, preferably repeated for a negative control) remains that it cannot distinguish survivor bias from a possible temporal biological effect of the exposure. To distinguish bias and a temporal effect, one should first look into the biology of the exposure toward the outcome. For this study, it seems that stratification on time is a good method to study survivor bias, as it is biologically implausible that statins do increase the risk for bleedings in the first year of use and then become protective.

A limitation of our example study is that numbers became small, especially after stratification on time between bleeding event and inclusion of cases. Therefore, the risk estimate (that was not more protective after adjustment for confounding factors in the stratified analyses) should be interpreted with caution. A way to overcome issues of survivor bias includes restriction of the analysis to cases who were included within a certain period in the study (dependent on the exposure and outcome). This analysis would be preferable when survivor bias is deemed as present. A negative control (when available) could be used to verify the presence of the bias if these are likely to occur due to chance, as was the case in our study. Another solution could be a study design where the exposure (statin use) and the outcome (major bleeding) are not related to inclusion in a study, for example, a cohort study.

In summary, when both the exposure and outcome are related to death or severe illness, overoptimistic associations could be found in case-control studies. Therefore,

we recommend using the method presented in this study (i.e., stratification on time between the event and inclusion in the study, preferably for both the exposure of interest and, in case of low numbers, also repeated in a negative control) to check for selective survival in cases.

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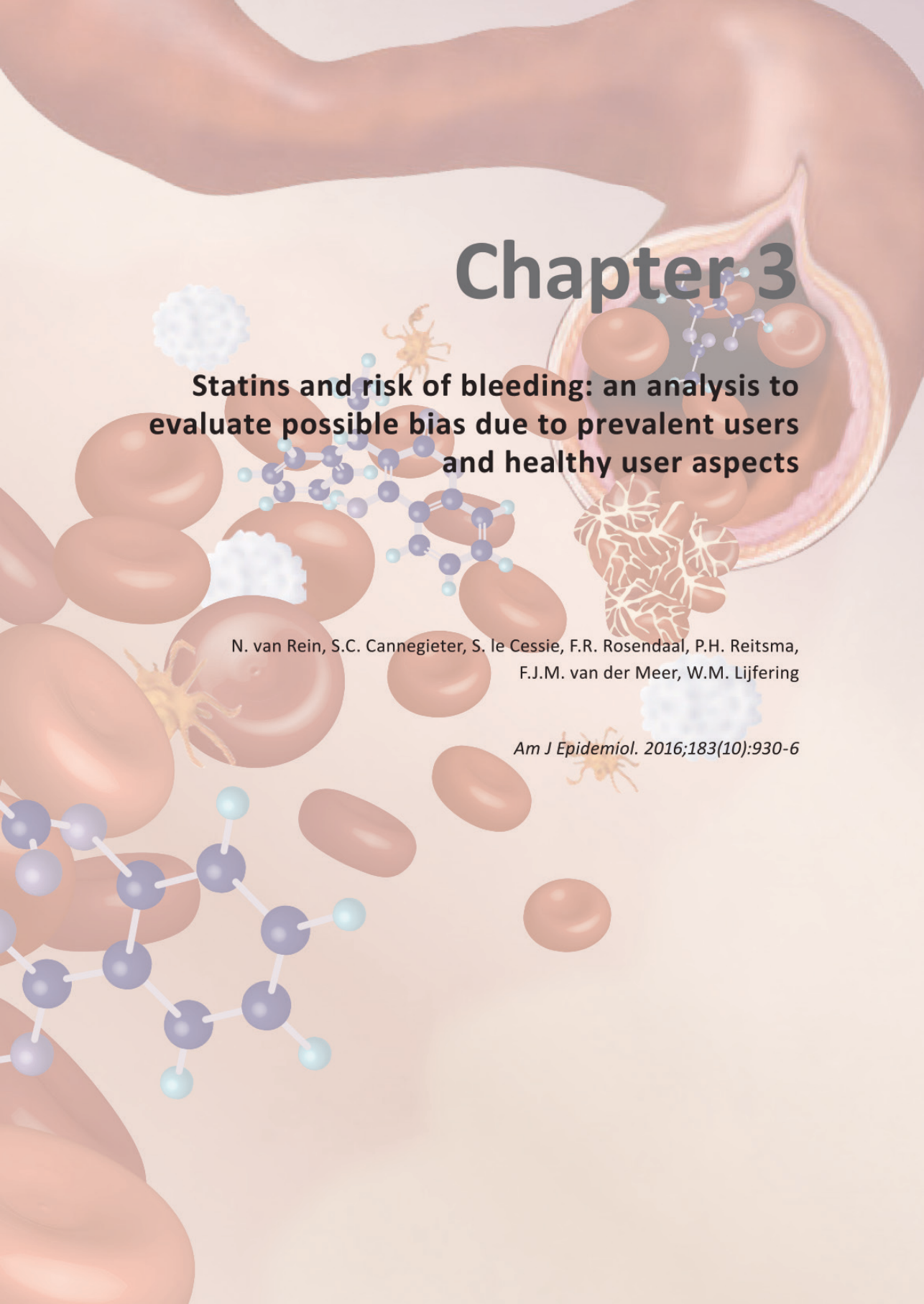
REFERENCES

1. Vandenbroucke JP. When are observational studies as credible as randomised trials? Lancet 2004;363:1728e31.
2. Hernan MA, Hernandez-Diaz S, Robins JM. A structural approach to selection bias. Epidemiology 2004;15:615e25.
3. Bryson A, Massey J. Tamoxifen's effect in women with breast cancer. Lancet 2001;357:1291e2.
4. van der Heijden JF, Rekk_e B, Hutten BA, van der Meer FJ, Remkes MG, Vermeulen M, et al. Non-fatal major bleeding during treatment with vitamin K antagonists: influence of soluble thrombomodulin and mutations in the propeptide of coagulation factor IX. J Thromb Haemost 2004;2:1104e9.
5. van der Meer FJ, Rosendaal FR, Vandenbroucke JP, Bri€et E. Bleeding complications in oral anticoagulant therapy. An analysis of risk factors. Arch Intern Med 1993;153:1557e62.
6. Palareti G, Leali N, Coccheri S, Poggi M, Manotti C, D'Angelo A, et al. Bleeding complications of oral anticoagulant treatment: an inception-cohort, prospective collaborative study (ISCOAT). Italian Study on Complications of Oral Anticoagulant Therapy. Lancet 1996;348:423e8.
7. Lipsitch M, Tchetgen TE, Cohen T. Negative controls: a tool for detecting confounding and bias in observational studies. Epidemiology 2010;21:383e8.
8. Rosendaal FR. Statins and venous thrombosis: a story too good to be true? PLoS Med 2012;9(9):e1001311. http://dx.doi.org/10.1371/ journal.pmed.1001311.
9. Pai M, Evans NS, Shah SJ, Green D, Cook D, Crowther MA. Statins in the prevention of venous thromboembolism: a meta-analysis of observational studies. Thromb Res 2011;128:422e30.
10. Lijfering WM, Flinterman LE, Vandenbroucke JP, Rosendaal FR, Cannegieter SC. Relationship between venous and arterial thrombosis: a review of the literature from a causal perspective. Semin Thromb Hemost 2011;37:885e96.



Chapter 3

Statins and risk of bleeding: an analysis to evaluate possible bias due to prevalent users and healthy user aspects

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ABSTRACT

Statins are said to protect against a wide range of diseases. We studied to what extent potential bias influences the results of studies on beneficial side effects of statins. We selected 8,188 atrial fibrillation patients who started treatment with anticoagulants at the Leiden Anticoagulation Clinic in the Netherlands between 2003 and 2009 and experienced 1,683 minor and 451 major bleeds during 18,105 person-years of follow-up. Statins were associated with a risk reduction of 9% for bleeds (hazard ratio 0.91, 95%confidence interval: 0.82, 1.00). Additionally, analyses were stratified by age, incident users (patients who started statins during follow-up, i.e., an inception cohort), and prevalent statin users (statin users at baseline), as restriction to incident users avoids overoptimistic risk estimates. After stratification, the protective associations disappeared or reversed (range of hazard ratios 0.99–3.22), except for patients aged 75 years or older. This remaining association could be due to another bias as, according to guidelines, in the elderly, statins should be prescribed only to those with a reasonable life expectancy. This could have resulted in a comparison of fit statin users with less fit non-statin users (healthy user effect). The apparent protective association of statins on bleeds may be due to bias. We recommend stratification by age and incident and prevalent statin use when studying associations of statins with disease outcomes to avoid overoptimistic risk estimates.

INTRODUCTION

Statins reduce atherosclerosis[1] and, consequently, arterial cardiovascular disease[2], but they are also known for their wide range of unintended beneficial effects. Examples in which statin use is associated with lower risk for disease include, among others, Alzheimer disease[3], Parkinson disease[4], depression[5], chronic obstructive pulmonary disease[6], venous thrombosis[7, 8], pneumonia[9], and fractures[10]. It has been argued that, if many cures are attributed to a single drug, the drug may in fact be ineffective, and noncausal explanations such as bias should be sought.[11]

A possible bias that may lead to lower risk estimates in observational studies is so-called "prevalent user bias." This type of bias was described by Danaei *et al.*[12] when studying associations of statins with mortality. In a meta-analysis, they showed that the pooled, multivariate-adjusted mortality hazard ratio for statin use was 0.54 (95% confidence interval (CI): 0.45, 0.66) when comparing prevalent statin users (statin users at baseline) with nonusers. However, in order to get the most valid results, observational studies should attempt to mimic clinical trials. When selecting the observational studies that, like the randomized trial, compared patients who started using statins during follow-up (an inception cohort design or incident statin users) with nonusers, researchers have found that risk estimates of studies with incident statin users (hazard ratio 0.77, 95% CI: 0.65, 0.91) were similar to those found in randomized controlled trials (hazard ratio 0.84, 95% CI: 0.77, 0.91).[12, 13]

Another type of bias may occur as the result of the prescription guidelines of statins: The Dutch guideline of 1999 states that statins should be prescribed only to individuals with a life expectancy of at least 5 years.[14] The renewed guideline from 2006 states that statins should be prescribed to individuals with a reasonable life expectancy who are not severely ill.[15] A comparison of those who start using statins during follow-up (incident statin user) with nonusers among the elderly may therefore result in a comparison of elderly persons with a reasonable life expectancy with those with a short life expectancy, resulting in a so-called "healthy user effect".

The present study was performed to examine to what extent these potential biases influence its results and conclusions. For this purpose, we performed a cohort study on the association of statin use and bleeding risk during treatment with vitamin K antagonists.

METHODS

Study population

A cohort was selected consisting of all patients who were 50 years or older and treated for atrial fibrillation at the Leiden Anticoagulation Clinic starting between January 2003 and December 2009 (8,188 patients). As patients could interrupt their treatment with vitamin K antagonist and start again, these patients had 8,853 treatment periods

altogether. Data collection Patients' characteristics and outcomes were collected from the computerized patient records from the Leiden Anticoagulation Clinic.[16] At the anticoagulation clinic, a blood sample is collected every 1–6 weeks to measure the international normalized ratio (INR). A standard short history is obtained with every venipuncture regarding comedications, intercurrent illness, planned surgery, and bleeding complications.[16] Baseline data of the cohort include age at start of vitamin K antagonist therapy, sex, main indication for vitamin K antagonist therapy, type of vitamin K antagonist (acenocoumarol or phenprocoumon), INR target range (2.5–3.5 or 3.0–4.0), and concomitant medication use. The starting date of medication use (for this study: statins, antiplatelet drugs, antidiabetic agents, and antihypertensive medications) was reported by the patient or the drug-dispensing pharmacy. Diabetes mellitus and hypertension were defined from the first date of use of any antidiabetic agent (oral and/or parenteral) or antihypertensive drug, respectively. Patients were considered exposed to statins and antiplatelet drugs from the first date of use of the drugs until the end date of the study period. Neither informed consent nor approval by a medical ethics committee is, according to Dutch law, required for studies in which data are collected from the records by a member of the treatment team.

Outcomes

Nontraumatic major bleeding events were classified by physicians of the Leiden Anticoagulation Clinic. Bleeding events that required hospitalization or blood transfusion, that were symptomatic in a critical area or organ, or led to death were classified as major.[17] Nontraumatic minor bleeding events included all haemorrhages that were not classified as major and were categorized as such by the Anticoagulant Clinic's physicians and/or nurses. We excluded traumatic bleeding events because statins could protect against nontraumatic bleeds, while their pharmacological actions are not likely to protect against bleeding events caused by trauma.

Statistical analyses

For every treatment with vitamin K antagonists, person-time was calculated from the start of treatment with vitamin K antagonists until the bleeding event, death, moving to a city that was not covered by the Leiden Anticoagulation Clinic, end of treatment, or end of the study period (December 31, 2009), whichever occurred first. When calculating the person-time for major bleeds, we ignored the occurrence of minor bleeds and vice versa. We used a Cox regression model, with time-dependent covariates to estimate hazard ratios and 95% confidence intervals. The proportionality of the hazards assumption was tested by analysis of Schoenfeld residuals, and for this analysis statistical significance was set at the 5% level.

Patients were classified in 3 age groups (50–64 years, 65– 74 years, and 75 years or older) to study age-specific associations. Age-specific associations were expected

as a potential healthy user effect in elderly patients may be present as a result of the prescription guidelines.[14, 15]

We adjusted for the confounding factors sex, INR target range (3.0–4.0 vs. 2.5–3.5), diabetes mellitus (time dependently), hypertension (time dependently), and use of antiplatelet drugs (time dependently) in the simple adjusted model. The fully adjusted model also included age (by the abovementioned age categories for the overall risk estimates and continuously for analyses within age groups, because confounding due to age can still be present within an age group). We performed 3 different analyses. First, we compared all statin users (as a time-dependent variable) with nonstatin users. Then, incident users were compared with nonusers by using only the patients who did not use statins at baseline. In the last analysis, prevalent users were compared with nonusers; if a patient started to use statins after baseline, his follow-up was censored.

All analyses were performed with the R, version 2.15.2, language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Of 8,188 patients, 7,592 had 1, 536 had 2, 53 had 3, 5 patients had 4, and 2 patients had 5 treatment periods during the study period. Patients with multiple treatment periods were treated for a period with vitamin K antagonists, which was discontinued for some time and then restarted. A common reason to discontinue vitamin K antagonist treatment was a successful cardioversion (either chemical or mechanical).[18]

The mean age at baseline was 74 (range, 50–103) years, and 4,847 patients (55%) were men. The INR target range was 2.5–3.5 in 8,659 (98%) treatment periods as recommended for atrial fibrillation in the Netherlands[19], and phenprocoumon was used during 8,009 (91%) treatment periods. Statin users were more frequently male than were nonstatin users. The prevalence of hypertension, diabetes, and use of antiplatelet drugs was higher in statin users than nonstatin users (Table 1). Stratification of incident statin users by age showed that patients aged 50–64 years were more frequently male and had a lower prevalence of diabetes mellitus than patients aged 75 years or older.

During a total follow-up of 15,008 person-years for all bleeds, 15,231 person-years for minor bleeding events, and 18,105 person-years for major bleeding events, 1,991 patients experienced any bleed, 1,683 experienced a minor bleed, and 451 patients experienced a major bleeding event. The incidence rate was 133 (95%CI: 127, 139) per 1,000 person-years for all, 110 (95% CI: 105, 116) per 1,000 person-years for minor, and 25 (95% CI: 23, 27) per 1,000 person-years for major bleeding complications. The incidence rates of all, major, and minor bleeding complications increased with age (Figure 1), and the most common sites of major bleeds were the gastrointestinal tract and cranium (Appendix 1).

Table 1. Baseline Characteristics of the Cohort by Type of Statin User Who Started Treatment With Vitamin K Antagonists in Leiden, the Netherlands, Between 2003 and 2009

					Incident Statin Users							
	Nonstatin Users		Prevalent Statin Users		50–64 Years		65–74 Years		≥75 Years		Total	
Characteristic	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
General characteristics												
Patients	5,919		2,367		250		308		282		840	
Treatment periods	6,365	100	2,488	100	250	100	309	100	283	100	842	100
Men	3,306	52	1,541	62	188	75	212	69	134	47	534	63
Age, years[a]	74 (11)		73 (9)		61 (4)		72 (3)		82 (4)		72 (9)	
INR target range, 2.5–3.5	6,242	98	2,417	97	243	97	302	98	271	96	816	97
Vitamin K antagonists												
Phenprocoumon	5,774	91	2,243	90	236	94	289	94	261	92	786	93
Acenocoumarol	591	9	245	10	14	6	20	6	22	8	56	7
Comedication and comorbidity												
Hypertension	3,528	55	1,739	70	206	82	254	82	237	84	697	83
Diabetes	535	8	518	21	44	18	72	23	80	28	196	23
Use of antiplatelet drugs	173	3	305	12	14	6	33	11	27	10	74	9

Abbreviation: INR, international normalized ratio. [a]Mean (standard deviation).

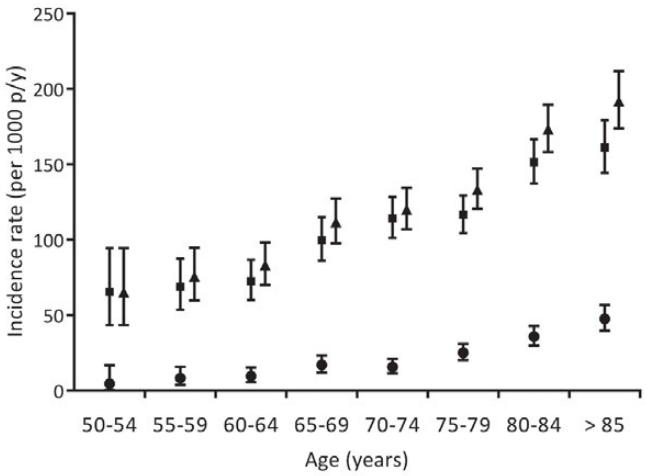


Figure 1. Incidence rates per 1,000 person-years of bleeding complications stratified by age of patients who started treatment with vitamin K antagonists in Leiden, Netherlands, between 2003 and 2009. Circles, major bleeding events; squares, minor bleeding events; triangles, all bleeding events; bars, 95% confidence intervals.

Statins were used during 3,330 treatment periods and initiated during 842 treatment periods (the latter were considered incident statin users). Overall, the crude hazard ratio of statin use (prevalent and incident statin use combined) versus non-statin use was 0.90 (95% CI: 0.81, 0.99) for all bleeds, 0.91 (95% CI: 0.82, 1.01) for minor bleeds, and 0.90 (95% CI: 0.75, 1.11) for major bleeds (Table 2). Adjustment for sex, INR target range, diabetes, hypertension, and use of antiplatelet drugs resulted in hazard ratios of 0.88 (95% CI: 0.80, 0.97) for all, 0.83 (95% CI: 0.67, 1.02) for major, and 0.90 (95% CI: 0.81, 1.01) for minor bleeds (Table 2). After further adjustment for age, these hazard ratios increased toward unity (Table 2).After stratification by age, no protective risk estimates were found in patients aged 50–64 years and 65–74 years, but protective risk estimates were still found in the oldest patients for all outcomes (Table 3). Restriction to incident statin users (Figure 2) and nonusers yielded higher risk estimates than did restriction to prevalent statin users and nonusers for all outcomes (incident users vs. nonusers for all bleeding complications: fully adjusted hazard ratio = 1.08, 95% CI: 0.90, 1.32; minor bleeding complications fully adjusted hazard ratio = 1.18, 95% CI: 0.96, 1.45; major bleeding complications fully adjusted hazard ratio = 0.93, 95% CI: 0.63, 1.36). Similar results were found in all age groups, except for major bleeds in patients 75 years or older (prevalent users fully adjusted hazard ratio = 0.89, 95% CI: 0.68, 1.18; incident users fully adjusted hazard ratio = 0.73, 95% CI: 0.45, 1.20).

Table 2. Association of Statin Use (Prevalent and Incident Statin Use Combined) With Major and Minor Bleeding Complications in a Cohort of Patients Who Started Treatment With Vitamin K Antagonists in Leiden, the Netherlands, Between 2003 and 2009

Outcome	Person-Time, years	No. of Cases	Events/ 100 Person-Years	HR[a]	95% CI	HR[b]	95% CI	HR[c]	95% CI
All bleeding complications									
Nonstatin users	9,584	1,357	14.16	1.00	Referent	1.00	Referent	1.00	Referent
Statin users	5,424	634	11.69	0.90	0.81, 0.99	0.88	0.80, 0.97	0.91	0.82, 1.00
Minor bleeding complications									
Nonstatin users	9,732	1,146	11.78	1.00	Referent	1.00	Referent	1.00	Referent
Statin users	5,499	537	9.77	0.91	0.82, 1.01	0.90	0.81, 1.01	0.92	0.83, 1.03
Major bleeding complications									
Nonstatin users	11,516	302	2.62	1.00	Referent	1.00	Referent	1.00	Referent
Statin users	6,589	149	2.26	0.91	0.75, 1.11	0.83	0.67, 1.02	0.90	0.73, 1.11

Abbreviations: CI, confidence interval; HR, hazard ratio; INR, international normalized ratio.
[a] Time-dependent analysis. [b] Time-dependent analysis adjusted for sex, INR target range, diabetes, hypertension, and antiplatelet drugs. [c] Time-dependent analysis adjusted for sex, INR target range, diabetes, hypertension, antiplatelet drugs, and age.

Table 3. Association of Statin Use (Prevalent and Incident Statin Use Combined) With Major and Minor Bleeding Complications Stratified by Age in a Cohort of Patients Who Started Treatment With Vitamin K Antagonists in Leiden, the Netherlands, Between 2003 and 2009

	Statin Users			Nonstatin Users								
Complication by Age, years	Person-Time, years	No. of Cases	Events/100 Person-Years	Person-Time, years	No. of Cases	Events/100 Person-Years	HR[a]	95% CI	HR[b]	95% CI	HR[c]	95% CI
All bleeding complications												
Age 50–64	1,116	84	7.53	1,891	154	8.14	1.05	0.80, 1.37	1.09	0.82, 1.45	1.08	0.81, 1.43
Age 65–74	1,808	201	11.12	2,563	317	12.37	1.00	0.84, 1.20	0.98	0.81, 1.18	0.98	0.81, 1.18
Age ≥75	2,500	349	13.96	5,130	886	17.27	0.88	0.78, 0.99	0.84	0.73, 0.95	0.89	0.78, 1.01
Minor bleeding complications												
Age 50–64	1,136	72	6.34	1,900	142	7.47	0.96	0.72, 1.28	1.03	0.76, 1.39	1.01	0.75, 1.37
Age 65–74	1,830	178	9.73	2,583	288	11.15	0.98	0.81, 1.19	0.99	0.81, 1.21	0.98	0.80, 1.21
Age ≥75	2,534	287	11.33	5,248	716	13.64	0.90	0.79, 1.03	0.86	0.75, 1.00	0.91	0.79, 1.06
Major bleeding complications												
Age 50–64	1,256	15	1.19	2,102	14	0.67	2.09	1.00, 4.34	1.93	0.88, 4.25	1.92	0.87, 4.23
Age 65–74	2,181	38	1.74	2,998	47	1.57	1.16	0.75, 1.79	0.96	0.59, 1.55	0.96	0.59, 1.55
Age ≥75	3,152	96	3.05	6,417	241	3.76	0.85	0.67, 1.08	0.79	0.62, 1.02	0.87	0.67, 1.12

Abbreviations: CI, confidence interval; HR, hazard ratio; INR, international normalized ratio. [a] Time-dependent analysis. [b] Time-dependent analysis adjusted for sex, INR target range, diabetes, hypertension, and antiplatelet drugs. [c] Time-dependent analysis adjusted for sex, INR target range, diabetes, hypertension, antiplatelet drugs, and age.

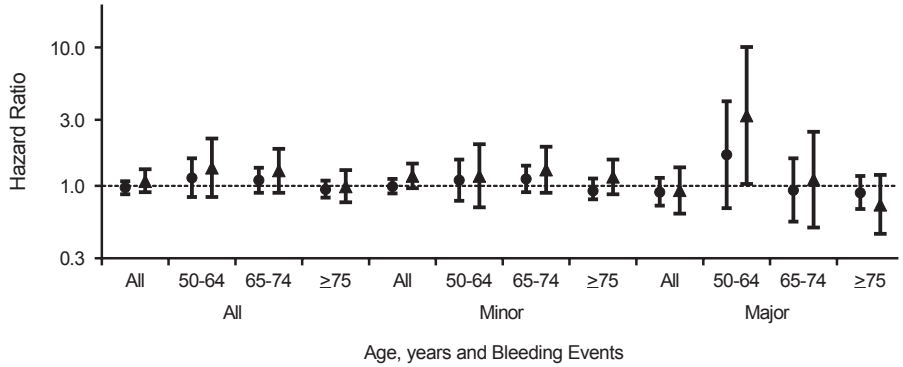


Figure 2. Fully adjusted hazard ratios for association of statins with bleeding complications, stratified by age and incident or prevalent statin use, in patients who started treatment with vitamin K antagonists in Leiden, Netherlands, between 2003 and 2009. Circles, prevalent statin users; triangles, incident statin users; bars, 95% confidence intervals.

DISCUSSION

This study shows that statin use initially seemed to reduce the risk of minor and major bleeding events by 9% in the overall straightforward analysis (i.e., before we stratified the analysis by age and incident and prevalent statin users). However, after restriction to incident statin users, protective risk estimates largely disappeared or even reversed. After stratification by age, the risk estimates showed a protective association in patients of 75 years or older. Associations in (pharmaco)epidemiologic studies can be due to causal mechanisms but also to confounding or bias.[20] As potential explanations for the associations found in this study, causal mechanisms, confounding, and bias were considered.

The association of statin use with bleeding events during treatment with vitamin K antagonists in the initial analysis of this study (fully adjusted hazard ratio = 0.91, 95% CI: 0.82, 1.00) was similar to the association that was previously found by Douketis *et al.*[21] (adjusted odds ratio 0.91, 95% CI: 0.77, 1.07). Douketis *et al.* suspected that this protective association was due to bias. As shown in this study, bias could partially be due to a comparison of prevalent statin users with nonusers, which is in line with what was described in the meta-analysis by Danaei *et al.*[12] This bias is not restricted only to statin therapy. Hernán *et al.*[22] showed that it is also present in studies on the effects of hormone replacement therapy on coronary heart disease when current (prevalent) drug users instead of initiators (incident drug users) are compared with nonusers.[23] The results of these studies[12, 22] along with those of the present study suggest that comparisons of drug initiators with nonusers yield more reliable risk estimates than comparisons of prevalent drug users with nonusers when studying associations of drugs on disease outcomes.

Another explanation for an association may be a causal effect, which is usually based on potential mechanisms from literature. Statins are known for their inhibitory effects on platelet function and their raising levels of procoagulant factors.[24, 25] These effects may explain the risk estimates indicative of an increased risk for bleeds of incident statin users as compared with nonusers in patients aged 50–64 years (hazard ratio 1.18–3.22) and 65–74 years (hazard ratio 1.11–1.31). However, this potential explanation of the found associations is not in line with the results in patients aged 75 years or older.

Another bias we will consider is healthy user bias due to prescription guidelines. The guidelines state that starting statins in elderly patients should be restricted to relatively healthy individuals.[14, 15] Especially in the elderly who started using a statin, these guidelines may result in a comparison of healthy elderly who started using a statin with less healthy ones who did not start using a statin, resulting in healthy user bias. The guidelines may explain why risk estimates of statin use for major bleeds remained protective in the oldest patients (aged 75 years or older) when considering only incident statin use (fully adjusted hazard ratio 0.73).

The last source of spurious associations we will discuss is confounding.[20] In this study, we were not able to fully adjust for confounding by indication and life style–dependent confounding factors (because, e.g., body mass index and cholesterol levels were unknown). The increased risk estimates found in patients aged 50–64 years may be attributable to residual confounding, as these patients may have received statins as the result of an unhealthy life style. In addition, diabetes and hypertension were classified by first date of use of antidiabetic or antihypertensive drugs. Because diabetic or hypertensive treatment could have started a long time after patients developed these conditions, misclassification could have occurred. Therefore, adjustments for hypertension and diabetes may not have been perfect. Furthermore, comparing the results of our study with findings in randomized clinical trials (i.e., a “gold standard”) in similar populations could give some indication of the amount of residual confounding, but unfortunately these trials have not been conducted.

In summary, a comparison of incident statin users with nonusers may yield most reliable risk estimates. Additionally we found that a healthy user bias may be present in older patients, suggesting that restriction to incident statin users alone will not prevent all biases. These biases may (partially) explain the wide range of unintended protective effects attributed to statins. Examples are protections against depression[5], chronic obstructive pulmonary disease[6], and pneumonia[9] that were found in case-control studies where often only prevalent users are considered in the analysis. Examples from cohort studies include Parkinson disease[4], venous thrombosis[7, 8], and fractures[10], where statin users (prevalent and incident combined) were compared with nonusers or never users. We therefore recommend stratification for types of statin

users and age in cohort studies. This could prevent overoptimistic risk estimates and false positive findings.

This study consisted of a large homogenous and consecutive cohort of atrial fibrillation patients with registration of more than 450 major and 1,650 minor bleeding events. Still, as a limitation of our sample study, numbers became small after stratification by age and incident and prevalent statin users. Additionally, without results from a randomized controlled trial in the population under study, we cannot rule out that our final adjusted findings are attributable to a causal effect, residual confounding, or sampling error. Another potential limitation of this study is that no data were present on the end date of statin use, which may have resulted in a dilution of the risk estimates.

Our findings support the hypothesis that comparing prevalent statin users with nonusers in observational studies leads to biased risk estimates.[12] In addition, we observed that restriction to incident statin users does not necessarily prevent all bias in observational studies on the associations of statins with disease outcomes, because a healthy user bias may be present when comparing older incident statin users with nonusers. Both biases give overoptimistic views on the pleiotropic effects of statins that are similar to what was found in earlier studies on hormone replacement therapy.[22, 23] To conclude, when studying the associations of statins with disease outcomes in cohorts, we recommend stratification by age and by incident and prevalent statin use to avoid overoptimistic risk estimates.

REFERENCES

1. Nissen SE, Nicholls SJ, Sipahi I, et al. Effect of very high intensity statin therapy on regression of coronary atherosclerosis: the ASTEROID trial. JAMA. 2006;295(13):1556–1565.
2. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). Lancet. 1994;344(8934):1383–1389.
3. Wolozin B, Kellman W, Ruosseau P, et al. Decreased prevalence of Alzheimer disease associated with 3-hydroxy-3-methyglutaryl coenzyme A reductase inhibitors. Arch Neurol. 2000;57(10):1439–1443.
4. Gao X, Simon KC, Schwarzschild MA, et al. Prospective study of statin use and risk of Parkinson disease. Arch Neurol. 2012; 69(3):380–384.
5. Yang CC, Jick SS, Jick H. Lipid-lowering drugs and the risk of depression and suicidal behavior. Arch Intern Med. 2003; 163(16):1926–1932.
6. García Rodríguez LA, Wallander MA, Tolosa LB, et al. Chronic obstructive pulmonary disease in UK primary care: incidence and risk factors. COPD. 2009;6(5):369–379.
7. Ray JG, Mamdani M, Tsuyuki RT, et al. Use of statins and the subsequent development of deep vein thrombosis. Arch Intern Med. 2001;161(11):1405–1410.

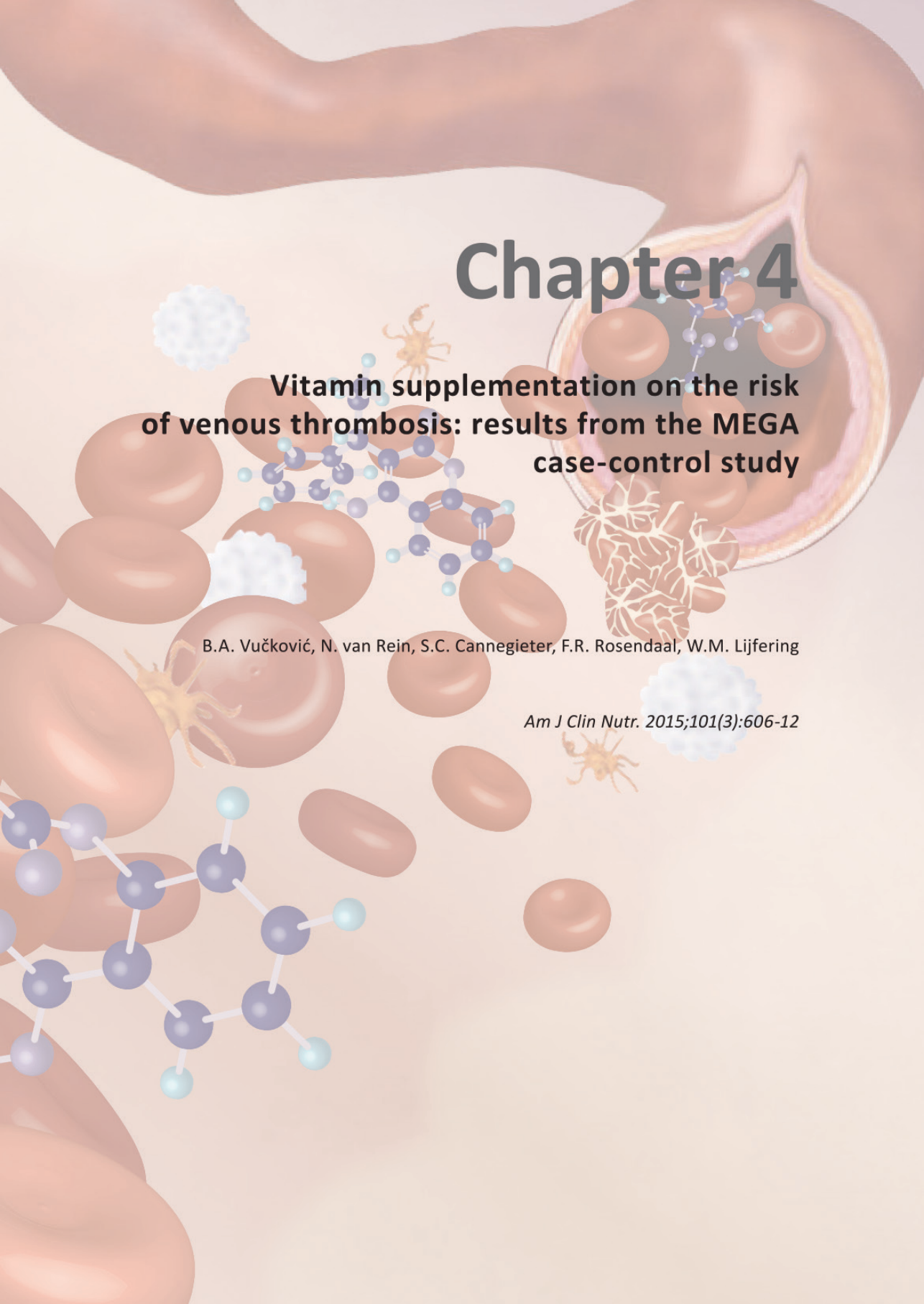
8. Biere-Rafi S, Hutten BA, Squizzato A, et al. Statin treatment and the risk of recurrent pulmonary embolism. Eur Heart J. 2013;34(24):1800–1806.
9. Schlienger RG, Fedson DS, Jick SS, et al. Statins and the risk of pneumonia: a population-based, nested case-control study. Pharmacotherapy. 2007;27(3):325–332.
10. Scranton RE, Young M, Lawler E, et al. Statin use and fracture risk: study of a US veterans population. Arch Intern Med. 2005; 165(17):2007–2012.
11. Rosendaal FR. Statins and venous thrombosis: a story too good to be true? PLoS Med. 2012;9(9):e1001311.
12. Danaei G, Tavakkoli M, Hernán MA. Bias in observational studies of prevalent users: lessons for comparative effectiveness research from a meta-analysis of statins. Am J Epidemiol. 2012;175(4):250–262.
13. Ray WA. Evaluating medication effects outside of clinical trials: new-user designs. Am J Epidemiol. 2003;158(9): 915–920.
14. van der Laan JR, Thomas S. Summary of 'cholesterol' guideline (first revision) of the Dutch Society of Family Physicians [in Dutch]. Ned Tijdschr Geneeskd. 2000;144(9): 421–427.
15. Bouma M, Rutten GE, de Grauw WJ, et al. Summary of the practice guideline 'diabetes mellitus type 2' (second revision) from the Dutch College of General Practitioners [in Dutch]. Ned Tijdschr Geneeskd. 2006;150(41):2251–2256.
16. van der Meer FJ, Rosendaal FR, Vandenbroucke JP, et al. Bleeding complications in oral anticoagulant therapy. An analysis of risk factors. Arch Intern Med. 1993;153(13):1557–1562.
17. Schulman S, Kearon C; Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. J Thromb Haemost. 2005;3(4):692–694.
18. You JJ, Singer DE, Howard PA, et al. Antithrombotic therapy for atrial fibrillation: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest. 2012; 141(2 suppl):e531S–e575S.
19. Opstelten W, Boode BS, Heeringa J, et al. Summary of the practice guideline 'atrial fibrillation' (first revision) from the Dutch College of General Practitioners [in Dutch]. Ned Tijdschr Geneeskd. 2010;154:A1570.
20. Hernan MA, Robins JM. Confounding. In: Causal Inference. Boston, MA: The President and Fellows of Harvard College; 2015:83–94. https://cdn1.sph.harvard.edu/wp-content/uploads/ sites/1268/2015/08/hernanrobins_v1.10.30.pdf. Accessed September 1, 2015.
21. Douketis JD, Melo M, Bell CM, et al. Does statin therapy decrease the risk for bleeding in patients who are receiving warfarin? Am J Med. 2007;120(4):369.e9–369.e14.
22. Hernán MA, Alonso A, Logan R, et al. Observational studies analyzed like randomized experiments: an application to postmenopausal hormone therapy and coronary heart disease. Epidemiology. 2008;19(6):766–779.

23. Vandenbroucke JP. The HRT controversy: observational studies and RCTs fall in line. Lancet. 2009;373(9671): 1233–1235.
24. Szapary L, Horvath B, Marton Z, et al. Short-term effect of low-dose atorvastatin on haemorrheological parameters, platelet aggregation and endothelial function in patients with cerebrovascular disease and hyperlipidaemia. CNS Drugs. 2004;18(3):165–172.
25. Adams NB, Lutsey PL, Folsom AR, et al. Statin therapy and levels of hemostatic factors in a healthy population: the Multi-Ethnic Study of Atherosclerosis. J Thromb Haemost. 2013;11(6):1078–1084.

APPENDIX 1

Table 1. Distribution and Type of Bleeding Events, Stratified by age Among Patients who Started Treatment With Vitamin K Antagonists in Leiden Between 2003 and 2009

	All	Age 50-64 years	Age 65-74 years	Age > 75 years
Major non-fatal bleeding complications, n (%)				
Total	311 (100)	27 (100)	70 (100)	214 (100)
Cerebral	46 (15)	6 (22)	16 (23)	24 (11)
Gastrointestinal	136 (44)	8 (30)	24 (34)	104 (49)
Muscle and joint	36 (12)	4 (15)	6 (18)	26 (12)
Skin	24 (8)	2 (7)	4 (6)	18 (8)
Respiratory	11 (4)	1 (4)	3 (4)	7 (3)
Epistaxis	7 (2)	2 (7)	3 (4)	2 (1)
Eye	20 (6)	0	5 (7)	15 (7)
Urogenital	28 (9)	4 (15)	9 (13)	15 (7)
Other	3 (1)	0	0	3 (1)
Major fatal bleeding complications, n (%)				
Total	140 (100)	2 (100)	15 (100)	123 (100)
Cerebral	99 (71)	2 (100)	13 (87)	84 (68)
Gastrointestinal	20 (14)	0	0	20 (16)
Aneurism	9 (6)	0	0	9 (7)
Respiratory	8 (6)	0	2 (13)	6 (5)
Other	4 (3)	0	0	4 (3)
Minor bleeding complications, n (%)				
Total	1705 (100)	219 (100)	471 (100)	1015 (100)
Gastrointestinal	155 (9)	18 (8)	33 (7)	104 (10)
Conjunctiva	458 (27)	65 (30)	153 (32)	240 (24)
Large bruising	404 (24)	51 (23)	87 (18)	266 (26)
Epistaxis	277 (16)	30 (14)	72 (15)	175 (17)
Urogenital	23 (1)	3 (1)	2 (0)	18 (2)
Respiratory	25 (1)	3 (1)	3 (1)	19 (2)
Haematuria	341 (20)	42 (19)	117 (25)	182 (18)



Chapter 4

Vitamin supplementation on the risk of venous thrombosis: results from the MEGA case-control study

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ABSTRACT

Background: Whether vitamin supplements decrease venous thrombosis risk is controversial. Previous reports did not all take confounding fully into account, either by randomization or by extensive adjustment.

Objective: The aim of our study was to determine whether vitamin supplementation decreases the risk of venous thrombosis.

Design: A large case-control study included 2506 patients with venous thrombosis, 2506 partner controls, and 2684 random-digit dialing (RDD) controls. When patients were compared with RDD controls, unconditional logistic regression was used to calculate ORs with 95% CIs. When patients were compared with partner controls, conditional logistic regression was used, providing further adjustment for unmeasured confounding.

Results: Vitamin use yielded a 37% lower risk of venous thrombosis than no vitamin use (OR: 0.63; 95% CI: 0.57, 0.70) when comparing patients with RDD controls. Adjustment for several putative confounders did not change the estimate (OR: 0.68; 95% CI: 0.61, 0.77). The fully adjusted ORs for vitamin A, vitamin B-6, vitamin B-12, folic acid, vitamin C, vitamin D, vitamin E, and multivitamin use were in the same range. However, when patients were compared with partner controls, ORs attenuated to unity. Results were similar for provoked and unprovoked events, as well as for deep vein thrombosis and pulmonary embolism.

Conclusions: After extensive adjustments, vitamin supplementation was no longer associated with a decreased risk of venous thrombosis in this study. Previous positive results may have been spurious as a result of uncontrolled confounding.

INTRODUCTION

Venous thrombosis is one of the leading causes of morbidity and mortality worldwide, occurring each year in about one in 1000 people in industrialized countries.[1] The condition can be prevented and treated with anticoagulants, but as a side effect, bleeding often occurs.[2] Therefore, strategies for the prevention of venous thrombosis that are not based on oral anticoagulant treatment are needed. Both basic research and observational epidemiologic studies have supported the hypothesis that vitamins may inhibit venous thrombosis. For example, based on early findings that elevated homocysteine concentrations are associated with thrombotic disease[3, 4], as well as the knowledge that homocysteine concentrations depend on a series of intracellular metabolic reactions in which folate acts as a substrate and vitamin B-12 as a coenzyme, it was believed that adequate supplementation of B vitamins could lower homocysteine and thus decrease the risk of thrombotic events.[5] However, initial therapeutic trials with vitamin B supplements that induce a decrease in homocysteine concentration have not resulted in an improvement of the thrombotic risk[6–8], probably because of the existence of a more complicated metabolic network than what was assumed at first or the absence of a causal relation between hyperhomocysteinemia and thrombotic risk.[9] As a consequence, multivitamin supplementation became of interest irrespective of the homocysteine concentration, and several studies dealing with the possible connection of different vitamins and thrombotic risk have been designed. Most of these studies investigated the risk of arterial thrombosis and yielded inconsistent results.[10-12] For venous thrombosis, studies are scarce. Some showed that vitamin D or E supplementation decreases the risk of venous thrombosis[13, 14]; others showed no effect.[15] As far as we know, no other observational studies or trials have analyzed whether other vitamins such as vitamin A and vitamin C are associated with a decreased risk of venous thrombosis.

One issue to keep in mind when studying the effect of vitamin use on venous thrombosis is that studies on vitamin therapy are generally not randomized and lack proper adjustment for the many lifestyle-related factors that could confound the relation.

In this study, we used data from the Multiple Environmental and Genetic Assessment (MEGA) case-control study to analyse whether use of vitamin A, vitamin B-6, vitamin B-12, vitamin C, vitamin D, vitamin E, folic acid, or multivitamins decreased the risk of venous thrombosis. This study provided an excellent opportunity to study this because both measured and unmeasured confounding factors could be taken into account by comparing patients with population-derived random-digit dialing (RDD) controls and with patients' partners, which formed the 2 control groups of the study.

METHODS

Study design

The design of the MEGA case-control study is described elsewhere.[16] In short, 4956 consecutive patients aged 18–70 y, with a first diagnosis of deep vein thrombosis or pulmonary embolism, were included from 6 anticoagulation clinics in The Netherlands (Amersfoort, Amsterdam, The Hague, Leiden, Rotterdam, and Utrecht) between March 1999 and September 2004. Diagnostic information was obtained from hospital discharge reports and general practitioners. The diagnosis of deep vein thrombosis was confirmed with Doppler ultrasonography, whereas the diagnosis of pulmonary embolism was confirmed with a ventilation perfusion lung scan, spiral computed tomography, or angiogram. Patients' partners were invited to participate as controls if they were aged 18–70 y and had no history of venous thrombosis. In total, 3297 partners participated, forming a first control group. Also, from January 2002 through September 2004, a total of 3000 additional controls, who were recruited by using an RDD method, formed a second control group. These participants were also aged 18–70 y with no previous history of venous thrombosis.

All participants gave written informed consent. The study was approved by the Medical Ethics Committee of the Leiden University Medical Center, Leiden, The Netherlands.

Data collection and definitions

The index date for patients and partner controls was defined as the date of diagnosis of the thrombotic event. For RDD controls, the index date was the date of informed consent signing. Participants completed a standardized questionnaire, including items on demographic and lifestyle factors, as well as on potential risk factors for venous thrombosis.[17] Also, self-reported information was obtained on weight, height, and smoking habits, according to which participants were classified as current smokers, previous smokers, or nonsmokers.[18] BMI was calculated according to the following formula: weight (kg)/height squared (m^2), and participants were classified into 3 categories (in kg/m^2): normal weight (<25), overweight (25–30), and obese (>30). A structured questionnaire was taken from all participants regarding, among others, medication use. Participants were classified as vitamin users if they reported regular use of one or more of the following: folic acid, multivitamins, or vitamins A, B-6, B-12, C, D, or E in the 12 mo before the onset of venous thrombosis (for patients) or before enrollment in the MEGA study (controls). No information was obtained on the dosage of vitamin intake.

Provoked venous thrombosis was defined as venous thrombosis preceded by surgery, plaster cast immobilization, bed rest, leg injury, or hospitalization in the 3 mo before the index date or long-distance travel in the 2 mo before the index date.

Inclusion/exclusion criteria

Of the 4956 patients, we excluded 182 women who were pregnant at the index date or within the previous 3 mo. These women were excluded as guidelines recommend that women should take folic acid during pregnancy, and pregnancy itself affects risk.[19] Next we excluded 517 patients from this study for whom information on vitamin consumption was missing, which left 4257 patients. Of these patients, 2506 had a partner who fulfilled the inclusion criteria and was willing to participate, so 2506 complete couples remained. After application of the same exclusion criteria on the RDD control group, 2684 RDD control participants could be included in our analysis.

Statistical analysis

Participants were analyzed as current vitamin users or nonusers but also as users and nonusers of different types of vitamins (folic acid, multivitamins, and vitamins A, B-6, B-12, C, D, and E). Because the control population consisted of either RDD controls or partners of patients, we could perform 2 analyses. In the first analysis, we compared patients with RDD controls and adjusted for all measured confounding factors. In the second analysis, we used the partners of the patients as control individuals.

When patients were compared with RDD controls, unconditional logistic regression was used to calculate ORs with 95% CIs as a measure of the relative risks for venous thrombosis in vitamin users compared with nonusers. This analysis is unconditional, because controls were not individually matched to the patients, apart for frequency matching for age and sex, for which we adjusted. In the unconditional logistic regression analysis, all patients (n = 4257) were compared with all RDD controls (n = 2684). In a separate analysis, we also compared patients who had a partner (n = 2506) with RDD controls to see whether this would affect our risk estimates. Analyses were adjusted for age, sex, BMI, smoking habits, and prevalent arterial cardiovascular diseases, which included prior myocardial infarction or ischemic stroke. To avoid that effects of vitamin use on the risk of venous thrombosis were attributable to residual lifestyle-related confounders that are associated with vitamin use[20–22], we also included statin use, hormonal drug use (defined as oral contraception or postmenopausal hormone therapy), and physical activity as potential sources of confounding. Hormonal drugs were added to the model as a dichotomous variable in which all men were classified as unexposed.[23, 24] Hyperhomocysteinemia was not added as a confounding variable in the models because vitamin supplementation in hyperhomocysteinemia is not common in The Netherlands. Patients with provoked and unprovoked venous thrombosis, as well as patients with deep vein thrombosis and pulmonary embolism, were combined in most analyses but also analyzed separately.

Vitamin use may be related to a general health-conscious behavior, which may affect the risk of thrombosis and therefore act as a confounder. Such behavior is not easily adjusted for and measured. Partners of patients are likely to resemble the

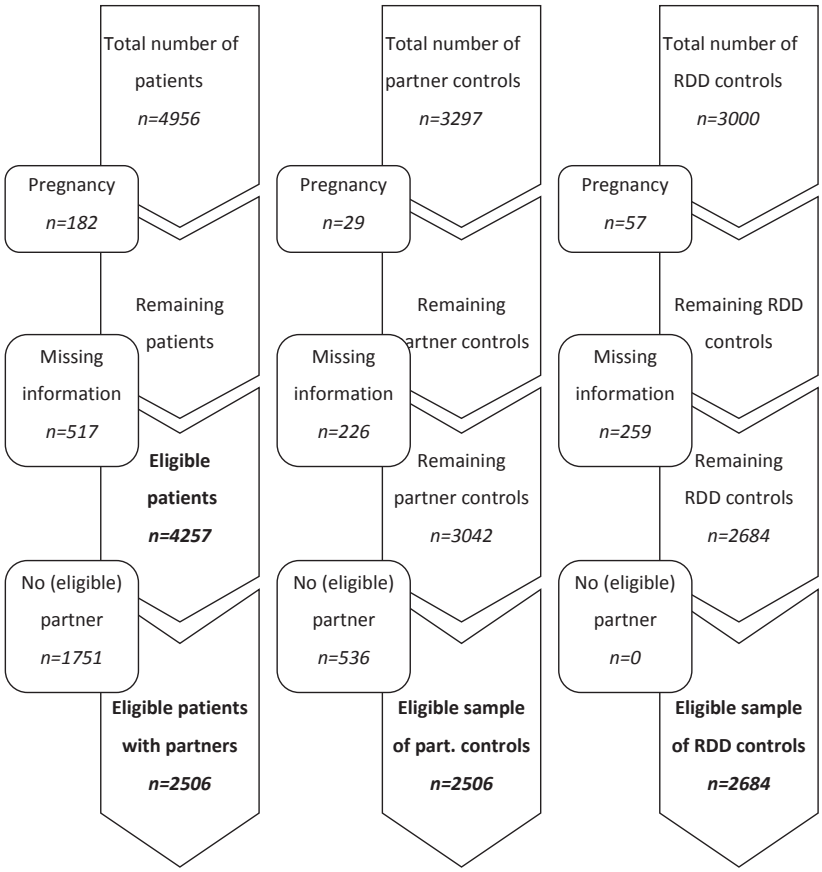

Figure 1. Selection of patients and controls in the Multiple Environmental and Genetic Assessment study. part., partner; RDD, random-digit dialing.

patients in health consciousness more than RDD controls, and therefore we performed a 1:1 matched analysis by conditional logistic regression, which adjusts for associations within matched pairs. This method provides adjustment for all unmeasured factors for which couples tend to be similar.[25] The analysis is conditional because many clinical characteristics of controls, who are individually matched to the patients, are likely to be similar to patient characteristics. One needs to take this into account in the analysis because otherwise, the frequency (of vitamin intake, for example) would become similar in cases and controls, leading to biased null findings. In this analysis, we also adjusted for all aforementioned potential confounding factors. Although using partners as controls results in most controls having the opposite sex as their matched case, one can adjust for sex in a partner-matched case-control study by allowing for sex with an indicator variable.[24]

Table 1. Clinical characteristics of the MEGA case-control study[1]

	Patients		Partner controls		RDD controls	
	(n = 4257)		(n=2506)		(n=2684)	
Vitamin use[2]	**No[3]**	**Yes[3]**	**No[3]**	**Yes[3]**	**No[3]**	**Yes[3]**
General characteristics						
Total	2844 (67)	1413 (33)	1688 (67)	818 (33)	1503 (56)	1181 (44)
Men	1479 (52)	544 (38)	905 (54)	294 (36)	772 (51)	404 (34)
Age at enrollment, y	49 (18-70)	49 (18-70)	49 (18-70)	49 (18-70)	46 (18-71)	45(18-70)
Body mass index, kg/m^2	27 (14-63)	26 (15-55)	26 (17-48)	25 (17-45)	25 (16-53)	25 (16-50)
Malignancy	232 (8)	153 (11)	28 (2)	13 (2)	25 (2)	29 (3)
Classical venous thrombosis risk factors						
Present[4]	1465 (52)	844 (61)	255 (16)	169 (23)	347 (23)	302 (26)
Without hormonal risk factors	655 (33)	346 (39)	70 (5)	45 (7)	75 (6)	54 (6)
With hormonal risk factors (in women)	798 (60)	483 (58)	179 (26)	119 (26)	271 (38)	243 (32)
Absent[4]	1359 (48)	549 (39)	1343 (84)	581 (77)	1142 (77)	858 (74)
Arterial cardiovascular risk factors						
Overweight	1240 (45)	516 (38)	707 (43)	282 (36)	514 (36)	357 (31)
Obesity	585 (21)	260 (19)	259 (16)	111 (14)	192 (13)	97 (9)
Previous smoking	854 (30)	421 (30)	502 (30)	260 (32)	410 (27)	317 (27)
Current smoking	1036 (37)	482 (34)	552 (33)	247 (31)	466 (31)	359 (31)
Self-reported prior CVD[5]	112 (4)	49 (4)	44 (3)	11 (1)	38 (2)	26 (2)
Statin use	120 (4)	30 (2)	97 (6)	37 (4)	98 (7)	61 (5)
Regular sports activity	845 (33)	470 (37)	539 (36)	319 (44)	595 (45)	550 (53)

[1]Values are n (%) unless otherwise indicated. CVD, cardiovascular disease; MEGA, Multiple Environmental and Genetic Assessment; RDD, random-digit dialing. [2]Use of either vitamin A, B-6, B-12, folic acid, vitamin C, D, E or multivitamins [3]Continuous variables denoted as mean (range), categorical variables as number (%) [4]Classical risk factors include surgery, malignancy, immobilization, trauma, plaster cast, oral contraceptive, hormonal replacement therapy and recent travel [5]CVD denotes self-reported myocardial infarction or ischemic stroke

All statistical analyses were performed with SPSS for Windows, version 20.0 (SPSS Inc.). Conditional logistic regression was performed by using the COXREG procedure, as explained on the SPSS tutorial page at http://www-01.ibm.com/support/docview.wss?uid=swg21477360.

Table 2. Risk of venous thrombosis by categories of vitamin supplementation[1]

	All Patients n (%)	RDD controls n (%)	Odds ratio[2] (95% CI)	Odds ratio[3] (95% CI)	Odds ratio[4] (95% CI)	Odds ratio[5] (95% CI)
Total venous thrombosis						
No Vitamin therapy	2844 (67)	1503 (56)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Vitamin therapy	1413 (33)	1181 (44)	0.63 (0.57,0.70)	0.60 (0.53,0.68)	0.68 (0.61,0.76)	0.68 (0.61,0.77)
Vitamin A	40 (1)	43 (2)	0.57 (0.37,0.89)	0.61 (0.38,0.97)	0.63 (0.38,1.03)	0.65 (0.38,1.12)
Vitamin B-6	112 (3)	109 (4)	0.63 (0.48,0.83)	0.63 (0.48,0.84)	0.64 (0.48,0.85)	0.61 (0.44,0.84)
Vitamin B-12	141 (3)	128 (5)	0.67 (0.52,0.86)	0.69 (0.53,0.89)	0.68 (0.52,0.89)	0.65 (0.48,0.87)
Folic acid	143 (5)	147 (3)	0.67 (0.53,0.85)	0.65 (0.50,0.83)	0.80 (0.62,1.05)	0.85 (0.64,1.13)
Vitamin C	364 (9)	396 (15)	0.55 (0.47,0.64)	0.57 (0.49,0.68)	0.57 (0.48,0.67)	0.57 (0.48,0.69)
Vitamin D	86 (2)	57 (2)	0.90 (0.64,1.27)	0.98 (0.69,1.40)	1.06 (0.72,1.54)	1.16 (0.77,1.75)
Vitamin E	96 (2)	79 (3)	0.71 (0.53,0.97)	0.73 (0.53,1.00)	0.81 (0.58,1.13)	0.87 (0.60,1.25)
Multivitamins	792 (19)	782 (29)	0.57 (0.51,0.64)	0.60 (0.53,0.68)	0.60 (0.53,0.68)	0.63 (0.55,0.72)

[1]ORs (95% CIs) were estimated by means of unconditional logistic regression. RDD, random-digit dialing. [2]Adjusted for age, sex, and partnership where applicable [3]Adjusted for age, sex, BMI, smoking, and partnership where applicable [4]Adjusted for age, sex, BMI, smoking, statin use, hormones use, and partnership where applicable [5]Adjusted for age, sex, BMI, smoking, statin use, hormones use, cardiovascular disease, sports activity, and partnership where applicable

RESULTS

A total of 7696 participants (2506 patients, 2506 partner controls, and 2684 RDD controls) were included in this study (Figure 1). The main characteristics of participants are presented in Table 1 and Appendix 1 Table 1. The mean age was 49 y (range, 18–70 y) in patients and partner controls and 46 y (range, 18–70 y) in RDD controls. Vitamin supplements were used by 796 (32%) patients, 818 (33%) partner controls, and 1181 (44%) RDD controls. Because patients were matched to their partners, only exposure-discordant couples (i.e., couples in whom vitamin consumption differs between patient and partner) were relevant to the univariable risk analyses.[26] In total, there were 744 discordant couples (i.e., in whom only one of the two used vitamins). Participants who used vitamins and those who did not were of similar age. Female participants used vitamins more frequently than did men. Patients and RDD controls with malignancy used vitamins more frequently (40% compared with 54%) than did participants without malignancy (32% compared with 44%), respectively, while in partner controls, there was no difference regarding vitamin use between those with and without malignancy. Also, participants with classic venous thrombosis risk factors used vitamins more frequently

than did those without these risk factors, but this was mainly associated with hormone use. In vitamin users, the prevalence of hormone use and sporting was higher, whereas the prevalence of statin use was lower than in nonusers. Also, participants who used vitamins were less likely to be overweight or obese than participants who did not use vitamins. No other characteristics were associated with vitamin use.

Overall, vitamin use was associated with a decreased risk of venous thrombosis when comparing all patients with RDD controls (OR: 0.63; 95% CI: 0.57, 0.70) (Table 2). Adjustment for age, sex, BMI, smoking, statin use, and hormone use yielded an OR of 0.68 (95% CI: 0.61, 0.77). The fully adjusted ORs for vitamin A, vitamin B-6, vitamin B-12, folic acid, vitamin C, vitamin D, vitamin E, and multivitamin use were in the same range. The results were the same when we compared patients with a partner with RDD controls (Appendix 1 Table 2).

When we compared patients with their partner controls, 361 couples were present in whom the patient had been taking vitamins but the partner had not, as well as 383 couples in whom it was the other way around, resulting in ORs close to unity; the fully adjusted ORs also were in the same range. An exception was vitamin A therapy, in which the OR was 0.47 (95%CI: 0.24, 0.91), and the fully adjusted OR was 0.46 (95% CI: 0.18, 1.20) (Table 3). In a further analysis, we restricted the outcome to deep vein thrombosis or pulmonary embolism and analyzed patients with and without classic provocative risk factors for venous thrombosis. These analyses showed similar results (Tables 4 and 5 and Appendix 1 Table 3).

DISCUSSION

We analyzed data from a large case-control study with the aim to investigate whether vitamin supplements decrease venous thrombosis risk by using both population and partner controls and found that vitamin B, C, D, and E supplementation is not associated with a decreased risk.

In the initial analyses, in which patients were compared with RDD controls, we observed a 37% decrease in risk for venous thrombosis in vitamin users compared with no vitamin users. After adjustment for many lifestyle-related factors such as BMI, smoking, statin use, hormone use, and sports activity, this decrease was still 32%. For the individual vitamins, a protective effect was found for vitamin B-6, vitamin B-12, folic acid, and vitamin C, with a 39%, 35%, 35%, and 43% decrease in risk after adjustment, respectively. On the basis of these results, we could have concluded that one should prescribe vitamins to prevent venous thrombosis, especially considering the popular opinion that vitamins are otherwise harmless.[27] However, comparison of patients with venous thrombosis and their partners showed that the above-mentioned risk estimates (with the possible exception of vitamin A) were likely to be confounded, because in this analysis, no decreased risk estimates were found for venous thrombosis in vitamin users

Table 3. Risk of venous thrombosis by categories of vitamin supplementation for all patients with partner controls[1]

	Patients with partners n (%)	Partner controls n (%)	Odds ratio[2] (95% CI)	Odds ratio[3] (95% CI)	Odds ratio[4] (95% CI)	Odds ratio[5] (95% CI)
No Vitamin therapy	1710 (68)	1688 (67)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Vitamin therapy	796 (32)	818 (33)	0.98 (0.84,1.14)	1.03 (0.88,1.20)	1.13 (0.95,1.35)	1.11 (0.91,1.35)
Vitamin A	38 (1)	23 (1)	0.47 (0.24,0.91)	0.47 (0.23,0.95)	0.58 (0.26,1.25)	0.46 (0.18,1.20)
Vitamin B-6	56 (2)	59 (2)	0.97 (0.65,1.44)	0.96 (0.63,1.46)	0.97 (0.61,1.54)	0.86 (0.49,1.49)
Vitamin B-12	75 (3)	65 (3)	1.22 (0.85,1.77)	1.24 (0.84,1.84)	1.04 (0.67,1.61)	0.94 (0.55,1.61)
Folic acid	73 (3)	69 (3)	1.10 (0.78,1.56)	1.08 (0.74,1.56)	1.19 (0.79,1.79)	1.04 (0.65,1.67)
Vitamin C	193 (8)	218 (9)	0.82 (0.64,1.06)	0.88 (0.68,1.15)	0.95 (0.71,1.28)	0.96 (0.68,1.36)
Vitamin D	39 (2)	45 (2)	0.85 (0.53,1.38)	1.01 (0.61,1.70)	0.87 (0.48,1.55)	1.23 (0.61,2.45)
Vitamin E	53 (2)	46 (2)	1.21 (0.77,1.91)	1.38 (0.85,2.25)	1.36 (0.79,2.33)	1.34 (0.73,2.47)
Multivitamins	454 (18)	488 (19)	0.89 (0.74,1.07)	0.94 (0.77,1.14)	0.98 (0.79,1.12)	0.99 (0.77,1.26)

[1]ORs (95% CIs) were estimated by means of unconditional logistic regression. RDD, random-digit dialing. [2]Adjusted for age, sex, and partnership where applicable [3]Adjusted for age, sex, BMI, smoking, and partnership where applicable [4]Adjusted for age, sex, BMI, smoking, statin use, hormones use, and partnership where applicable [5]Adjusted for age, sex, BMI, smoking, statin use, hormones use, cardiovascular disease, sports activity, and partnership where applicable

compared with nonusers of vitamins (fully adjusted OR: 1.11; 95% CI: 0.91, 1.35). Similar results were obtained for all individual vitamins. This could be due to the influence of lifestyle-related confounders, which the comparison with partners adjusts for.

Most studies investigating the influence of vitamin intake on the risk of venous thrombosis focused on vitamin B-6, vitamin B-12, and folic acid. Because folic acid decreases homocysteine concentration, appears to interact with the metabolism of nitric oxide, and reduces superoxide anion generation[5, 28], it was assumed that supplementation of this vitamin would lead to a decreased risk of venous thrombosis. Our results showed no decrease in risk for venous thrombotic events in vitamin B users, which is consistent with the results of large clinical trials on first and recurrent venous thrombosis, such as the Heart Outcomes Prevention Evaluation and the Vitamins and Thrombosis trial[7, 29], which showed no benefit of homocysteine-lowering therapy. Regarding the other vitamins, some studies showed that combined antioxidant treatment with vitamins C and E for 4 wk improves endothelial function and decreases the plasminogen activator inhibitor 1/tissue plasminogen activator ratio.[30] The Women's Health Study reported a 21% risk reduction of venous thrombosis in women taking vitamin E over a 10-y follow-up period (14). However, results of this

Table 4. Risk of venous thrombosis by vitamin supplementation for all patients and RDD controls, subgroup analysis

	All Patients n (%)	**RDD controls n (%)**	**Odds ratio[1] (95% CI)**
Provoked venous thrombosis			
No vitamin therapy	1465 (63)	1503 (57)	1.0 (Reference)
Vitamin therapy	844 (37)	1181 (44)	0.77 (0.66,0.89)
Unprovoked venous thrombosis			
No vitamin therapy	1359 (71)	1503 (56)	1.0 (Reference)
Vitamin therapy	549 (29)	1181 (44)	0.61 (0.52,0.72)
Deep vein thrombosis only			
No vitamin therapy	1664 (68)	1503 (57)	1.0 (Reference)
Vitamin therapy	795 (32)	1181 (44)	0.67 (0.59,0.77)
Pulmonary embolism +/- deep vein thrombosis			
No vitamin therapy	1180 (66)	1503 (56)	1.0 (Reference)
Vitamin therapy	618 (34)	1181 (44)	0.71 (0.61,0.82)

[1]Estimated by means of unconditional logistic regression and adjusted for age, sex, BMI, smoking, statin use, hormone use, cardiovascular disease, and sports activity. RDD, random-digit dialing.

Table 5. Risk of venous thrombosis by vitamin supplementation for patients with partner controls, subgroup analysis

	Patients with partners n (%)	**Partner Controls n (%)**	**Odds ratio[1] (95% CI)**
Provoked venous thrombosis			
No vitamin therapy	865 (64)	1688 (67)	1.0 (Reference)
Vitamin therapy	486 (36)	818 (33)	1.05 (0.93,1.19)
Unprovoked venous thrombosis			
No vitamin therapy	837 (74)	1688 (67)	1.0 (Reference)
Vitamin therapy	302 (26)	818 (33)	0.94 (0.81,1.09)
Deep vein thrombosis only			
No vitamin therapy	991 (69)	1688 (67)	1.0 (Reference)
Vitamin therapy	447 (31)	818 (33)	0.98 (0.86,1.11)
Pulmonary embolism +/- deep vein thrombosis			
No vitamin therapy	719 (67)	1688 (67)	1.0 (Reference)
Vitamin therapy	349 (33)	818 (33)	1.02 (0.88,1.18)

[1]Estimated by means of conditional logistic regression and adjusted for partnership, age, sex, BMI, smoking, statin use, hormone use, cardiovascular disease, and sports activity.

study are difficult to interpret. The Women's Health Study used a 2 X 2 factorial randomized design (vitamin E, aspirin, placebo) but failed to show an effect of vitamin E alone compared with placebo on venous thrombosis risk. It is therefore possible that it was not vitamin E but aspirin, or a combination of both, that decreased the risk of venous thrombosis and not vitamin E itself. No other evidence is present that supplementation with these antioxidant vitamins leads to a decreased risk for venous thrombosis. Our results showed no protective effect of vitamins C, E, or D on venous thrombotic risk, but vitamin A consumption showed a beneficial effect. This latter result should be interpreted with caution because numbers were small in this analysis. Also, in the argument to take vitamins or not, it should be considered that vitamin supplements may not be that harmless. For example, use of folic acid might promote progression of atherosclerosis[31] and increase the risk for carcinogenesis.[32] Moreover, antioxidant vitamins interfere with essential defensive mechanisms such as apoptosis, phagocytosis, and detoxification and might lead to increased mortality[33], which makes prescription of these supplements less attractive.

Limitations of our study are that data on vitamin use were self-reported without information on duration of vitamin use or exact dose for every single vitamin. Also, because we could only investigate associations between vitamin supplements and venous thrombosis, our findings should not be applied directly to natural vitamins (as in food). Given that we did not have data about vitamin status before or after vitamin consumption, we were unable to evaluate whether some vitamins would be beneficial against venous thrombosis in people with vitamin deficiency at baseline. Furthermore, it would have been interesting to consider vitamin K status in MEGA because vitamin K–dependent coagulation factors can determine venous thrombosis risk. Unfortunately, this information was not available. A strength of our research lies in the large study size and in the study design, which included both RDD and partner controls, making it possible to show that for this research question, protective risk estimates can easily be found if not all lifestyle-dependent confounding is accounted for and measured. We consider it unlikely that the accuracy or completeness of the recollections retrieved by study participants regarding vitamin use in the past is different among RDD controls, partner controls, and the patients with venous thrombosis. In addition, if somehow the patients did erroneously recall their vitamin use after venous thrombosis diagnosis, this would not explain the difference in risk estimates when patients were compared with RDD controls or partner controls. It should be noted, however, that using partner controls in case-control studies has drawbacks, too. First, patients without a partner are not included, which may lead to selection of a certain "type" of patient. This, however, should not compromise internal validity but could at most hamper generalization to other patients. Second, it is possible that partner controls are more similar to the cases with respect to the frequency of exposure than a random sample from the population.

This would lead to selection bias and to an underestimation of the effect. However, carrying out a matched analysis (such as we did) takes this into account and adjusts for such bias should it have occurred. Although the number of comparisons in this study was extensive, no adjustment for multiple testing was performed. We decided not to adjust for multiple testing because nearly all our analyses pointed toward a lower risk of venous thrombosis in vitamin users when patients were compared with RDD controls, whereas relative risks were all close to unity when patients were compared with partners. This consistent pattern agrees with our null hypothesis (i.e., no decreased risk of venous thrombosis in vitamin supplement users after extensive adjustments for confounding), and therefore there is no risk of falsely rejecting it (i.e., no risk of a type I error) and no need for adjustment for multiple testing. Of note, some of the estimates in this analysis had confidence intervals that were wide and sometimes included 1.0. However, our most robust estimates (in which we pooled all vitamin users together in one group) showed that the risk of venous thrombosis was lower (OR: 0.68; 95% CI: 0.61, 0.77) and still confounded when patients were compared with RDD controls as opposed to a more rigorous adjustment for confounding when patients were compared with partners (OR for venous thrombosis: 1.11; 95% CI: 0.91, 1.35). Results for the individual vitamin supplements pointed toward the same direction (i.e., lower risk of venous thrombosis compared with RDD controls as opposed to partners), indicating that relative risk estimates on any vitamin use were likely to be confounded by unmeasured confounding factors when patients were compared with population-derived RDD controls.

In conclusion, our findings confirm that vitamin B supplementation is not associated with a decreased risk of venous thrombosis and adds as a novelty that vitamins C, D, and E are not associated with this disease. Furthermore, our study demonstrated that initial protective risk estimates were found due to control selection, reinforcing that when studying health-conscious related exposures, the control group must be selected with care, and lifestyle-dependent confounding should be measured as much as possible.

REFERENCES

1. Næss IA, Christiansen SC, Romundstad P, Cannegieter SC, Rosendaal FR, Hammerstrøm J. Incidence and mortality of venous thrombosis: a population-based study. J Thromb Haemost 2007;5:692–9.
2. Veeger NJGM, Piersma-Wichers M, Tijssen JGP, Hillege HL, van der Meer J. Individual time within target range in patients treated with vitamin K antagonists: main determinant of quality of anticoagulation and predictor of clinical outcome. A retrospective study of 2300 consecutive patients with venous thromboembolism. Br J Haematol 2005; 128:513–9.
3. McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. Am J Pathol 1969;56:111–28.

4. Lijfering WM, Rosendaal FR, Cannegieter SC. Risk factors for venous thrombosis—current understanding from an epidemiological point of view. Br J Haematol 2010;149:824–33.
5. Das UN. Folic acid says no to vascular diseases. Nutrition 2003;19: 686–92.
6. Remacha AF, Souto JC, Pin˜ana JL, Sarda MP, Queralto JM, Martı´- Fabregas JM, Moll XG, Fe´rnandez C, Rodriguez A, Cuesta J. Vitamin B12 deficiency, hyperhomocysteinemia and thrombosis: a case and control study. Int J Hematol 2011;93:458–64.
7. den Heijer M, Willems HP, Blom HJ, Gerrits WB, Cattaneo M, Eichinger S, Rosendaal FR, Bos GMJ. Homocysteine lowering by B vitamins and the secondary prevention of deep vein thrombosis and pulmonary embolism: a randomized, placebo-controlled, double-blind trial. Blood 2007;109:139–44.
8. Ray JG, Kearon C, Yi Q, Sheridan P, Lonn E; Heart Outcomes Prevention Evaluation 2 (HOPE-2) Investigators. Homocysteine-lowering therapy and risk for venous thromboembolism: a randomized trial. Ann Intern Med 2007;146:761–7.
9. Loscalzo J. Homocysteine trials-clear outcomes for complex reasons. N Engl J Med 2006;354:1629–32.
10. Bhupathiraju SN, Tucker KL. Coronary heart disease prevention: nutrients, foods, and dietary patterns. Clin Chim Acta 2011;412:1493–514.
11. Lock K, Pomerleau J, Causer L, Altmann DR, McKee M. The global burden of disease attributable to low consumption of fruit and vegetables: implications for the global strategy on diet. Bull World Health Organ 2005;83:100–8.
12. Myung SK, JuW, Cho B, Oh SW, Park SM, Koo BW, Park BJ. Efficacy of vitamin and antioxidant supplements in prevention of cardiovascular disease: systemic review and meta-analysis of randomized controlled trials. BMJ 2013;346:f10.
13. Brøndum-Jacobsen P, Benn M, Tybjaerg-Hansen A, Nordestgaard BG. 25-Hydroxyvitamin D concentrations and risk of venous thromboembolism in the general population with 18.791 participants. J Thromb Haemost 2013;11:423–31.
14. Glynn RJ, Ridker PM, Goldhaber SZ, Zee RYL, Buring JE. Effects of random allocation to vitamin E supplementation on the occurrence of venous thromboembolism. Circulation 2007;116:1497–503.
15. Brodin E, Lerstad G, Grimnes G, Brækkan SK, Vik A, Brox J, Svartberg J, Jorde R, Hansen JB. Serum levels of vitamin D are not associated with future risk of venous thromboembolism. Thromb Haemost 2013;109:885–90.
16. Pomp ER, van Stralen KJ, Le Cessie S, Vandenbroucke JP, Rosendaal FR, Doggen CJ. Experience with multiple control groups in a large population-based case-control study on genetic and environmental risk factors. Eur J Epidemiol 2010;25:459–66.
17. Ocak G, Vossen CY, Verdujin M, Dekker FW, Rosendaal FR, Cannegieter SC, Lijfering WM. Risk of venous thrombosis in patients with major illnesses: results from the MEGA study. J Thromb Haemost 2013;11:116–23.
18. Pomp ER, Rosendaal FR, Doggen CJ. Smoking increases the risk of venous thrombosis and acts synergistically with oral contraceptive use. Am J Hematol 2008;83:97–102.

19. Van Allen MI, Fraser FC, Dallaire L, Allanson J, McLeod DR, Andermann E, Friedman JM. Recommendations on the use of folic acid supplementation to prevent the recurrence of neural tube defects. Clinical Teratology Committee, Canadian College of Medical Geneticists. CMAJ 1993;149:1239–43.
20. Vasankari T, Ahotupa M, Viiakri J, Nuotio I, Strandberg T, Vanhanen H, Gylling H, Miettinen T, Tikkanen MJ. Effect of 12-month statin therapy on antioxidant potential of LDL and serum antioxidant vitamin concentrations. Ann Med 2004;36:618–22.
21. Lussana F, Zighletti ML, Bucciarelli P, Cugno M, Cattaneao M. Blood levels of homocysteine, folate, vitamin B6 and B12 in women using oral contraceptives to non-users. Thromb Res 2003;112:37–41.
22. Scragg R, Camargo CA Jr. Frequency of leisure-time physical activity and serum 25-hydroxyvitamin D levels in the US population: results from the Third National Health and Nutrition Examination Survey. Am J Epidemiol 2008;168:577–86.
23. Cannegieter SC. Thrombosis after travel: reply to a perspective. PLoS Med 2009;3:e307.
24. Rothman KJ. Thrombosis after travel. PLoS Med 2006;3:e300.
25. Breslow NE, Day NE. Statistical methods in cancer research. Lyon (France): International Agency for Research on Cancer; 1980.
26. Rothman KJ, Greenland S. Modern epidemiology. Philadelphia (PA): Lippincott Williams & Wilkins; 1988.
27. Messerer M, Johansson SE, Wolk A. Use of dietary supplements and natural remedies increased dramatically during the 1990s. J Intern Med 2001;250:160–6.
28. Spence JD, Stampfer MJ. Understanding the complexity of homocysteine lowering with vitamins: the potential role of subgroup analyses. JAMA 2011;306:2610–1.
29. Lonn E, Yusuf S, Arnold MJ, Sheridan P, Pogue J, Micks M, McQueen MJ, Probstfield J, Fodor G, Held C, et al. Homocysteine lowering with folic acid and B vitamins in vascular disease. N Engl J Med 2006;354: 1567–77.
30. Antoniades C, Tousoulis D, Tentolouris C, Toutouzo M, Marinou K, Goumas G, Tsioufis C, Toutouzas P, Stefanadis C. Effects of antioxidant vitamins C and E on endothelial function and thrombosis/fibrinolysis system in smokers. Thromb Haemost 2003;89:990–5.
31. Miller ER, Juraschek S, Pastor-Barviuso R, Bazzano LA, Appel LJ, Guallar E. Meta-analysis of folic acid supplementation trials on risk of cardiovascular disease and risk interaction with baseline homocysteine levels. Am J Cardiol 2010;106:517–27.
32. Figueiredo JC, Grau MV, Haile RW, Sandler RS, Summers RW, Bresalier RS. Folic acid and risk of prostate cancer: results from a randomized clinical trial. J Natl Cancer Inst 2009;101:432–5.
33. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. Cochrane Database Syst Rev 2012; 3:CD007176.

APPENDIX 1

Table 1. Clinical characteristics of MEGA patients who had a partner

	Patients with partners	
	(n=2506)	
Vitamin use[1]	**No**	**Yes**
General characteristics		
Total	1710 (68)	796 (32)
Men	965 (56)	348 (44)
Age at enrollment,y	50 (18-70)	49 (18-70)
Body mass index, kg/m^2	27 (16-57)	26 (16-45)
Malignancy	143 (8)	92 (12)
Classical venous thrombosis risk factors		
Present[2]	865 (51)	486 (62)
Without hormonal risk factors	401 (32)	205 (40)
With hormonal risk factors (in women)	460 (63)	276 (63)
Absent[2]	837 (49)	302 (38)
Arterial cardiovascular risk factors		
Overweight	798 (48)	307 (40)
Obesity	336 (20)	141 (18)
Previous smoking	611 (36)	272 (35)
Current smoking	555 (33)	241 (31)
Self-reported prior CVD‡	64 (4)	32 (4)
Statin use	84 (5)	15 (2)
Regular sports activity	533 (34)	274 (38)

[1]Use of either vitamin A, B-6, B-12, folic acid, vitamin C, D, E or multivitamins. [2]Classical risk factors include surgery, malignancy, immobilization, trauma, plaster cast, oral contraceptive, hormonal replacement therapy and recent travel. ‡CVD denotes self reported myocardial infarction or ischemic stroke.

Table 2. Risk of venous thrombosis by categories of vitamin supplementation in patients with partners vs RDD controls

	Patients with partners n (%)	RDD controls n (%)	Odds ratio[1,2] (95% CI)	Odds ratio[1,3] (95% CI)	Odds ratio[1,4] (95% CI)	Odds ratio[1,5] (95% CI)
Total venous thrombosis						
No Vitamin therapy	1710 (68)	1503 (56)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Vitamin therapy	796 (32)	1181 (44)	0.61 (0.55,0.69)	0.64 (0.56,0.72)	0.65 (0.58,0.74)	0.64 (0.56,0.74)
Vitamin A	23 (1)	43 (2)	0.56 (0.34,0.95)	0.58 (0.34,0.99)	0.66 (0.37,1.17)	0.66 (0.35,1.25)
Vitamin B-6	56 (2)	109 (4)	0.56 (0.40,0.78)	0.56 (0.40,0.79)	0.58 (0.41,0.83)	0.53 (0.36,0.79)
Vitamin B-12	75 (3)	128 (5)	0.63 (0.47,0.85)	0.65 (0.48,0.89)	0.67 (0.49,0.93)	0.57 (0.40,0.82)
Folic acid	73 (3)	143 (5)	0.60 (0.45,0.80)	0.57 (0.42,0.77)	0.73 (0.53,1.01)	0.76 (0.54,1.07)
Vitamin C	193 (8)	396 (15)	0.50 (0.42,0.60)	0.53 (0.44,0.64)	0.53 (0.43,0.65)	0.52 (0.42,0.64)
Vitamin D	39 (2)	57 (2)	0.72 (0.47,1.09)	0.79 (0.51,1.23)	0.83 (0.52,1.32)	0.88 (0.54,1.46)
Vitamin E	53 (2)	79 (3)	0.67 (0.47,0.96)	0.68 (0.47,0.98)	0.74 (0.50,1.09)	0.83 (0.54,1.26)
Multivitamins	454 (18)	782 (29)	0.57 (0.50,0.65)	0.60 (0.52,0.68)	0.60 (0.52,0.69)	0.61 (0.52,0.71)

[1]Estimated by means of unconditional logistic regression. [2]Adjusted for age and sex. [3]Adjusted for age, sex, BMI and smoking. [4]Adjusted for age, sex, BMI, smoking, statin use and hormones use. [5]Adjusted for age, sex, BMI, smoking, statin use, hormones use, cardiovascular disease and sports activity.

Table 3. Risk of venous thrombosis by vitamin supplementation, subgroup analysis

	Patients with partners n (%)	RDD controls n (%)	Odds ratio[1,2] (95% CI)
Provoked venous thrombosis			
No vitamin therapy	865 (64)	1503 (56)	1.0 (Reference)
Vitamin therapy	486 (36)	1181 (44)	0.73 (0.62,0.87)
Unprovoked venous thrombosis			
No vitamin therapy	837 (74)	1503 (56)	1.0 (Reference)
Vitamin therapy	302 (26)	1181 (44)	0.57 (0.48,0.69)
Deep vein thrombosis only			
No vitamin therapy	991 (69)	1503 (56)	1.0 (Reference)
Vitamin therapy	447 (31)	1181 (43)	0.63 (0.54,0.75)
Pulmonary embolism +/- deep vein thrombosis			
No vitamin therapy	719 (67)	1503 (56)	1.0 (Reference)
Vitamin therapy	349 (33)	1181 (44)	0.68 (0.57,0.81)

[1]Estimated by means of unconditional logistic regression. [2]Adjusted for age, sex, BMI, smoking, statin use, hormones use, cardiovascular disease and sports activity.

Chapter 5

Discrepancies between risk estimates when using different control groups in a case-control study

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In preparation

5

ABSTRACT

Background: Control selection in case-control studies is challenging and can result in bias when done inappropriately. Literature that compares odds ratios (ORs) between different control groups is scarce.

Objective: To investigate whether ORs differ when using partner and random digit dialling (RDD) controls. We hypothesized that the difference in ORs would increase when comorbidity related quality of life of the control decreased.

Methods: We used data from the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis study, with patients with first venous thrombosis and partner- and RDD controls who were included between 1999 and 2004. Exposures to comorbidity were any self-reported chronic diseases, acute arterial thromboembolic events and malignancies. Quality of life associated with comorbidity (i.e. exposure of interest) was based on prior validated EQ-5D scores, where 0 indicates that a person is dead and 1 that a person is completely healthy. ORs and 95% confidence intervals (CIs) were estimated by means of logistic regression and adjusted for sex and age. The ratio of ORs ($OR_{RDD}/OR_{Partner}$) was calculated to quantify the difference between ORs when using both control groups.

Results: 4956 Patients, 2917 partner controls and 3000 RDD controls were enrolled. Eighteen exposures were included in the analyses with $OR_{RDD}/OR_{Partner}$ between 0.35 and 2.03. A low quality of life (i.e. EQ-5D score of the exposure) was associated with a decreased $OR_{RDD}/OR_{Partner}$. This differences in risk estimates, and therefore the ratio between the risk estimates, may be due to lower participation rates among severely diseased (i.e. exposed) partners compared with RDD controls.

Conclusions: Discrepancies between risk estimates obtained with a partner control group as compared with a RDD control group increase with exposures that affect quality of life.

INTRODUCTION

A case-control study design is commonly used as an efficient way to study disease outcomes.[1] The principle of the design is that frequencies of exposure among diseased (cases) and referent subjects (controls) are compared to determine whether an association exists between the exposure and disease. Ideally, selection of cases and controls occurs from the same study base, where controls represent the exposure rate of the study base that the cases arose from.[2,3] When performed correctly, case-control studies can be as credible as randomised studies[4], but as all study designs, they are susceptible to bias.[2]

Defining the study base, and thereby a representative control group, can be challenging, especially when the study base is not clear, for example when hospital-based cases are used.[3] Even when an appropriate control group is selected, bias can still occur in case of different participation rates among cases and controls which are related to the exposure of interest and if the tendency to participate is stronger (or weaker) in cases than controls.[5,6]

Literature where risk estimates in case-control studies are compared between different control groups is scarce, since the majority of case-control studies only include one control group. However, some studies contain two or more control groups, such as the 'Multiple Environmental Genetic Assessment (MEGA) case-control study to assess risk factors for venous thrombosis'.[7] The two control groups included in the MEGA study were a partner- and random digit dialling (RDD) control group. Partner controls were included as control subjects as the main focus was on genetic risk factors for venous thrombosis and their interaction with environmental and life-style factors. However, as there was only a small group of young male patients which yielded an even smaller control group of young female partners, it was difficult to analyse women-specific risk factors. To remedy the case-control imbalance in sex and age and to boost statistical power, the MEGA study also included an RDD control group at a later stage.[8]

During participant selection of a recently published trial from us[9], we came to the impression that patients were less willing to participate in the trial if they and a relative (e.g. partner, child), were also ill. However, if this tendency for non-participation in a study really exists has as far as we know not been empirically studied. We therefore hypothesized that partner controls are less willing to participate in a study if they, next to their partner (i.e. the case), are (seriously) ill. In addition, we hypothesized that RDD controls with chronic diseases that impair quality of life may be overrepresented as these control individuals will be more at home and therefore more accessible to contact by telephone as compared to healthy RDD controls. If true, differences in participation rates can lead to discrepancies between risk estimates (odds ratios [ORs]) when exposures that impair quality of life are studied and partner- or RDD controls are used.

METHODS

Study population

The design of the MEGA case-control study is described elsewhere.[7] In short, 4956 consecutive patients aged 18–70 years, with a first objectively diagnosed deep vein thrombosis or pulmonary embolism, were included from 6 anticoagulation clinics in The Netherlands (Amersfoort, Amsterdam, The Hague, Leiden, Rotterdam, and Utrecht) between March 1999 and September 2004.

Two control groups were selected using the same exclusion criteria as for the patients. The first control group consisted of partners of the patients. Partners who were aged 18 to 70 years and had no history of venous thrombosis were asked to participate from March 1999 to September 2004. In total, 3586 partners were eligible, 18 suffered from end stage disease and 651 refused to participate, leaving 2917 participating partner controls.

From January 2002 to September 2004, a second control group was recruited using RDD as described by Waksberg.[10] 4346 Eligible controls from the same geographical area as the patients were asked to participate, and were frequency matched to the patients on age and sex. With each telephone call we asked a specific person within a household to participate depending on our needs to fulfill age and sex criteria. The RDD method is only useful if the vast majority of individuals live in households with a fixed (land-line) telephone. In December 2005 fixed (land-line) telephone coverage in the Netherlands was very high (96%), and sufficient for RDD selection.[8]

15 potential RDD controls suffered from end stage disease and 1331 refused to take part, leaving 3000 RDD controls. All participants gave written informed consent in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of the Leiden University Medical Center, Leiden, the Netherlands and has been described in detail elsewhere.[8]

Data collection

All participants were asked to fill in a standardized questionnaire within a few weeks after inclusion in the study. The questionnaire was returned by 4543 (92%) patients, 2757 (95%) partner controls and 2789 (93%) RDD controls. The index date was defined as the date of venous thrombosis for the patients, and the date of completing the questionnaire for the control subjects. The questionnaire provided information on current comorbidity, weight, height, use of medication, and risk factors for venous thrombosis, such as malignancies in the five years prior to the index date.

At least three months after anticoagulation therapy was discontinued, or during anticoagulation therapy in patients who were treated for over one year, patients and controls visited the anticoagulation clinic for a blood sample. From December 1999, self-administered buccal swabs were obtained by mail when participants were not able

or willing to give a blood sample. From June 2002, buccal swabs were the only used method to obtain DNA. DNA was obtained from 4290 (87%) cases, 2544 (92%) partner controls and 2023 (67%) RDD controls. Common genetic risk factors, such as factor V Leiden and ABO blood group, were determined, as has been described previously.[7,11]

Exposures of interest were all chronic diseases, acute arterial thromboembolic events (i.e. ischemic stroke, myocardial infarction) and malignancies. To classify quality of life when having a comorbidity (i.e. an exposure of interest), we used EQ-5D scores. EQ-5D scores are a standardized validated measure of health status for clinical and economical applications. For every disease (expressed as ICD-9 code), a value between 0 and 1 is determined, where 1 indicates that a person is alive and completely healthy and 0 indicates that a person is dead (see Supplementary Appendix 1).[12,13] In addition, ORs for the association of Factor V Leiden and ABO-blood group on venous thrombosis were estimated and compared when taking partner and RDD controls. Both genetic variants are not related with changes in quality of life and we therefore expected that both control groups should yield similar ORs (i.e. a 'negative control of the analysis'[14,15]), as neither is associated with changes in quality of life, and therefore should yield similar ORs according to the hypothesis.

Statistical analysis

Self-reported information from the questionnaire was used to classify whether participants had been exposed to chronic diseases, acute arterial thromboembolic events (i.e. ischemic stroke, myocardial infarction) and malignancies. Dates of cancer diagnosis were available and participants were considered exposed to cancer if they reported having a cancer diagnosis a year before the index date. ORs and 95% confidence intervals (CIs) were estimated by means of conditional logistic regression for the partner controls and adjusted for age and sex. RDD controls were compared to patients using (unconditional) logistic regression and ORs were also adjusted for age and sex.

The ratio of ORs ($OR_{RDD}/OR_{Partner}$) was calculated to quantify the difference between ORs when using the partner- or the RDD control group. This ratio was plotted against the EQ-5D of that exposure to show a potential trend between discrepancies of ORs and the quality of life. All statistical analyses were performed in R 2.15.2.

RESULTS

In total, 4956 patients and 5917 controls were enrolled, of which 2917 were partner controls and 3000 were RDD controls. Patients and partner controls were slightly older (49 years, range 18 to 70 and 50 years, range 18 to 70 years respectively) than RDD controls (45 years, range 18 to 70). The prevalence of all types of cancer (i.e. breast,

Table 1. Clinical characteristics

	Cases	Cases with partner	Partner controls	RDD controls
General characteristics				
Patients	4956	2917	2917	3000
Men	2682 (54)	1449 (50)	1463 (50)	1719 (57)
Age	49 (13)	50 (12)	50 (12)	45 (13)
BMI	27 (5)	27 (5)	26 (4)	25 (4)
Cancer				
Prostate	24 (0)	14 (0)	2 (0)	2 (0)
Breast	39 (1)	14 (0)	4 (0)	5 (0)
Colorectal	65 (1)	29 (1)	3 (0)	2 (0)
Chronic diseases				
Hyperthyroidism	40 (1)	16 (1)	22 (1)	15 (1)
Hypothyroidism	91 (2)	64 (2)	55 (2)	54 (2)
Bronchitis	253 (5)	134 (5)	72 (2)	82 (3)
Emphysema	66 (1)	35 (1)	16 (1)	16 (1)
Diabetes mellitus	183 (4)	89 (3)	92 (3)	88 (3)
Paralysis	46 (1)	24 (1)	4 (0)	12 (0)
Liver disease	27 (1)	14 (0)	8 (0)	11 (0)
Nephropathy	60 (1)	27 (1)	7 (0)	12 (0)
Rheumatoid arthritis	145 (3)	75 (3)	61 (2)	59 (2)
Multiple sclerosis	30 (1)	20 (1)	5 (0)	12 (0)
Heart failure	76 (2)	42 (1)	23 (1)	32 (1)
Angina pectoris	64 (1)	35 (1)	28 (1)	17 (1)
Previous acute events				
Myocardial infarction	137 (3)	79 (3)	46 (2)	54 (2)
Stroke	54 (1)	29 (1)	21 (1)	23 (1)
Intracranial haemorrhage	36 (1)	24 (1)	6 (0)	4 (0)
Kidney function, eGFR				
0-15	11 (0)	3 (0)	2 (0)	2 (0)
15-30	11 (0)	8 (5)	2 (0)	2 (0)
30-60	421 (18)	317 (21)	178 (13)	224 (15)
60-90	761 (32)	480 (32)	523 (39)	497 (34)
> 90	1176 (49)	676 (46)	628 (47)	731 (50)

Continuous variables denoted as mean (SD), categorical variables as number (%)

colorectal and prostate) was higher among patients than controls. Acute events were more prevalent among patients than controls, as well as chronic diseases, except for hypo- and hyperthyroidism (Table 1).

Eighteen exposures were included in the analyses which yielded ORs between 0.72 (95% CI 0.38 to 1.38) and 16.04 (95% CI 3.93 to 65.45). The ratio of ORs ($OR_{RDD}/OR_{Partner}$) was between 0.35 and 0.79 of eight exposures, between 0.80 and 1.19 of five exposures and between 1.20 and 2.03 of five exposures (Table 2).

Table 2. Association between exposure and venous thrombosis when using partner and RDD controls

	Adjusted odds ratios* (95% confidence interval)		Ratio ORs
Exposure	**RDD controls**	**Partner controls**	**OR_{RDD}*/$OR_{Partner}$***
Bronchitis	1.82 (1.41 to 2.35)	1.98 (1.46 to 2.69)	0.95
Emphysema	2.01 (1.16 to 3.49)	2.32 (1.24 to 4.33)	0.79
Angina	1.77 (1.03 to 3.03)	1.23 (0.74 to 2.04)	1.44
Myocardial infarction	1.23 (0.89 to 1.70)	1.74 (1.19 to 2.54)	0.71
Heart failure	1.19 (0.78 to 1.80)	1.84 (1.10 to 3.09)	0.65
Haemorrhagic stroke	4.91 (1.74 to 13.84)	3.97 (1.62 to 9.72)	1.24
Ischemic stroke	1.22 (0.74 to 2.00)	1.37 (0.77 to 2.43)	0.89
Paralysis	2.19 (1.16 to 4.17)	5.85 (2.02 to 16.88)	0.37
Multiple sclerosis	1.37 (0.70 to 2.68)	3.94 (1.48 to 10.53)	0.35
Rheumatoid arthritis	1.35 (0.99 to 1.84)	1.25 (0.88 to 1.79)	1.08
Diabetes	1.05 (0.81 to 1.37)	0.95 (0.70 to 1.30)	1.11
Hypothyroidism	0.95 (0.67 to 1.34)	0.97 (0.66 to 1.42)	0.98
Hyperthyroidism	1.46 (0.80 to 2.66)	0.72 (0.38 to 1.38)	2.03
Renal failure	3.01 (1.61 to 5.62)	4.34 (1.79 to 10.56)	0.69
Liver disease	1.32 (0.65 to 2.68)	1.73 (0.73 to 4.13)	0.76
Breast cancer	2.66 (1.24 to 5.72)	3.68 (1.49 to 9.11)	1.38
Bowel cancer	16.04 (3.93 to 65.45)	11.22 (3.44 to 36.57)	0.69
Prostate cancer	2.14 (0.81 to 5.64)	3.94 (1.31 to 11.82)	1.45

*Odds ratios were adjusted for age and sex. RDD, random-digit-dialling.

Figure 1 shows that declining EQ-5D scores (i.e. quality of life) of the exposure was associated with a decreased ratio between the ORs of the partner controls and ORs of the RDD controls, with a r^2 of the regression line of 0.54. The lowest ratio of ORs was found for the exposures paralysis and heart failure, which are both associated with a low quality of life (EQ-5D scores 0.35 and 0.49 respectively). The highest ratios of the ORs were found for hyperthyroidism and prostate cancer, which are both associated with a high quality of life (EQ-5D scores 0.74 and 0.90 respectively).

The ratio of ORs of Factor V Leiden was 1.01 (OR_{RDD} 3.35, 95% CI 2.72 to 4.14; $OR_{Partner}$ 3.31, 95% CI 2.68 to 4.09). The ratio of ORs of blood group non-O was 0.95 (OR_{RDD} 2.10, 95% CI 1.88 to 2.35; $OR_{Partner}$ 2.21, 95% CI 1.95 to 2.49)

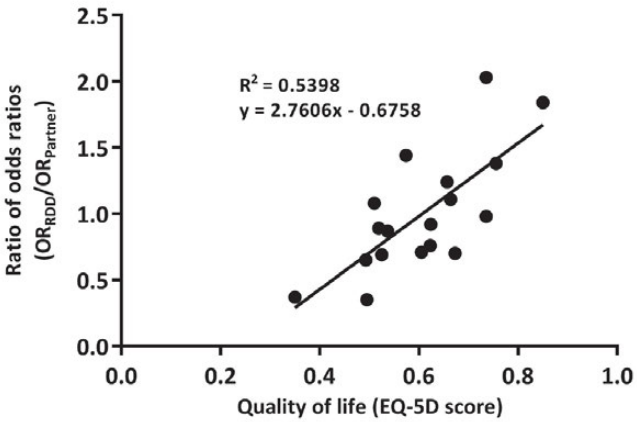


Figure 1. Association between quality of life and ratio between the odds ratios when using two different control groups

DISCUSSION

The results of this study showed that decreased quality of life due to exposure to a chronic condition was associated with higher ORs when using RDD controls as compared with partner controls. These results indicate that, consistent with our hypothesis, differences in risk estimates between the two control groups were associated with impaired quality of life. This hypothesis was confirmed by our second analysis that showed that two exposures not associated with changes in quality of life (genetic variants), gave similar risk estimates for both control groups.

Exposures associated with a low quality of life showed lower risk estimates when using RDD controls than partner controls. These differences in risk estimates and therefore differences of the ratio between the risk estimates could be due to lower

participation rates among partner controls than RDD controls when being severely ill. These lower participation rates would result in higher risk estimates when using partner controls instead of RDD controls. The reason for the differences between the risk estimates could not be determined in this study as data on reasons not to participate were not available.

To our knowledge, there is no literature that describes differences between participation rates of partner- and RDD- or other control groups. However, RDD control groups have been studied extensively in the past, especially in comparison with other population based controls. One study found that RDD controls, as compared with controls who were recruited by mail and visited at home, were subject to more screening tests and had a slightly higher prevalence of health outcomes such as a high cholesterol.[16] These differences could indicate that ill subjects recruited by RDD may participate more frequently as compared with other control groups, potentially explaining the differences found between the partner- and RDD controls. Another explanation for the different risk estimates found between the partner- and RDD controls may be that the burden of care for the patient by a very ill partner (control) is high and may result in declining to participate in a study.

This is the first study that looks into differences between partner controls and RDD controls. Due to the recruitment of two control groups, it was possible to study potential differences in risk estimates when using partner controls as compared with RDD controls. Due to the large numbers of participants and the extensive questionnaire, it was possible to study a wide range of exposures (rare, common, severe and less severe illnesses). A limitation is that exposures were self-reported, which may have resulted in non-reliable risk estimates. However, it is not expected that patients would answer differently as compared with the controls regarding their current illnesses. Additionally, it is unlikely that RDD controls would give less reliable answers than partner controls (or the other way around) on their current illnesses, meaning that the results are unlikely to be explained by differential misclassification. Another limitation is that it is not possible to study the cause of the difference in ORs. Therefore, a recommendation cannot be given on the most appropriate control group when studying research questions with exposures that impair the quality of life considerably. Future research is needed to determine what the source of these discrepancies is and show what control group is most appropriate under these circumstances.

In summary, results of this study showed that discrepancies between risk estimates obtained with a partner control group as compared with a RDD control group increase with exposures that considerably affect quality of life.

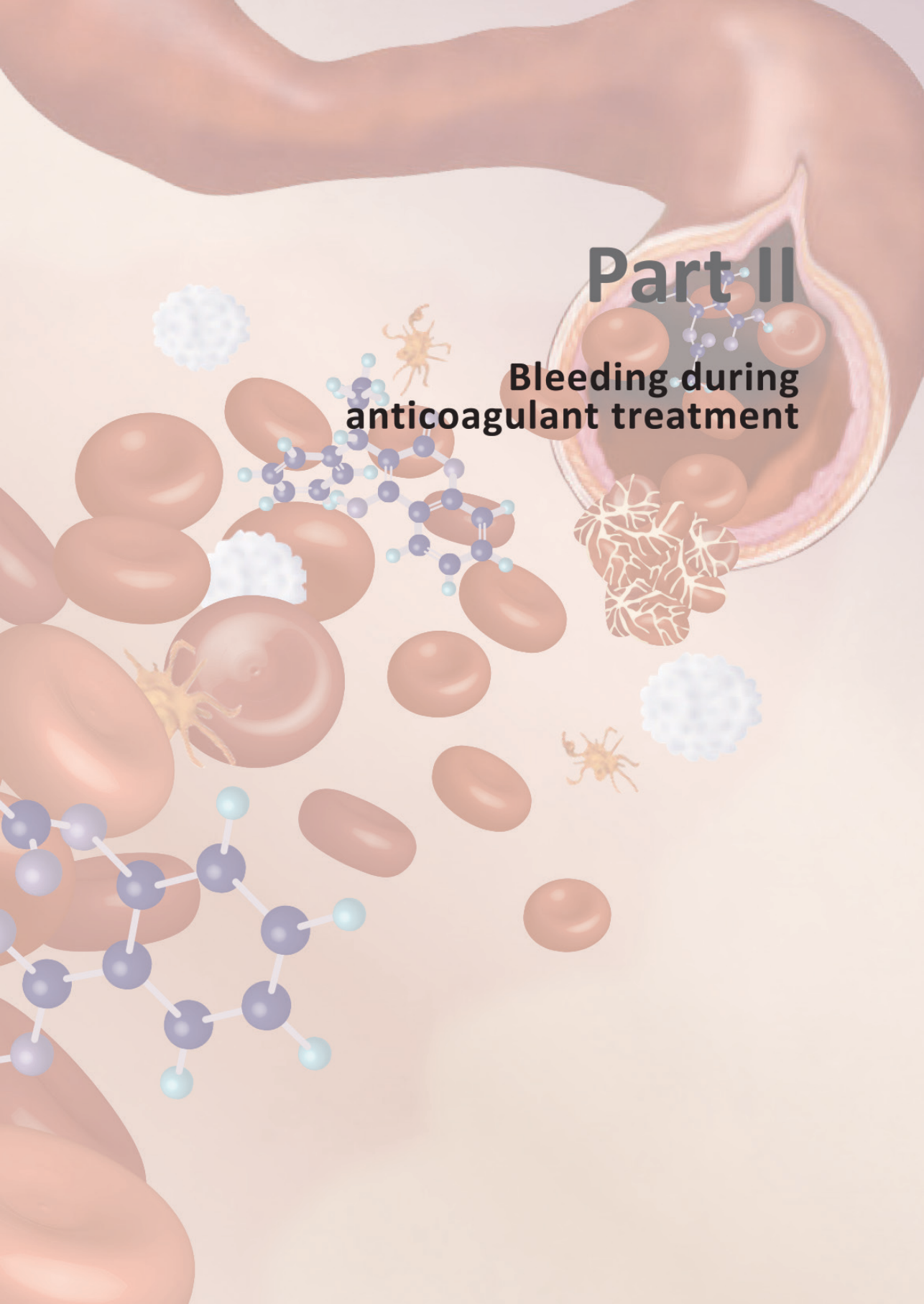
REFERENCES

1. Rothman K, Greenland S. *Modern Epidemiology.* Philadelphia: Lippincott-Raven publishers; 1998.
2. Hernan MA, Hernandez-Diaz S, Robins JM. A structural approach to selection bias. *Epidemiology.* Sep 2004;15(5):615-625.
3. Vandenbroucke JP, Pearce N. Case-control studies: basic concepts. *International journal of epidemiology.* Oct 2012;41(5):1480-1489.
4. Vandenbroucke JP. When are observational studies as credible as randomised trials? *Lancet.* May 22 2004;363(9422):1728-1731.
5. Austin MA, Criqui MH, Barrett-Connor E, Holdbrook MJ. The effect of response bias on the odds ratio. *American journal of epidemiology.* Jul 1981;114(1):137-143.
6. Morabia A. Case-control studies in clinical research: mechanism and prevention of selection bias. *Preventive medicine.* Sep-Oct 1997;26(5 Pt 1):674-677.
7. Blom JW, Doggen CJ, Osanto S, Rosendaal FR. Malignancies, prothrombotic mutations, and the risk of venous thrombosis. *Jama.* Feb 09 2005;293(6):715-722.
8. Pomp ER, Van Stralen KJ, Le Cessie S, Vandenbroucke JP, Rosendaal FR, Doggen CJ. Experience with multiple control groups in a large population-based case-control study on genetic and environmental risk factors. *European journal of epidemiology.* Jul 2010;25(7):459-466.
9. van Rein N, Gebuis EPA, Lijfering WM, et al. Vitamin K1 in oral solution or tablets: a crossover trial and two randomized controlled trials to compare effects. *J Thromb Haemost.* Dec 2014;12(12):2017-2023.
10. Waksberg J. Sampling Methods for Random Digit Dialing. *J Am Stat Assoc.* 1978;73(361):40-46.
11. van Langevelde K, Lijfering WM, Rosendaal FR, Cannegieter SC. Increased risk of venous thrombosis in persons with clinically diagnosed superficial vein thrombosis: results from the MEGA study. *Blood.* Oct 13 2011;118(15):4239-4241.
12. Bertaccini A, Ceccarelli R, Urbinati M, et al. BSP-PC (Bononian Satisfaction Profile--Prostate Cancer): development and validation of a "disease-specific" questionnaire for the evaluation of health-related quality of life in patients with prostate cancer. *Archivio italiano di urologia, andrologia : organo ufficiale [di] Societa italiana di ecografia urologica e nefrologica.* Dec 2003;75(4):187-194.
13. Sullivan PW, Slejko JF, Sculpher MJ, Ghushchyan V. Catalogue of EQ-5D scores for the United Kingdom. *Medical decision making : an international journal of the Society for Medical Decision Making.* Nov-Dec 2011;31(6):800-804.
14. Lipsitch M, Tchetgen Tchetgen E, Cohen T. Negative controls: a tool for detecting confounding and bias in observational studies. *Epidemiology.* May 2010;21(3):383-388.
15. van Rein N, Cannegieter SC, Rosendaal FR, Reitsma PH, Lijfering WM. Suspected survivor bias in case-control studies: stratify on survival time and use a negative control. *Journal of clinical epidemiology.* Feb 2014;67(2):232-235.
16. Olson SH, Kelsey JL, Pearson TA, Levin B. Evaluation of Random Digit Dialing as a Method of Control Selection in Case-Control Studies. *American journal of epidemiology.* Jan 15 1992;135(2):210-222.

APPENDIX 1

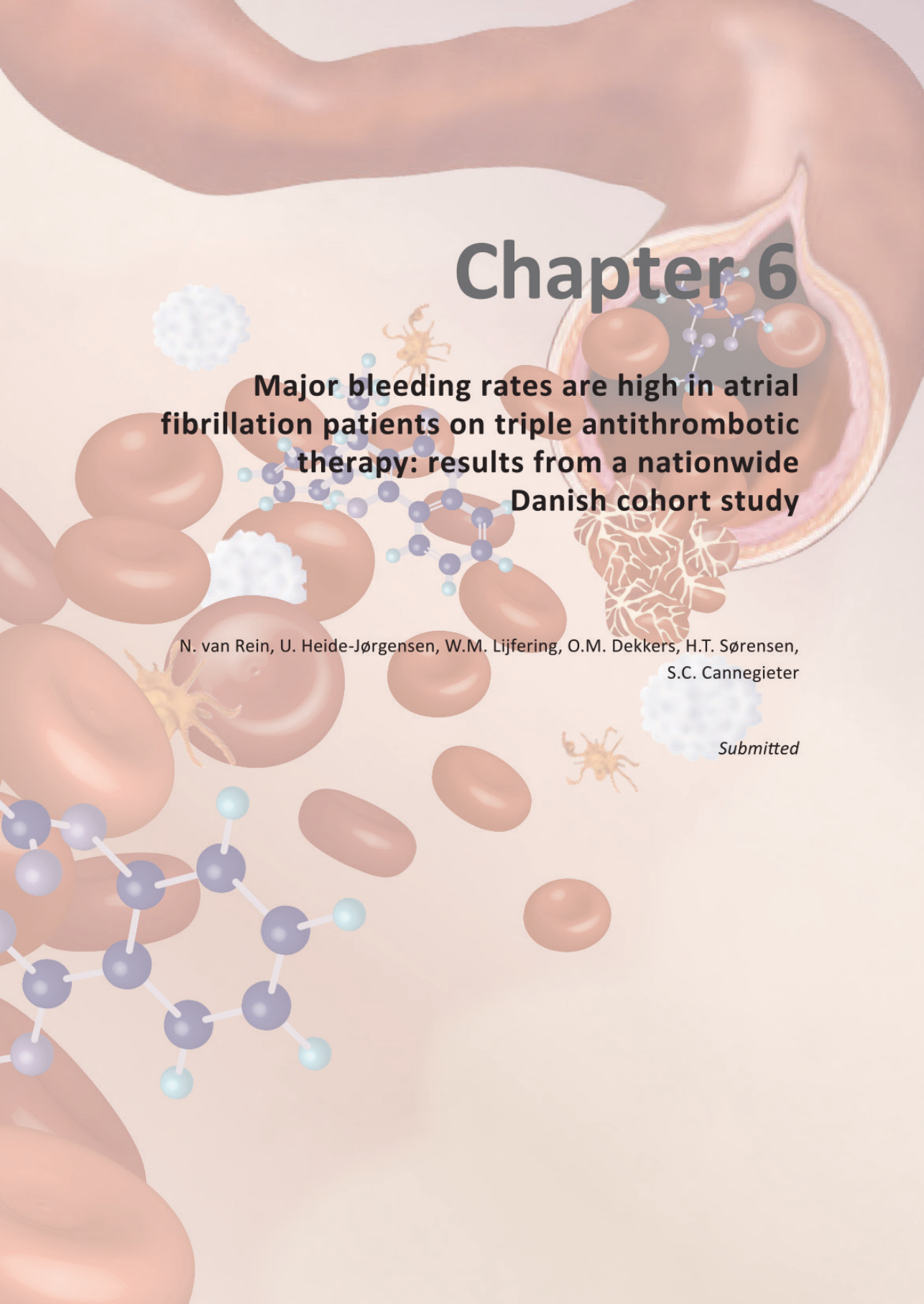
Table 1. EQ-5D scores used in the study

Disease	EQ-5D score	Reference
Bronchitis	0.624	Sullivan *et al.* 2011
Emphysema	0.537	Sullivan *et al.* 2011
Angina	0.574	Sullivan *et al.* 2011
Myocardial infarction	0.605	Sullivan *et al.* 2011
Heart failure	0.493	Sullivan *et al.* 2011
Haemorrhagic stroke	0.657	Sullivan *et al.* 2011
Ischemic stroke	0.519	Sullivan *et al.* 2011
Paralysis	0.35	Sullivan *et al.* 2011
Multiple sclerosis	0.495	Sullivan *et al.* 2011
Rheumatoid arthritis	0.51	Sullivan *et al.* 2011
Diabetes	0.664	Sullivan *et al.* 2011
Hypothyroidism	0.736	Sullivan *et al.* 2011
Hyperthyroidism	0.736	Sullivan *et al.* 2011
Renal failure	0.525	Sullivan *et al.* 2011
Liver disease	0.623	Sullivan *et al.* 2011
Breast cancer	0.756	Sullivan *et al.* 2011
Bowel cancer	0.673	Sullivan *et al.* 2011
Prostate cancer	0.85	Sullivan *et al.* 2011 Bertaccini *et al.* 2003



Part II

Bleeding during anticoagulant treatment



Chapter 6

Major bleeding rates are high in atrial fibrillation patients on triple antithrombotic therapy: results from a nationwide Danish cohort study

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Submitted

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ABSTRACT

Background: Patients with atrial fibrillation generally require treatment with vitamin K antagonists (VKAs) and at times with additional platelet aggregation inhibitors. Data are scarce on bleeding rates in high-risk groups receiving combnation therapy, such as the elderly or patients with a high CHA_2DS_2-VASc score.

Methods: We conducted a nationwide cohort study of Danish atrial fibrillation patients aged 50 years or older. Treatments were ascertained from a prescription database. These included no anticoagulant treatment and treatment with VKAs, aspirin, clopidogrel, and combinations of anticoagulant drugs. Incidence rates (IRs) of major bleeding and hazard ratios were estimated, overall and stratified by treatment modality, age, CHA_2DS_2-VASc score, and comorbidity.

Results: We identified 216,109 patients with atrial fibrillation. Median age was 75 years and 48% were women. Over a total follow-up period of 854,914 patient-years (py), 24,414 major bleeds occurred [incidence rate (IR) 2.9/100 pys, 95% confidence interval (CI) 2.8-2.9/100 pys]. Compared with VKA monotherapy, adjusted hazard ratios of major bleeding were 1.52 (95% CI 1.37-1.69) for dual antiplatelet therapy, 1.78 (95% CI 1.71-1.86) for therapy with a VKA and an antiplatelet drug, and 3.73 (95% CI 3.23-4.31) for triple therapy. Subgroup analyses showed similar patterns. The IR for major bleeding was 11.9/100 pys among triple-therapy patients. Very high major bleeding rates occurred among patients over 90 years (IR 50.0/100 pys, 95% CI 24.4-91.8) and in patients with a CHA_2DS_2-VASc score over 6 (IR 20.0/100 pys, 95% CI 10.2-35.7).

Conclusions: Patients with atrial fibrillation on triple therapy experienced high rates of major bleeding compared with patients on dual therapy or monotherapy. The exceptionally high bleeding rates observed in patients on triple therapy over the age of 90 years or with a CHA_2DS_2-VASc score over 6 suggest that such therapy should be carefully considered in these patients.

INTRODUCTION

Persistent atrial fibrillation often requires long-term treatment with oral anticoagulants.[1] As patients with atrial fibrillation often have other underlying cardiovascular diseases, concurrent treatment with platelet inhibitors also may be indicated.[1,2] Previous research has shown that concurrent use of vitamin K antagonists (VKAs) with a single platelet inhibitor increases the risk of bleeding complications twofold to threefold compared with VKA monotherapy.[3] Triple therapy with VKA, aspirin, and clopidogrel has been associated with an almost fourfold increased risk of major bleeds compared with VKA monotherapy.[3] Although these relative risks are high, they do not provide sufficient information to assess clinical safety implications. For this, knowledge of absolute rates is needed, especially in patient groups with risk factors for major bleeding complications.[4] As well, sufficient numbers of patients are required to allow comparison of bleeding rates associated with several combinations of anticoagulant drugs.

We therefore conducted a cohort study in a nationwide setting (*i.e.*, the entire population of Denmark) to determine rates of major bleeds in patients with atrial fibrillation who used combinations of anticoagulant and antiplatelet drugs. Our approach took several high-risk groups into account.

METHODS

Setting and databases

The Danish National Health Service provides tax-funded medical care to all Danish residents.[5] The Danish Civil Registration System (CRS) issues a unique Civil Personal Register (CPR) number to all Danish residents at birth or upon immigration, which permits patient-level linkage of data among all Danish medical databases.[5] The data sources used in this study were the Danish National Patient Registry (DNPR)[5], the Danish Registry of Medicinal Product Statistics (DRMPS)[6], and the Danish Registry of Causes of Death.[7]

The DNPR is a nationwide registry containing information on all inpatient hospitalizations since 1977 and on all hospital specialist outpatient clinic and emergency room visits since 1995. Each record contains the patient's CPR number, dates of hospital inpatient and outpatient encounters, the discharge date (if applicable), and one or more discharge diagnoses, including a dedicated field for the primary diagnosis. Diagnoses were coded according to the *International Classification of Diseases, Eighth Revision* (ICD-8) from 1977 to 1993 and according to the *Tenth Revision* (ICD-10) thereafter.[8]

The nationwide DRMPS contains information on all prescriptions dispensed at community pharmacies in Denmark since 1995. All records contain the patient's CPR number, date of dispensing, quantity of drugs dispensed, and the Anatomical Therapeutic Chemical (ATC) code of the dispensed drug.[9]

The nationwide Danish Registry of Causes of Death contains information on all deaths in Denmark since 1875. Each record from 1994 on contains the deceased person's CPR number, date of death, and cause(s) of death classified by ICD-10 codes, including a code for the primary cause of death.[7]

Study population

The study included all patients in Denmark aged 50 years or older with a first-time primary or secondary hospital inpatient or outpatient discharge diagnosis of atrial fibrillation or flutter registered in the DNPR between 1 January 1995 and 31 December 2012. Younger patients were not included, as atrial fibrillation is rare in persons under age 50.[10] Patients with an atrial fibrillation diagnosis in an acute setting (*e.g.,* emergency room) were not eligible for inclusion. The diagnosis of atrial fibrillation and flutter has a positive predictive value of 99% in the DNPR.[11]

Exposure

Data on redeemed prescriptions for VKAs (warfarin and phenprocoumon), and platelet inhibitors (aspirin and clopidogrel) were obtained from the DRMPS using ATC codes (see Appendix 1 for codes). Patients were considered exposed starting on the day they filled a prescription for a VKA or platelet inhibitor. Length of exposure to VKAs was assumed to be 90 days per prescription, as drugs for chronic conditions are seldomly provided for more than three months in Denmark. Length of exposure to antiplatelet drugs was assumed to be one day per pill dispensed plus an extra 14 days as a wash-out period. The wash-out period was used to account for delay in picking up a prescribed drug from a pharmacy as well as the duration of action of individual drugs. Among the anticoagulant and antiplatelet drugs examined in this study, the only over-the-counter medicine is low-dose aspirin. However, patients treated long-term with low-dose aspirin usually receive a prescription to allow financial reimbursement, as reported in other studies.[3,12] Therefore aspirin use was included and coded as a prescription.

Based on medication use, seven categories of exposure were identified: no anticoagulant treatment; monotherapy with a VKA; monotherapy with aspirin; monotherapy with clopidogrel; dual therapy with a VKA and one antiplatelet drug (clopidogrel or aspirin); dual antiplatelet therapy with aspirin and clopidogrel; and triple therapy (VKA, aspirin, and clopidogrel).

Outcomes, comorbidities and comedications

Outcomes of interest were major bleeds (primary outcome), ischemic strokes, myocardial infarctions (MIs), and all-cause mortality (secondary outcomes). The DNPR and the Danish Registry of Causes of Death were used to ascertain outcomes, classified according to ICD-10 codes (see Appendix 1). Outcomes included both primary and secondary diagnoses recorded in the DNPR (excluding diagnoses made during

emergency room visits). The outcomes of fatal bleed, fatal ischemic stroke, and fatal MI were included only if the event was recorded as the primary cause of death in the Danish Registry of Causes of Death.

Diagnostic codes in the DNPR were used to identify comorbidities, defined as the presence, at any time, in a patient's record of ischemic heart disease, valvular heart disease, hypertension, MI, ischemic stroke, diabetes, liver disease, renal failure, malignancy, and previous major bleeds (see Appendix 1). Based on these diagnostic codes and clinical characteristics, we computed CHA_2DS_2-VASc scores. This score is based on age, sex, a history of congestive heart failure, hypertension, stroke/transient ischemic attack/thromboembolism, vascular disease, and diabetes mellitus.[13]

Use of anticoagulants during the 180 days preceding diagnosis of atrial fibrillation was ascertained from the DRMPS (see ATC codes in Appendix 1).

Statistical analysis

Patients were followed from the date of their atrial fibrillation diagnosis until occurrence of each of the study outcomes (major bleeding event, ischemic stroke, and MI), death or end of the study period (31 December 2013). When calculating follow-up time until a major bleed or another outcome, we did not consider the occurrence of the other outcomes. For example, when major bleeding events were studied, MIs were disregarded in the analysis even if a patient had an MI before the bleeding event.

Rates [incidence rates per 100 person-years (pys)] of the outcomes were estimated and further stratified by risk groups defined *a-priori* (*i.e.,* age in 10-year categories, CHA_2DS_2-VASc score, sex, previous ischemic heart disease, previous major bleeds, previous ischemic stroke, and previous MI). Exposure was considered as a time-dependent variable in all analyses.

In a secondary analysis, relative risk estimates of major bleeds were estimated for the different exposure groups using VKA monotherapy as the reference category. Hazard ratios (HRs) along with 95% confidence intervals (CIs) were estimated using a time-dependent Cox model. HRs were adjusted for the following confounding factors: sex and, as time-dependent variables, ischemic heart disease, valvular heart disease, liver disease, kidney failure, and cancer. HRs were not estimated for secondary outcomes (*i.e.*, ischemic stroke, MI, and all-cause mortality), as confounding by indication for these outcomes would make such comparative results difficult to interpret. A sensitivity analysis was performed in which outcomes from the Danish Registry of Causes of Death were excluded. The rationale was that causes of death are more prone to misclassification than diagnoses and thus could influence the parameter estimates.

All analyses were performed using R version 2.15.2 (R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/).

Table 1. Baseline characteristics of all patients in Denmark aged 50 years or older with a first-time primary or secondary hospital inpatient or outpatient discharge diagnosis of atrial fibrillation or flutter between 1 January 1995 and 31 December 2012, by type of therapy.

	All patients	No anticoagulant treatment	VKA monotherapy	Aspirin monotherapy	Clopidogrel monotherapy	Two Antiplatelet drugs	VKA+ Antiplatelet drug	Triple therapy
Patients	216,109	71,796	52,953	57,511	1962	3625	26,971	1291
Age, median (IQR)	75 (67-83)	75 (65-83)	72 (65-79)	80 (71-86)	78 (70-85)	77 (69-84)	74 (68-80)	74 (68-80)
Female sex	103,430 (48)	37,086 (52)	21,517 (41)	30,547 (53)	1025 (52)	2107 (58)	10,763 (40)	385 (30)
Comorbidities								
Ischemic heart disease	52,894 (24)	15,361 (21)	9396 (18)	21,578 (38)	1079 (55)	3203 (88)	11,190 (42)	1158 (90)
Valvular heart disease	17,961 (8)	4324 (6)	5006 (10)	4426 (8)	184 (9)	421 (12)	3432 (13)	168 (13)
Hypertension	66,318 (31)	17,168 (24)	15,091 (29)	19,467 (34)	1109 (57)	1,793 (50)	11,056 (41)	634 (49)
Diabetes	23,654 (11)	6689 (9)	4757 (9)	7163 (13)	321 (16)	670 (19)	3809 (14)	245 (19)
Liver disease	3686 (2)	1645 (2)	676 (1)	909 (2)	41 (2)	55 (2)	344 (1)	16 (1)
Renal failure	7405 (3)	2850 (4)	1085 (2)	2196 (4)	124 (6)	237 (7)	840 (3)	73 (6)
Malignancy	41,127 (19)	15,656 (22)	8271 (16)	11,530 (20)	464 (24)	695 (19)	4309 (16)	202 (16)
Previous ischemic stroke	32,327 (15)	7984 (11)	5718 (11)	11,080 (19)	980 (50)	851 (24)	5404 (20)	310 (24)
Previous myocardial infarction	36,124 (17)	8480 (12)	4979 (9)	12,134 (21)	716 (37)	2,649 (73)	6247 (24)	919 (71)
Previous major bleeds	26,887 (12)	9693 (14)	5022 (10)	7895 (14)	460 (23)	571 (16)	3081 (11)	165 (13)
Previous anticoagulant therapy								
VKA	31,368 (15)	2897 (4)	19,970 (38)	1044 (2)	57 (3)	60 (2)	7073 (26)	267 (21)
Aspirin	71,828 (33)	7101 (10)	5524 (10)	36,034 (63)	442 (23)	2384 (66)	19,487 (72)	856 (66)
Clopidogrel	4505 (2)	321 (0)	244 (1)	399 (1)	1097 (56)	1153 (32)	777 (3)	514 (40)

RESULTS

Characteristics

We identified 216,109 patients aged 50 years or older who were admitted to a hospital or who had an outpatient visit in a hospital clinic with a first-time diagnosis of atrial fibrillation between 1995 and 2013 (see Table 1). Median age was 75 years [interquartile range (IQR) 67-83 years] and 103,430 patients (48%) were women. The most common treatments were monotherapy with a VKA [52,953 patients (25%)] or aspirin [57,511 patients (27%)] or dual therapy with a VKA and an antiplatelet drug [26,971 patients (12%)]. Triple therapy was prescribed to 1962 patients (0.9%). The prevalence of a history of ischemic heart disease or a MI was highest among patients treated with aspirin and clopidogrel or with aspirin, clopidogrel, and a VKA (see Table 1).

Major bleeding by type of therapy

Median follow-up was three years (IQR 1-7 years), resulting in total follow-up time of 854,914 pys. A total of 24,414 major bleeds occurred during follow-up. Of these, 1141 (4.6%) were fatal. Major bleeding rates were lowest in patients not treated with an anticoagulant and increased with the number of anticoagulants or antiplatelet drugs used concurrently (incidence rates between 1.4 and 11.9 per 100 pys; see Table 2). Incidence rates and adjusted HRs for major bleeding, using VKA monotherapy as reference, were slightly lower in aspirin users than in VKA users, but higher in clopidogrel users. Compared with VKA monotherapy, adjusted HRs of major bleeding were 1.52 (95% CI 1.37-1.69) for dual antiplatelet therapy, 1.78 (95% CI 1.71-1.86) for therapy with both a VKA and an antiplatelet drug, and 3.73 (95% CI 3.23-4.31) for triple therapy.

Table 2. Incidence rate and hazard ratio of major bleeding associated with single, dual, and triple therapy.

	Bleeds (no.)	Exposure time (py)	Incidence rate per 100 py (95% CI)	Hazard ratio (95% CI)	Hazard ratio* (95% CI)
No anticoagulant therapy	6147	310,859	2.0 (1.9-2.0)	0.81 (0.79-0.84)	0.82 (0.80-0.86)
VKA monotherapy	6070	249,559	2.4 (2.4-2.5)	reference	reference
Aspirin monotherapy	7409	271,917	2.7 (2.7-2.8)	1.12 (1.09-1.16)	0.93 (0.89-0.96)
Clopidogrel monotherapy	336	8427	4.0 (3.6-4.4)	1.62 (1.45-1.81)	1.11 (1.00-1.24)
Dual antiplatelet therapy	397	7296	5.4 (4.9-6.0)	2.06 (1.86-2.28)	1.52 (1.37-1.69)
VKA+ antiplatelet drug	3862	77,994	5.0 (4.8-5.1)	1.98 (1.90-2.06)	1.78 (1.71-1.86)
Triple therapy	193	1617	11.9 (10.3-13.7)	4.24 (3.67-4.89)	3.73 (3.23-4.31)

* Adjusted for sex and the following comorbidities: ischemic heart disease, valvular heart disease, liver disease, kidney failure, and cancer.

Risk groups

Rates of major bleeding were lowest in the youngest age group (incidence rates between 0.7 and 9.6 per 100 pys) (see Table 3) and in the group with a CHA_2DS_2-VASc score of 0 (incidence rates between 0.6 and 2.6 per 100 pys) (see Table 4). As in the overall analysis, major bleeding rates increased with age and the number of anticoagulants used concurrently. For each 10-year increase in age, major bleeding rates in patients on triple therapy increased concurrently (9.6 per 100 pys for persons aged 50-59, 9.3 per 100 pys for persons aged 60-69, 12.6 per 100 pys for persons aged 70-79, 13.2 per 100 pys for persons aged 80-89, and 50.0 per 100 pys for those aged 90 and over). When incidence rates were contrasted with monotherapy as the reference group, the adjusted HRs closely followed the pattern of increased major bleeding risk with age. Similar results were found for the CHA_2DS_2-VASc scores. Absolute rates of major bleeds were highest in patients who used triple therapy and who had a CHA_2DS_2-VASc score above 6 (IR 20.0, 95% CI 10.2-35.7).

Table 3. Incidence rate and hazard ratio of major bleeding associated with single, dual, and triple therapy, stratified by age.

	Bleeds no.	Exposure time (py)	Incidence rate per 100 py (95% CI)	Hazard ratio (95% CI)	Hazard ratio* (95% CI)
Age 50-59 yrs					
No anticoagulant therapy	271	40,093	0.7 (0.6-0.8)	0.55 (0.46-0.66)	0.62 (0.52-0.75)
VKA monotherapy	211	17,289	1.2 (1.1-1.4)	reference	reference
Aspirin monotherapy	156	15,614	1.0 (0.9-1.2)	0.85 (0.69-1.04)	0.84 (0.68-1.04)
Clopidogrel monotherapy	6	348	1.7 (0.7-3.6)	1.19 (0.49-2.90)	0.99 (0.41-2.42)
Dual antiplatelet therapy	14	426	3.3 (1.9-5.4)	2.27 (1.30-3.98)	1.83 (1.03-3.24)
VKA+ antiplatelet drug	112	4303	2.6 (2.2-3.1)	2.13 (1.69-2.68)	1.83 (1.45-2.32)
Triple therapy	11	114	9.6 (5.1-16.8)	6.75 (3.67-12.39)	5.35 (2.88-9.95)
Age 60-69 yrs					
No anticoagulant therapy	874	83,284	1.0 (1.0-1.1)	0.65 (0.59-0.71)	0.69 (0.63-0.76)
VKA monotherapy	1033	64,284	1.6 (1.5-1.7)	reference	reference
Aspirin monotherapy	687	53,125	1.3 (1.2-1.4)	0.80 (0.73-0.89)	0.79 (0.71-0.87)
Clopidogrel monotherapy	39	1532	2.5 (1.8-3.4)	1.57 (1.14-2.17)	1.22 (0.88-1.69)
Dual antiplatelet therapy	43	1557	2.8 (2.0-3.7)	1.62 (1.19-2.20)	1.29 (0.95-1.76)
VKA+ antiplatelet drug	637	20,014	3.2 (2.9-3.4)	1.95 (1.77-2.16)	1.72 (1.56-1.91)
Triple therapy	45	484	9.3 (6.9-12.3)	5.10 (3.78-6.88)	4.18 (3.08-5.66)
Age 70-79 yrs					
No anticoagulant therapy	1890	90,445	2.1 (2.0-2.2)	0.87 (0.81-0.92)	0.89 (0.83-0.94)
VKA monotherapy	2311	97,775	2.4 (2.3-2.5)	reference	reference
Aspirin monotherapy	1907	80,940	2.4 (2.3-2.5)	0.98 (0.92-1.04)	0.92 (0.86-0.98)

Table 3. (*Continued*)

	Bleeds no.	Exposure time (py)	Incidence rate per 100 py (95% CI)	Hazard ratio (95% CI)	Hazard ratio* (95% CI)
Clopidogrel monotherapy	97	2610	3.7 (3.0-4.5)	1.49 (1.21-1.84)	1.16 (0.94-1.14)
Dual antiplatelet therapy	121	2462	4.9 (4.1-5.9)	1.89 (1.57-2.27)	1.47 (1.22-1.77)
VKA+ antiplatelet drug	1541	32,301	4.8 (4.5-5.0)	1.96 (1.84-2.09)	1.74 (1.63-1.86)
Triple therapy	86	684	12.6 (10.1-15.5)	4.71 (3.80-5.85)	3.87 (3.11-4.81)
Age 80-89 yrs					
No anticoagulant therapy	2366	77,094	3.1 (2.9-3.2)	0.83 (0.78-0.88)	0.84 (0.79-0.89)
VKA monotherapy	2252	64,053	3.5 (3.4-3.7)	reference	reference
Aspirin monotherapy	3378	93,034	3.6 (3.5-3.8)	0.98 (0.93-1.04)	0.95 (0.90-1.00)
Clopidogrel monotherapy	157	3094	5.1 (4.3-5.9)	1.34 (1.13-1.59)	1.15 (0.97-1.37)
Dual antiplatelet therapy	174	2367	7.4 (6.3-8.5)	1.92 (1.64-2.25)	1.61 (1.38-1.89)
VKA+ antiplatelet drug	1453	19,992	7.3 (6.9-7.6)	2.01 (1.88-2.15)	1.87 (1.74-2.00)
Triple therapy	42	317	13.2 (9.7-17.7)	3.32 (2.45-4.51)	2.82 (2.08-3.84)
Age > 90 years					
No anticoagulant therapy	746	19,942	3.7 (3.5-4.0)	0.81 (0.70-0.94)	0.84 (0.72-0.98)
VKA monotherapy	263	6158	4.3 (3.8-4.8)	reference	reference
Aspirin monotherapy	1281	29,204	4.4 (4.2-4.6)	0.99 (0.86-1.14)	1.02 (0.89-1.18)
Clopidogrel monotherapy	37	834	4.4 (3.2-6.1)	0.96 (0.66-1.38)	0.95 (0.66-1.38)
Dual antiplatelet therapy	45	485	9.3 (6.8-12.3)	1.80 (1.28-2.54)	1.72 (1.22-2.44)
VKA+ antiplatelet drug	119	1484	8.0 (6.7-9.6)	1.93 (1.55-2.41)	1.88 (1.41-2.35)
Triple therapy	9	18	50.0 (24.4-91.8)	9.34 (4.61-18.94)	8.43 (4.15-17.13)

* Adjusted for sex and the following comorbidities: ischemic heart disease, valvular heart disease, liver disease, kidney failure, and cancer.

Compared with male patients, female patients had higher major bleeding rates (see Table 5). Patients with ischemic heart disease and patients who experienced a MI had similar rates of major bleeding. Rates were higher in patients with a history of ischemic stroke or a history of major bleeding. Results of the sensitivity analysis (see Appendix 2, Tables 1 to 4) were similar to those of the overall analysis.

Ischemic events and death

Rates of MI, ischemic stroke, and death increased with age and were highest among individuals who received clopidogrel monotherapy or two antiplatelet drugs with or without a VKA. Rates of ischemic stroke varied between 0.0 to 7.0 per 100 pys, rates of MIs varied between 0.0 to 14.2 per 100 pys, and death rates ranged from 0.0 to 55.0 per 100 pys (see Appendix 2 Figure 1).

Table 4. Incidence rate and hazard ratio of major bleeding associated with single, dual and triple therapy, stratified by CHA_2DS_2-VASc score.

	Bleeds (no.)	Exposure time (py)	Incidence rate per 100 py (95% CI)	Hazard ratio (95% CI)	Hazard ratio* (95% CI)
CHA_2DS_2-VASc 0					
No anticoagulant therapy	156	26,955	0.6 (0.5-0.7)	0.57 (0.45-0.73)	0.55 (0.43-0.70)
VKA monotherapy	115	10,973	1.0 (0.9-1.3)	reference	reference
Aspirin monotherapy	59	7066	0.8 (0.6-1.1)	0.83 (0.61-1.14)	0.81 (0.59-1.11)
Clopidogrel monotherapy	0	30	NA	NA	NA
Dual antiplatelet therapy	0	6	NA	NA	NA
VKA+ antiplatelet drug	32	1238	2.6 (1.8-3.6)	2.34 (1.58-3.47)	2.35 (1.58-3.47)
Triple therapy	0	1	NA	NA	NA
CHA_2DS_2-VASc 1,2					
No anticoagulant therapy	1417	118,405	1.2 (1.1-1.3)	0.67 (0.62-0.72)	0.72 (0.67-0.77)
VKA monotherapy	1559	85,894	1.8 (1.7-1.9)	reference	reference
Aspirin monotherapy	1085	63,262	1.7 (1.6-1.8)	0.96 (0.89-1.04)	0.93 (0.86-1.00)
Clopidogrel monotherapy	27	1043	2.6 (1.7-3.7)	1.42 (0.97-2.08)	1.38 (0.94-2.02)
Dual antiplatelet therapy	27	984	2.7 (1.8-3.9)	1.33 (1.91-1.94)	1.59 (1.08-2.34)
VKA+ antiplatelet drug	612	17,802	3.4 (3.2-3.7)	1.81 (1.65-1.99)	1.87 (1.70-2.05)
Triple therapy	16	274	5.8 (3.5-9.3)	2.59 (1.58-4.24)	3.29 (2.00-5.42)
CHA_2DS_2-VASc 3,4					
No anticoagulant therapy	3144	126,358	2.5 (2.4-2.6)	0.95 (0.91-1.00)	0.93 (0.88-0.98)
VKA monotherapy	2921	113,539	2.6 (2.5-2.7)	reference	reference
Aspirin monotherapy	3854	134,483	2.9 (2.8-3.0)	1.10 (1.05-1.16)	1.01 (0.96-1.06)
Clopidogrel monotherapy	148	3511	4.2 (3.6-4.9)	1.59 (1.35-1.88)	1.39 (1.18-1.64)
Dual antiplatelet therapy	156	3064	5.1 (4.3-5.9)	1.76 (1.50-2.07)	1.64 (1.39-1.93)
VKA+ antiplatelet drug	1915	38,304	5.0 (4.8-5.2)	1.87 (1.76-1.98)	1.83 (1.72-1.94)
Triple therapy	84	808	10.4 (8.3-12.8)	3.80 (3.09-4.67)	3.99 (3.24-4.91)
CHA_2DS_2-VASc 5					
No anticoagulant therapy	897	26,593	3.4 (3.2-3.6)	0.94 (0.86-1.03)	0.92 (0.84-1.01)
VKA monotherapy	935	26,680	3.5 (3.3-3.7)	reference	Reference
Aspirin monotherapy	1484	43,266	3.4 (3.3-3.6)	0.96 (0.88-1.04)	0.90 (0.83-0.98)
Clopidogrel monotherapy	78	2075	3.8 (3.0-4.7)	1.03 (0.82-1.30)	0.90 (0.71-1.13)
Dual antiplatelet therapy	111	1764	6.3 (5.2-7.5)	1.64 (1.34-2.00)	1.48 (1.21-1.80)
VKA+ antiplatelet drug	784	13,389	5.9 (5.5-6.3)	1.62 (1.47-1.78)	1.57 (1.42-1.73)
Triple therapy	45	314	14.3 (10.6-19.0)	3.47 (2.57-4.68)	3.30 (2.44-4.46)

Table 4. (*Continued*)

	Bleeds (no.)	Exposure time (py)	Incidence rate per 100 py (95% CI)	Hazard ratio (95% CI)	Hazard ratio* (95% CI)
CHA_2DS_2-VASc 6					
No anticoagulant therapy	416	9901	4.2 (3.8-4.6)	0.97 (0.85-1.11)	0.94 (0.82-1.08)
VKA monotherapy	419	9837	4.3 (3.9-4.7)	reference	reference
Aspirin monotherapy	704	18,364	3.8 (3.6-4.1)	0.89 (0.79-1.00)	0.86 (0.76-0.97)
Clopidogrel monotherapy	57	1240	4.6 (3.5-5.9)	1.05 (0.80-1.39)	0.97 (0.73-1.28)
Dual antiplatelet therapy	73	1080	6.8 (5.3-8.5)	1.48 (1.15-1.90)	1.37 (1.07-1.76)
VKA+ antiplatelet drug	383	5717	6.7 (6.1-7.4)	1.53 (1.34-1.76)	1.49 (1.30-1.72)
Triple therapy	28	170	16.5 (11.2-23.5)	3.39 (2.31-4.98)	3.13 (2.13-4.61)
CHA_2DS_2-VASc 7-9					
No anticoagulant therapy	117	2648	4.4 (3.7-5.3)	0.94 (0.73-1.21)	0.90 (0.70-1.17)
VKA monotherapy	121	2637	4.6 (3.8-5.5)	reference	reference
Aspirin monotherapy	223	5475	4.1 (3.6-4.6)	0.87 (0.70-1.09)	0.84 (0.67-1.05)
Clopidogrel monotherapy	26	528	4.9 (3.3-7.1)	1.03 (0.68-1.58)	0.98 (0.64-1.51)
Dual antiplatelet therapy	30	398	7.5 (5.2-10.6)	1.49 (1.00-2.23)	1.39 (0.92-2.08)
VKA+ antiplatelet drug	136	1545	8.8 (7.4-10.4)	1.84 (1.44-2.35)	1.76 (1.38-2.26)
Triple therapy	10	50	20.0 (10.2-35.7)	3.55 (1.85.6.79)	3.23 (1.68-6.20)

* Adjusted by sex and the following comorbidities: ischemic heart disease, valvular heart disease, liver disease, kidney failure, and cancer.

DISCUSSION

Our study showed that the incidence rate of major bleeding increased with the number of prescribed anticoagulants. Nearly all groups treated with triple therapy experienced high rates of bleeding complications, up to 50.0 per 100 pys in the oldest age group. Relative risk estimates did not change greatly after adjustment for confounding factors, indicating that triple therapy was associated with a 2.8- to 8.4-fold increased risk of major bleeding complications compared with VKA monotherapy.

Major bleeding

We found that triple therapy was associated with a four-fold average increased risk of major bleeding, compared with VKA monotherapy. This was consistent across subgroups and agrees with the literature.[3] The clinical impact of relative risks depends on their absolute values. We expected that groups with a low baseline bleeding risk (*e.g.,* patients aged 50 to 60 years or with a CHA_2DS_2-VASc score of 1 to 2) would experience low rates of major bleeding complications during triple therapy. However,

Table 5. Incidence rate and hazard ratio of major bleeding associated with single, dual, and triple therapy, stratified by sex and by comorbidity.

	Bleeds (no.)	Exposure time (py)	Incidence rate per 100 py (95% CI)	Hazard ratio (95% CI)	Hazard ratio* (95% CI)
Female					
No anticoagulant therapy	2659	153,008	1.7 (1.7-1.8)	0.78 (0.74-0.82)	0.78 (0.74-0.82)
VKA monotherapy	2322	104,588	2.2 (2.1-2.3)	reference	reference
Aspirin monotherapy	3355	138,171	2.4 (2.3-2.5)	1.09 (1.04-1.15)	0.89 (0.84-0.94)
Clopidogrel monotherapy	135	4485	3.0 (2.5-3.6)	1.34 (1.13-1.60)	0.92 (0.77-1.09)
Dual antiplatelet therapy	159	3048	5.2 (4.5-6.1)	2.16 (1.84-2.54)	1.57 (1.33-1.85)
VKA+ antiplatelet drug	1328	27,786	4.8 (4.5-5.0)	2.07 (1.93-2.21)	1.91 (1.78-2.04)
Triple therapy	65	415	15.7 (12.2-19.8)	5.91 (4.62-7.57)	5.18 (4.04-6.64)
Male					
No anticoagulant therapy	3488	157,851	2.2 (2.1-2.3)	0.86 (0.82-0.90)	0.86 (0.82-0.90)
VKA monotherapy	3748	144,971	2.6 (2.5-2.7)	reference	reference
Aspirin monotherapy	4054	133,746	3.0 (2.9-3.1)	1.18 (1.13-1.24)	0.96 (0.92-1.00)
Clopidogrel monotherapy	201	3942	5.1 (4.4-5.8)	1.95 (1.69-2.25)	1.30 (1.13-1.50)
Dual antiplatelet therapy	238	4248	5.6 (4.9-6.3)	2.00 (1.75-2.28)	1.49 (1.31-1.71)
VKA+ antiplatelet drug	2534	50,208	5.0 (4.9-5.2)	1.91 (1.82-2.01)	1.74 (1.65-1.83)
Triple therapy	128	1202	10.6 (8.9-12.6)	3.60 (3.02-4.30)	3.33 (2.79-3.98)
Previous MI					
No anticoagulant therapy	948	33,594	2.8 (2.6-3.0)	0.86 (0.79-0.95)	0.86 (0.78-0.94)
VKA monotherapy	785	23,922	3.3 (3.1-3.5)	reference	reference
Aspirin monotherapy	1639	60,705	2.7 (2.6-2.8)	0.83 (0.77-0.91)	0.78 (0.71-0.85)
Clopidogrel monotherapy	122	2804	4.4 (3.6-5.2)	1.30 (1.08-1.58)	1.09 (0.90-1.32)
Dual antiplatelet therapy	254	4923	5.2 (4.6-5.8)	1.45 (1.26-1.67)	1.29 (1.12-1.49)
VKA+ antiplatelet drug	1148	21,649	5.3 (5.0-5.6)	1.59 (1.46-1.75)	1.59 (1.45-1.75)
Triple therapy	134	1052	12.7 (10.7-15.0)	3.35 (2.79-4.04)	3.47 (2.89-4.18)
Previous major bleed					
No anticoagulant therapy	1542	32,508	4.7 (4.5-5.0)	0.96 (0.89-1.04)	0.95 (0.88-1.03)
VKA monotherapy	1179	25,004	4.7 (4.5-5.0)	reference	reference
Aspirin monotherapy	1573	30,444	5.2 (4.9-5.4)	1.06 (0.99-1.15)	0.96 (0.89-1.04)
Clopidogrel monotherapy	114	1633	7.0 (5.8-8.4)	1.39 (1.15-1.69)	1.15 (0.95-1.40)
Dual antiplatelet therapy	87	1069	8.1 (6.6-10.0)	1.49 (1.20-1.85)	1.26 (1.01-1.57)
VKA+ antiplatelet drug	763	9297	8.2 (7.6-8.8)	1.67 (1.53-1.83)	1.61 (1.46-1.76)
Triple therapy	35	199	17.6 (12.4-24.2)	2.98 (2.13-4.18)	2.82 (2.01-3.96)

Table 5. (*Continued*)

	Bleeds (no.)	Exposure time (py)	Incidence rate per 100 py (95% CI)	Hazard ratio (95% CI)	Hazard ratio* (95% CI)
Previous ischemic stroke					
No anticoagulant therapy	1236	32,084	3.9 (3.6-4.1)	1.17 (1.08-1.26)	1.08 (1.00-1.17)
VKA monotherapy	1329	40,909	3.2 (3.1-3.4)	reference	reference
Aspirin monotherapy	2040	52,283	3.9 (3.7-4.1)	1.19 (1.11-1.27)	1.05 (0.97-1.12)
Clopidogrel monotherapy	170	4108	4.1 (3.6-4.8)	1.24 (1.05-1.45)	1.10 (0.93-1.29)
Dual antiplatelet therapy	155	2318	6.7 (5.7-7.8)	1.95 (1.65-2.31)	1.69 (1.43-2.01)
VKA+ antiplatelet drug	1100	17,573	6.3 (5.9-6.6)	1.86 (1.72-2.01)	1.80 (1.66-1.95)
Triple therapy	50	313	16.0 (12.0-20.9)	4.12 (3.10-5.47)	3.86 (2.90-5.13)
Ischemic heart disease					
No anticoagulant therapy	2165	81,222	2.7 (2.6-2.8)	0.88 (0.83-0.94)	0.86 (0.81-0.91)
VKA monotherapy	2059	68,794	3.0 (2.9-3.1)	reference	reference
Aspirin monotherapy	3396	125,011	2.7 (2.6-2.8)	0.90 (0.85-0.95)	0.83 (0.78-0.87)
Clopidogrel monotherapy	214	5188	4.1 (3.6-4.7)	1.34 (1.17-1.55)	1.09 (0.95-1.26)
Dual antiplatelet therapy	337	6429	5.2 (4.7-5.8)	1.58 (1.40-1.77)	1.41 (1.25-1.58)
VKA+ antiplatelet drug	2195	44,540	4.9 (4.7-5.1)	1.60 (1.51-1.70)	1.59 (1.50-1.69)
Triple therapy	185	1539	12.0 (10.4-13.9)	3.42 (2.94-3.98)	3.58 (3.07-4.16)

*Adjusted for age at baseline, sex, and the following comorbidities: ischemic heart disease, valvular heart disease, liver disease, kidney failure, and cancer.

this was not the case, as major bleeding rates were at least 5.8 per 100 pys. One explanation is that triple therapy causes major bleeding. An alternate explanation may be that the indication for this therapy, *i.e.,* high risk of atherothrombosis, is also associated with a high risk of bleeding.[14] All other subgroups experienced very high major bleeding rates (at least 9.3 per 100 pys) while receiving triple therapy. Bleeding rates gradually increased with age, as is well known. Bleeding rates also increased with higher CHA_2DS_2-VASc scores, which is to be expected since elements of the score, such as age, diabetes mellitus, hypertension, and a history of ischemic stroke, are risk factors for bleeding.[14]

We also observed that female patients experienced higher major bleeding rates than male patients, in contrast to the findings of previous studies.[14] This makes it likely that there is an alternate explanation, such as confounding, for the sex difference in bleeding rates.

High major bleeding rates also were observed among patients on triple therapy with ischemic heart disease or a history of a major bleeding or ischemic event. In addition, patients with a history of major bleeding or an ischemic stroke experienced higher rates

of major bleeding than patients with a history of MI or with ischemic heart disease. The reason may be that ischemic strokes and major bleeds are risk factors for future major bleeding. This has not been reported for ischemic heart disease or history of MI.[14]

Clinical implications

The high rates of major bleeding found among patients receiving triple therapy raises the question whether concomitant use of three anticoagulants is advisable. However, risk factors for ischemic events and major bleeding overlap[15], making it hard to distinguish which patients are at high risk for major bleeding, but not at risk for ischemic events, and vice versa. In addition, due to confounding by indication, this non-randomized study does not permit evaluation of the effectiveness of combinations of antithrombotic drugs (*i.e.*, medication could have been indicated due to high risk of thromboembolic outcomes). Still, two important findings in our study were that among patients receiving triple therapy, half of those aged over 90 years experienced a major bleed per year and that patients with a CHA_2DS_2-VASc scores of 7 to 9 had a bleeding rate of 20.0 per 100 pys. These very high bleeding rates suggest that triple therapy may be contraindicated in these groups.

Strengths and limitations

This population-based cohort study contained data from over 200,000 patients with large numbers of outcome events, making the results robust and generalizable to the currently treated population and allowing multiple subgroup analyses. A limitation is its reliance on dispensed prescriptions as recorded in a pharmacy registry, as filled prescriptions do not imply that patients actually took the medications. Still, if patients did not take their medications, results would have been diluted. The rates and risk estimates of bleeding complications would likely have been higher if patients had been compliant. Another limitation is the study's observational design, which precludes strong recommendations about optimal treatment choices for patients. Another potential limitation is that ICD codes do not distinguish between paroxysmal, persistent, and permanent atrial fibrillation[16], and these specific diagnoses may have influenced choice of treatment. In addition, only bleeding events that resulted in hospital admissions or were fatal were considered major. This may have resulted in underestimation of rates of major bleeding.

CONCLUSION

This study showed that patients with atrial fibrillation on triple therapy experienced a high rate of major bleeding. Some subgroups, such as patients over 90 years of age and patients with a CHA_2DS_2-VASc of 7 to 9, had very high bleeding rates, suggesting that triple therapy should be carefully considered in these patients.

REFERENCES

1. Lansberg MG, O'Donnell MJ, Khatri P, et al. Antithrombotic and thrombolytic therapy for ischemic stroke: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest.* Feb 2012;141(2 Suppl):e601S-636S.
2. Paikin JS, Wright DS, Crowther MA, Mehta SR, Eikelboom JW. Triple antithrombotic therapy in patients with atrial fibrillation and coronary artery stents. *Circulation.* May 11 2010;121(18):2067-2070.
3. Hansen ML, Sorensen R, Clausen MT, et al. Risk of bleeding with single, dual, or triple therapy with warfarin, aspirin, and clopidogrel in patients with atrial fibrillation. *Archives of internal medicine.* Sep 13 2010;170(16):1433-1441.
4. Levine MN, Raskob G, Beyth RJ, Kearon C, Schulman S. Hemorrhagic complications of anticoagulant treatment: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest.* Sep 2004;126(3 Suppl):287S-310S.
5. Schmidt M, Schmidt SA, Sandegaard JL, Ehrenstein V, Pedersen L, Sorensen HT. The Danish National Patient Registry: a review of content, data quality, and research potential. *Clinical epidemiology.* 2015;7:449-490.
6. Kildemoes HW, Sorensen HT, Hallas J. The Danish National Prescription Registry. *Scandinavian journal of public health.* Jul 2011;39(7 Suppl):38-41.
7. Helweg-Larsen K. The Danish Register of Causes of Death. *Scandinavian journal of public health.* Jul 2011;39(7 Suppl):26-29.
8. Lynge E, Sandegaard JL, Rebolj M. The Danish National Patient Register. *Scandinavian journal of public health.* Jul 2011;39(7 Suppl):30-33.
9. Gaist D, Sorensen HT, Hallas J. The Danish prescription registries. *Danish medical bulletin.* Sep 1997;44(4):445-448.
10. van Rein N, Cannegieter SC, le Cessie S, et al. Statins and Risk of Bleeding: An Analysis to Evaluate Possible Bias Due to Prevalent Users and Healthy User Aspects. *American journal of epidemiology.* May 15 2016;183(10):930-936.
11. Frost L, Vukelic Andersen L, Vestergaard P, Husted S, Mortensen LS. Trends in risk of stroke in patients with a hospital diagnosis of nonvalvular atrial fibrillation: National Cohort Study in Denmark, 1980-2002. *Neuroepidemiology.* 2006;26(4):212-219.
12. Sorensen R, Hansen ML, Abildstrom SZ, et al. Risk of bleeding in patients with acute myocardial infarction treated with different combinations of aspirin, clopidogrel, and vitamin K antagonists in Denmark: a retrospective analysis of nationwide registry data. *Lancet.* Dec 12 2009;374(9706):1967-1974.
13. Lip GY, Nieuwlaat R, Pisters R, Lane DA, Crijns HJ. Refining clinical risk stratification for predicting stroke and thromboembolism in atrial fibrillation using a novel risk factor-based approach: the euro heart survey on atrial fibrillation. *Chest.* Feb 2010;137(2):263-272.
14. Schulman S, Beyth RJ, Kearon C, Levine MN, American College of Chest P. Hemorrhagic complications of anticoagulant and thrombolytic treatment: American College of Chest

Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest.* Jun 2008;133(6 Suppl):257S-298S.

15. Lip GY, Lane DA, Buller H, Apostolakis S. Development of a novel composite stroke and bleeding risk score in patients with atrial fibrillation: the AMADEUS Study. *Chest.* Dec 2013;144(6):1839-1847.
16. Sundboll J, Adelborg K, Munch T, et al. Positive predictive value of cardiovascular diagnoses in the Danish National Patient Registry: a validation study. *BMJ open.* Nov 18 2016;6(11):e012832.

APPENDIX 1.

Diagnosis and pharmaceutical codes used in the study

Study population - Danish National Registry of Patients
Atrial fibrillation ICD-10 code 148

Baseline drug use - Danish Registry of Medicinal Product Statistics
Warfarin ATC code B01AA03
Phenprocoumon ATC code B01AA04
Aspirin ATC code B01AC06
Clopidogrel ATC code B01AC04

Baseline comorbidities - Danish National Registry of Patients
Ischemic heart disease ICD-10 code I20-I25; ICD-8 code 409-415
Valvular heart disease ICD-10 code I34-I37; ICD-8 code 393-398, 424
Heart failure ICD-10 code I50; ICD-8 code 427.0, 427.1
Hypertension ICD-10 code I10-I15; ICD-8 code 399-405
Diabetes ICD-10 code E10-E14; ICD-8 code 249, 250
Liver disease ICD-10 code K70-K77, R16 and R17; ICD-8 code 570-573, 782.8, 785.1, 785.2
Renal failure ICD-10 code N17-N19 and R34; ICD-8 code 403, 404, 579-585
Malignancy ICD-10 code C00-C97; ICD-8 code 139-240
Systemic embolism ICD-10 code I26 and I74; ICD-8 code 444, 450
Ischemic stroke ICD-10 code I63-I66, I69.3 and I69.4; ICD-8 code 431, 439
Myocardial infarction ICD-10 code I21; ICD-8 code 410
Major bleed ICD-10 code D62, I60-I62, I69.0, I69.1, I69.2, J94.2, K25.0, K25.2, K25.4, K25.6, K26.0, K26.2, K26.4, K26.6, K27.0, K27.2, K28.0, K28.2, K28.4, K28.6, K92.0, K92.1, K92.2, N02, R04, R31, S06.4, S06.5 and S06.6; ICD-8 code 430, 431, 531.0, 531.2, 532.0, 532.2, 533.0, 534.0, 534.2, 783.0, 783.1, 784.5, 785.7, 789.3

Exposure - Danish Registry of Medicinal Product Statistics
Warfarin ATC code B01AA03
Phenprocoumon ATC code B01AA04
Aspirin ATC code B01AC06
Clopidogrel ATC code B01AC04

Outcomes - Danish National Registry of Patients and Danish Registry of Causes of Death
Major bleeds ICD 10 codes D62, I60-I62, I69.0, I69.1, I69.2, J94.2, K25.0, K25.2, K25.4, K25.6, K26.0, K26.2, K26.4, K26.6, K27.0, K27.2, K28.0, K28.2, K28.4, K28.6, K92.0, K92.1, K92.2, N02, R04, R31, S06.4, S06.5 and S06.6
Ischemic stroke ICD-10 code I63
Myocardial infarction ICD-10 code I21

APPENDIX 2

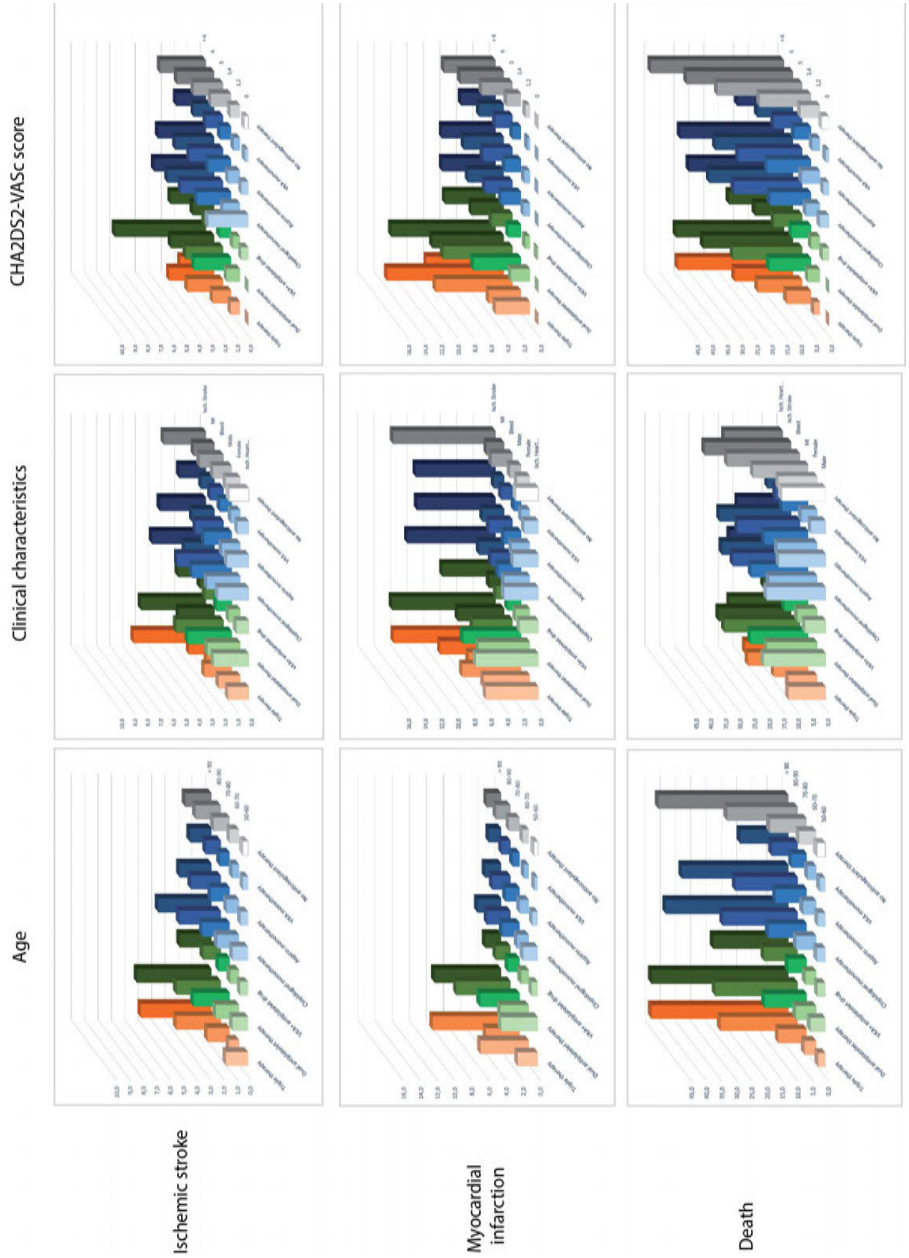

Figure 1. Incidence rates per 100 person-years of secondary outcomes by subgroups

Table 1. Sensitivity analysis excluding cause of death: incidence rate and hazard ratio of non-fatal major bleeding associated with single, dual, and triple therapy.

	Bleeds (no.)	Exposure time (py)	Incidence rate per 100 py (95% CI)	Hazard ratio (95% CI)	Hazard ratio* (95% CI)
No anticoagulant therapy	5775	310,859	1.9 (1.8-1.9)	0.79 (0.76-0.82)	0.80 (0.77-0.83)
VKA monotherapy	5883	249,559	2.4 (2.3-2.4)	reference	reference
Aspirin monotherapy	6961	271,917	2.6 (2.5-2.6)	1.09 (1.05-1.13)	0.90 (0.87-0.94)
Clopidogrel monotherapy	311	8427	3.7 (3.3-4.1)	1.55 (1.38-1.74)	1.08 (0.96-1.21)
Dual antiplatelet therapy	380	7296	5.2 (4.7-5.7)	2.04 (1.84-2.26)	1.51 (1.36-1.68)
VKA+ antiplatelet drug	3771	77,994	4.8 (4.7-5.0)	2.00 (1.92-2.08)	1.80 (1.72-1.87)
Triple therapy	192	1617	11.9 (10.2-13.7)	4.36 (3.78-5.04)	3.82 (3.30-4.42)

* Adjusted for sex and the following comorbidities: ischemic heart disease, valvular heart disease, liver disease, kidney failure, and cancer.

Table 2. Sensitivity analysis excluding cause of death: incidence rate of non-fatal major bleeding associated with single, dual, and triple therapy, stratified by age.

	Bleeds no.	Exposure time (py)	Incidence rate per 100 py (95% CI)	Hazard ratio (95% CI)	Hazard ratio* (95% CI)
Age 50-59 yrs					
No anticoagulant therapy	259	40,093	0.65 (0.57-0.73)	0.55 (0.46-0.66)	0.62 (0.52-0.75)
VKA monotherapy	210	17,289	1.21 (1.06-1.39)	reference	reference
Aspirin monotherapy	153	15,614	0.98 (0.83-1.15)	0.85 (0.69-1.04)	0.84 (0.68-1.04)
Clopidogrel monotherapy	5	348	1.44 (0.53-3.19)	1.19 (0.49-2.90)	0.99 (0.41-2.42)
Dual antiplatelet therapy	13	426	3.05 (1.70-5.09)	2.27 (1.30-3.98)	1.83 (1.03-3.24)
VKA+ antiplatelet drug	110	4203	2.62 (2.16-3.14)	2.13 (1.69-2.68)	1.83 (1.45-2.32)
Triple therapy	11	114	9.65 (5.07-16.77)	6.75 (3.67-12.39)	5.35 (2.88-9.95)
Age 60-69 yrs					
No anticoagulant therapy	832	83,284	1.00 (0.93-10.69)	0.65 (0.59-0.71)	0.69 (0.63-0.76)
VKA monotherapy	1009	64,284	1.57 (1.48-1.67)	reference	reference
Aspirin monotherapy	657	53,125	1.24 (1.15-1.33)	0.80 (0.73-0.89)	0.79 (0.71-0.87)
Clopidogrel monotherapy	38	1532	2.48 (1.78-3.37)	1.57 (1.14-2.17)	1.22 (0.88-1.69)
Dual antiplatelet therapy	43	1557	2.76 (2.02-3.69)	1.62 (1.19-2.20)	1.29 (0.95-1.76)
VKA+ antiplatelet drug	625	20,014	3.12 (2.89-3.38)	1.95 (1.77-2.16)	1.72 (1.56-1.91)
Triple therapy	45	484	9.30 (6.86-12.33)	5.10 (3.78-6.88)	4.18 (3.08-5.66)
Age 70-79 yrs					
No anticoagulant therapy	1811	90,445	2.00 (1.91-2.10)	0.86 (0.81-0.92)	0.89 (0.83-0.94)
VKA monotherapy	2253	97,775	2.30 (2.21-2.40)	reference	reference
Aspirin monotherapy	1829	80,940	2.26 (2.16-2.37)	0.98 (0.92-1.04)	0.92 (0.86-0.98)
Clopidogrel monotherapy	91	2610	3.49 (2.82-4.26)	1.49 (1.21-1.84)	1.16 (0.94-1.44)
Dual antiplatelet therapy	117	2462	4.75 (3.95-5.67)	1.89 (1.57-2.27)	1.47 (1.22-1.77)
VKA+ antiplatelet drug	1503	32,301	4.65 (4.42-4.89)	1.96 (1.84-2.09)	1.74 (1.63-1.86)
Triple therapy	86	684	12.57 (10.12-15.45)	4.71 (3.80-5.85)	3.87 (3.11-4.81)
Age 80-89 yrs					
No anticoagulant therapy	2217	77,094	2.88 (2.76-3.00)	0.83 (0.78-0.88)	0.84 (0.79-0.89)
VKA monotherapy	2171	64,053	3.39 (3.25-3.53)	reference	reference
Aspirin monotherapy	3154	93,034	3.39 (3.27-3.51)	0.98 (0.93-1.04)	0.95 (0.90-1.00)
Clopidogrel monotherapy	144	3094	4.65 (3.94-5.46)	1.34 (1.13-1.59)	1.15 (0.97-1.37)
Dual antiplatelet therapy	169	2367	7.14 (3.12-8.28)	1.92 (1.64-2.25)	1.61 (1.38-1.89)
VKA+ antiplatelet drug	1416	19,992	7.08 (6.72-7.46)	2.01 (1.88-2.15)	1.87 (1.74-2.00)
Triple therapy	42	317	13.25 (9.67-17.74)	3.32 (2.45-4.51)	2.82 (2.07-3.84)

Table 2. (*Continued*)

	Bleeds no.	Exposure time (py)	Incidence rate per 100 py (95% CI)	Hazard ratio (95% CI)	Hazard ratio* (95% CI)
Age ≥ 90 yrs					
No anticoagulant therapy	656	19,942	3.29 (3.05-3.55)	0.81 (0.70-0.94)	0.84 (0.72-0.98)
VKA monotherapy	240	6158	3.90 (3.43-4.41)	reference	reference
Aspirin monotherapy	1168	29,204	4.00 (3.78-4.23)	0.99 (0.86-1.14)	1.02 (0.89-1.18)
Clopidogrel monotherapy	33	843	3.91 (2.74-5.43)	0.96 (0.66-1.38)	0.95 (0.66-1.38)
Dual antiplatelet therapy	38	485	7.84 (5.62-10.64)	1.80 (1.28-2.54)	1.72 (1.22-2.44)
VKA+ antiplatelet drug	117	1484	7.88 (6.55-9.41)	1.93 (1.55-2.41)	1.88 (1.51-2.35)
Triple therapy	8	18	44.44 (20.64-84.40)	9.34 (4.61-18.94)	8.43 (4.15-17.13)

* Adjusted by sex and the following comorbidities: ischemic heart disease, valvular heart disease, liver disease, kidney failure, and cancer.

Table 3. Sensitivity analysis excluding cause of death: incidence rate of non-fatal major bleeding associated with single, dual, and triple therapy, stratified by sex and by comorbidity.

	Bleeds (no.)	Exposure time (py)	Incidence rate per 100 py (95% CI)	Hazard ratio (95% CI)	Hazard ratio* (95% CI)
Female					
No anticoagulant therapy	2456	153,008	1.61	0.75 (0.71-0.79)	0.75 (0.71-0.79)
VKA monotherapy	2237	104,588	2.14	reference	reference
Aspirin monotherapy	3122	138,171	2.26	1.05 (1.00-1.11)	0.86 (0.82-0.92)
Clopidogrel monotherapy	122	4485	2.72	1.26 (1.05-1.51)	0.88 (0.73-1.06)
Dual antiplatelet therapy	151	3048	4.95	2.14 (1.81-2.52)	1.57 (1.33-1.86)
VKA+ antiplatelet drug	1292	27,786	4.65	2.09 (1.95-2.24)	1.93 (1.80-2.07)
Triple therapy	64	415	15.42	6.10 (4.75-7.82)	5.34 (4.16-6.87)
Male					
No anticoagulant therapy	3319	157,851	2.10	0.84 (0.80-0.88)	0.84 (0.80-0.88)
VKA monotherapy	3646	144,971	2.51	reference	reference
Aspirin monotherapy	3839	133,746	2.87	1.15 (1.10-1.20)	0.94 (0.89-0.98)
Clopidogrel monotherapy	189	3942	4.79	1.89 (1.63-2.18)	1.26 (1.09-1.46)
Dual antiplatelet therapy	229	4248	5.39	1.98 (1.73-2.26)	1.48 (1.29-1.69)
VKA+ antiplatelet drug	2479	50,208	4.94	1.93 (1.83-2.03)	1.74 (1.66-1.84)
Triple therapy	128	1202	10.65	3.70 (3.10-4.41)	3.40 (2.85-4.07)
Previous MI					
No anticoagulant therapy	902	33,594	2.69	0.84 (0.77-0.93)	0.84 (0.76-0.92)
VKA monotherapy	765	23,922	3.20	reference	reference
Aspirin monotherapy	1547	60,705	2.55	0.81 (0.74-0.88)	0.76 (0.69-0.83)
Clopidogrel monotherapy	115	2804	4.10	1.26 (1.04-1.53)	1.07 (0.88-1.30)
Dual antiplatelet therapy	241	4923	4.90	1.41 (1.22-1.63)	1.27 (1.10-1.47)
VKA+ antiplatelet drug	1122	21,649	5.18	1.60 (1.46-1.75)	1.60 (1.46-1.76)
Triple therapy	134	1052	12.74	3.46 (2.87-4.16)	3.60 (2.99-4.33)
Previous bleed					
No anticoagulant therapy	1446	32,508	4.45	0.93 (0.86-1.00)	0.92 (0.85-1.00)
VKA monotherapy	1148	25,004	4.59	reference	reference

Table 3. (*Continued*)

	Bleeds (no.)	Exposure time (py)	Incidence rate per 100 py (95% CI)	Hazard ratio (95% CI)	Hazard ratio* (95% CI)
Aspirin monotherapy	1482	30,444	4.87	1.03 (0.95-1.11)	0.94 (0.87-1.01)
Clopidogrel monotherapy	109	1633	6.67	1.37 (1.12-1.66)	1.15 (0.94-1.40)
Dual antiplatelet therapy	86	1069	8.04	1.51 (1.21-1.88)	1.29 (1.03-1.61)
VKA+ antiplatelet drug	746	9297	8.02	1.68 (1.53-1.84)	1.61 (1.47-1.77)
Triple therapy	35	199	17.59	3.07 (2.19-4.30)	2.89 (2.06-4.06)
Previous ischemic stroke					
No anticoagulant therapy	1135	32,084	3.54	1.11 (1.03-1.21)	1.04 (0.96-1.12)
VKA monotherapy	1279	40,909	3.13	reference	reference
Aspirin monotherapy	1842	52,283	3.52	1.11 (1.04-1.20)	0.99 (0.92-1.07)
Clopidogrel monotherapy	154	4108	3.75	1.17 (0.99-1.38)	1.04 (0.88-1.23)
Dual antiplatelet therapy	150	2318	6.47	1.97 (1.66-2.33)	1.71 (1.44-2.03)
VKA+ antiplatelet drug	1075	17,573	6.12	1.89 (1.74-2.05)	1.82 (1.68-1.98)
Triple therapy	50	313	15.97	4.30 (3.24-5.71)	4.00 (3.01-5.32)
Ischemic heart disease					
No anticoagulant therapy	2043	81,222	2.52	0.85 (0.80-0.91)	0.84 (0.79-0.89)
VKA monotherapy	2001	68,794	2.91	reference	reference
Aspirin monotherapy	3213	125,011	2.57	0.88 (0.83-0.93)	0.81 (0.76-0.85)
Clopidogrel monotherapy	197	5188	3.80	1.27 (1.10-1.47)	1.05 (0.90-1.21)
Dual antiplatelet therapy	321	6429	4.99	1.55 (1.37-1.74)	1.39 (1.23-1.56)
VKA+ antiplatelet drug	2142	44,540	4.81	1.61 (1.52-1.71)	1.59 (1.50-1.69)
Triple therapy	184	1539	11.96	3.52 (3.02-4.10)	3.66 (3.14-4.26)

* Adjusted for sex and the following comorbidities: ischemic heart disease, valvular heart disease, liver disease, kidney failure, and cancer.

Table 4. Sensitivity analysis excluding cause of death: incidence rate of non-fatal major bleeding associated with single, dual, and triple therapy, stratified by CHA_2DS_2-VASc score.

	Bleeds (no.)	Exposure time (py)	Incidence rate per 100 py (95% CI)	Hazard ratio (95% CI)	Hazard ratio* (95% CI)
CHA_2DS_2-VASc 0					
No anticoagulant therapy	151	26,955	0.56	0.57 (0.44-0.72)	0.54 (0.42-0.69)
VKA monotherapy	113	10,973	1.03	reference	reference
Aspirin monotherapy	56	7066	0.79	0.80 (0.58-1.11)	0.78 (0.57-1.08)
Clopidogrel monotherapy	0	30	NA	NA	NA
Dual antiplatelet therapy	0	6	NA	NA	NA
VKA+ antiplatelet drug	32	1238	2.58	2.39 (1.61-3.54)	2.39 (1.61-3.55)
Triple therapy	0	1	NA	NA	NA
CHA_2DS_2-VASc 1,2					
No anticoagulant therapy	1342	118,405	1.13	0.65 (0.60-0.70)	0.70 (0.65-0.75)
VKA monotherapy	1522	85,894	1.77	reference	reference
Aspirin monotherapy	1033	63,262	1.63	0.93 (0.86-1.01)	0.90 (0.83-0.98)
Clopidogrel monotherapy	26	1043	2.49	1.40 (0.95-2.07)	1.36 (0.92-2.01)
Dual antiplatelet therapy	27	984	2.74	1.36 (0.93-1.99)	1.62 (1.10-2.38)
VKA+ antiplatelet drug	602	17,802	3.38	1.83 (1.66-2.01)	1.88 (1.71-2.07)
Triple therapy	16	274	5.84	2.66 (1.63-4.36)	3.34 (2.03-5.51)
CHA_2DS_2-VASc 3,4					
No anticoagulant therapy	2954	126,358	2.34	0.92 (0.87-0.97)	0.90 (0.86-0.95)
VKA monotherapy	2841	113,539	2.50	reference	reference
Aspirin monotherapy	3646	134,483	2.71	1.07 (1.02-1.12)	0.99 (0.94-1.04)
Clopidogrel monotherapy	150	3511	4.27	1.55 (1.31-1.83)	1.36 (1.15-1.62)
Dual antiplatelet therapy	151	3064	4.93	1.75 (1.49-2.06)	1.62 (1.38-1.92)
VKA+ antiplatelet drug	1876	38,304	4.90	1.88 (1.78-2.00)	1.83 (1.73-1.94)
Triple therapy	94	808	11.63	3.92 (3.19-4.81)	4.04 (3.28-4.98)
CHA_2DS_2-VASc 5					
No anticoagulant therapy	831	26,593	3.12	0.91 (0.83-1.01)	0.89 (0.81-0.98)
VKA monotherapy	893	26,680	3.35	reference	reference
Aspirin monotherapy	1376	43,266	3.18	0.93 (0.86-1.01)	0.88 (0.81-0.96)
Clopidogrel monotherapy	73	3075	2.37	1.01 (0.80-1.28)	0.89 (0.70-1.13)
Dual antiplatelet therapy	106	1764	6.01	1.64 (1.34-2.00)	1.48 (1.20-1.81)
VKA+ antiplatelet drug	758	13,389	5.66	1.64 (1.49-1.80)	1.58 (1.43-1.74)
Triple therapy	45	314	14.33	3.63 (2.69-4.91)	3.41 (2.52-4.62)

Table 4. (*Continued*)

	Bleeds (no.)	Exposure time (py)	Incidence rate per 100 py (95% CI)	Hazard ratio (95% CI)	Hazard ratio* (95% CI)
CHA_2DS_2-VASc 6					
No anticoagulant therapy	393	9901	3.97	0.96 (0.84-1.11)	0.94 (0.81-1.08)
VKA monotherapy	399	9837	4.06	reference	reference
Aspirin monotherapy	650	18,364	3.54	0.86 (0.76-0.98)	0.84 (0.74-0.95)
Clopidogrel monotherapy	51	1239	4.12	0.99 (0.74-1.33)	0.92 (0.69-1.23)
Dual antiplatelet therapy	66	1080	6.11	1.41 (1.09-1.83)	1.31 (1.00-1.71)
VKA+ antiplatelet drug	370	5717	6.47	1.56 (1.35-1.80)	1.51 (1.31-1.74)
Triple therapy	27	170	15.88	3.46 (2.34-5.13)	3.17 (2.17-4.70)
CHA_2DS_2-VASc 7-9					
No anticoagulant therapy	104	2648	3.93	0.88 (0.67-1.15)	0.85 (0.65-1.12)
VKA monotherapy	115	2637	4.36	reference	reference
Aspirin monotherapy	200	5475	3.65	0.82 (0.65-1.04)	0.80 (0.63-1.01)
Clopidogrel monotherapy	21	528	3.98	0.88 (0.55-1.41)	0.85 (0.53-1.36)
Dual antiplatelet therapy	30	398	7.54	1.59 (1.06-2.37)	1.48 (0.99-2.23)
VKA+ antiplatelet drug	133	1545	8.61	1.90 (1.48-2.44)	1.82 (1.41-2.34)
Triple therapy	10	50	20.00	3.83 (2.00-7.34)	3.47 (1.80-6.67)

* Adjusted by sex and the following comorbidities: ischemic heart disease, valvular heart disease, liver disease, kidney failure, and cancer.

Chapter 7

Major bleeding risks of different Low-Molecular-Weight-Heparin agents: a cohort study in 12 934 patients treated for acute venous thrombosis

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ABSTRACT

Background: Low-molecular-weight-heparins (LMWHs) are considered members of a class of drugs with similar anticoagulant properties. However, pharmacodynamics and pharmacokinetics between LMWHs differ, which may result in different bleeding risks. As these agents are used by many patients, small differences may lead to a large effect on numbers of major bleeding events.

Objectives: To determine major bleeding risks for different LMWH agents and dosing schedules.

Methods: Cohort with acute venous thrombosis patients from four anticoagulation clinics who used a LMWH and a vitamin K antagonist. Patients were followed until they ceased LMWH treatment or until major bleeding. Exposures were classified according to different types of LMWHs and for b.i.d. and o.d. use. Cumulative incidences for major bleeding per 1000 patients and risk ratios were calculated and adjusted for study center.

Results: The study comprised of 12,934 patients with a mean age of 59 years and 6218 (48%) men. The cumulative incidence for major bleeding was 2.5 per 1000 patients (95% confidence interval [CI] 1.7-3.5). Enoxaparin b.i.d. or o.d. was associated with a relative bleeding risk of 1.7 (95%CI 0.2-17.5) compared with nadroparin o.d. In addition, a nadroparin b.i.d. dosing schedule was associated with a 2.0-fold increased major bleeding risk (95%CI 0.8-5.1) as compared with a nadroparin o.d. dosing schedule.

Conclusions: Absolute major bleeding rates were low for all LMWH agents and dosing schedules in a large, unselected cohort. Twice daily dosing with nadroparin appeared to be associated with an increased major bleeding risk as compared with once daily dosing as also suggested in a meta-analysis of controlled clinical trials.

BACKGROUND

Low-molecular-weight-heparins (LMWH) are widely used for prevention and treatment of venous thrombosis (the composite of deep vein thrombosis and pulmonary embolism).[1] The LMWHs currently on the market are considered members of a class of drugs with similar anticoagulant properties. However, pharmacodynamics and pharmacokinetics between LMWHs differ. For example, the half-life of the anti-Xa activity is 4.3 hours for enoxaparin as compared with 2.4 hours for dalteparin.[2] Such differences in duration of anticoagulant effect could result in different bleeding risks between LMWH agents. For the acute treatment of venous thrombosis, a head to head trial was conducted that compared two different LMWHs, i.e. dalteparin omni die (o.d., i.e. once daily) versus tinzaparin o.d. In this randomized study, with 505 patients, dalteparin appeared to have a lower bleeding risk than tinzaparin (relative risk 0.40, 95% confidence interval [CI] 0.08 to 2.07) yet confidence intervals included unity and the authors concluded that the bleeding rate was similar for both LMWHs.[3] To prevent venous thrombosis after a spinal cord injury with a LMWH, head-to-head trials were performed to compare dalteparin with enoxaparin which showed that overall bleeding rates were similar[4,5], while others showed that enoxaparin may in favour with respect to major bleeding.[6] It is currently unclear whether one LMWH should be preferred over the other in the treatment of venous thrombosis even though small differences in major bleeding rates may lead to a large reduction in major bleeding events because these agents are used by many patients on an annual basis.

We therefore set out to perform a cohort study in 12 934 patients with acute venous thrombosis who were treated with LMWH (and concurrently received VKA) to determine major bleeding risk for several LMWHs agents and dosage schedules.

METHODS

Study population and data collection

All patients over 18 years of age, with a new onset of venous thrombosis between 2006 and 2013 who received initial treatment with a LMWH (nadroparin o.d., nadroparin bis in die [b.i.d., i.e. twice daily], tinzaparin o.d., enoxaparin, dalteparin o.d.) and a VKA and were treated at one of the four participating anticoagulation clinics in the Netherlands (Leiden, Rotterdam, The Hague and Utrecht) were included. Enoxaparin treatment was not stratified by o.d. and b.i.d. use because numbers were too small. New onset was defined as an acute diagnosis of a first or recurrent venous thrombosis. Diagnoses were made at hospitals and were based on the international diagnosis criteria, after which patients received therapeutic dosages of LMWHs for at least 5 days.[7] At the anticoagulation clinics, patients with acute venous thrombosis are registered. There, the INR is measured at least every

three days when VKA and LMWH treatment are combined. LMWH treatment is ceased after at least five days of treatment and when two consecutive INRs are in target range.[7] At each appointment blood is drawn to measure the INR and a standard short questionnaire is taken (and electronically stored) by a nurse to list any changes in co-medication or onset of new diseases and to enquire if patients experienced bleeding events or have any surgical procedures planned.[8] If patients missed their appointment, they are contacted by nurses of the anticoagulation clinic and a new appointment is made on short notice.

Patient characteristics and information regarding major bleeding events were derived from the computerized medical records of the anticoagulation clinics. Data included age, sex, indication for VKA treatment, type of LMWH, duration of LMWH exposure, concomitant drug use and date of major bleeding events. Patients were considered exposed to a LMWH from the subscription date at the anticoagulant clinic until ceasing treatment with the LMWH plus an additional two days wash-out period, or until a patient died, changed anticoagulation clinic or experienced a major bleeding, whichever occurred first. Of note, in this study it was not planned that the patient records were used before the data collection took place. However, the data was necessary for patient care and collected during treatment.

Outcome

The outcome of our study was non-traumatic major bleeding which was notified through the routine procedures of the anticoagulation clinic. If patients mentioned any bleeding event or hospitalization related to a bleeding event during the visits or calls, information was obtained from the hospital, patient or general practitioner to classify the bleeding event as minor or major. Bleeding events were considered major if these required blood transfusion, were symptomatic in a critical area or organ, or led to death.[9] In addition, bleedings for which patients were hospitalized were also considered major bleedings. These bleedings were classified according to a standardized protocol by trained physicians of the anticoagulation clinics, who were not involved in the current study.

Statistical analysis

Patients were considered exposed to a LMWH from the subscription date at the anticoagulant clinic until date of ceasing treatment with LMWHs plus an additional two days wash-out period. Risks (cumulative incidences) and risk ratios were calculated and adjusted for study center by Mantel–Haenszel methods.[10] Nadroparin o.d. was a-priori chosen as the reference category in these analyses, as this was the most frequently used type of LMWH treatment. We adjusted only for study center as the choice of a LMWH agent is determined hospital wide and therefore not related to patient characteristics. Furthermore, the choice of the vitamin K antagonist is strongly associated with the

anticoagulation clinic on which basis we assume that no other confounding factors than the anticoagulation clinic are present. A sensitivity analysis was performed where results were restricted to first venous thrombotic events because patients with a recurrent venous thrombotic event have different patient characteristics that also relate to bleeding risk.[11] As a second sensitivity analysis, we computed incidence rates per 100 person-years to confirm that incidence rates would yield a similar pattern of risk estimates as cumulative incidences.

All analyses were performed with R version 2.15.2 (R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/).

RESULTS

The study cohort comprised 12 934 patients who experienced an acute venous thrombotic event (Figure 1 and Table 1). The mean age at baseline was 59 years (standard deviation 17 years) and 6218 (48%) patients were male. Most patients were treated at the anticoagulation clinic in Rotterdam (3883 patients; 30%). A deep vein thrombosis (DVT) was the indication of treatment for 8058 patients (62%) and 4889 patients (38%) experienced a pulmonary embolism (PE). In 11 237 (87%) patients the thrombotic episode concerned a first event of whom 6735 experienced a DVT. The remaining 1697 (13%) patients had a recurrent thrombotic episode, of whom 1326 experienced a DVT. The most frequently used LMWHs were nadroparin o.d. (5317 patients) and tinzaparin o.d. (3338 patients).

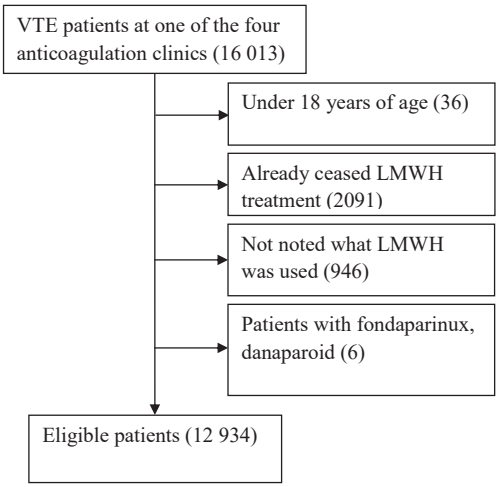


Figure 1. Flow chart of patients

Table 1. Patient characteristics

	Nadroparin o.d.	Nadroparin b.i.d.	Tinzaparin o.d.	Enoxaparin	Dalteparin o.d.
General characteristics					
Patients	5317	2076	3338	264	1939
Male sex, n (%)	2657 (50)	904 (44)	1638 (49)	125 (47)	894 (46)
Age, years, mean (SD)	58 (17)	61 (18)	59 (16)	57 (17)	57 (17)
Indication VKA treatment					
Deep vein thrombosis, n (%)	3402 (64)	1302 (63)	2042 (61)	137 (52)	1175 (61)
First, n (%)	2735 (80)	1093 (84)	1735 (85)	115 (84)	1057 (90)
Recurrent, n (%)	667 (20)	212 (16)	307 (15)	22 (16)	118 (10)
Pulmonary embolism, n (%)	1924 (36)	776 (37)	1298 (39)	127 (48)	764 (39)
First, n (%)	1695 (88)	731 (93)	1220 (94)	122 (96)	761 (100)
Recurrent, n (%)	229 (12)	59 (8)	78 (6)	5 (4)	3 (0)
Vitamin K antagonist					
Acenocoumarol, n (%)	1399 (26)	1059 (51)	2204 (66)	223 (84)	1861 (96)
Phenprocoumon, n (%)	3918 (74)	1017 (49)	1134 (34)	41 (16)	78 (4)
Co-medication					
Antihypertensive(s), n (%)	1137 (21)	373 (18)	511 (15)	38 (14)	54 (3)
Antidiabetic(s), n (%)	376 (7)	179 (9)	248 (7)	20 (8)	151 (8)
NSAID(s), n (%)	384 (7)	135 (7)	248 (7)	21 (8)	187 (10)
Anti-platelet drug(s), n (%)	152 (3)	100 (5)	138 (4)	13 (5)	112 (6)
Anticoagulation clinic					
The Hague, n (%)	2409 (45)	611 (29)	587 (18)	18 (7)	20 (1)
Leiden, n (%)	1873 (35)	544 (26)	729 (22)	62 (23)	30 (2)
Rotterdam, n (%)	347 (7)	561 (27)	1079 (32)	89 (34)	1807 (93)
Utrecht, n (%)	688 (13)	360 (17)	943 (28)	95 (36)	82 (4)

The median duration of treatment with LMWH was 5 days (interquartile range 2 to 9 days, Table 2). In total, 32 of the 12 934 patients (corresponding cumulative incidence 2.47 per 1000 patients, 95% CI 1.74 to 3.49) experienced a major bleeding event during combined VKA and LMWH treatment, which is similar to cumulative incidences reported in clinical trials.[12] The cumulative incidence (per 1000 patients) of major bleeding during combined treatment was 2.07 (95% CI 1.11 to 3.75) in nadroparin o.d. users, 3.37 (95% CI 1.48 to 7.10) in nadroparin b.i.d. users; 2.70 (95% CI 1.33 to 5.20) in tinzaparin o.d. users, 3.79 (95% CI 0.00 to 23.34) in enoxaparin o.d. or b.i.d. users and 2.06 (95% CI 0.60 to 5.50) in dalteparin o.d. users (Table 2). The sensitivity analysis with incidence rates showed similar patterns of risk estimates as analysis with cumulative

Table 2. Bleeding events associated with LMWH treatment

	Patients	Events	Median LMWH treatment period (IQR), days	Cumulative incidence per 1000	Risk ratio (95% CI)	Risk ratio* (95% CI)
All venous thrombotic events						
Nadroparin o.d.	5317	11	6 (3 to 10)	2.07 (1.11-3.75)	reference	reference
Nadroparin b.i.d.	2076	7	5 (2 to 10)	3.37 (1.48-7.10)	1.63 (0.63-4.20)	1.98 (0.76-5.14)
Tinzaparin o.d.	3338	9	5 (2 to 8)	2.70 (1.33-5.20)	1.30 (0.54-3.14)	1.24 (0.46-3.58)
Enoxaparin	264	1	5 (2 to 8)	3.79 (0.00-23.34)	1.83 (0.24-14.13)	1.74 (0.17-17.46)
Dalteparin o.d.	1939	4	4 (2 to 7)	2.06 (0.60-5.50)	1.00 (0.32-3.13)	4.19 (0.47-37.00)
First venous thrombotic event						
Nadroparin o.d.	4425	9	6 (3 to 10)	2.03 (1.01-3.93)	reference	reference
Nadroparin b.i.d.	1805	7	5 (2 to 9)	3.88 (1.70-8.16)	1.91 (0.71-5.11)	2.32 (0.85-6.31)
Tinzaparin o.d.	2953	8	5 (2 to 8)	2.71 (1.27-5.44)	1.33 (0.51-3.45)	2.30 (0.92-5.78)
Enoxaparin	273	1	5 (2 to 8)	3.66 (0.00-22.58)	1.80 (0.23-14.16)	1.98 (0.19-21.09)
Dalteparin o.d.	1819	3	4 (2 to 7)	1.65 (0.32-5.08)	0.81 (0.22-2.99)	3.97 (0.42-37.59)

*Adjusted for study center and academic hospital by Mantel–Haenszel method

incidences (Supplementary Table 1). Risk ratios for the different LMWH agents were between 1.00 and 1.83 using nadroparin o.d. as reference LMWH and increased after adjustment for study center (Table 2). The relative risk estimate for nadroparin b.i.d. was 1.98 (95% CI 0.76 to 5.14) as compared with nadroparin o.d. treatment. When we compared the absolute risk difference in b.i.d. dosing nadroparin as compared with o.d. dosing, we observed an absolute risk increase of 0.13% (95% CI -0.15% to 0.41%). Patients with a first venous thrombotic event had similar cumulative incidences of major bleeding as patients with recurrent events.

DISCUSSION

In this study, the absolute risk of major bleeding was low among patients who were registered at the anticoagulation clinic with an acute venous thrombotic event (between 2.0 to 3.5 per 1000 patients during combined treatment with VKA and LMWH), indicating that the combination of a VKA and LMWH for a short period is relatively safe for these patients. The relative risk estimates for major bleeding events were highest in patients treated with nadroparin b.i.d. and enoxaparin o.d. or b.i.d. These results should nevertheless be interpreted with caution since numbers were small and confidence intervals showed that a similar risk of bleeding events

for these LMWHs as compared with the other LMWHs cannot be ruled out. Despite the small numbers, enoxaparin o.d. or b.i.d. treatment was associated with a higher bleeding risk than nadroparin o.d., tinzaparin o.d. and dalteparin o.d. treatment. These results are similar to what was found in a meta-analysis[12], where all LMWHs gave lower bleeding risk as compared with UFH (nadroparin odd ratio [OR] 0.41, 95% CI 0.14 to 1.17; tinzaparin OR 0.30, 95% CI 0.12 to 0.73 and dalteparin OR 0.15, 95% CI 0.02 to 1.44), while enoxaparin gave risk estimates around unity (OR 1.14, 95% CI 0.50-2.16).

Our results also indicate that nadroparin o.d. users were at lower risk for bleeding complications than nadroparin b.i.d. users. While we cannot exclude that these results are a chance finding or due to confounding, they are in line with a Cochrane review from previous trials in acute venous thrombosis patients. This review suggested that b.i.d. treatment with a LMWH results in higher bleeding rates as compared with o.d. treatment (relative risk 1.29; 95% CI, 0.79-2.50).[13] In addition, one trial showed that nadroparin b.i.d. gave higher rates of bleeding complications as compared with o.d. (relative risk 1.64; 95% CI 0.74-3.57).[14] When combining results from our study and the latter study in a post-hoc meta-analysis with a random effects model, the OR indicates a 1.77 increased risk (95% CI 0.97-3.23) for patients using b.i.d. nadroparin as compared with o.d. nadroparin.

Some methodological aspects of our study need comment. First, this study evaluated bleeding risks in a large population of unselected venous thrombosis patients from four anticoagulation clinics, which makes our results generalizable to the community. However, as a limitation, patients were included after registration at the anticoagulation clinic, which is usually a couple of days after the diagnosis of a venous thrombotic event at the hospital. During these few days, we could have missed the bleeding events. If so, this would have influenced the absolute bleeding rates found in our study. Therefore, our results are only applicable to patients discharged from hospital. A second limitation is that few bleeding events occurred which prevented us from performing several subgroup analyses in patients who are potentially at high risk of major bleeding. The small numbers also hamper the robustness of our results and may have inflated the risk estimate of bleeding events in the enoxaparin o.d. or b.i.d. group. In addition, we were not able to stratify enoxaparin treatment by o.d. and b.i.d. use due to small numbers and we had no patients who used a dalteparin b.i.d regimen. Another limitation is that we were only able to adjust for study center. However, the choice of LMWH is not based on patient characteristics, but based on the preference of the hospital for a specific type of LMWH that the patient presents him or herself. Therefore, we assume that patient characteristics are not associated with the type of LMWH prescribed and therefore consider it unlikely that residual confounding has influenced our results.

In conclusion, the absolute risk for major bleeding complications during treatment with LMWH and VKA in patients with an acute venous thrombosis who were treated at an anticoagulation clinic was low, with an approximate risk of 2.5 per 1000 patients. These small numbers prevent us from concluding whether one LMWH should be preferred over the other. Furthermore, twice daily dosing with nadroparin appeared to be associated with an increased major bleeding risk as compared with once daily dosing, which is in accordance with the literature.

REFERENCES

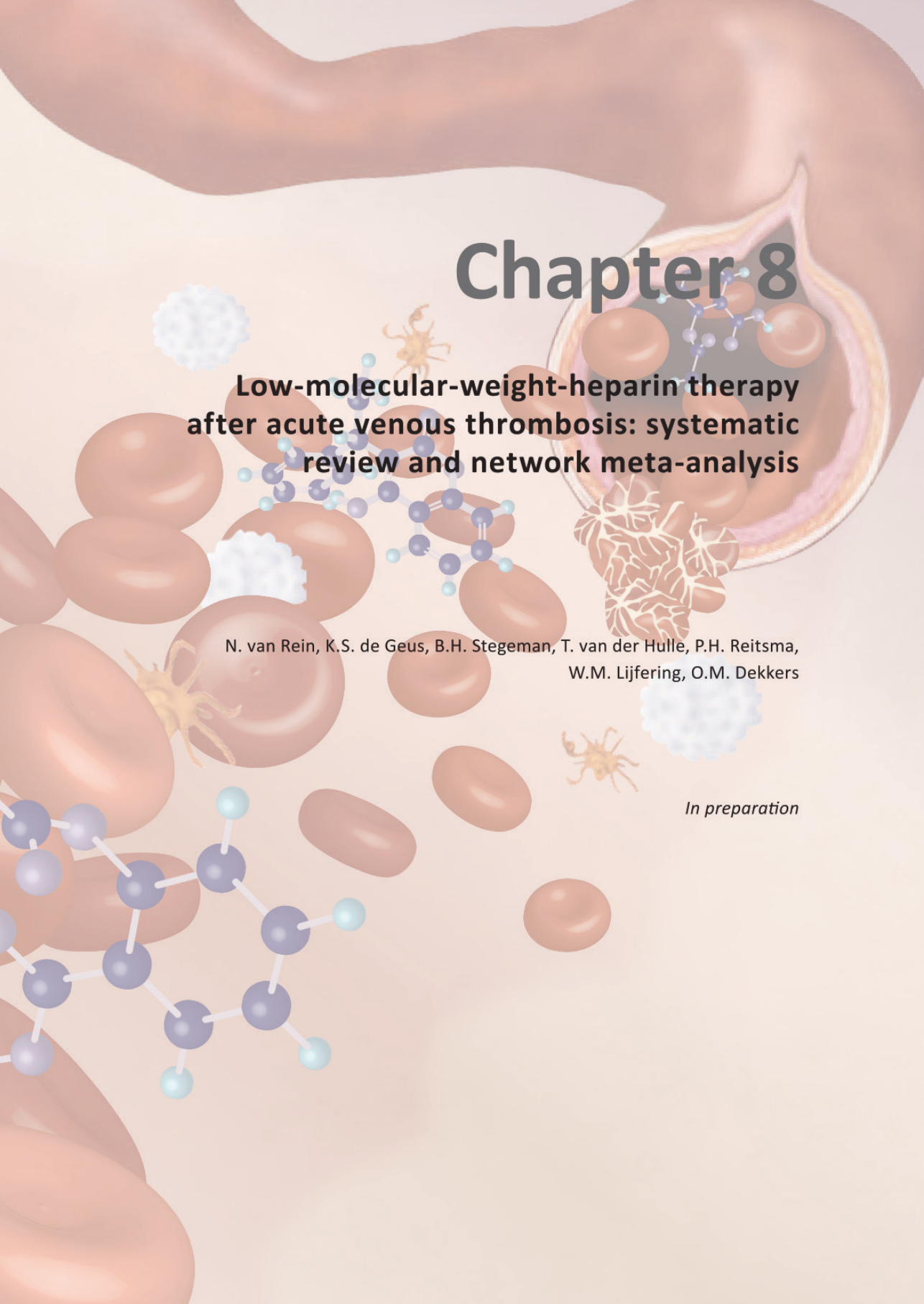
1. Kearon C, Akl EA, Comerota AJ, et al. Antithrombotic therapy for VTE disease: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest.* Feb 2012;141(2 Suppl):e419S-494S.
2. White RH, Ginsberg JS. Low-molecular-weight heparins: are they all the same? *British journal of haematology.* Apr 2003;121(1):12-20.
3. Wells PS, Anderson DR, Rodger MA, et al. A randomized trial comparing 2 low-molecular-weight heparins for the outpatient treatment of deep vein thrombosis and pulmonary embolism. *Archives of internal medicine.* Apr 11 2005;165(7):733-738.
4. Slavik RS, Chan E, Gorman SK, et al. Dalteparin versus enoxaparin for venous thromboembolism prophylaxis in acute spinal cord injury and major orthopedic trauma patients: 'DETECT' trial. *The Journal of trauma.* May 2007;62(5):1075-1081; discussion 1081.
5. Michalis LK, Katsouras CS, Papamichael N, et al. Enoxaparin versus tinzaparin in non-ST-segment elevation acute coronary syndromes: the EVET trial. *American heart journal.* Aug 2003;146(2):304-310.
6. Chiou-Tan FY, Garza H, Chan KT, et al. Comparison of dalteparin and enoxaparin for deep venous thrombosis prophylaxis in patients with spinal cord injury. *American journal of physical medicine & rehabilitation.* Sep 2003;82(9):678-685.
7. Bates SM, Greer IA, Middeldorp S, et al. VTE, thrombophilia, antithrombotic therapy, and pregnancy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest.* Feb 2012;141(2 Suppl):e691S-736S.
8. van der Meer FJ, Rosendaal FR, Vandenbroucke JP, Briet E. Bleeding complications in oral anticoagulant therapy. An analysis of risk factors. *Archives of internal medicine.* Jul 12 1993;153(13):1557-1562.
9. Schulman S, Kearon C, Haemostasis IST. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. *J Thromb Haemost.* Apr 2005;3(4):692-694.
10. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *Journal of the National Cancer Institute.* Apr 1959;22(4):719-748.

11. Roach RE, Lijfering WM, Rosendaal FR, Cannegieter SC, le Cessie S. Sex difference in risk of second but not of first venous thrombosis: paradox explained. *Circulation.* Jan 7 2014;129(1):51-56.
12. van Dongen CJ, van den Belt AG, Prins MH, Lensing AW. Fixed dose subcutaneous low molecular weight heparins versus adjusted dose unfractionated heparin for venous thromboembolism. *The Cochrane database of systematic reviews.* 2004(4):CD001100.
13. van Dongen CJ, MacGillavry MR, Prins MH. Once versus twice daily LMWH for the initial treatment of venous thromboembolism. *The Cochrane database of systematic reviews.* 2005(3):CD003074.
14. Charbonnier BA, Fiessinger JN, Banga JD, Wenzel E, d'Azemar P, Sagnard L. Comparison of a once daily with a twice daily subcutaneous low molecular weight heparin regimen in the treatment of deep vein thrombosis. FRAXODI group. *Thrombosis and haemostasis.* May 1998;79(5):897-901.

APPENDIX 1

Table 1. Bleeding events associated with LMWH treatment

	Follow-up (years)	Events	Cumulative incidence per 1000	Incidence rate per 100 person-years
All venous thrombotic events				
Nadroparin o.d.	151	11	2.07 (1.11-3.75)	7.3 (3.8-12.7)
Nadroparin b.i.d.	59	7	3.37 (1.48-7.10)	11.9 (5.2-23.5)
Tinzaparin o.d.	82	9	2.70 (1.33-5.20)	11.0 (5.4-20.1)
Enoxaparin	6	1	3.79 (0.00-23.34)	16.7 (8.3-82.2)
Dalteparin o.d.	40	4	2.06 (0.60-5.50)	10.0 (31.8-24.1)



Chapter 8

Low-molecular-weight-heparin therapy after acute venous thrombosis: systematic review and network meta-analysis

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In preparation

ABSTRACT

Background: Low-molecular-weight heparin (LMWH) are often referred to as one class of drugs which are all equally effective. However, pharmacokinetic and pharmacodynamics between various LMWHs do differ, which may result in different efficacy (preventing thrombosis) and safety (major bleeding) profiles. Therefore, it remains unknown whether different LMWHs are indeed equally effective and safe.

Objective: To compare the efficacy and safety of different LMWHs among patients with acute venous thrombosis.

Methods: We performed a systematic review and network meta-analysis including randomized controlled trials that assessed the effects of unfractionated heparin (UFH) and/or LMWHs in venous thrombosis patients. Outcomes were recurrent venous thrombosis and major bleeding. An extension of frequentist random effects models for mixed multiple treatment comparisons to estimate risk ratios (RRs) and 95% confidence intervals (CIs) was used with UFH as reference category. Treatment with LMWHs was stratified by dosing frequency (once daily [o.d.] or twice a day [b.i.d.]) and treatment duration (whole study period versus initial period as bridging towards treatment with vitamins K antagonists). Risk of bias analysis was performed according to the Cochrane tool.

Results: 4120 Publications were retrieved through a search strategy and 49 randomized controlled trials were included. Three studies compared LMWHs directly, while other studies compared an LMWH with UFH. The risk of bias assessments indicated for all studies at least one category with a risk of bias, but for most studies at least two, which may bias the risk estimates. Treatments during the initial period with the most beneficial risk-benefit profile were dalteparin b.i.d. (RR recurrent venous thrombosis 0.48, 95% CI 0.10 to 2.31; RR major bleeding 0.55, 95% CI 0.07 to 4.68), reviparin o.d. (RR recurrent VT 0.54, 95% CI 0.28 to 1.05; RR major bleeding 0.50, 95% CI 0.05 to 5.51) and nadroparin o.d. (RR recurrent VT 0.57, 95% CI 0.29 to 1.14; RR major bleeding 0.46, 95% CI 0.12 to 1.81).

Conclusion: Results suggest that dalteparin b.i.d., reviparin o.d. and nadroparin o.d. during the initial treatment period may give the fewest recurrent venous thrombotic- and major bleeding events in patients with an acute venous thrombosis. Results should be interpreted with caution as confidence intervals were wide, comparisons were mainly indirect and risk of bias was considerable.

INTRODUCTION

Heparins are widely used for prevention and acute treatment of venous thrombosis.[1] In the past, the most commonly prescribed heparin was unfractionated heparin (UFH), which was a time-consuming treatment as it required constant monitoring and dosage changing accordingly.[2] In the 1980s, the fixed dose low-molecular-weight heparin (LMWH) treatment was introduced, which was later shown to be superior to UFH.[3]

LMWHs are often referred to as one class of drugs all of which are equally effective.[4] However, pharmacokinetic and pharmacodynamics between various LMWHs do differ, which may result in different efficacy (preventing thrombosis) and safety (major bleeding) profiles.[4] Several trials compared treatment of a single LMWH with UFH in terms of efficacy and safety. However, only three trials directly compared LMWHs, but these trials were underpowered to determine whether one LMWH is superior to another.[5-7] Therefore, it remains unknown whether all LMWHs are equally effective and safe.

Because LMWHs are mostly compared with UFHs, standard meta-analytic techniques will not be able to assess the optimal LMWH; in contrast, a network meta-analysis allows evidence from direct and indirect comparisons to be used for all possible comparisons.[8] We therefore aimed to compare the efficacy and safety of different LMWH agents among patients with acute venous thrombosis in a network meta-analysis.

METHODS

Data sources and searches

A systematic literature search was conducted to identify studies on PubMed (695 hits), Medline (483), Embase (1497), Web of Science (617) and the Cochrane Register of Controlled Trials (828). The databases were searched for randomized controlled trials without any language restrictions from inception until 20 December 2013. The search string consisted of MeSH headings and subheadings, text words, and word variations for 'randomized controlled trials', 'venous thromboembolism' and 'low-molecular-weight-heparin'. The search strategy was amended for every database. Appendix 1 shows the full search string.

Study selection

All publications were independently screened on title and abstract by two investigators (NvR and TvdH). Potentially relevant publications were independently reviewed in full length by two investigators (NvR and KSG) and included in the analysis if they met the following criteria: 1) Randomized controlled trial with adult venous thrombosis patients (deep vein thrombosis, pulmonary embolism or a combination of both). 2) Patients were randomised to one regiment of subcutaneous LMWHs and intravenous or subcutaneous UFH or to two regimens of subcutaneous LMWHs. Also, the LMWHs had

still be present on today's market (2016). 3) At least one of the primary or secondary outcomes was reported. In case of multiple publications from the same study, the most updated or extensively described study was included. Potential disagreements were resolved by consensus.

Data extraction and risk of bias assessment

Extracted data included baseline characteristics, treatment regimens, study duration and outcomes. LMWHs were classified according to treatment duration (three months, i.e. whole study period [W] or the initial [I] period as bridging therapy to vitamin K antagonist therapy) and dosage scheme (twice a day (b.i.d.) or once a day (o.d.)). The treatment regimens available were: UFH, I certoparin b.i.d., I dalteparin b.i.d., I dalteparin o.d., W dalteparin b.i.d., W dalteparin o.d., I danaparoid b.i.d., I enoxaparin b.i.d., I enoxaparin o.d., W enoxaparin o.d., I fondaparinux o.d., I nadroparin b.i.d., I nadorparin o.d., W nadroparin o.d., W parnaparin o.d., I reviparin o.d., I reviparin b.i.d., I tinzaparin o.d. and W tinzaparin o.d.

Two reviewers (NvR and KSG) independently assessed the risk of bias of the publications and extracted all data. Risk of bias of included publications was determined based on the Cochrane risk of bias assessment tool.[9] This risk bias assessment includes the following items: allocation sequence generation, allocation concealment, participant masking, personnel and outcome assessors, completeness of outcome data, and selective outcome reporting. We also scored whether diagnoses were made according to standard procedures. These were the following for venous thrombosis: (1) a new constant intraluminal filling defect not present on the last available venogram; (2) if the venogram was not diagnostic, either an abnormal 125I-fibrinogen leg scan or abnormal impedance plethysmogramor ultrasound result that had been normal before the suspected recurrent episode.[10] For diagnosis of pulmonary embolism we considered the following as standard: (1) a segmental defect on the perfusion lung scan that was unmatched on the ventilation scan or chest roentgenogram; (2) positive pulmonary angiography; (3) pulmonary embolism at autopsy.[3] Bleeds were classified as major if they were intracranial, retroperitoneal, led directly to death, necessitated transfusion or they led to the interruption of antithrombotic treatment or (re)operation.[3]

Outcome measures

Outcomes were symptomatic recurrent venous thrombosis and major bleeding events. Recurrent venous thrombosis was considered an event if occurred within the first three months of treatment and allocated according to the intention to treat principle. Major bleeding events were considered during the treatment with LMWHs or during the first fourteen days of treatment. If these data were not available, we considered the number of bleeds during the shortest period that was reported.

Statistical analysis

A network meta-analysis was conducted to compare different LMWHs (stratified by period of LMWH treatment (initial or whole study period) and by dosing schedule (o.d. or b.i.d.)). We used an extension of frequentist random effects models for mixed multiple treatment comparisons to estimate risk ratios (RRs) and 95% confidence intervals (CIs). The network meta-analysis was performed with the mvmeta command for Stata, as described by White and colleagues.[11] We used crude data from a 2×2 table in the analysis, which is reasonable given the randomized design of all studies. For publications with zero events in one cell of a 2×2 table, all cells of that 2×2 table were inflated by adding 0.5. If more than one study provided data for the same stratum we checked consistency of the results. An interaction term was added to the model to estimate the difference in results from direct and indirect evidence. All potential interactions were tested in an overall test for inconsistency in our network meta-analysis. All statistical analyses were performed with Stata, version 12.0 (Statacorp LP).

RESULTS

Study selection

The electronic searches yielded 4120 publications, which were 2223 unique publications (Figure 1). 2051 Studies were excluded based on the title and abstract, and another 124 were excluded after assessment of the full text after which 49 studies were included.

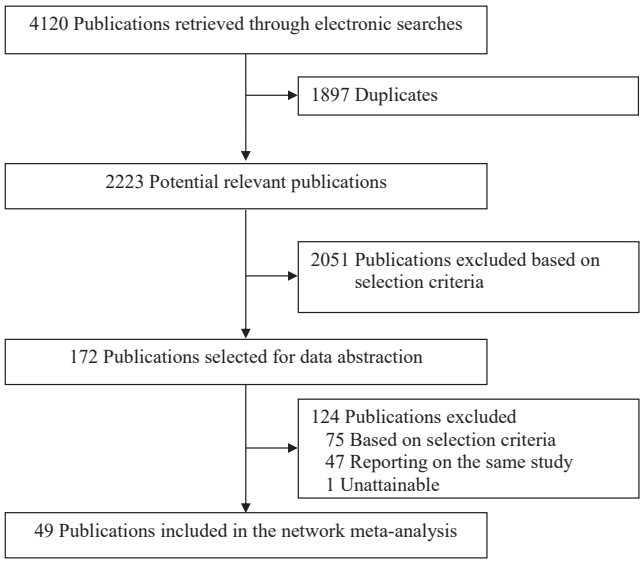


Figure 1. Flow diagram of included and excluded publications

Study characteristics

All studies were randomized controlled trials carried out between 1986 and 2011. 12 Trials were single centre, 4 studies did not report the number of study centres and 33 were multicentre trials (Table 1). The total number of participants was 19,487 and ranged from 40 to 2205 per study. There were 9 studies that included patients with pulmonary embolism (PE), 30 that included patients with deep vein thrombosis (DVT) and 10 studies that included both PE and DVT. 48 Studies reported recurrent venous thrombosis as an outcome and 48 studies reported major bleeding as an outcome.

Risk of bias

The random sequence generation was adequate in 24 (49%) of the 49 included studies (Figure 2). Assigned treatment was adequately concealed prior to allocation in 13 studies (27%), while concealment of allocation was not present in three studies (6%) and unclear in 33 studies (67%). The treatment was not blinded in most studies (43 studies, 88%) due to the difference in route of administration between low molecular weight heparin (i.e. subcutaneous) and unfractionated heparin (i.e. intravenous). Outcome assessment was blinded in 31 studies (63%), 21 studies (43%) reported incomplete data and 47 studies (96%) obtained industry sponsored funding, while two studies (4%) obtained independent funding.

Network meta-analysis

The full network is shown in Figure 3. Most comparisons of LMWHs were against UFH, with one head-to-head comparison of dalteparin with tinzaparin, one head-to-head comparison of nadroparin with parniparin and one head-to-head comparison of enoxaparin with fondaparinux. In addition, 8 studies compared LMWH treatment during the initial period and the whole study period and 7 studies compared an o.d. dosing schedule directly with a b.i.d. schedule. Because none of the potential strata in the network contained two or more studies, it was not possible to check inconsistency of the network.

Network analysis of individual LMWH agents

39 Trials were included to compare different LMWH agents. 10 Studies were not considered for this analysis as these did not compare two heparins, but treatment during the initial and whole period or an o.d versus b.i.d. dosing schedule of the same LMWH. Table 2 and 3 show the results of the network analysis for recurrent venous thrombosis and major bleeding respectively. Results regarding recurrent venous thrombosis indicate that, with UFH as a reference, dalteparin (RR 1.06, 95% CI 0.53 to 2.14), danaparoid (RR 0.92, 95% CI 0.06 to 14.66) and nadroparin (RR 0.85, 95% CI 0.53 to 1.38) were associated with the highest relative risks for recurrent venous thrombosis which was similar to UFH. The other LMWHs gave risk relative risk in the

Table 1. Characteristics of included studies

Authors	Study period	Centers	Country	Blinding	Patients enrolled	Patients	Deep vein thrombosis/ Pulmonary embolism	Mean age	Heparins
Nakamura *et al.* 2011	2007 to 2009	34	Japan	Open-label	NR	80	Both	67	UFH vs I fonda o.d.
Pérez-de-Llano *et al.* 2010	2005 to 2008	4	Spain	Open-label	Consec.	102	PE	72	I tinza o.d. vs W tinza o.d.
Hull *et al.* 2009	1997 to 1998	22	Canada	Open-label	Consec.	480	DVT	< 60 240; > 60 240*	I tinza o.d. vs W tinza o.d.
Romera *et al.* 2009	2002 to 2005	2	Spain	Open-label	Consec.	241	DVT	61, 59**	I tinza o.d. vs W tinza o.d.
Bellosta *et al.* 2007	2002 to 2005	1	Italy	Open-label	NR	91	DVT	63, 67**	I nad o.d. vs W par b.i.d.
Hull *et al.* 2007	1994-2003	30	Canada	Open-label	Consec.	737	DVT	< 60: 338; > 60 399*	UFH vs W tinza o.d.
Hull *et al.* 2006	1994-2003	23	Canada	Open-label	Consec.	200	DVT	< 60: 62; > 60 138*	UFH vs W tinza o.d.
Kucher *et al.* 2005	NR	1	USA	Open-label	NR	40	PE	52, 51**	W enoxa o.d. vs I enoxa b.i.d.
Chong *et al.* 2005	1996 to 2000	18	Multiple	Open-label	NR	298	DVT	56, 55**	UFH vs I enoxa b.i.d.
Daskalopoulos *et al.* 2005	NR	1	Greece	Open-label	Consec.	102	DVT	59, 58**	UFH vs W tinza o.d.
Wells *et al.* 2005	1991 to 2001	4	Canada	Single- blind	NR	505	Both	59, 57**	I tinza o.d. vs I dalte o.d.
Büller *et al.* 2004	2000 to 2001	154	Multiple	Double-blind	Consec.	2205	DVT	61, 62**	I fonda o.d. vs I enoxa b.i.d.
Writing Committee for the Galilei Investigators. 2004	1998 to 2001	19	Italy	Open-label	Consec.	720	Both	68, 67**	UFH vs I fonda o.d.
The Matisse Investigators. 2003	2000 to 2002	235	Multiple	Open-label	Consec.	2213	PE	63, 62**	UFH vs I fonda o.d.
Pérez-de-Llano *et al.* 2003	NR	3	Spain	Open-label	NR	56	PE	66	UFH vs I enoxa b.i.d.

Table 1 continues on next page

Table 1. (*Continued*)

Authors	Study period	Centers	Country	Blinding	Patients enrolled	Patients	Deep vein thrombosis/ Pulmonary embolism	Mean age	Heparins
Riess *et al.* 2003	NR	121	Germany, Czech Rep.	Open-label	NR	1220	DVT	61	UFH vs I certo b.i.d.
Lee *et al.* 2003	1999 to 2001	48	Multiple	Open-label	NR	676	Both	62, 63**	W dalte o.d. vs I dalte o.d.
Beckman *et al.* 2003	1999 to 2001	1	USA	Open-label	Consec.	60	PE	56, 55**	UFH vs W enoxa o.d.
Findik *et al.* 2002	1998 to 2000	1	Turkey	Open-label	Consec.	59	PE	49, 51**	UFH vs W enoxa o.d.
Meyer *et al.* 2002	1995 to 1999	25	France	Open-label	Consec.	146	Both	66, 65**	I enoxa o.d. vs W enoxa o.d.
Peternel *et al.* 2002	1999	1	Slovenia	Open-label	Consec.	59	DVT	68, 69**	UFH vs I dalte o.d.
Breddin *et al.* 2001	1996 to 1998	104	Multiple	Open-label	Consec.	1137	DVT	59	UFH vs I revip o.d.
Merli *et al.* 2001	NR	74	Multiple	Partially blinded	Consec.	900	Both	61	UFH vs W enoxa o.d. vs I enoxa b.i.d.
Harenberg *et al.* 2000	1996 to 1998	33	Multiple	Open-label	NR	538	DVT	61, 63**	UFH vs I certo b.i.d.
Nicolaides *et al.* 1999	1992 to 1995	NR	NR	Open-label	Consec.	294	DVT	54, 54, 53**	UFH vs I nadro b.i.d.
Lopaciuk *et al.* 1999	1995 to 1996	11	Poland	Open-label	NR	193	DVT	57, 58**	W nadro o.d. vs I nadro b.i.d.
Charbonnier *et al.* 1998	1993 to 1995	70	Europe	Double-blind	NR	651	DVT	59, 60**	I nadro o.d. vs I nadro b.i.d.
Decousus *et al.* 1998	1991 to 1995	44	France	Open-label	Consec.	400	DVT	73, 72**	UFH vs I enoxa b.i.d.
Simonneaui *et al.* 1997	1995 to 1996	57	Europe	Open-label	Consec.	612	PE	67	UFH vs I tinza o.d.
Partsch *et al.* 1996	1993 to 1994	1	Austria	Open-label	Consec.	140	DVT	69, 72**	I dalte o.d. vs I dalte b.i.d.
Fiessinger *et al.* 1996	NR	16	Europe	Open-label	Consec.	253	DVT	62, 61**	UFH vs I dalte o.d.
Luomanmaki *et al.* 1996	NR	7	Multiple	Open-label	Consec.	248	DVT	58, 61**	UFH vs I dalte o.d.
Koopman *et al.* 1996	NR	10	Multiple	Open-label	Consec.	400	DVT	62, 59**	UFH vs I nadro b.i.d.

Table 1. (*Continued*)

Authors	Study period	Centers	Country	Blinding	Patients enrolled	Patients	Deep vein thrombosis/ Pulmonary embolism	Mean age	Heparins
Levine *et al.* 1996	1992 to 1995	15	Canada	Open-label	Consec.	500	DVT	57, 59**	UFH vs I enoxa b.i.d.
Meyer *et al.* 1995	NR	NR	France	Open-label	NR	60	PE	60, 61**	UFH vs I dalte b.i.d.
de Valk *et al.* 1995	1991 to 1992	3	Netherlands	Open-label	Consec.	188	Both	63, 61, 50**	UFH vs I dana b.i.d.
Lindmarker *et al.* 1994	1992 to 1992	5	Sweden	Open-label	Consec.	204	DVT	62, 60	UFH vs I dalte o.d.
Simonneaui *et al.* 1993	1998 to 1990	16	Europe	Open-label	Consec.	134	DVT	64, 61**	UFH vs I enoxa b.i.d.
Lopaciuk *et al.* 1992	1989 to 1990	6	Poland	Open-label	Consec.	149	DVT	49, 48**	UFH vs I nadro b.i.d.
Holstrom *et al.* 1992	NR	1	Sweden	Open-label	Consec.	101	DVT	63, 60**	I dalte b.i.d. vs I dalte o.d.
Hull *et al.* 1992	NR	15	N-America	Double-blind	Consec.	432	DVT	< 60: 162; > 60: 270*	UFH vs I tinza o.d.
Prandoni *et al.* 1992	1986 to 1991	1	Italy	Open-label	Consec.	170	DVT	< 65: 84 > 65: 86*	UFH vs I nadro o.d.
Prandoni *et al.* 1990	NR	1	Italy	Open-label	Consec.	90	DVT	NR	UFH vs I nadro b.i.d.
Holm *et al.* 1986	NR	2	Norway	Double-blind	Consec.	56	DVT	60, 61**	UFH vs W dalte b.i.d.
Hafeli *et al.* 2001	1998 to 1999	1	Switzerland	Open-label	Consec.	125	Both	62, 68**	UFH vs I dalte o.d.
Columbus investigators 1997	1994 to 1995	31	Multiple	Open-label	Consec.	1021	Both	59, 62**	UFH vs I revip o.d.
Súarez et al. 1995	NR	NR	NR	Open-label	NR	48	DVT	75, 76**	UFH vs W dalte o.d.
Rapti et al. 2001	NR	NR	NR	Open-label	NR	52	PE	NR	UFH vs I enoxa b.i.d.
Deitcher et al. 2006	2001 to 2002	27	USA	Open-label	NR	101	Both	64	W enoxa o.d. vs I enoxa b.i.d.

Certo, Certoparin; Consec, Consecutively; Dalte, Dalteparin; DVT, Deep Vein Thrombosis; Enoxa, Enoxaparin; Fonda, Fondaparinux; Nadro, Nadroparin; NR, Not reported; Par, Parniparin; PE, Pulmonary Embolism; Revip, Reviparin; Tinza, Tinzaparin; UFH, Unfractionated Heparin; vs, versus, *Numbers of patients per age category, ** Mean age per arm of the trial

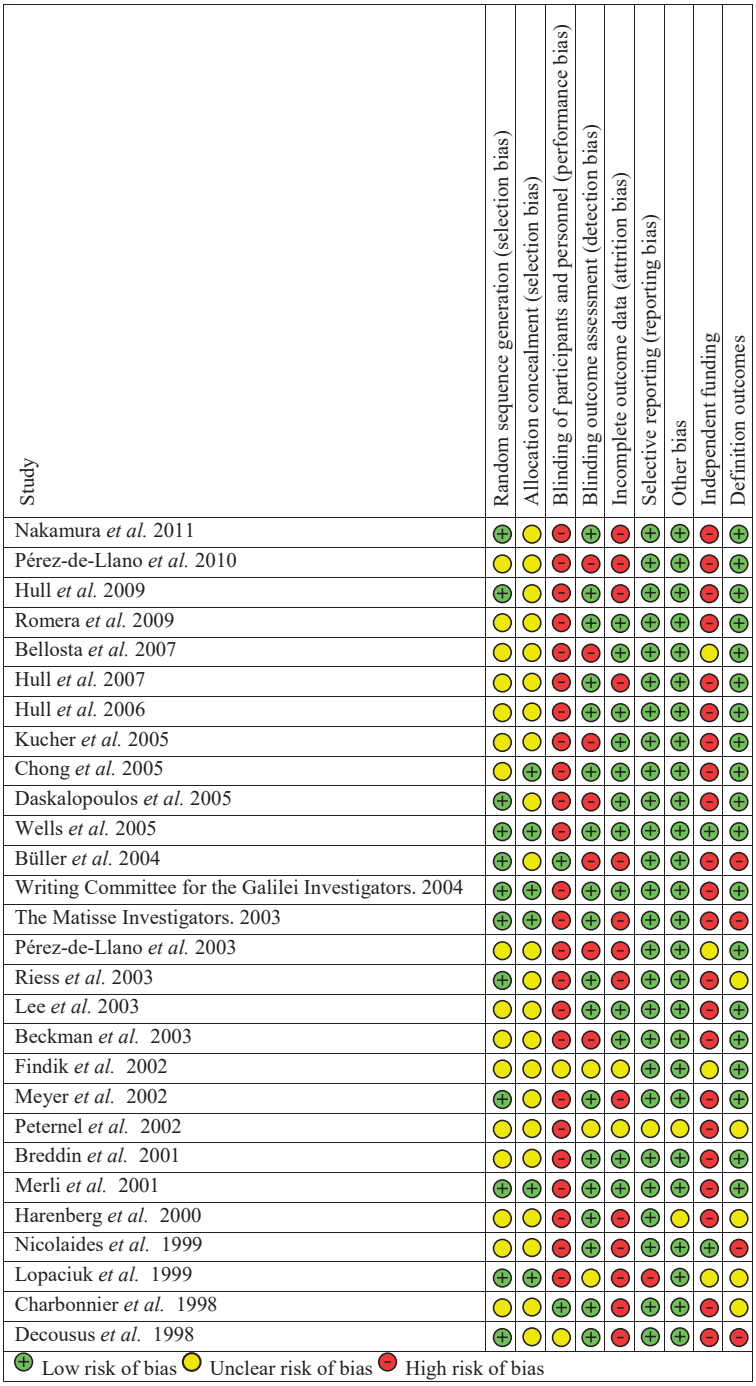
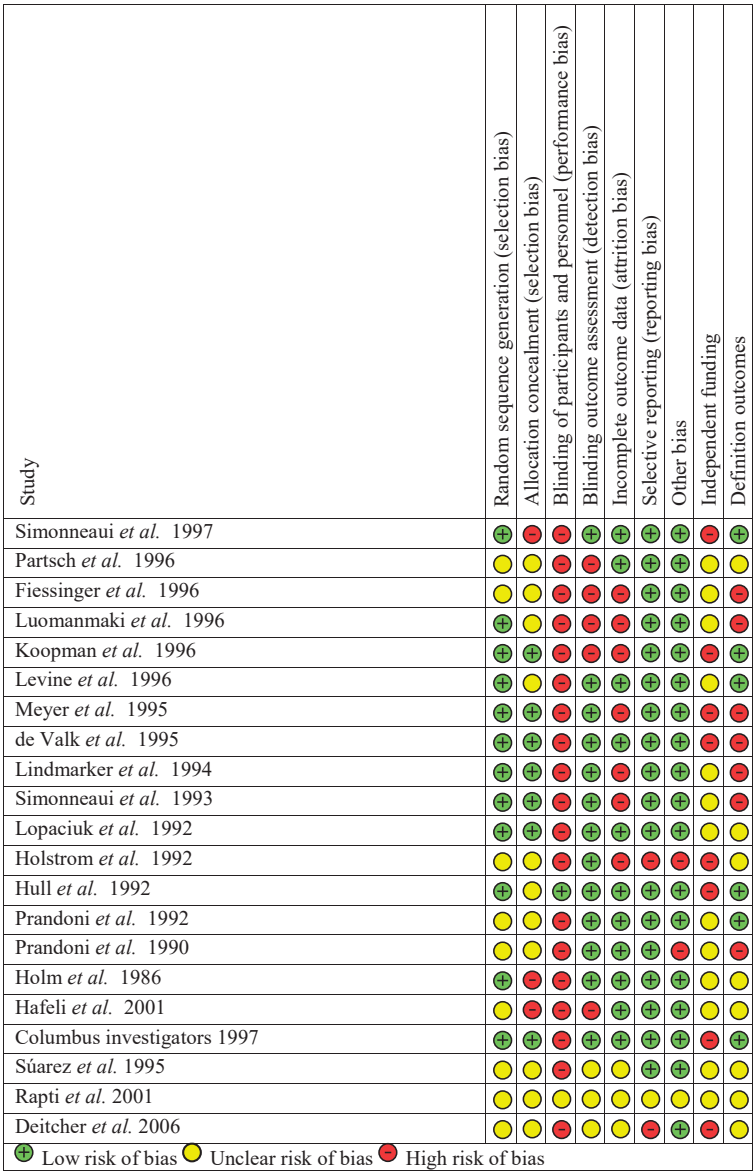


Figure 2. Risk of bias summary



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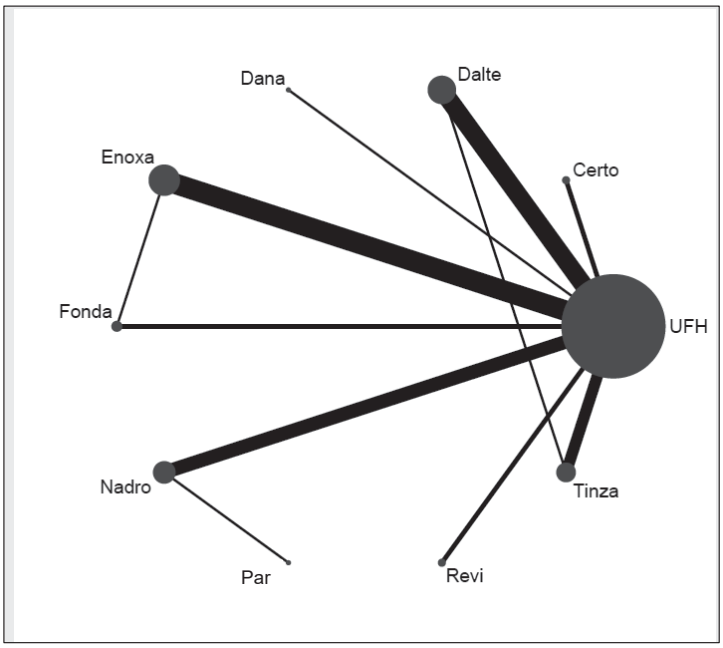


Figure 3. Networks of included LMWH and UFH trials.
Each line represents a direct comparison between two treatments from one or more trials. Certo, Certoparin; Dalte, Dalteparin; Enoxa, Enoxaparin; Fonda, Fondaparinux; Nadro, Nadroparin; Par, Parniparin; Revi, Reviparin; Tinza, Tinzaparin; UFH, Unfractionated Heparin

range of 0.65 to 0.75, however with broad confidence intervals. Results with respect to major bleeding with UFH as a reference category indicated that reviparin (RR 1.11, 95% CI 0.47 to 2.63), parniparin (RR 1.14, 95% CI 0.04 to 31.33) fondaparinux (RR 1.04, 95% CI 0.57 to 1.88) and enoxaparin (RR 0.98, 95% CI 0.58 to 1.65) were associated with the highest major bleeding risks, while the other LMWHs were associated with lower risk estimates (RRs 0.45 to 0.69).

LMWH agents stratified by period and dosing schedule

46 Trials were included to compare different LMWH agents stratified by treatment period. Treatment periods were stratified according to duration: treatment during the whole study period (three months) or the initial period as bridging therapy to vitamin K antagonist therapy. Results stratified by the period of LMWH treatment indicated that a combination of a LMWH and VKA for the whole study period gives lower risks for recurrent venous thrombosis, but increased risks for major bleeding complications as compared with the LMWH treatment during the initial period only (Figure 4A, Appendix 2 Table 1 and 2). Initial treatment with enoxaparin (RR 0.71, 95% CI 0.50 to 1.00) and certoparin (RR 0.66,

Table 2. Association of different LMWHs with recurrent venous thrombosis (RR > 1 indicates an increased recurrence risk)

UFH	1.54 (0.89-2.67)	0.94 (0.47-1.90)	1.08 (0.07-17.20)	1.34 (0.94-1.91)	1.36 (0.92-2.02)	1.17 (0.73-1.89)	1.49 (0.09-24.51)	1.42 (0.82-2.47)	1.32 (0.86-2.01)
0.65 (0.37-1.13)	**Certo**	0.61 (0.25-1.51)	0.70 (0.04-11.79)	0.87 (0.46-1.66)	0.89 (0.45-1.73)	0.76 (0.37-1.57)	0.97 (0.06-16.77)	0.92 (0.44-1.94)	0.86 (0.43-1.71)
1.06 (0.53-2.14)	1.64 (0.66-4.05)	**Dalte**	1.15 (0.07-19.94)	1.42 (0.64-3.16)	1.45 (0.65-3.25)	1.24 (0.53-2.92)	1.59 (0.09-28.41)	1.51 (0.60-3.79)	1.40 (0.69-2.83)
0.92 (0.06-14.66)	1.42 (0.08-23.84)	0.87 (0.05-15.07)	**Dana**	1.24 (0.08-20.11)	1.26 (0.08-20.56)	1.08 (0.07-17.90)	1.38 (0.03-70.44)	1.31 (0.08-22.03)	1.22 (0.07-19.97)
0.75 (0.52-1.06)	1.15 (0.60-2.18)	0.70 (0.32-1.56)	0.81 (0.05-13.12)	**Enoxa**	1.02 (0.69-1.51)	0.87 (0.48-1.58)	1.11 (0.07-18.67)	1.06 (0.56-1.99)	0.98 (0.57-1.71)
0.73 (0.50-1.08)	1.13 (0.58-2.20)	0.69 (0.31-1.55)	0.79 (0.05-12.95)	0.98 (0.66-1.46)	**Fonda**	0.86 (0.46-1.59)	1.09 (0.06-18.44)	1.04 (0.54-2.02)	0.97 (0.54-1.72)
0.85 (0.53-1.38)	1.31 (0.64-2.72)	0.80 (0.34-1.89)	0.92 (0.06-15.30)	1.14 (0.63-2.07)	1.16 (0.63-2.16)	**Nadro**	1.28 (0.08-20.07)	1.21 (0.59-2.50)	1.13 (0.59-2.13)
0.67 (0.04-10.98)	1.03 (0.06-17.83)	0.63 (0.04-11.29)	0.73 (0.01-37.04)	0.90 (0.05-15.05)	0.91 (0.05-15.39)	0.78 (0.05-12.35)	**Parni**	0.95 (0.06-16.46)	0.88 (0.05-14.95)
0.70 (0.41-1.22)	1.08 (0.52-2.27)	0.66 (0.26-1.66)	0.76 (0.05-12.76)	0.94 (0.50-1.77)	0.96 (0.50-1.85)	0.82 (0.40-1.69)	1.05 (0.06-18.13)	**Revi**	0.93 (0.46-1.85)
0.76 (0.50-1.16)	1.17 (0.58-2.34)	0.71 (0.35-1.44)	0.82 (0.05-13.47)	1.02 (0.59-1.77)	1.03 (0.58-1.84)	0.89 (0.47-1.68)	1.13 (0.07-19.18)	1.08 (0.54-2.16)	**Tinza**

certo, certoparin; dalte, dalteparin; dana, danaparoid; enoxa, enoxaparin; fonda, fondaparinux; nadro, nadroparin; parni, parnaparin; revi, reviparin; tinza, tinzaparin; UFH, unfractionated heparin

Table 3. Association of different LMWHs with major bleeding (RR > 1 indicates an increased bleeding risk)

UFH	1.61 (0.73-3.56)	2.23 (0.74-6.71)	2.10 (0.20-22.56)	1.03 (0.61-1.73)	0.96 (0.53-1.75)	2.08 (0.80-5.38)	0.88 (0.03-24.12)	0.90 (0.38-2.12)	1.44 (0.87-2.41)
0.62 (0.28-1.38)	**Certo**	1.39 (0.36-5.39)	1.31 (0.11-15.97)	0.64 (0.25-1.65)	0.60 (0.22-1.62)	1.29 (0.37-4.46)	0.55 (0.02-16.48)	0.56 (0.17-1.80)	0.90 (0.35-2.31)
0.45 (0.15-1.35)	0.72 (0.19-2.80)	**Dalte**	0.94 (0.07-12.90)	0.46 (0.14-1.56)	0.43 (0.12-1.51)	0.93 (0.22-3.99)	0.39 (0.01-12.92)	0.40 (0.10-1.63)	0.65 (0.20-2.07)
0.48 (0.04-5.12)	0.77 (0.06-9.36)	1.06 (0.08-14.55)	**Dana**	0.49 (0.04-5.56)	0.46 (0.04-5.31)	0.99 (0.08-12.76)	0.42 (0.01-24.63)	0.43 (0.03-5.35)	0.69 (0.06-7.80)
0.98 (0.58-1.65)	1.57 (0.61-4.06)	2.18 (0.64-7.37)	2.05 (0.18-23.30)	**Enoxa**	0.94 (0.51-1.72)	2.02 (0.68-6.00)	0.86 (0.03-24.51)	0.88 (0.32-2.40)	1.41 (0.68-2.93)
1.04 (0.57-1.88)	1.67 (0.62-4.49)	2.31 (0.66-8.08)	2.18 (0.19-25.16)	1.06 (0.58-1.95)	**Fonda**	2.15 (0.70-6.60)	0.91 (0.03-26.36)	0.93 (0.33-2.65)	1.50 (0.68-3.28)
0.48 (0.19-1.25)	0.77 (0.22-2.68)	1.07 (0.25-4.61)	1.01 (0.08-13.06)	0.49 (0.17-1.46)	0.46 (0.15-1.43)	**Nadro**	0.42 (0.02-10.11)	0.43 (0.12-1.56)	0.70 (0.24-2.05)
1.14 (0.04-31.33)	1.83 (0.06-55.34)	2.54 (0.08-83.53)	2.39 (0.04-141.09)	1.17 (0.04-33.49)	1.10 (0.04-31.87)	2.37 (0.10-56.56)	**Parni**	1.02 (0.03-31.42)	1.65 (0.06-47.06)
1.11 (0.47-2.63)	1.79 (0.56-5.77)	2.48 (0.61-10.04)	2.34 (0.19-29.21)	1.14 (0.42-3.13)	1.07 (0.38-3.05)	2.31 (0.64-8.33)	0.98 (0.03-29.97)	**Revi**	1.61 (0.59-4.37)
0.69 (0.42-1.15)	1.11 (0.43-2.86)	1.54 (0.48-4.94)	1.45 (0.13-16.49)	0.71 (0.34-1.48)	0.67 (0.31-1.46)	1.44 (0.49-4.23)	0.61 (0.02-17.37)	0.62 (0.23-1.69)	**Tinza**

certo, certoparin; dalte, dalteparin; dana, danaparoid; enoxa, enoxaparin; fonda, fondaparinux; nadro, nadroparin; parni, parnaparin; revi, reviparin; tinza, tinzaparin; UFH, unfractionated heparin

95% CI 0.39 to 1.11) with UFH as a reference category gave the lowest risks of recurrent venous thrombosis, while initial treatment with nadroparin gave slightly higher recurrent venous thrombosis risks (RR 0.88, 95% CI 0.55 to 1.42), but lower major bleeding risks than enoxaparin (RR 0.59, 95% CI 0.20 to 1.70 as compared with RR 0.96, 95% CI 0.57 to 1.63) and similar risks as compared with certoparin (RR 0.62, 95% CI 0.28 to 1.38).

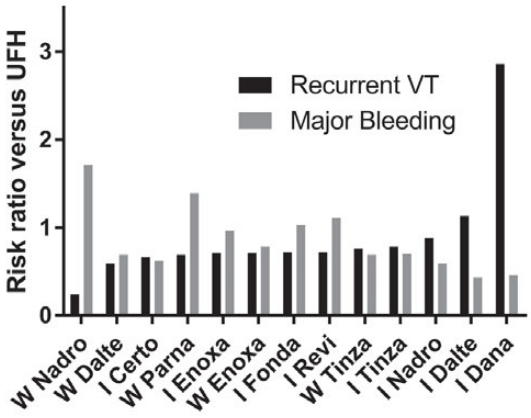
After further stratification by o.d. and b.i.d. dosing schedules, results still indicated that treatment of a LMWH combined with a vitamin K antagonist during the whole study period was associated with the lowest risk of recurrent venous thrombosis, but also the highest risk for major bleeding (Figure 4B, Appendix 2 Table 3 and 4). Treatments during the initial period with the most beneficial risk-benefit profile with UFH as a reference were dalteparin b.i.d. (RR recurrent venous thrombosis 0.48, 95% CI 0.10 to 2.31; RR major bleeding 0.55, 95% CI 0.07 to 4.68), reviparin o.d. (RR recurrent VT 0.54, 95% CI 0.28 to 1.05; RR major bleeding 0.50, 95% CI 0.05 to 5.51) and nadroparin o.d. (RR recurrent VT 0.57, 95% CI 0.29 to 1.14; RR major bleeding 0.46, 95% CI 0.12 to 1.81).

DISCUSSION

Results of this network meta-analysis for the treatment of venous thrombosis including over 19,000 patients showed that risks of recurrent venous thrombosis and major bleeding may differ between LMWHs. However, it should be underlined that confidence intervals were wide, which hampers an ultimate verdict regarding the optimal treatment. The network analysis on the comparison of individual LMWHs showed that nadroparin and dalteparin are associated with higher risks of recurrent venous thrombosis than the other LMWHs, although the imprecision of the estimates should clearly be taken into account. Results from a previous (standard) meta-analysis[3] showed that tinzaparin, dalteparin and reviparin were the LMWHs with the highest risk of recurrent venous thrombosis. These differences may be explained by the higher number of studies included in the present network analysis (49 versus 22). The rates of bleeding complications were highest among users of parniparin, reviparin, fondaparinux and enoxaparin users, which is in line with the results from the meta-analyses from van Dongen *et al.*[3]

After stratifying by treatment period and b.i.d. and o.d. treatment regimens, results indicated that dalteparin b.i.d., reviparin o.d. and nadroparin o.d. gave the lowest recurrent venous thrombotic- and major bleeding risk. The results may suggest that treatment with dalteparin b.i.d., reviparin o.d. and nadroparin o.d. may be preferable over the other LMWH treatment regimens. Still, we should interpret these results with caution as confidence intervals were wide and risk estimates therefore captured with uncertainty. It should also be noted that most evidence comes from indirect comparisons.

A



B

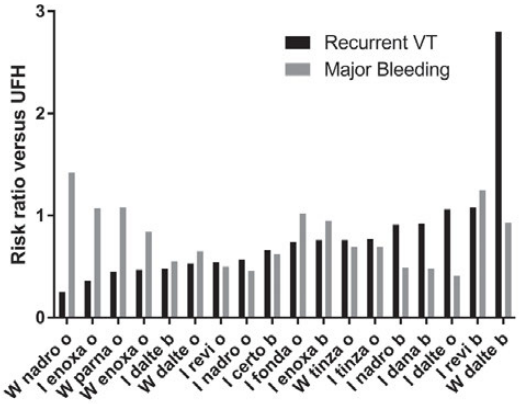


Figure 4. Risk ratios stratified by (A) initial and whole treatment duration and (B) further by once daily versus twice daily schedule

The present network meta-analysis included studies that compared UFHs and/or LMWHs with or without a vitamin K antagonist. The current guideline of the American college of Chest Physicians recommends direct oral anticoagulants to treat venous thrombosis, where LMWHs have to be administered before treating with dabigatran or edoxaban, but not when using apixaban or rivaroxaban[1]. These treatment regimens differ from the treatments in included studies in this network meta-analysis. However, as we do not expect vitamin K antagonists or direct oral anticoagulants to influence the effects of LMWHs and populations treated with LMWHs have not changed, results of this study will in all probability be generalizable to the current venous thrombosis population.

This network meta-analysis included 49 randomized controlled trials, which enabled us to study differences between LMWHs, but also between LMWH regimens. Still, the number of events was small, which hampers the robustness of the results and did not enable us to compare dosages of LMWHs. In addition, differences in risk estimates may not be clinically relevant as the relative risks between LMWHs were not very high and absolute risks of recurrent venous thrombosis and major bleeding were low. Also, UFH treatment was similar in the studies, but not identical: different reference values were used for the activated partial thromboplastin time (activated partial thromboplastin time ranging from 1.5 to 3.5 times the control value). In addition, three head-to-head trials were performed to compare LMWHs directly, which were all underpowered and made us mainly rely on indirect evidence for the comparisons of individual LMWHs. Due to the limited number of head-to-head trials, we were not able to test the consistency of the network statistically, which did not enable us to confirm the robustness of our results. In addition, risk of bias assessments indicated for all studies at least one category with a risk of bias, but for most studies at least two, which may bias the risk estimates.

To conclude, results from this network meta-analysis suggest that dalteparin b.i.d., reviparin o.d. and nadroparin o.d. during the initial treatment period may give the fewest recurrent venous thrombotic- and major bleeding events in patients with an acute venous thrombosis. Results should be interpreted with caution as confidence intervals were wide, comparisons were mainly indirect and risk of bias was considerable.

REFERENCES

1. Kearon C, Akl EA, Ornelas J, et al. Antithrombotic Therapy for VTE Disease: CHEST Guideline and Expert Panel Report. *Chest.* Feb 2016;149(2):315-352.
2. Simonneau G, Charbonnier B, Decousus H, et al. Subcutaneous low-molecular-weight heparin compared with continuous intravenous unfractionated heparin in the treatment of proximal deep vein thrombosis. *Archives of internal medicine.* Jul 12 1993;153(13):1541-1546.
3. van Dongen CJ, van den Belt AG, Prins MH, Lensing AW. Fixed dose subcutaneous low molecular weight heparins versus adjusted dose unfractionated heparin for venous thromboembolism. *The Cochrane database of systematic reviews.* Oct 18 2004(4):CD001100.
4. White RH, Ginsberg JS. Low-molecular-weight heparins: are they all the same? *British journal of haematology.* Apr 2003;121(1):12-20.
5. Wells PS, Anderson DR, Rodger MA, et al. A randomized trial comparing 2 low-molecular-weight heparins for the outpatient treatment of deep vein thrombosis and pulmonary embolism. *Archives of internal medicine.* Apr 11 2005;165(7):733-738.
6. Bellosta R, Ferrari P, Luzzani L, et al. Home therapy with LMWH in deep vein thrombosis: randomized study comparing single and double daily administrations. *Angiology.* Jun-Jul 2007;58(3):316-322.
7. Buller HR, Davidson BL, Decousus H, et al. Fondaparinux or enoxaparin for the initial treatment of symptomatic deep venous thrombosis: a randomized trial. *Annals of internal medicine.* Jun 01 2004;140(11):867-873.
8. Lumley T. Network meta-analysis for indirect treatment comparisons. *Statistics in medicine.* Aug 30 2002;21(16):2313-2324.
9. Higgins JP, Altman DG, Gotzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *Bmj.* Oct 18 2011;343:d5928.
10. Buller HR, Lensing AW, Hirsh J, ten Cate JW. Deep vein thrombosis: new non-invasive diagnostic tests. *Thrombosis and haemostasis.* Jul 12 1991;66(1):133-137.
11. White IR, Barrett JK, Jackson D, Higgins JP. Consistency and inconsistency in network meta-analysis: model estimation using multivariate meta-regression. *Research synthesis methods.* Jun 2012;3(2):111-125.

APPENDIX 1

Pubmed

("Heparin, Low-Molecular-Weight"[Mesh] OR "low molecular weight heparin"[all fields] OR "low molecular weight heparins"[all fields] OR "LMWHs"[all fields] OR "LMWH"[all fields] OR "Dalteparin"[all fields] OR "Enoxaparin"[all fields] OR "Nadroparin"[all fields] OR ("low molecular weight"[all fields] AND heparin*[all fields]) OR **"LMW Heparin"[all fields] OR "LMW Heparins"[all fields]**) AND ("Venous Thrombosis"[Mesh] OR "Venous Thrombosis"[all fields] OR "Venous Thromboses"[all fields] OR "venous thrombotic"[all fields] OR "Phlebothrombosis"[all fields] OR "Phlebothromboses"[all fields] OR "Vein Thromboses"[all fields] OR "Vein Thrombosis"[all fields] OR "Deep-Venous Thrombosis"[all fields] OR "Deep-Venous Thromboses"[all fields] OR "Deep-Vein Thrombosis"[all fields] OR "Deep-Vein Thromboses"[all fields] OR "DVT"[all fields] OR "Thrombophlebitis"[all fields] OR "Thrombophlebitides"[all fields] OR "venous thromboembolism"[all fields] OR "venous thromboembolic"[all fields] OR "Venous Thromboembolism"[Mesh] OR "Pulmonary Embolism"[Mesh] OR "Pulmonary Embolisms"[all fields] OR "Pulmonary Thromboembolisms"[all fields] OR "Pulmonary Thromboembolism"[all fields] OR "pe"[tw])

MEDLINE

(exp *Heparin, Low-Molecular-Weight/ OR "low molecular weight heparin".ti OR "low molecular weight heparins".ti OR "LMWHs".ti OR "LMWH".ti OR "Dalteparin".ti OR "Enoxaparin".ti OR "Nadroparin".ti OR "Clexane".ti OR "Fragmin".ti OR "Tedelparin".ti OR "Danaparoid".ti OR "Orgaran".ti OR "Fondaparinux".ti OR "Fondaparin".ti OR "Arixtra".ti OR "Fraxodi".ti OR "Fraxiparin".ti OR "Tinzaparin".ti OR "Innohep".ti OR "Certoparin".ti OR "Sandoparin".ti OR "Reviparin".ti OR ("low molecular weight".ti AND heparin*.ti) OR "LMW Heparin".ti OR "LMW Heparins".ti) AND (exp *Venous Thrombosis/ OR "Venous Thrombosis".ti OR "Venous Thromboses".ti OR "venous thrombotic".ti OR "Phlebothrombosis".ti OR "Phlebothromboses".ti OR "Vein Thromboses".ti OR "Vein Thrombosis".ti OR "Deep-Venous Thrombosis".ti OR "Deep-Venous Thromboses".ti OR "Deep-Vein Thrombosis".ti OR "Deep-Vein Thromboses".ti OR "DVT".ti OR "Thrombophlebitis".ti OR "Thrombophlebitides".ti OR "venous thromboembolism".ti OR "venous thromboembolic".ti OR exp *Venous Thromboembolism/ OR exp *Pulmonary Embolism/ OR "Pulmonary Embolisms".ti OR "Pulmonary Thromboembolisms".ti OR "Pulmonary Thromboembolism".ti OR "pe".ti) AND Clinical Trial.pt

EMBASE

(exp *low molecular weight heparin/ OR “low molecular weight heparin”.ti OR ”low molecular weight heparins”.ti OR ”LMWHs”.ti OR ”LMWH”.ti OR ”Dalteparin”.ti OR ”Enoxaparin”.ti OR ”Nadroparin”.ti OR ”Clexane”.ti OR ”Fragmin”.ti OR ”Tedelparin”.ti OR ”Danaparoid”.ti OR ”Orgaran”.ti OR ”Fondaparinux”.ti OR ”Fondaparin”.ti OR ”Arixtra”.ti OR ”Fraxodi”.ti OR ”Fraxiparin”.ti OR ”Tinzaparin”.ti OR ”Innohep”.ti OR ”Certoparin”.ti OR ”Sandoparin”.ti OR ”Reviparin”.ti OR (”low molecular weight”.ti AND heparin*.ti) OR ”LMW Heparin”.ti OR ”LMW Heparins”.ti) AND (exp *Vein Thrombosis/ OR “Venous Thrombosis”.ti OR “Venous Thromboses”.ti OR “venous thrombotic”.ti OR “Phlebothrombosis”.ti OR “Phlebothromboses”.ti OR “Vein Thromboses”.ti OR “Vein Thrombosis”.ti OR “Deep-Venous Thrombosis”.ti OR “Deep-Venous Thromboses”.ti OR “Deep-Vein Thrombosis”.ti OR “Deep-Vein Thromboses”.ti OR “DVT”.ti OR “Thrombophlebitis”.ti OR “Thrombophlebitides”.ti OR “venous thromboembolism”.ti OR “venous thromboembolic”.ti OR exp * venous thromboembolism/ OR exp *Lung Embolism/ OR “Pulmonary Embolisms”.ti OR “Pulmonary Thromboembolisms”.ti OR “Pulmonary Thromboembolism”.ti OR “pe”.ti) AND (exp Clinical Trial/ OR trial.ti OR RCT.ti OR random*.ti)

Web of Science

TI=(low molecular weight heparin OR “low molecular weight heparin” OR ”low molecular weight heparins” OR ”LMWHs” OR ”LMWH” OR ”Dalteparin” OR ”Enoxaparin” OR ”Nadroparin” OR ”Clexane” OR ”Fragmin” OR ”Tedelparin” OR ”Danaparoid” OR ”Orgaran” OR ”Fondaparinux” OR ”Fondaparin” OR ”Arixtra” OR ”Fraxodi” OR ”Fraxiparin” OR ”Tinzaparin” OR ”Innohep” OR ”Certoparin” OR ”Sandoparin” OR ”Reviparin” OR (”low molecular weight” AND heparin*) OR ”LMW Heparin” OR ”LMW Heparins”) AND TI=(Vein Thrombosis OR Venous Thrombosis OR “Venous Thrombosis” OR “Venous Thromboses” OR “venous thrombotic” OR “Phlebothrombosis” OR “Phlebothromboses” OR “Vein Thromboses” OR “Vein Thrombosis” OR “Deep-Venous Thrombosis” OR “Deep-Venous Thromboses” OR “Deep-Vein Thrombosis” OR “Deep-Vein Thromboses” OR “DVT” OR “Thrombophlebitis” OR “Thrombophlebitides” OR “venous thromboembolism” OR “venous thromboembolic” OR venous thromboembolism OR Lung Embolism OR “Pulmonary Embolisms” OR “Pulmonary Thromboembolisms” OR “Pulmonary Thromboembolism” OR “pe”) AND TS=(trial OR trials OR trial* OR RCT OR RCTS OR random OR randomised OR randomised OR random* OR placebo OR placebo*)

Cochrane

(low molecular weight heparin OR low molecular weight heparin OR low molecular weight heparins OR LMWHs OR LMWH OR Dalteparin OR Enoxaparin OR Nadroparin OR Clexane OR Fragmin OR Tedelparin OR Danaparoid OR Orgaran OR Fondaparinux OR Fondaparin OR Arixtra OR Fraxodi OR Fraxiparin OR Tinzaparin OR Innohep OR Certoparin OR Sandoparin OR Reviparin OR (low molecular weight AND heparin*) OR LMW Heparin OR LMW Heparins) AND (Vein Thrombosis OR Venous Thrombosis OR Venous Thrombosis OR Venous Thromboses OR venous thrombotic OR Phlebothrombosis OR Phlebothromboses OR Vein Thromboses OR Vein Thrombosis OR Deep-Venous Thrombosis OR Deep-Venous Thromboses OR Deep-Vein Thrombosis OR Deep-Vein Thromboses OR DVT OR Thrombophlebitis OR Thrombophlebitides OR venous thromboembolism OR venous thromboembolic OR venous thromboembolism OR Lung Embolism OR Pulmonary Embolisms OR Pulmonary Thromboembolisms OR Pulmonary Thromboembolism OR pe)

APPENDIX 2

Table 1. Association of LMWH, stratified by duration, with recurrent venous thrombosis

UFH	1.53 (0.90-2.58)	0.89 (0.43-1.81)	1.70 (0.74-3.93)	0.35 (0.04-3.30)	1.41 (1.00-2.00)	1.40 (0.44-4.52)	1.40 (0.98-2.00)	1.13 (0.70-1.83)	4.09 (0.80-20.91)	1.44 (0.09-23.52)	1.40 (0.82-2.37)	1.28 (0.76-2.17)	1.32 (0.83-2.10)
0.66 (0.39-1.11)	**I Cer**	0.58 (0.24-1.43)	1.12 (0.41-3.05)	0.23 (0.02-2.30)	0.93 (0.50-1.71)	0.92 (0.26-3.30)	0.92 (0.49-1.71)	0.74 (0.37-1.51)	2.68 (0.48-14.87)	0.95 (0.06-16.18)	0.91 (0.45-1.86)	0.84 (0.40-1.77)	0.86 (0.43-1.74)
1.13 (0.55-2.31)	1.72 (0.70-4.24)	**I Dal**	1.92 (1.19-3.08)	0.39 (0.04-4.16)	1.59 (0.71-3.56)	1.58 (0.40-6.26)	1.57 (0.70-3.53)	1.28 (0.54-3.02)	4.61 (0.78-27.40)	1.63 (0.09-29.03)	1.57 (0.63-3.95)	1.45 (0.71-2.94)	1.48 (0.67-3.30)
0.59 (0.25-1.36)	0.90 (0.33-2.45)	0.52 (0.32-0.84)	**W Dal**	0.21 (0.02-2.26)	0.83 (0.33-2.08)	0.83 (0.19-3.50)	0.82 (0.33-2.06)	0.67 (0.25-1.75)	2.41 (0.38-15.06)	0.85 (0.05-15.64)	0.82 (0.29-2.30)	0.75 (0.33-1.74)	0.77 (0.31-1.93)
2.86 (0.30-26.96)	4.36 (0.43-43.70)	2.53 (0.24-26.73)	4.86 (0.44-53.37)	**I Dana**	4.03 (0.42-39.07)	4.01 (0.32-50.44)	3.99 (0.41-38.75)	3.24 (0.33-32.14)	11.69 (0.73-187.45)	4.13 (0.12-148.19)	3.99 (0.40-40.02)	3.67 (0.37-36.78)	3.76 (0.38-37.26)
0.71 (0.50-1.00)	1.08 (0.58-2.00)	0.63 (0.28-1.41)	1.21 (0.48-3.03)	0.25 (0.03-2.40)	**I Enox**	1.00 (0.32-3.10)	0.99 (0.69-1.42)	0.80 (0.45-1.45)	2.90 (0.55-15.36)	1.02 (0.06-17.02)	0.99 (0.54-1.81)	0.91 (0.48-1.71)	0.93 (0.52-1.66)
0.71 (0.22-2.29)	1.09 (0.30-3.90)	0.63 (0.16-2.50)	1.21 (0.29-5.13)	0.25 (0.02-3.13)	1.00 (0.32-3.13)	**W Enox**	0.99 (0.30-3.25)	0.81 (0.23-2.85)	2.91 (0.39-21.67)	1.03 (0.05-21.18)	0.99 (0.28-3.55)	0.91 (0.25-3.29)	0.94 (0.27-3.30)
0.72 (0.50-1.03)	1.09 (0.58-2.04)	0.64 (0.28-1.42)	1.22 (0.49-3.06)	0.25 (0.03-2.43)	1.01 (0.70-1.45)	1.01 (0.31-3.29)	**I Fon**	0.81 (0.45-1.48)	2.93 (0.55-15.56)	1.03 (0.06-17.23)	1.00 (0.54-1.86)	0.92 (0.49-1.74)	0.94 (0.52-1.70)
0.88 (0.55-1.42)	1.35 (0.66-2.73)	0.78 (0.33-1.85)	1.50 (0.57-3.95)	0.31 (0.03-3.07)	1.25 (0.69-2.25)	1.24 (0.35-4.38)	1.23 (0.68-2.24)	**I Nad**	3.61 (0.76-17.17)	1.28 (0.08-19.91)	1.23 (0.60-2.51)	1.13 (0.56-2.30)	1.16 (0.60-2.27)
0.24 (0.05-1.25)	0.37 (0.07-2.07)	0.22 (0.04-1.29)	0.42 (0.07-2.60)	0.09 (0.01-1.37)	0.34 (0.07-1.83)	0.34 (0.05-2.55)	0.34 (0.06-1.81)	0.28 (0.06-1.32)	**W Nad**	0.35 (0.01-8.32)	0.34 (0.06-1.89)	0.31 (0.06-1.74)	0.32 (0.06-1.76)
0.69 (0.04-11.26)	1.06 (0.06-18.04)	0.61 (0.03-10.94)	1.18 (0.06-21.68)	0.24 (0.01-8.69)	0.98 (0.06-16.24)	0.97 (0.05-20.02)	0.97 (0.06-16.10)	0.78 (0.05-12.25)	2.83 (0.12-66.74)	**W Par**	0.97 (0.06-16.51)	0.89 (0.05-15.19)	0.91 (0.05-15.42)
0.72 (0.42-1.22)	1.09 (0.54-2.22)	0.64 (0.25-1.60)	1.22 (0.44-3.41)	0.25 (0.02-2.52)	1.01 (0.55-1.85)	1.01 (0.28-3.60)	1.00 (0.54-1.86)	0.81 (0.40-1.65)	2.93 (0.53-16.28)	1.04 (0.06-17.71)	**I Revi**	0.92 (0.43-1.95)	0.94 (0.47-1.90)
0.78 (0.46-1.32)	1.19 (0.56-2.50)	0.69 (0.34-1.40)	1.33 (0.57-3.06)	0.27 (0.03-2.73)	1.10 (0.59-2.06)	1.09 (0.30-3.94)	1.09 (0.58-2.06)	0.88 (0.43-1.80)	3.19 (0.57-17.68)	1.13 (0.07-19.24)	1.09 (0.51-2.30)	**I Tin**	1.03 (0.59-1.78)
0.76 (0.48-1.21)	1.16 (0.58-2.33)	0.67 (0.30-1.49)	1.29 (0.52-3.22)	0.27 (0.03-2.63)	1.07 (0.60-1.91)	1.07 (0.30-3.75)	1.06 (0.59-1.91)	0.86 (0.44-1.68)	3.11 (0.57-16.95)	1.10 (0.06-18.56)	1.06 (0.53-2.12)	0.97 (0.56-1.69)	**W Tin**

cer=certoparin; dal=dalteparin; dana=danaparoid; enox=enoxaparin; fon=fondaparinux; i= treated initial period; nad= nadroparin; par=parnaparin; revi=reviparin; tin= tinzaparin; UFH= unfractionated heparin; w=treated whole study period

Table 2. Association of LMWH, stratified by duration, with major bleeding

UFH	1.61 (0.73-3.56)	2.31 (0.76-7.06)	1.45 (0.40-5.22)	2.17 (0.20-23.29)	1.04 (0.62-1.77)	1.28 (0.39-4.18)	0.97 (0.54-1.76)	1.70 (0.59-4.92)	0.59 (0.02-16.85)	0.72 (0.03-20.45)	0.90 (0.38-2.12)	1.44 (0.60-3.42)	1.45 (0.83-2.52)
0.62 (0.28-1.38)	**I Cer**	1.44 (0.37-5.66)	0.90 (0.20-4.07)	1.35 (0.11-16.48)	0.65 (0.25-1.68)	0.80 (0.19-3.31)	0.60 (0.22-1.63)	1.06 (0.28-3.98)	0.36 (0.01-11.49)	0.45 (0.01-13.95)	0.56 (0.17-1.80)	0.89 (0.28-2.90)	0.90 (0.34-2.37)
0.43 (0.14-1.32)	0.70 (0.18-2.73)	**I Dal**	0.63 (0.31-1.25)	0.94 (0.07-12.91)	0.45 (0.13-1.55)	0.55 (0.11-2.81)	0.42 (0.12-1.49)	0.74 (0.16-3.43)	0.25 (0.01-8.72)	0.31 (0.01-10.59)	0.39 (0.09-1.59)	0.62 (0.17-2.24)	0.63 (0.18-2.13)
0.69 (0.19-2.50)	1.11 (0.25-5.03)	1.60 (0.80-3.21)	**W Dal**	1.50 (0.10-22.28)	0.72 (0.18-2.89)	0.89 (0.15-5.07)	0.67 (0.16-2.77)	1.18 (0.22-6.22)	0.40 (0.01-14.76)	0.50 (0.01-17.93)	0.62 (0.13-2.91)	0.99 (0.24-4.17)	1.00 (0.25-3.98)
0.46 (0.04-4.96)	0.74 (0.06-9.08)	1.07 (0.08-14.72)	0.67 (0.04-9.93)	**I Dana**	0.48 (0.04-5.48)	0.59 (0.04-8.39)	0.45 (0.04-5.19)	0.79 (0.06-10.59)	0.27 (0.00-16.53)	0.33 (0.01-20.12)	0.41 (0.03-5.18)	0.66 (0.05-8.32)	0.67 (0.06-7.66)
0.96 (0.57-1.63)	1.54 (0.59-4.00)	2.22 (0.65-7.62)	1.39 (0.35-5.56)	2.08 (0.18-23.66)	**I Enox**	1.23 (0.39-3.86)	0.93 (0.51-1.71)	1.63 (0.50-5.34)	0.56 (0.02-16.84)	0.69 (0.02-20.44)	0.86 (0.31-2.36)	1.38 (0.50-3.80)	1.39 (0.65-2.98)
0.78 (0.24-2.55)	1.26 (0.30-5.22)	1.81 (0.36-9.17)	1.13 (0.20-6.47)	1.69 (0.12-24.01)	0.81 (0.26-2.56)	**W Enox**	0.76 (0.22-2.67)	1.33 (0.27-6.51)	0.46 (0.01-16.10)	0.56 (0.02-19.55)	0.70 (0.16-3.03)	1.12 (0.26-4.86)	1.13 (0.31-4.17)
1.03 (0.57-1.86)	1.65 (0.61-4.46)	2.38 (0.67-8.42)	1.49 (0.36-6.12)	2.23 (0.19-23.76)	1.07 (0.59-1.96)	1.32 (0.38-4.62)	**I Fon**	1.75 (0.52-5.90)	0.60 (0.02-18.25)	0.74 (0.02-22.15)	0.92 (0.32-2.63)	1.48 (0.52-4.23)	1.49 (0.66-3.36)
0.59 (0.20-1.70)	0.94 (0.25-3.55)	1.36 (0.29-6.33)	0.85 (0.16-4.49)	1.27 (0.09-17.15)	0.61 (0.19-2.00)	0.75 (0.15-3.68)	0.57 (0.17-1.93)	**I Nad**	0.34 (0.01-8.33)	0.42 (0.02-10.11)	0.53 (0.13-2.07)	0.84 (0.21-3.32)	0.85 (0.26-2.81)
1.71 (0.06-49.19)	2.75 (0.09-86.78)	3.95 (0.11-136.26)	2.47 (0.07-90.19)	3.70 (0.06-226.65)	1.78 (0.06-53.46)	2.19 (0.06-77.09)	1.66 (0.05-50.41)	2.91 (0.12-70.54)	**W Nad**	1.23 (0.01-110.60)	1.54 (0.05-49.25)	2.46 (0.08-78.97)	2.48 (0.08-74.60)
1.39 (0.05-39.47)	2.23 (0.07-69.65)	3.21 (0.09-109.41)	2.01 (0.06-72.43)	3.01 (0.05-182.31)	1.45 (0.05-42.90)	1.78 (0.05-61.90)	1.35 (0.05-40.46)	2.37 (0.10-56.56)	0.81 (0.01-73.12)	**W Par**	1.25 (0.04-39.54)	2.00 (0.06-63.40)	2.01 (0.07-59.87)
1.11 (0.47-2.63)	1.79 (0.56-5.77)	2.57 (0.63-10.53)	1.61 (0.34-7.55)	2.41 (0.19-30.14)	1.16 (0.42-3.18)	1.43 (0.33-6.15)	1.08 (0.38-3.08)	1.89 (0.48-7.42)	0.65 (0.02-20.90)	0.80 (0.03-25.37)	**I Revi**	1.60 (0.47-5.43)	1.61 (0.58-4.48)
0.70 (0.29-1.65)	1.12 (0.35-3.62)	1.61 (0.45-5.79)	1.01 (0.24-4.22)	1.51 (0.12-18.87)	0.73 (0.26-2.00)	0.89 (0.21-3.86)	0.68 (0.24-1.93)	1.18 (0.30-4.66)	0.41 (0.01-13.07)	0.50 (0.02-15.88)	0.62 (0.18-2.12)	**I Tin**	1.01 (0.41-2.50)
0.69 (0.40-1.20)	1.11 (0.42-2.92)	1.60 (0.47-5.42)	1.00 (0.25-3.96)	1.49 (0.13-17.11)	0.72 (0.34-1.54)	0.88 (0.24-3.26)	0.67 (0.30-1.51)	1.17 (0.36-3.88)	0.40 (0.01-12.16)	0.50 (0.02-14.76)	0.62 (0.22-1.72)	0.99 (0.40-2.46)	**W Tin**

cer=certoparin; dal=dalteparin; dana=danaparoid; enox=enoxaparin; fon=fondaparinux; i= treated initial period; nad= nadroparin; par=parnaparin; revi=reviparin; tin= tinzaparin; UFH= unfractionated heparin; w=treated whole study period

Table 3. Association of LMWH, stratified by duration and dosing schedule, with recurrent

UFH	1.51 (0.9-2.5)	2.09 (0.4-10.1)	0.94 (0.5-1.9)	0.36 (0.0-8.4)	1.87 (0.8-4.3)	1.08 (0.1-16.9)	1.32 (0.9-1.8)	2.79 (1.0-7.5)
0.66 (0.4-1.1)	**I cer b**	1.39 (0.3-7.2)	0.63 (0.3-1.5)	0.24 (0.0-5.8)	1.24 (0.5-3.2)	0.72 (0.0-11.7)	0.87 (0.5-1.6)	1.85 (0.6-5.6)
0.48 (0.1-2.3)	0.72 (0.1-3.8)	**I dal b**	0.45 (0.1-1.9)	0.17 (0.0-5.8)	0.89 (0.2-4.1)	0.52 (0.0-12.3)	0.63 (0.1-3.1)	1.33 (0.2-8.6)
1.06 (0.5-2.2)	1.60 (0.7-3.8)	2.22 (0.5-9.5)	**I dal o**	0.38 (0.0-9.6)	1.98 (1.3-3.1)	1.15 (0.1-19.7)	1.39 (0.6-3.1)	2.96 (0.9-10.0)
2.80 (0.1-65.9)	4.23 (0.2-103.3)	5.86 (0.2-200.2)	2.64 (0.1-67.4)	**W dal b**	5.24 (0.2-137.0)	3.03 (0.0-199.8)	3.68 (0.2-88.3)	7.82 (0.3-213.9)
0.53 (0.2-1.2)	0.81 (0.3-2.1)	1.12 (0.2-5.1)	0.50 (0.3-0.8)	0.19 (0.0-5.0)	**W dal o**	0.58 (0.0-10.2)	0.70 (0.3-1.7)	1.49 (0.4-5.4)
0.92 (0.1-14.4)	1.39 (0.1-22.7)	1.93 (0.1-46.0)	0.87 (0.1-14.9)	0.33 (0.0-21.7)	1.73 (0.1-30.5)	**I dan b**	1.21 (0.1-19.4)	2.58 (0.1-47.8)
0.76 (0.5-1.1)	1.15 (0.6-2.1)	1.59 (0.3-8.0)	0.72 (0.3-1.6)	0.27 (0.0-6.5)	1.42 (0.6-3.5)	0.82 (0.1-13.1)	**I en b**	2.12 (0.8-5.9)
0.36 (0.1-1.0)	0.54 (0.2-1.6)	0.75 (0.1-4.8)	0.34 (0.1-1.1)	0.13 (0.0-3.5)	0.67 (0.2-2.4)	0.39 (0.0-7.2)	0.47 (0.2-1.3)	**I en o**
0.47 (0.1-1.6)	0.71 (0.2-2.7)	0.99 (0.1-7.4)	0.45 (0.1-1.9)	0.17 (0.0-5.0)	0.88 (0.2-3.9)	0.51 (0.0-10.5)	0.62 (0.2-2.2)	1.32 (0.4-4.8)
0.74 (0.5-1.0)	1.12 (0.6-2.0)	1.55 (0.3-7.8)	0.70 (0.3-1.5)	0.26 (0.0-6.3)	1.39 (0.6-3.4)	0.80 (0.1-12.8)	0.98 (0.7-1.4)	2.07 (0.7-5.8)
0.91 (0.6-1.4)	1.37 (0.7-2.7)	1.90 (0.4-9.8)	0.86 (0.4-2.0)	0.32 (0.0-7.9)	1.70 (0.7-4.4)	0.98 (0.1-16.0)	1.20 (0.7-2.1)	2.54 (0.9-7.5)
0.57 (0.3-1.1)	0.86 (0.4-2.0)	1.19 (0.2-6.7)	0.54 (0.2-1.5)	0.20 (0.0-5.2)	1.07 (0.4-3.1)	0.62 (0.0-10.5)	0.75 (0.3-1.6)	1.59 (0.5-5.3)
0.25 (0.1-1.3)	0.38 (0.1-2.0)	0.53 (0.1-5.0)	0.24 (0.0-1.4)	0.09 (0.0-3.1)	0.47 (0.1-2.9)	0.27 (0.0-6.6)	0.33 (0.1-1.7)	0.70 (0.1-4.7)
0.45 (0.0-7.6)	0.68 (0.0-11.9)	0.94 (0.0-23.8)	0.42 (0.0-7.8)	0.16 (0.0-11.1)	0.84 (0.0-15.9)	0.48 (0.0-25.0)	0.59 (0.0-10.1)	1.25 (0.1-24.9)
0.54 (0.3-1.1)	0.82 (0.4-1.9)	1.14 (0.2-6.3)	0.51 (0.2-1.4)	0.19 (0.0-4.9)	1.02 (0.4-2.9)	0.59 (0.0-9.9)	0.71 (0.3-1.5)	1.52 (0.5-5.0)
1.08 (0.6-1.8)	1.63 (0.8-3.3)	2.27 (0.4-12.0)	1.02 (0.4-2.5)	0.39 (0.0-9.5)	2.02 (0.8-5.4)	1.17 (0.1-19.3)	1.42 (0.8-2.7)	3.02 (1.0-9.3)
0.77 (0.5-1.3)	1.16 (0.6-2.3)	1.61 (0.3-7.8)	0.72 (0.4-1.4)	0.27 (0.0-6.7)	1.43 (0.6-3.2)	0.83 (0.1-13.6)	1.01 (0.5-1.9)	2.14 (0.7-6.5)
0.76 (0.5-1.2)	1.14 (0.6-2.2)	1.59 (0.3-8.0)	0.71 (0.3-1.6)	0.27 (0.0-6.6)	1.42 (0.6-3.4)	0.82 (0.1-13.3)	1.00 (0.6-1.7)	2.11 (0.7-6.3)

b=twice daily; cer=certoparin; dal=dalteparin; dan=danaparoid; en=enoxaparin; fon=fondaparinux; UFH= unfractionated heparin; w=treated whole study period

venous thrombosis

2.12 (0.6-7.4)	1.35 (1.0-1.9)	1.10 (0.7-1.7)	1.75 (0.9-3.5)	3.97 (0.8-19.9)	2.24 (0.1-37.7)	1.84 (1.0-3.6)	0.92 (0.5-1.6)	1.30 (0.8-2.2)	1.32 (0.8-2.1)
1.40 (0.4-5.4)	0.89 (0.5-1.6)	0.73 (0.4-1.4)	1.16 (0.5-2.7)	2.63 (0.5-14.2)	1.48 (0.1-26.0)	1.22 (0.5-2.8)	0.61 (0.3-1.3)	0.86 (0.4-1.8)	0.87 (0.5-1.7)
1.01 (0.1-7.6)	0.64 (0.1-3.2)	0.53 (0.1-2.7)	0.84 (0.1-4.7)	1.90 (0.2-18.1)	1.07 (0.0-27.1)	0.88 (0.2-4.9)	0.44 (0.1-2.3)	0.62 (0.1-3.0)	0.63 (0.1-3.2)
2.24 (0.5-9.4)	1.43 (0.7-3.1)	1.17 (0.5-2.7)	1.86 (0.7-5.0)	4.21 (0.7-24.6)	2.37 (0.1-43.7)	1.95 (0.7-5.2)	0.98 (0.4-2.4)	1.38 (0.7-2.8)	1.40 (0.6-3.1)
5.93 (0.2-177.0)	3.78 (0.2-90.4)	3.08 (0.1-75.0)	4.91 (0.2-124.5)	11.13 (0.3-386.1)	6.26 (0.1-433.7)	5.16 (0.2-129.9)	2.59 (0.1-63.7)	3.65 (0.1-89.7)	3.70 (0.2-89.9)
1.13 (0.3-5.1)	0.72 (0.3-1.7)	0.59 (0.2-1.5)	0.94 (0.3-2.7)	2.12 (0.3-13.0)	1.20 (0.1-22.7)	0.98 (0.3-2.8)	0.49 (0.2-1.3)	0.70 (0.3-1.6)	0.71 (0.3-1.7)
1.95 (0.1-40.0)	1.25 (0.1-19.8)	1.02 (0.1-16.5)	1.62 (0.1-27.5)	3.67 (0.2-88.9)	2.06 (0.0-106.4)	1.70 (0.1-28.7)	0.85 (0.1-14.0)	1.20 (0.1-19.8)	1.22 (0.1-19.8)
1.61 (0.5-5.6)	1.03 (0.7-1.4)	0.84 (0.5-1.5)	1.33 (0.6-2.9)	3.02 (0.6-15.7)	1.70 (0.1-29.2)	1.40 (0.7-2.9)	0.70 (0.4-1.3)	0.99 (0.5-1.8)	1.00 (0.6-1.8)
0.76 (0.2-2.8)	0.48 (0.2-1.4)	0.39 (0.1-1.2)	0.63 (0.2-2.1)	1.42 (0.2-9.4)	0.80 (0.0-16.0)	0.66 (0.2-2.2)	0.33 (0.1-1.0)	0.47 (0.2-1.4)	0.47 (0.2-1.4)
W en o	0.64 (0.2-2.3)	0.52 (0.1-2.0)	0.83 (0.2-3.4)	1.88 (0.2-14.4)	1.06 (0.0-23.2)	0.87 (0.2-3.6)	0.44 (0.1-1.7)	0.62 (0.2-2.4)	0.62 (0.2-2.4)
1.57 (0.4-5.6)	**I fon o**	0.82 (0.5-1.4)	1.30 (0.6-2.8)	2.94 (0.6-15.3)	1.66 (0.1-28.5)	1.36 (0.7-2.8)	0.68 (0.4-1.3)	0.97 (0.5-1.8)	0.98 (0.6-1.7)
1.92 (0.5-7.3)	1.23 (0.7-2.2)	**I nad b**	1.59 (0.9-2.9)	3.61 (0.8-16.9)	2.03 (0.1-33.5)	1.67 (0.7-3.7)	0.84 (0.4-1.7)	1.19 (0.6-2.4)	1.20 (0.6-2.3)
1.21 (0.3-5.0)	0.77 (0.4-1.6)	0.63 (0.3-1.1)	**I nad o**	2.27 (0.4-11.9)	1.28 (0.1-19.8)	1.05 (0.4-2.7)	0.53 (0.2-1.3)	0.74 (0.3-1.8)	0.75 (0.3-1.7)
0.53 (0.1-4.1)	0.34 (0.1-1.8)	0.28 (0.1-1.3)	0.44 (0.1-2.3)	**W nad o**	0.56 (0.0-13.8)	0.46 (0.1-2.6)	0.23 (0.0-1.3)	0.33 (0.1-1.8)	0.33 (0.1-1.8)
0.95 (0.0-20.8)	0.60 (0.0-10.4)	0.49 (0.0-8.1)	0.78 (0.1-12.2)	1.78 (0.1-43.7)	**W pa o**	0.82 (0.0-15.0)	0.41 (0.0-7.3)	0.58 (0.0-10.3)	0.59 (0.0-10.3)
1.15 (0.3-4.7)	0.73 (0.4-1.5)	0.60 (0.3-1.3)	0.95 (0.4-2.5)	2.16 (0.4-12.3)	1.21 (0.1-22.1)	**I rev o**	0.50 (0.2-1.2)	0.71 (0.3-1.6)	0.72 (0.3-1.6)
2.29 (0.6-8.9)	1.46 (0.8-2.7)	1.19 (0.6-2.4)	1.90 (0.8-4.5)	4.30 (0.8-23.5)	2.42 (0.1-42.9)	1.99 (0.9-4.6)	**I rev b**	1.41 (0.7-3.0)	1.43 (0.7-2.9)
1.62 (0.4-6.3)	1.03 (0.6-1.9)	0.84 (0.4-1.7)	1.34 (0.6-3.2)	3.05 (0.6-16.6)	1.71 (0.1-30.3)	1.41 (0.6-3.3)	0.71 (0.3-1.5)	**I tin o**	1.01 (0.6-1.7)
1.60 (0.4-6.0)	1.02 (0.6-1.8)	0.83 (0.4-1.6)	1.33 (0.6-3.0)	3.01 (0.6-16.1)	1.69 (0.1-29.6)	1.39 (0.6-3.1)	0.70 (0.3-1.4)	0.99 (0.6-1.7)	**W tin o**

i= treated initial period; nad= nadroparin; o=once daily; pa=parnaparin; rev=reviparin; tin= tinzaparin;

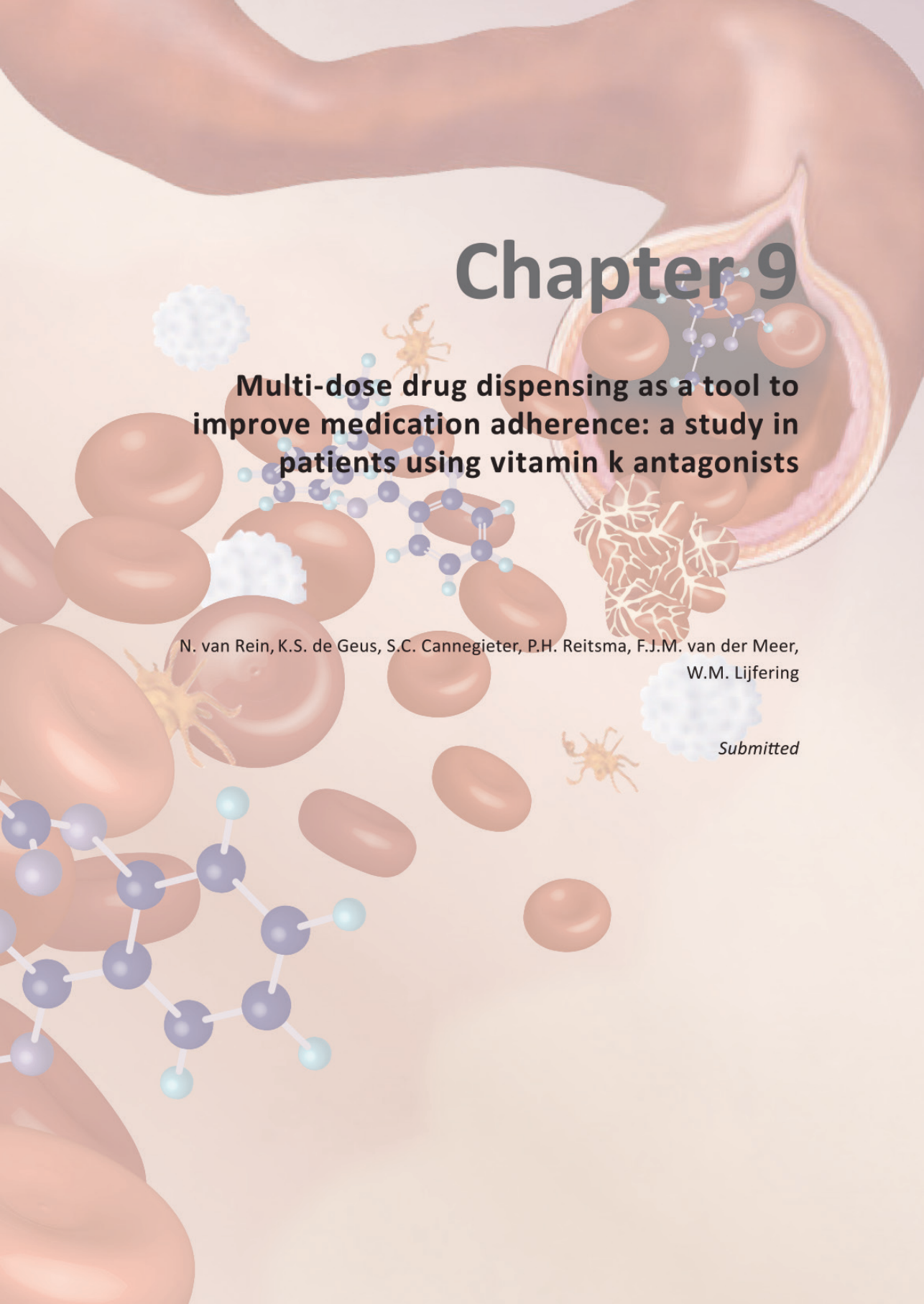
Table 4. Association of LMWH, stratified by duration and dosing schedule, with major bleeding

UFH	1.61 (0.7-3.6)	1.81 (0.2-15.4)	2.44 (0.9-7.9)	1.07 (0.0-52.2)	1.54 (0.4-6.1)	2.10 (0.2-22.6)	1.05 (0.6-1.8)	0.93 (0.1-6.5)
0.62 (0.3-1.4)	**I cer b**	1.13 (0.1-11.0)	1.52 (0.4-6.3)	0.67 (0.0-35.2)	0.96 (0.2-4.7)	1.31 (0.1-16.0)	0.65 (0.3-1.7)	0.58 (0.1-4.7)
0.55 (0.1-4.7)	0.89 (0.1-8.7)	**I dal b**	1.35 (0.2-9.6)	0.59 (0.0-49.9)	0.85 (0.1-6.9)	1.16 (0.0-28.3)	0.58 (0.1-5.3)	0.20 (0.0-9.2)
0.41 (0.1-1.3)	0.66 (0.2-2.7)	0.74 (0.1-5.3)	**I dal o**	0.44 (0.0-25.5)	0.63 (0.3-1.3)	0.86 (0.1-12.2)	0.43 (0.1-1.6)	0.38 (0.0-3.7)
0.93 (0.0-45.5)	1.50 (0.0-79.2)	1.69 (0.0-142.6)	2.28 (0.0-132.0)	**W dal b**	1.44 (0.0-88.6)	1.96 (0.0-186.2)	0.98 (0.0-49.7)	0.87 (0.0-66.9)
0.65 (0.2-2.6)	1.04 (0.2-5.1)	1.18 (0.1-9.5)	1.58 (0.8-3.2)	0.70 (0.0-42.8)	**W dal o**	1.36 (0.1-21.1)	0.68 (0.2-3.0)	0.61 (0.1-6.5)
0.48 (0.0-5.1)	0.77 (0.1-9.4)	0.86 (0.0-21.0)	1.16 (0.1-16.4)	0.51 (0.0-48.5)	0.73 (0.0-11.4)	**I dan b**	0.50 (0.0-5.7)	0.44 (0.0-9.5)
0.95 (0.6-1.6)	1.53 (0.6-4.0)	1.72 (0.2-15.6)	2.32 (0.6-8.4)	1.02 (0.0-51.5)	1.46 (0.3-6.4)	2.00 (0.2-22.8)	**I en b**	0.89 (0.1-6.3)
1.07 (0.2-7.4)	1.72 (0.2-14.0)	1.94 (0.1-34.8)	2.62 (0.3-25.2)	1.15 (0.0-88.3)	1.65 (0.2-17.7)	2.25 (0.1-48.2)	1.13 (0.2-8.1)	**I en o**
0.84 (0.2-3.1)	1.36 (0.3-6.2)	1.53 (0.1-18.5)	2.06 (0.4-11.8)	0.90 (0.0-54.2)	1.30 (0.2-8.5)	1.77 (0.1-26.4)	0.89 (0.2-3.2)	0.79 (0.1-4.8)
1.02 (0.6-1.9)	1.65 (0.6-4.4)	1.85 (0.2-17.1)	2.50 (0.7-9.3)	1.10 (0.0-55.9)	1.58 (0.4-7.0)	2.15 (0.2-24.9)	1.08 (0.6-2.0)	0.95 (0.1-7.1)
0.49 (0.2-1.3)	0.78 (0.2-2.8)	0.88 (0.1-9.3)	1.19 (0.3-5.5)	0.52 (0.0-28.7)	0.75 (0.1-4.0)	1.02 (0.1-13.3)	0.51 (0.2-1.6)	0.45 (0.1-4.0)
0.46 (0.1-1.8)	0.74 (0.2-3.6)	0.83 (0.1-10.5)	1.12 (0.2-6.8)	0.49 (0.0-30.2)	0.71 (0.1-4.9)	0.96 (0.1-14.9)	0.48 (0.1-2.1)	0.43 (0.0-4.6)
1.42 (0.1-39.8)	2.28 (0.1-70.2)	2.57 (0.0-134.8)	3.46 (0.1-118.7)	1.52 (0.0-254.2)	2.18 (0.1-80.4)	2.98 (0.0-178.4)	1.49 (0.1-43.7)	1.32 (0.0-62.5)
1.08 (0.0-34.4)	1.74 (0.1-60.5)	1.96 (0.0-114.4)	2.64 (0.1-102.0)	1.16 (0.0-210.8)	1.67 (0.0-68.9)	2.28 (0.0-150.9)	1.14 (0.0-37.8)	1.01 (0.0-53.2)
0.50 (0.0-5.5)	0.81 (0.1-10.1)	0.91 (0.0-22.5)	1.22 (0.1-17.6)	0.54 (0.0-51.6)	0.77 (0.0-12.2)	1.05 (0.0-30.7)	0.53 (0.0-6.2)	0.47 (0.0-10.2)
1.25 (0.5-3.1)	2.01 (0.6-6.8)	2.27 (0.2-23.3)	3.06 (0.7-13.6)	1.34 (0.0-72.8)	1.93 (0.4-10.1)	2.63 (0.2-33.6)	1.32 (0.5-3.8)	1.17 (0.1-10.0)
0.69 (0.3-1.6)	1.11 (0.3-3.6)	1.25 (0.1-11.8)	1.68 (0.4-6.3)	0.74 (0.0-39.6)	1.06 (0.2-4.8)	1.45 (0.1-18.2)	0.73 (0.3-2.0)	0.64 (0.1-5.4)
0.69 (0.4-1.2)	1.11 (0.4-2.9)	1.25 (0.1-11.2)	1.68 (0.5-6.0)	0.74 (0.01-37.4)	1.06 (0.2-4.6)	1.45 (0.1-16.6)	0.73 (0.3-1.6)	0.64 (0.1-4.8)

b=twice daily; cer=certoparin; dal=dalteparin; dan=danaparoid; en=enoxaparin; fon=fondaparinux; UFH= unfractionated heparin; w=treated whole study period

1.19 (0.3-4.3)	0.98 (0.5-1.8)	2.05 (0.8-5.4)	2.18 (0.6-8.6)	0.71 (0.0-19.8)	0.92 (0.0-29.3)	1.99 (0.2-21.9)	0.80 (0.3-2.0)	1.45 (0.6-3.5)	1.45 (0.8-2.5)
0.74 (0.2-3.3)	0.61 (0.2-1.6)	1.28 (0.4-4.5)	1.36 (0.3-6.6)	0.44 (0.0-13.5)	0.57 (0.0-19.9)	1.24 (0.1-15.5)	0.50 (0.1-1.7)	0.90 (0.3-2.9)	0.90 (0.3-2.4)
0.65 (0.1-7.9)	0.54 (0.1-5.0)	1.13 (0.1-11.9)	1.20 (0.1-15.3)	0.39 (0.0-20.4)	0.51 (0.0-29.7)	1.10 (0.0-27.3)	0.44 (0.0-4.5)	0.80 (0.1-7.5)	0.80 (0.1-7.2)
0.49 (0.1-2.8)	0.40 (0.1-1.5)	0.84 (0.2-3.9)	0.89 (0.1-5.4)	0.29 (0.0-9.9)	0.38 (0.0-14.6)	0.82 (0.1-11.8)	0.33 (0.1-1.5)	0.59 (0.2-2.2)	0.59 (0.2-2.1)
1.11 (0.0-66.3)	0.91 (0.0-46.5)	1.92 (0.0-105.3)	2.04 (0.0-125.5)	0.66 (0.0-110.2)	0.86 (0.0-156.4)	1.86 (0.0-178.9)	0.75 (0.0-40.4)	1.35 (0.0-72.5)	1.35 (0.0-68.6)
0.77 (0.1-5.0)	0.63 (0.1-2.8)	1.33 (0.2-7.2)	1.42 (0.2-9.9)	0.46 (0.0-16.8)	0.60 (0.0-24.7)	1.29 (0.1-20.5)	0.52 (0.1-2.7)	0.94 (0.2-4.2)	0.94 (0.2-4.0)
0.56 (0.0-8.4)	0.47 (0.0-5.4)	0.98 (0.1-12.7)	1.04 (0.1-16.1)	0.34 (0.0-20.1)	0.44 (0.0-29.1)	0.95 (0.0-27.7)	0.38 (0.0-4.9)	0.69 (0.1-8.6)	0.69 (0.1-7.9)
1.13 (0.3-4.1)	0.93 (0.5-1.7)	1.95 (0.6-5.9)	2.07 (0.5-9.0)	0.67 (0.0-19.6)	0.88 (0.0-29.0)	1.90 (0.2-22.1)	0.76 (0.3-2.2)	1.38 (0.5-3.8)	1.38 (0.6-3.0)
1.27 (0.2-7.7)	1.05 (0.1-7.8)	2.20 (0.3-19.3)	2.34 (0.2-25.1)	0.76 (0.0-35.8)	0.99 (0.0-52.1)	2.14 (0.1-46.6)	0.86 (0.1-7.3)	1.55 (0.2-13.0)	1.55 (0.2-11.7)
W en o	0.82 (0.2-3.3)	1.73 (0.3-8.7)	1.84 (0.3-12.1)	0.60 (0.0-21.2)	0.78 (0.0-31.2)	1.68 (0.1-25.5)	0.67 (0.1-3.3)	1.22 (0.3-5.8)	1.22 (0.3-5.0)
1.21 (0.3-4.8)	**I fon o**	2.10 (0.7-6.6)	2.23 (0.5-10.0)	0.72 (0.0-21.4)	0.94 (0.0-31.6)	2.04 (0.2-24.1)	0.82 (0.3-2.5)	1.48 (0.5-4.3)	1.48 (0.7-3.3)
0.58 (0.1-2.9)	0.48 (0.2-1.5)	**I nad b**	1.06 (0.3-3.5)	0.34 (0.0-8.3)	0.45 (0.0-13.4)	0.97 (0.1-12.9)	0.39 (0.1-1.5)	0.71 (0.2-2.6)	0.71 (0.2-2.2)
0.54 (0.1-3.6)	0.45 (0.1-2.0)	0.94 (0.3-3.1)	**I nad o**	0.32 (0.0-9.8)	0.42 (0.0-10.1)	0.91 (0.1-14.4)	0.37 (0.1-1.9)	0.66 (0.1-3.4)	0.66 (0.2-2.9)
1.68 (0.0-59.9)	1.38 (0.0-41.0)	2.91 (0.1-70.5)	3.09 (0.1-93.4)	**W nad** o	1.31 (0.0-137.8)	2.83 (0.0-171.6)	1.13 (0.0-36.0)	2.05 (0.1-64.4)	2.06 (0.1-60.4)
1.28 (0.0-51.4)	1.06 (0.0-35.4)	2.22 (0.1-66.3)	2.37 (0.1-56.6)	0.76 (0.0-80.6)	**W pa o**	2.16 (0.0-145.1)	0.87 (0.0-31.0)	1.57 (0.0-55.5)	1.57 (0.0-52.2)
0.59 (0.0-9.0)	0.49 (0.0-5.8)	1.03 (0.1-13.7)	1.09 (0.1-17.3)	0.35 (0.0-21.5)	0.46 (0.0-31.1)	**I rev o**	0.40 (0.0-5.2)	0.73 (0.1-9.3)	0.73 (0.1-8.5)
1.48 (0.3-7.2)	1.22 (0.4-3.7)	2.57 (0.7-9.8)	2.73 (0.5-14.3)	0.88 (0.0-28.1)	1.16 (0.0-41.4)	2.50 (0.2-32.6)	**I rev b**	1.82 (0.5-6.4)	1.82 (0.6-5.3)
0.82 (0.2-3.9)	0.67 (0.2-1.9)	1.42 (0.4-5.2)	1.51 (0.3-7.6)	0.49 (0.0-15.3)	0.64 (0.0-22.5)	1.38 (0.1-17.6)	0.55 (0.2-2.0)	**I tin o**	1.00 (0.4-2.5)
0.82 (0.2-3.3)	0.67 (0.3-1.5)	1.41 (0.5-4.3)	1.50 (0.3-6.6)	0.49 (0.0-14.3)	0.64 (0.0-21.1)	1.37 (0.1-16.1)	0.55 (0.2-1.6)	1.00 (0.4-2.5)	**W tin o**

i= treated initial period; nad= nadroparin; o=once daily; pa=parnaparin; rev=reviparin; tin= tinzaparin;



Chapter 9

Multi-dose drug dispensing as a tool to improve medication adherence: a study in patients using vitamin k antagonists

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Submitted

ABSTRACT

Background: Multidose drug dispensing (MDD) is a dosing aid that provides patients with disposable bags containing all drugs intended for one dosing moment. MDD is believed to increase medication adherence, but studies are based on self-reported data and results may depend on socially desirable answers.

Objectives: We aimed to determine the effect of MDD on medication adherence in non-compliant patients taking vitamin K antagonists (VKAs), and to compare with instructing patients on medication use.

Methods: VKA patients are regularly monitored and the time in therapeutic range (TTR) reflects the stability of treatment and was selected as measure for adherence. Non-compliant patients (TTR <75% six months prior to MDD) were included and the TTRs before and after start of MDD were compared within patients to estimate the change in adherence. Non-compliant patients usually receive letters or calls from nurses from the anticoagulation clinic to improve adherence. To analyze whether standard care compares to MDD, non-compliant patients without MDD were also followed to estimate their TTR change over time.

Results: 83 Non-compliant VKA patients started using MDD. The TTR increased by 13% (95%CI 6%-21%) within one month after starting MDD and remained stable during the next five months. The TTR of MDD-patients increased 10% (95%CI 2%-19%) more as compared with non-MDD patients within one month, but was similar after four months (TTR difference 3%, 95%CI -2%-9%). To conclude, MDD was associated with improved adherence.

Conclusions: Compared with instructing patients, MDD led to better adherence within one month but was associated with similar improvement after four months.

INTRODUCTION

Vitamin K antagonists are drugs used to treat and prevent thromboembolism.[1] The treatment of patients on vitamin K antagonists in the Netherlands is monitored on a regular basis by anticoagulation clinics. There the international normalized ratio (INR) is measured and, if necessary, dose adjustments are made based on the measured INR, the INR target range and the previous dosage.[2]

A measure that reflects how much time INRs of a patient are in therapeutic range is the time in therapeutic range (TTR). The TTR is calculated by interpolation between consecutive INRs[3] and is on average 75% in patients on long term anticoagulant treatment in the Netherlands.[4] A low TTR is associated with increased thromboembolic and bleeding risks[5] and can be due to low adherence or other mistakes with medication intake (for example because patients are not able to regulate their medication themselves).

Multi-dose drug dispensing (MDD) is a dosing aid that provides patients with robot-dispensed unit doses. All drugs that should be taken at the same moment are gathered in a disposable bag and labeled with the date and time for intake.[6] These are especially useful when patients are not able to regulate their medications or in case of low adherence. A cross-sectional study showed that MDD was associated with better medication adherence.[7] However, the improved medication adherence was self-reported and may also be due to socially desirable answers.

The aim of this study was to determine whether MDD initiation improves subsequent medication adherence in patients with low adherence. Second, we examined whether MDD is equally effective as routinely instructing patients by phone or letters from an anticoagulation clinic.

METHODS

Study population

The study base consisted of patients who were treated with vitamin K antagonists at the Leiden Anticoagulation Clinic. Patients were included for whom MDD was started between March 2012 and November 2013. MDD initiation and date of initiation is automatically registered in an electronic system of the anticoagulation clinic for all patients. MDD was started by the general practitioner or pharmacists for reasons that are not known by the anticoagulation clinic. As we were interested in improving medication adherence in non-compliant patients, we only included patients with a TTR $\leq$ 75% (i.e. a surrogate for low adherence) six months prior to start MDD. Furthermore, patients were only included if they started with started MDD after having at least one year of treatment with vitamin K antagonists. At the anticoagulation clinic, INRs are measured every 1 to 6 weeks in patients who are treated with vitamin K antagonists.

After determining the INR, a new appointment is scheduled and the frequency of these appointments depends on the measured INR and the stability of the INR values during previous appointments. Together with the venepuncture, a standard short history is obtained regarding, amongst others, change in co-medications.[2]

Patients who moved to a nursing home were excluded from the study because of uncertainty as to whether studied effects could be due to better caretaking at the nursing home or to MDD.

Data collection and outcomes

Patients' characteristics and outcomes were collected from the computerized patient records at the Leiden Anticoagulation Clinic. Data variables needed for the present study include age at initiation of MDD, sex, main indication for therapy with vitamin K antagonists, type of vitamin K antagonist (acenocoumarol or phenprocoumon), INR target range (2.5 to 3.5 or 3.0 to 4.0), and concomitant drug use at baseline, and during follow-up.

Two treatment periods were examined: six months before starting MDD (referred to as the 'before MDD' period) and six months after starting MDD (referred to as the MDD period). The TTR was used as a proxy for adherence to the treatment with vitamin K antagonists and was calculated using the method described by Rosendaal *et al*.[8] The outcome was the TTR during the MDD period, but also the difference in TTR after initiating MDD as compared with before MDD. Neither informed consent nor approval by a medical ethics committee is, according to Dutch law, required for studies in which data are collected from the records by a member of the treatment team.

Comparing treatment strategies

Patients who do not adhere to VKA treatment and have a non-therapeutic INR level routinely receive calls and letters from the nurses and doctors of the anticoagulation clinic. The calls and letters are used to create awareness among patients of the importance of adhering to the treatment. To compare the two strategies (i.e. MDD versus this standard treatment strategy), MDD patients were matched to 1 to 6 similar patients from the Leiden Anticoagulation Clinic who did not start MDD. Patients were matched on age (plus or minus 5 years), sex, main indication for vitamin K antagonists, duration of treatment with vitamin K antagonists and TTR (plus or minus 10%) of the patients during the six months prior to the MDD date of the match. As concurrent use of other medications could indicate that a patient is less able to adhere to medication or is less stable on vitamin K antagonists, we performed a sensitivity analysis where we additionally matched on use of other medication related to medication adherence (i.e. thiamine, antipsychotics, anxiolytics, antidepressants, antineoplastic agents, blood glucose lowering drugs, opioids and medicines to treat Alzheimer's disease).

Statistical analysis

The median (interquartile range [IQR]) time under, in and above therapeutic range was calculated for the standard treatment- as well as the MDD period. Also, the mean TTR difference and 95% confidence intervals (CIs) between the MDD- and standard treatment period were calculated. The MDD period was divided into several time frames, from MDD until 1, 2, 3, 4, 5, and 6 months after start of MDD. Additionally, the percentage of patients that was under, in or above therapeutic range was computed for every day and depicted per day with the MDD start date as a reference day (day 0). A mixed model with an unstructured covariance matrix was used to compare the instruction strategy with the MDD strategy.

RESULTS

From 302 patients who started MDD between March 2012 and October 2013, 83 moved to a nursing home, 1 had an uncertain MDD starting date and 135 had a TTR > 75%, leaving 83 patients for the analyses (Supplementary Figure 1). The mean age was 83 years (standard deviation [SD] 7) and 34 (41%) were male (Table 1). There were 77 patients (93%) who used phenprocoumon and the indication for treatment with vitamin K antagonists was atrial fibrillation in 63 (76%) patients. The median treatment duration with vitamin K antagonists before starting MDD was 5 years (IQR 3 to 10).

The median TTR before starting MDD was 63% (IQR 54 to 91) during the 6 months before MDD and was 73% (IQR 59 to 91) during the 6 months after starting MDD (Table 2). After initiating MDD, the percentage of patients in therapeutic range increased (Figure 1). On an individual basis, TTRs increased in 66 (80%) patients (Supplementary Figure 2). Additionally, the TTR increase within a person was on average between 13-16% after starting MDD (Table 3).

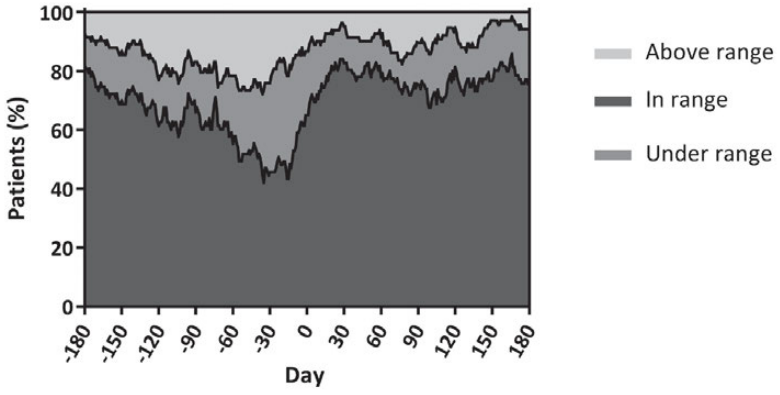


Figure 1. TTR (%) per day with day 0 as the date of starting MDD

Table 1. General characteristics*

	MDD patients	Matched MDD patients	Matched non-MDD patients
General characteristics			
Patients	83 (100)	63 (100)	333 (100)
Men	34 (41)	24 (38)	118 (30)
Age, mean (SD)	83 (7)	84 (7)	84 (7)
INR target range			
2.5-3.5	68 (82)	55 (87)	307 (92)
3.0-4.0	15 (18)	8 (13)	26 (8)
Treatment indication			
Atrial fibrillation	63 (76)	53 (84)	302 (91)
Venous thrombosis	3 (4)	1 (2)	4 (1)
Mechanical heart valves	2 (2)	1 (2)	2 (1)
Ischemic hearts disease	5 (6)	3 (5)	7 (2)
Peripheral arterial disease	7 (8)	5 (8)	19 (6)
Other	7 (8)	1 (2)	1 (0)
Vitamin K antagonist			
Phenprocoumon	77 (93)	61 (97)	326 (98)
Acenocoumarol	6 (7)	2 (3)	7 (2)
Medication			
Thiamine	4 (5)	3 (5)	5 (2)
Anxiolytic	5 (6)	2 (3)	20 (6)
Antidepressants	6 (7)	6 (10)	45 (14)
Antipsychotics	3 (4)	2 (3)	12 (4)
Anti-dementia drugs	0 (0)	0 (0)	6 (2)
Antineoplastic drugs	3 (4)	3 (5)	10 (3)
Opioids	10 (12)	9 (14)	20 (6)
Blood glucose lowering drugs	26 (31)	20 (32)	72 (22)

* Values are n (%) unless otherwise indicated. INR International Normalized Ratio; MDD Multidose drug dispensing; SD Standard Deviation

Matches were found in 63 (78%) of 83 patients who started MDD (Table 1) and for 43 (51%) patients in the sensitivity analyses of concurrent medication use. In total, 63 patients who started MDD were compared to 333 patients who received standard care in order to examine whether MDD was equally effective as instructing patients by the anticoagulation clinic. The analyses showed that patients who used MDD had a 10% (95% CI 2% to 19%) higher TTR increase as compared to patients who received standard care after one month (Table 4). At four months after starting MDD the TTR

Table 2. Median TTR (%) of all MDD patients before and after MDD

	Time under therapeutic range, median (IQR)	Time in therapeutic range, median (IQR)	Time above therapeutic range, median (IQR)
Before MDD	24 (0 to 30)	63 (54 to 69)	19 (0 to 24)
1 month after MDD	18 (0 to 28)	68 (58 to 74)	14 (0 to 14)
2 months after MDD	18 (0 to 31)	71 (55 to 76)	11 (0 to 16)
3 months after MDD	17 (0 to 29)	71 (58 to 81)	12 (0 to 19)
4 months after MDD	17 (0 to 25)	71 (57 to 85)	12 (0 to 19)
5 months after MDD	17 (0 to 28)	72 (57 to 89)	11 (0 to 16)
6 months after MDD	17 (0 to 24)	73 (57 to 91)	10 (0 to 16)

MDD Multidose drug dispensing; TTR time in therapeutic range

Table 3. Mean difference of TTR (%) within patients after MDD compared with before MDD by paired T test

	Time under therapeutic range mean difference (95%CI)	Time in therapeutic range mean difference (95%CI)	Time above therapeutic range mean difference (95%CI)
Before MDD	reference	reference	reference
1 month after MDD	-6 (-30 to 18)	13 (6 to 21)	-8 (-14 to -2)
2 months after MDD	-7 (-13 to -1)	16 (10 to 22)	-9 (-14 to -4)
3 months after MDD	-8 (-13 to -3)	15 (10 to 21)	-7 (-12 to -2)
4 months after MDD	-7 (-12 to -2)	14 (9 to 19)	-7 (-12 to -2)
5 months after MDD	-7 (-11 to -2)	13 (9 to 18)	-7 (-11 to -2)
6 months after MDD	-6 (-11 to -2)	14 (10 to 18)	-8 (-12 to -3)

MDD Multidose drug dispensing; TTR time in therapeutic range

increase was 3% (95% CI -2% to 9%) higher in the MDD as compared to the standard care group. The sensitivity analysis, where patients were also matched on co-medication, gave similar results (Table 4).

DISCUSSION

Results of this study showed that incompliant patients starting MDD had an average TTR increase of 14% (95% CI 10% to 18%). This indicates that MDD improved medication adherence. To our knowledge, the association between MDD and better medication adherence has only been studied by Kwint *et al.*[7] Results of that study showed that MDD was associated with improved medication adherence as 91% of MDD patients

Table 4. TTR difference (%) of patients starting MDD versus patients receiving standard care

	TTR difference (95% CI)	Sensitivity analysis TTR difference (95% CI)
Before MDD	reference	reference
1 month after MDD	10 (2 to 19)	11 (-1 to 22)
2 months after MDD	8 (2 to 15)	7 (2 to 16)
3 months after MDD	5 (-1 to 11)	4 (-4 to 12)
4 months after MDD	3 (-2 to 9)	2 (-6 to 9)
5 months after MDD	3 (-2 to 7)	2 (-5 to 9)
6 months after MDD	3 (-2 to 7)	2 (-5 to 9)

MDD Multidose drug dispensing; TTR time in therapeutic range

reported being adherent to all medications as compared with 58% among non-MDD patients.[7] However, the study by Kwint *et al.* was cross sectional and the results were self-reported, which could have resulted in socially desirable answers and an overestimation of medication adherence. Our study adds an objective outcome (i.e. TTR) to measure medication adherence.

The second aim of this study was to compare MDD with standard care (i.e. calling patients and writing letters). The results showed that the MDD group had a 10% (95% CI 2% to 19%) higher TTR increase in the first month after MDD as compared with the instruction group. The higher TTR increase indicated that MDD improved adherence to vitamin K antagonists faster than standard care. However, the TTR increase was not different between the MDD- and instruction group four months after starting MDD. A possible explanation could be regression to the mean. Regression to the mean occurs when patients are selected according to their low (or high) value of a measurement and when that measurement shows intraindividual variability (e.g. blood pressure or in our case TTR). The value of that measurement will be higher (or lower respectively) on re-measurement due to variation in the circadian patterns, measurement or other biological mechanisms.[9] An alternate explanation for the similar TTRs after four months is that MDD and instructing patients were equally effective in improving adherence in the long-run. Nevertheless, MDD may also improve adherence to other medications than vitamin K antagonists as these medications are all provided in the MDD in unit disposed bags. Our study used standard care of the anticoagulation clinic as comparison group, while such care is probably absent for other medications. The effect we found is therefore likely to be stronger for other medications. A potential disadvantage of MDD may be that MDD (when not further controlled) can lead to an increased prevalence of overmedication due to uncritical renewal of prescription.[6,10,11] but this limitation is beyond the scope

of our study in which we wanted to quantify if MDD led to better adherence in vitamin K antagonist users.

A strength of this study is that patients were compared with themselves thereby eliminating possible fixed confounding factors such as sex or chronic diseases. A potential limitation is that the association of MDD with adherence of patients in nursing homes could not be studied, making the results not generalizable to patients in nursing homes.

In summary, MDD was associated with improved adherence in this study. Compared with instructing patients, MDD led to better adherence within one month but was associated with similar improvement after four months.

REFERENCES

1. Lansberg MG, O'Donnell MJ, Khatri P, et al. Antithrombotic and thrombolytic therapy for ischemic stroke: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest.* Feb 2012;141(2 Suppl):e601S-636S.
2. van der Meer FJ, Rosendaal FR, Vandenbroucke JP, Briet E. Bleeding complications in oral anticoagulant therapy. An analysis of risk factors. *Arch.Intern.Med.* 7/12/1993 1993;153(13):1557-1562.
3. Rosendaal FR, Cannegieter SC, van der Meer FJ, Briet E. A method to determine the optimal intensity of oral anticoagulant therapy. *Thrombosis and haemostasis.* Mar 1 1993;69(3):236-239.
4. *Medisch jaarverslag 2014 Nederlandse Trombosediensten 'Medical annual report Dutch Anticoagulation Clinics 2014'.* Federation of Dutch Anticoagulation Clinics;2014.
5. Linkins LA. Bleeding risks associated with vitamin K antagonists. *Blood Rev.* 5/2013 2013;27(3):111-118.
6. Johnell K, Fastbom J. Multi-dose drug dispensing and inappropriate drug use: A nationwide register-based study of over 700,000 elderly. *Scandinavian journal of primary health care.* 2008;26(2):86-91.
7. Kwint HF, Stolk G, Faber A, Gussekloo J, Bouvy ML. Medication adherence and knowledge of older patients with and without multidose drug dispensing. *Age and ageing.* Sep 2013;42(5):620-626.
8. Rosendaal FR, Cannegieter SC, van der Meer FJ, Briet E. A method to determine the optimal intensity of oral anticoagulant therapy. *Thromb.Haemost.* 3/1/1993 1993;69(3):236-239.
9. Grobbee DE HA. Clinical Epidemiology. Jones And Bartlett Publishers, Inc; 2008:135.
10. Belfrage B, Koldestam A, Sjoberg C, Wallerstedt SM. Prevalence of suboptimal drug treatment in patients with and without multidose drug dispensing--a cross-sectional study. *European journal of clinical pharmacology.* Jul 2014;70(7):867-872.
11. Sinnemaki J, Sihvo S, Isojarvi J, Blom M, Airaksinen M, Mantyla A. Automated dose dispensing service for primary healthcare patients: a systematic review. *Systematic reviews.* 2013;2:1.

APPENDIX 1

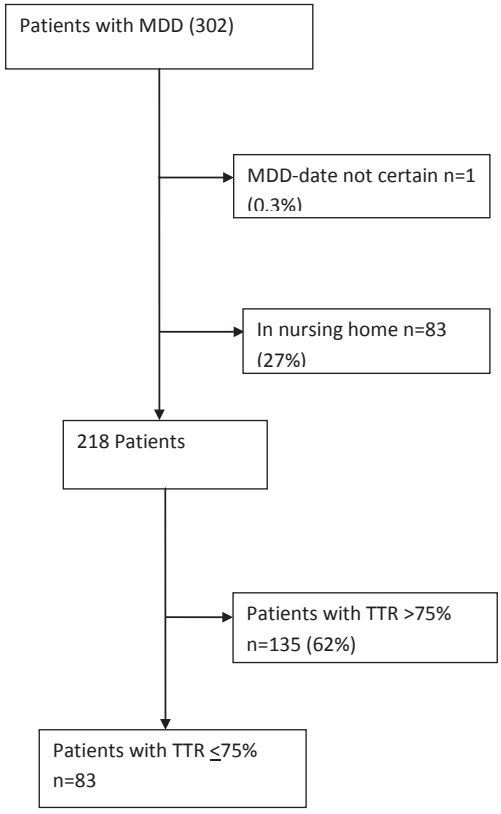


Figure 1. Flow chart of patients

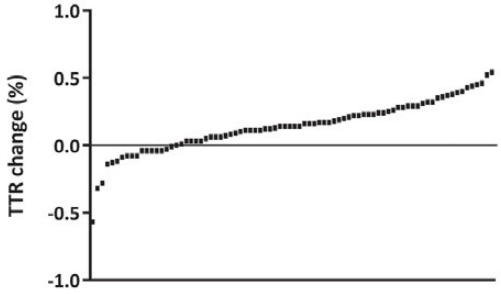
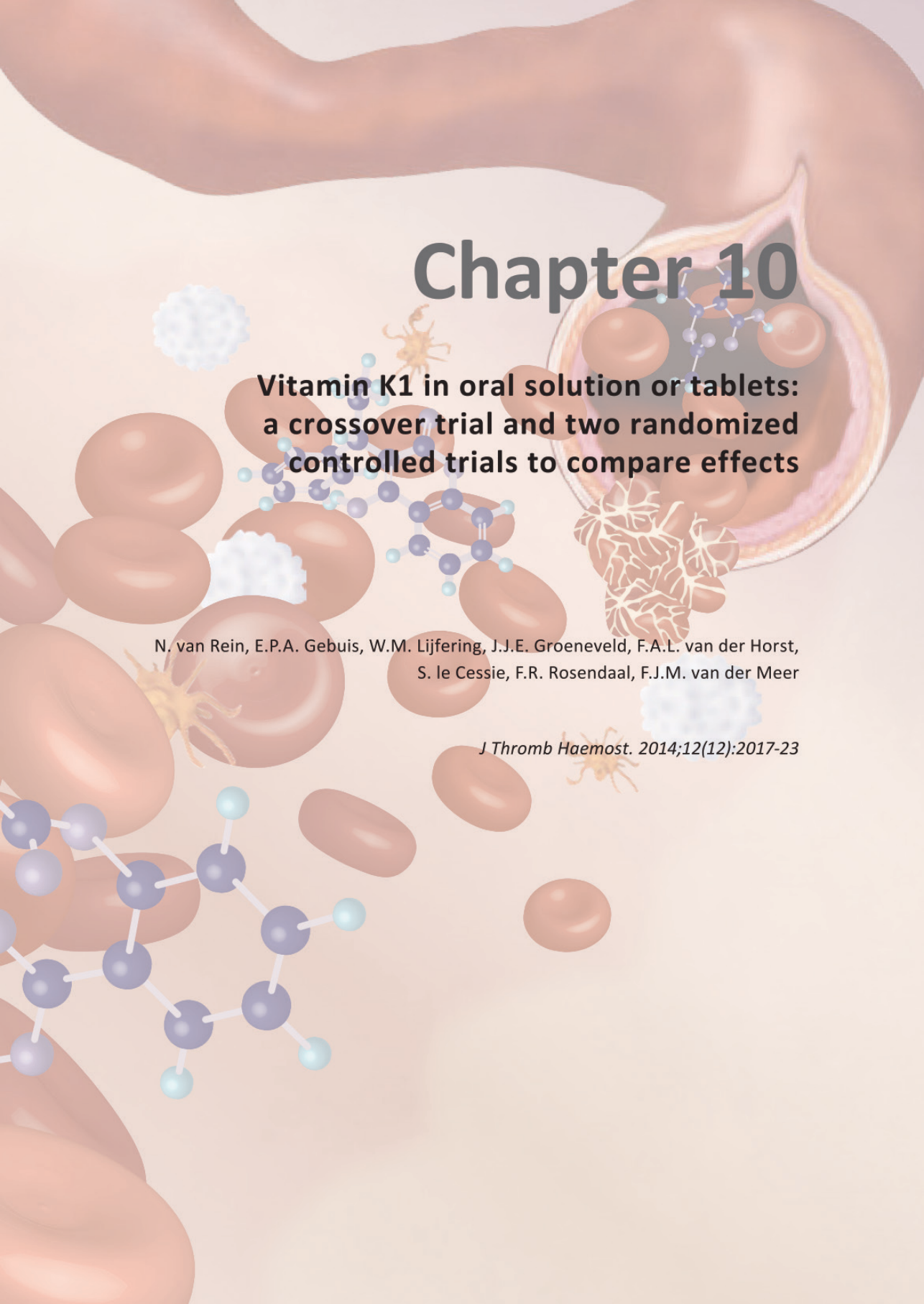


Figure 2. TTR change 6 months after MDD as compared with before MDD, where every line represents the result of a single patient



Chapter 10

Vitamin K1 in oral solution or tablets: a crossover trial and two randomized controlled trials to compare effects

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J Thromb Haemost. 2014;12(12):2017-23

ABSTRACT

Background: Vitamin K1 (VK1) reverses the effects of vitamin K antagonists (VKAs). The literature shows that the bioavailability from solutions might be higher than that from tablets, possibly resulting in different effects.

Objectives: To compare the bioavailability and effect on the International Normalized Ratio (INR) of 5-mg VK1 tablets and solution in three randomized clinical trials.

Methods and results: The bioavailability was determined in a crossover trial with 25 healthy volunteers. VK1 plasma concentrations were assessed at 0, 2, 4, 5, 6, 8, 10 and 24 h, and the area under the curve was higher in the solution group than in the tablet group (mean difference 365 μg L^{-1} h, 95% confidence interval [CI] 230–501, P < 0.0001). In the other two trials, the effects of both formulations on the INR were measured at 0, 24 and 48 h. In the second trial, on 72 patients on phenprocoumon with planned invasive procedures, both formulations were similarly effective, because all patients reached an INR of < 2.0, which was the primary endpoint. In the last trial, on 72 patients on phenprocoumon with an INR of 7.0–11.0, the INR decreased slightly more in the solution group (4.7, 95% CI 4.3–5.1) than in the tablet group (4.2, 95% CI 3.8–4.6). The solution group had a 3.3-fold increased likelihood (95% CI 0.7–15.1) of reaching an INR of < 2.0 at 48 h. Additionally, the increases in VK1 concentrations were similar (tablets, 3.2 μg L^{-1}; solution, 3.4 μg L^{-1}; P = 0.99) after 24 h.

Conclusions: VK1 tablets are at least as clinically effective as the solution in countering VKAs.

INTRODUCTION

The vitamin K family comprises fat-soluble proteins, consisting of menaquinones (vitamin K2) and phylloquinone (vitamin K1 [VK1]).[1,2] In order for the vitamin K-dependent coagulation proteins to become biologically active, the glutamic acid residues are carboxylated to c-carboxyglutamic acid (Gla). In this carboxylation reaction, the stable hydroquinone form of VK1 is catalysed to the epoxide form.[2,3] The epoxide form of vitamin K is then converted to the quinone form, and this is followed by reduction to the hydroquinone by vitamin K epoxide reductase (VKOR). These transformations of vitamin K are also known as the vitamin K epoxide cycle.[4]

Vitamin K antagonists (VKAs) are anticoagulant drugs that inhibit the activity of VKOR and thereby block the recycling of vitamin K.[5] This results in fewer Gla residues and therefore reduced coagulation.[5] The three vitamin K antagonists that are mainly used are warfarin, phenprocoumon, and acenocoumarol, which differ mainly in half-life: 120 h for phenprocoumon, 40 h for warfarin, and 7 h for acenocoumarol.[6] VKAs have a narrow therapeutic window, and the dosage depends on interindividual and intraindividual factors. Therefore, the International Normalized Ratio (INR) is measured on a regular basis to allow adjustment of the dose of VKAs, thereby maximizing their effectiveness in preventing thrombosis and minimizing the risk of bleeding complications.[7,8] An increased INR indicates an increased bleeding risk, which can be decreased with VK1. Other indications for decreasing the INR with VK1 in VKA-treated patients are invasive procedures.[7]

In The Netherlands, the main VKAs used are phenprocoumon and acenocoumarol. VK1 is available as an oral solution in oil[9], but tablets have recently become available. An obvious advantage of tablets is that administration is easier, but it is not known whether tablets are as effective as the solution. As the absorption of VK1 is stimulated by fat[10,11], it is possible that the oily solution has higher bioavailability and is therefore more effective in decreasing the INR than tablets.

To investigate the bioavailability of VK1 in an oral solution and tablets, we performed a crossover trial in healthy volunteers. Additionally, to compare the clinical effects of tablet and oral solution formulations of VK1 on the INR in patients treated with phenprocoumon, we compared the two treatments in a trial in patients who had to undergo an invasive procedure and in a third trial in patients who had a high INR (between 7.0 and 11.0).

METHODS

Design and participants

Three studies were performed to determine the bioavailability and to compare the effects of tablets and the oral solution, all carried out with 5 mg of VK1. The VK1 solution and tablets were manufactured by Tiofarma (Oud-Beijerland, The Netherlands).

The shelf-life of the tablets was adjusted from 36 to 12 months after the completion of the study. We subsequently verified the vitamin K1 content, which showed a mean reduction of 9% after 36 months (0.80 mg per tablet, standard deviation [SD] 0.01 mg) as compared with 12 months (0.88 mg per tablet, SD 0.005 mg), and a 20% decrease as compared with the intended 1 mg. The solution and tablets were provided by the hospital pharmacy of the Leiden University Medical Center. The solution consisted of VK1 at a concentration of 1 mg mL^{-1} in arachis oil. The tablets contained 1 mg of VK1, and mainly consisted of cellulose and lactose.

The first study was a crossover trial with healthy volunteers aged ≥ 18 years, in which the bioavailability of both VK1 formulations was studied. The healthy volunteers were randomized by the hospital pharmacy to start with either the solution or tablets, and allocation concealment was ensured through a sealed envelope procedure. Also, VK1 was ingested in the presence of the researcher to ensure compliance.

The second and third studies were single-center, openlabel, randomized controlled trials. Randomization was performed by the hospital pharmacy, and allocation concealment was ensured through a sealed envelope procedure. Additionally, VK1 was ingested in the presence of the researcher in both studies to ensure compliance. We recruited patients aged ≥ 18 years who were treated with phenprocoumon at the Leiden Anticoagulation Clinic. Patients suffering from liver failure and/or on dialysis were excluded. For the second study, patients were included who were scheduled to undergo an invasive procedure for which an INR of < 2.0 was required. For the third study, patients with a high INR (between 7.0 and 11.0) to whom 5 mg of VK1 was prescribed were included.

The study was performed in accordance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice, and all participants gave written informed consent prior to participation in the study. The study was approved by the Medical Ethics Committee of the Leiden University Medical Center, Leiden, The Netherlands, and was registered at www.trialregister.nl (NTR3485).

Interventions and outcomes

Healthy volunteer study The bioavailability from both formulations was determined in 25 healthy volunteers. They took 5 mg of VK1 in tablets or the oral solution, and crossed over to the other formulation after a washout period of 2 weeks. Blood (5 mL) was taken just before ingestion of either tablets or the oral solution, and 2, 4, 5, 6, 8, 10 and 24 h after ingestion. Breakfast was postponed until half an hour after ingestion of VK1, and all volunteers adhered to a low-VK1 diet (no green vegetables, vitamin tablets with VK1, or juice with VK1) during the days when blood draws were performed. VK1 concentrations were determined by HPLC with fluorescence detection.[12]

The primary outcomes were VK1 concentration after 4 h and area under the curve (AUC) during 24 h. The mean difference and 95% confidence intervals (CIs) were calculated between tablets and the oral solution.

Invasive procedures study Seventy-two patients from the Leiden Anticoagulation Clinic were included, who used phenprocoumon and to whom, in accordance with the standard protocol, 5 mg of VK1 was prescribed for an invasive procedure. They were randomized to receive 5 mg of VK1 in tablets or in the oral solution. INRs were measured with CoaguChek XS (Roche Diagnostics, Basel, Switzerland) before ingestion, and 24 and 48 h after ingestion, either at home, at the anticoagulation clinic, or at the hospital.

The primary endpoint was an INR of < 2.0 after 48 h, and effectiveness was considered to be similar if the proportion achieving this differed by < 10% between groups. Additionally, the INR decreases after 48 h and 95% CIs were calculated. Also, a repeated measures model was used with the INR as outcome and with an unstructured covariance matrix. This method deals with missing variables, and also estimates whether the decrease in INRs over time was different among users of the oral solution and users of tablets. Although the study was not powered to evaluate bleeding episodes and venous thrombotic events, both were studied from VK1 ingestion until 7 days afterwards. Scoring was performed by specialized physicians from the anticoagulation clinic who were not aware of treatment allocation.

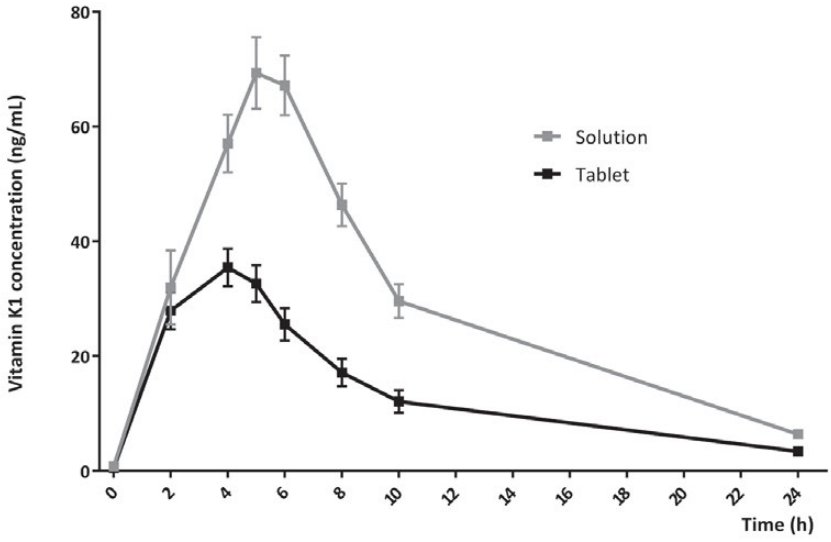


Figure 1. Mean concentrations with standard errors of means of vitamin K1 for the tablet and solution groups.

High-INR study Seventy-two patients from the Leiden Anticoagulation Clinic on phenprocoumon were recruited, with an INR between 7.0 and 11.0, to whom 5 mg of VK1 was prescribed to decrease the INR. They were enrolled on the day of the INR measurement, after which anticoagulant treatment was interrupted for at least 2 days. They were randomized to receive tablets or the oral solution. Blood was drawn by venepuncture (5 mL) to determine the concentration of VK1 in serum before ingestion of the drug. After 24 h, blood was drawn (10 mL) to determine the INR and the concentration of VK1 in serum. After 48 h, the INR was measured with CoaguChek XS (Roche Diagnostics), and phenprocoumon treatment was restarted. VK1 concentrations were determined by HPLC with fluorescence detection.[12]

The primary endpoint was the difference in INR (95% CIs) between 24 h and the first day. To ensure that INRs did not become too low, a secondary endpoint was defined as the percentage of INRs of < 2.0 after 24 h and the percentage of INRs of < 2.0 after 48 h for both groups. Another secondary outcome was the difference in VK1 concentration between 24 h after injection and just before ingestion tested with a Wilcoxon signed rank test. Also, a repeated measures model was used with, depending on the analysis, INR or VK1 concentrations as outcome and an unstructured covariance matrix. Bleeding episodes and venous thrombotic events were scored from the day of VK1 ingestion until 7 days after ingestion, although the study was not powered for these outcomes. Scoring was performed by specialized physicians from the anticoagulation clinic who were not aware of treatment allocation. To obtain insights into further decreases in INRs after the follow-up of the study (after 48 h), the first INR after the study was obtained from the patient records. Patients with a first INR of < 2.0 were classified as patients with a 'late low INR'. All statistical analyses were performed in R version 2.15.0 and SPSS version 20 (Chicago, IL, USA).

RESULTS

Healthy volunteer study

Between May 2013 and October 2013, 25 healthy volunteers were included in the study. Figure 1 shows the mean concentrations for tablets and the oral solution as a function of time. For both formulations, the peak values were seen 2–8 h after ingestion. The median VK1 concentrations after 4 h were 29 μg L^{-1} (interquartile range [IQR] 19–46) for tablets and 34 μg L^{-1} (IQR 20–95) for the oral solution, and the mean difference between tablets and the solution was 31 μg L^{-1} (95% CI 2–61). The median AUCs after 24 h were 241 μg L^{-1} h (IQR 180-390) for tablets and 584 μg L^{-1} h for the oral solution (IQR 491–771). The mean difference between tablets and the solution was 365 μg L^{-1} h (95% CI 230–501, P < 0.0001).

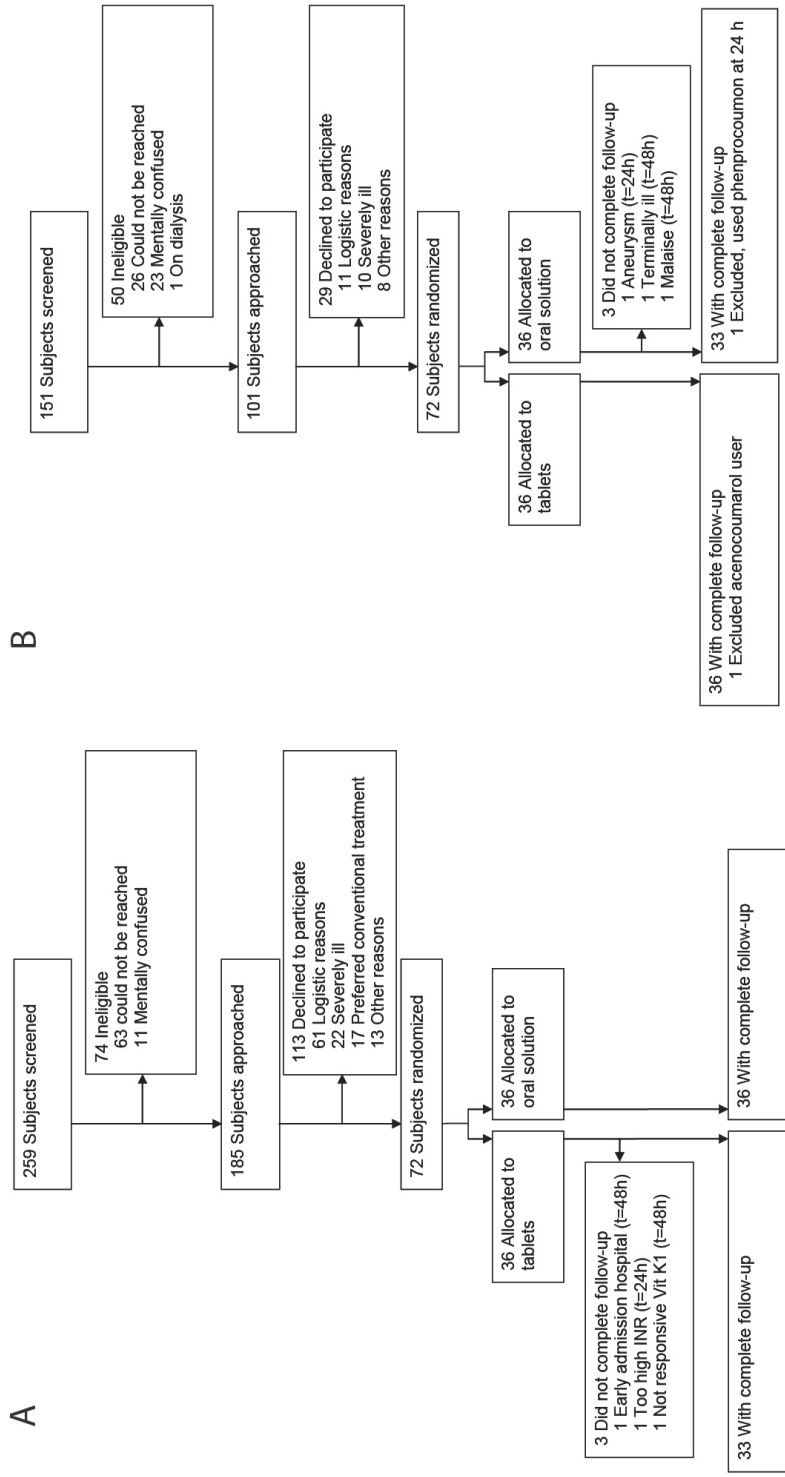


Figure 2. Flow chart of number of individuals included. (A) Invasive procedure study (B) High-INR study. VK1, vitamin K1.

Table 1. Characteristics cohort at baseline

	Healthy volunteers*		Invasive procedures		High INRs	
	solution	tablets	solution	tablets	solution	tablets
	25	25	36	36	35	35
Male sex	12 (48)	12 (48)	23 (64)	24 (67)	20 (57)	17 (49)
Age, years	34 (12)	34 (12)	72 (8)	74 (8)	77 (10)	78 (10)
INR at baseline	-	-	2.5 (0.6)	2.6 (0.5)	8.1 (0.8)	8.4 (1.0)
Indication VKA treatment						
Atrial fibrillation	-	-	23 (64)	28 (78)	25 (71)	27 (77)
Venous thrombosis	-	-	7 (19)	1 (3)	1 (3)	6 (17)
Other	-	-	6 (17)	7 (19)	9 (26)	2 (6)
Invasive procedure						
Minor skin excisions	-	-	10 (28)	12 (33)	-	-
Tooth extraction	-	-	9 (25)	7 (19)	-	-
Pain blockade	-	-	2 (6)	4 (11)	-	-
Biopsy (prostate, liver)	-	-	3 (8)	1 (3)	-	-
Gastro-and colonoscopy	-	-	3 (8)	3 (8)	-	-
Other	-	-	9 (25)	9 (25)	-	-

INR, International Normalized Ratio; VKA, vitamin K antagonist. *All healthy volunteers took the tablets and solution

Invasive procedures study

Between December 2011 and January 2013, 259 patients were screened for enrolment in the study. Sixty-three patients could not be reached on time, and 11 patients were ineligible because they were mentally confused. One hundred and eighty-five patients were asked to participate in the study, of whom 72 were enrolled (39%). Reasons for declining to participate are shown in Fig. 2A. Thirty-six patients were randomized to the tablet group and 36 to the oral solution group. Three patients on tablets did not complete the follow-up: one patient at 48 h, because of admission to hospital, one patient at 48 h, because he did not respond to VK1 and received an additional dose of 10 mg of VK1, and one patient because of a high INR at 24 h (INR 2.3), for which he received an additional 5 mg of VK1.

Patients were equally distributed in terms of age and sex (Table 1). The most frequently performed procedures were minor skin excisions (22 patients, 31%) and tooth extractions (16 patients, 22%). The mean INRs at baseline were 2.6 (SD 0.5) in the tablet group and 2.5 (SD 0.6) in the solution group. INRs of all patients for all time points are shown in Appendix 1 Fig. 1A. Median INRs for both study arms over time are shown in Fig. 3A. Table 2 shows the baseline INR and mean decrease in INR over time in

Table 2. INR decrease after VK1 ingestion invasive procedure and high INR group

	Mean INR at 0h (SD)	Mean INR decrease 0-24h (95% CI)	Mean INR decrease 24-48h (95% CI)
Invasive procedures			
Oral solution	2.5 (0.6)	0.9 (0.8-1.1)	0.2 (0.2-0.2)
Tablets	2.6 (0.5)	0.9 (0.7-1.0)	0.2 (0.2-0.3)
High INRs			
Oral Solution	8.1 (0.8)	4.7 (4.3-5.1)	0.6 (0.4-0.8)
Tablets	8.4 (1.0)	4.1 (3.6-4.6)	0.4 (0.1-0.8)

CI, confidence interval; SD, standard deviation.

both study arms. All participants (in both the tablet group and the oral solution group) had an INR of < 2.0 after 48 h, and all procedures were performed as scheduled. The INR decreases after 48 h were 43% (95% CI 40–46) in the tablet group and 43% (95% CI 40–46) in the solution group. The results did not change when a linear mixed model was used and missing values were taken into account (results not shown). Patients experienced no thrombotic and bleeding episodes from the day of VK1 ingestion until 7 days thereafter.

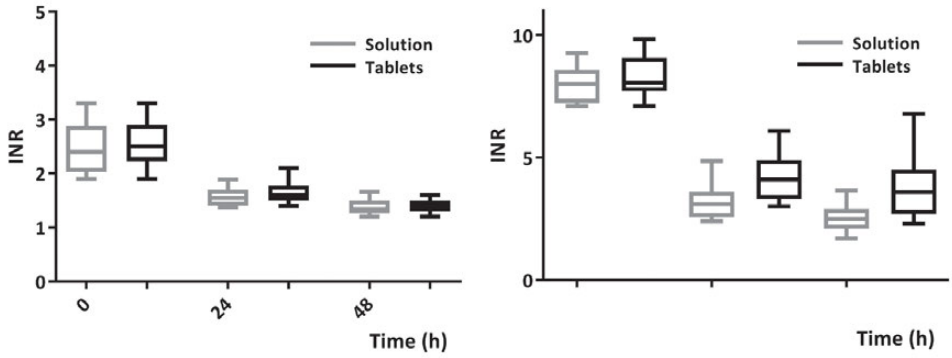


Figure 3. Median International Normalized Ratios (INRs) with the 25th and 75th percentiles (boxes) and the 10th to 90th percentiles (whiskers) over time for the tablet and solution groups. (A) Invasive procedure study (B) High-INR study.

High-INR study

Between December 2011 and April 2013, 151 patients were screened for enrolment in this study. Of these, 26 (17%) could not be reached on time, 23 (15%) were ineligible because they were mentally confused, and one (1%) was on dialysis. The remaining patients (n = 101) were asked to participate in the study, and of these, 72 (71%) agreed to take part. The reasons for declining to participate are shown in Fig. 2B. Seventy-two patients were randomized to the tablet group (n = 36) and the oral solution group (n = 36). Three patients from the solution group did not complete the study; one patient dropped out at 24 h because he was admitted to hospital; and two other patients dropped out at 48 h because of terminal illness and admittance to hospital. The VK1 concentrations of three patients at baseline (two on tablets and one on the solution) could not be determined, owing to technical errors. Two patients (one on tablets and one on the solution) were excluded from analysis because of protocol violations (that is, one acenocoumarol user was included and one patient did not stop using phenprocoumon during follow-up).

The mean age (78 years for the tablet group and 77 years for the solution group) and the proportion of males (49% for the tablet group and 57% for the solution group) were similar in both groups (Table 1). There were no signs or symptoms of active bleeding in any patient at the time of inclusion. The mean INRs at baseline were 8.4 (SD 1.0) in the tablet group and 8.1 (SD 0.8) the solution group. INRs of all patients at all time points are shown in

Appendix 1 Fig. 1B. Median INRs for both study arms and all time points are shown in Fig. 3B. The mean decreases in INRs after 24 h were 4.1 (95% CI 3.6–4.6) for the tablet group and 4.7 (95% CI 4.3–5.1) for the oral solution group, as shown in Table 2. There was one patient in the tablet group with an INR of < 2.0 (3%, 95% CI 0–8) and none in the solution group at 24 h. After 48 h, these numbers were two (6%, 95% CI 0–13) in the tablet group and six (19%, 95% CI 6–32) in the oral solution group (relative risk for an INR of < 2.0 at 48 h for solution vs. tablets: 3.3, 95% CI 0.7–15.1). The median increases in VK1 concentration after 24 h were 3.2 μg L^{-1} (IQR 1.7–5.2 μg L^{-1}) in the tablet group and 3.4 μg L^{-1} (IQR 2.0–4.8 μg L^{-1}) in the oral solution group (P = 0.99). The results did not change when a linear mixed model was used and missing values were taken into account (data not shown). During the 7-day follow-up, no patients experienced a thrombotic or major bleeding episode, and two patients experienced a minor bleeding episode (one in the tablet group and one in the solution group). The INRs after the study were measured, on average, 4 days after the study, and two patients from the solution group and three patients from the tablet group had a ‘late low INR’.

DISCUSSION

These studies show that the bioavailability from the VK1 oral solution was, on average, 365 µg L^{-1} h higher than that from tablets in healthy volunteers. Despite this difference in bioavailability, the decreases in INR for patients on phenprocoumon who had to undergo an invasive procedure were similar for both formulations. In the high-INR study, the INR decrease was slightly less in the tablet group than in the solution group (mean INR decrease 4.1 vs. 4.7, respectively), but satisfactory in both groups.

Despite the different compositions of the solution and tablets, peak concentrations of VK1 were found at similar time points for both formulations. Peak times were 2–8 h as compared with 4–6 h in a previous study.[10] This difference could be attributable to different VK1 sources (in our study, we used VK1 tablets and solution, whereas Gijsbers et al.[10] used spinach). However, in accordance with the study of Gijsbers *et al.*[10], this study shows that the use of a fatty substance such as the oily VK1 solution results in higher bioavailability of VK1.

The bioavailability from the solution was higher than that from tablets, but did not result in different clinical effects in the invasive procedure study. Here, no difference was found in INR decrease between the solution and tablet groups. Also, all INRs were < 2.0 at 48 h, and therefore the effects of both formulations can be considered to be similar, based on the prespecified primary endpoint. In the high-INR study, INRs decreased slightly more in the solution group than in the tablet group, and more patients had an INR of < 2.0 in the solution group after 48 h (relative risk for solution vs. tablets: 3.3, 95% CI 0.7–15.1). This difference in the numbers of patients with an INR of < 2.0 at 48 h could have been caused by patients with a 'relatively low' high INR at 0 h who received a too high dosage of VK1. However, this was not the case, as shown in Appendix 1 Fig. 1B: INRs at baseline of the patients with an INR of < 2.0 at 48 h were between 7.1 and 9.4, which are similar to the INRs of patients with an INR at 48 h of ≥ 2.0 (mean INR of 8.0 for the group with an INR of < 2.0 at 0 h as compared with a mean INR of 8.2 for the group with an INR of ≥ 2.0 at 0 h). This result suggests that the solution may have a stronger effect on INR reduction than tablets, which is in agreement with the higher bioavailability from the solution.

This is the first study to report the effect of 5 mg of VK1 on the INR in patients using phenprocoumon. We showed that the INR decreases most during the first 24 h after VK1 intake. Additionally, we showed that 5 mg of VK1 is effective and safe in patients who have either a too high INR or are scheduled for an invasive procedure. Considering the high number of patients with an INR of < 2.0 in the solution group of the high-INR study, a lower dose of VK1 solution may give a similar INR reduction and fewer patients with an INR of < 2.0. To our knowledge, no literature is available on the effects of different VK1 doses on patients using phenprocoumon, but a number of studies have investigated VK1 doses in healthy volunteers and patients on warfarin.[13-19] In our

study, fewer 'late low INRs' were found than in the literature on warfarin.[13,14,16,17] The difference from our findings may be explained by the longer half-life of phenprocoumon than of warfarin. When phenprocoumon is restarted on day 3, blood concentrations of phenprocoumon may be higher than those of warfarin, which may result in faster-rising INRs. However, measuring the INR after follow-up was not the primary goal of this study, and no other literature is available on patients using phenprocoumon with which to compare our results. Hence, obtaining more insights into the course of the INR after different VK1 dosages in patients on phenprocoumon may be a subject for future research.

A potential limitation of this study is that the bioavailability of both formulations was determined in healthy volunteers and is assumed to be similar in patients on VKAs. To our knowledge, there is no literature on why the pharmacokinetics of VK1 in patients on VKAs would be different from those in healthy volunteers. Furthermore, the results of the trial on high-INR patients showed that the INRs decreased slightly more in the solution group than in the tablet group, making the assumption that the oral solution also has a higher bioavailability in patients on VKAs more plausible. Second, all trials were not blinded, which could lead to biased estimates of the outcomes. However, the allocation was concealed and the outcomes were laboratory tests that were assessed by technicians unaware of the treatment allocation. Therefore, there is no reason to suspect that there is bias. Third, our study was performed in patients on phenprocoumon. Therefore, absolute values of the INR decrease cannot be applied to patients on warfarin and acenocoumarol. However, the trends observed in this study (the at least as good effectiveness of tablets and the greatest increase in INRs in the first 24 h after intake) are unlikely to be different in patients on warfarin or acenocoumarol and phenprocoumon. Also, the VK1 oral solutions in oil and tablets that we investigated are only available in The Netherlands; different preparations are used in other countries. The differences between various preparations limit our findings and conclusions to these specific preparations. Fourth, after completion of our study, the shelf-life of the tablets was adjusted by the manufacturer from 36 to 12 months. We verified the vitamin K1 contents of the tablets that we used in our study and compared them with those of recently produced tablets. Because only a 9% difference was found, we believe that the limitation of the shelf-life has not influenced our results.

In this study, two patients randomized to tablets had no INR decrease after VK1 ingestion. Both patients ingested the tablets in the presence of the researcher and also stopped using phenprocoumon. The patient in the invasive procedure study reported that he experienced no effect of VK1 on the previous occasion when he ingested VK1. The patient had an INR of 3.9 at 0 h, and ingested 5 mg of VK1. At 24 h, he had an INR of 3.9, after which he received an additional 10 mg of VK1 solution; after this, his INR was 3.2 at 48 h. Six months later, he was diagnosed with cholangiocarcinoma, which might

have contributed to the absence of response to VK1. The second patient participated in the high-INR study. She had an INR of 8.0 at 0 h, after which she ingested VK1. The INRs were 7.8 and 8.0 at 24 and 48 h, respectively. VK1 concentrations were not increased after 24 h, suggesting that VK1 was not absorbed. No explanation was found for why she did not respond to VK1.

In summary, the oral VK1 solution has a higher bioavailability than VK1 tablets. In patients on phenprocoumon, the INR decreases most in the first 24 h after ingestion of VK1, independently of the formulation. In patients with invasive procedures, the effects of both formulations on an INR level of < 2.0 were similar (reached in all patients after 48 h). However, in patients with a high INR, the INR decreased slightly more when they took the solution. This also resulted in a higher percentage of patients with a too low INR after 48 h. We conclude from this study that VK1 tablets are as effective and safe as the VK1 solution with regard to the ability to decrease the INR in patients on phenprocoumon.

REFERENCES

1. Berkner KL, Runge KW. The physiology of vitamin K nutriture and vitamin K-dependent protein function in atherosclerosis. J Thromb Haemost 2004; 2: 2118–32.
2. Furie B, Bouchard BA, Furie BC. Vitamin K-dependent biosynthesis of gamma-carboxyglutamic acid. Blood 1999; 15: 1798–808.
3. Wittkowsky AK. Warfarin and other coumarin derivatives: pharmacokinetics, pharmacodynamics, and drug interactions. Semin Vasc Med 2003; 3: 221–30.
4. Wu S, Liu S, Davis CH, Stafford DW, Kulman JD, Pedersen LG. A hetero-dimer model for concerted action of vitamin K carboxylase and vitamin K reductase in vitamin K cycle. J Theor Biol 2011; 21: 143–9.
5. Holmes MV, Hunt BJ, Shearer MJ. The role of dietary vitamin K in the management of oral vitamin K antagonists. Blood Rev 2012; 26: 1–14.
6. Ufer M. Comparative pharmacokinetics of vitamin K antagonists: warfarin, phenprocoumon and acenocoumarol. Clin Pharmacokinet 2005; 44: 1227–46.
7. Ageno W, Gallus AS, Wittkowsky A, Crowther M, Hylek EM, Palareti G. Oral anticoagulant therapy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest 2012; 141: e44S–88S.
8. van der Meer FJ, Rosendaal FR, Vandenbroucke JP, Briet E. Bleeding complications in oral anticoagulant therapy. An analysis of risk factors. Arch Intern Med 1993; 12: 1557–62.
9. Fytomenadiondrank 10 mg/ml. Formularium Nederlandse Apothekers. 2013 edn 2009.
10. Gijsbers BL, Jie KS, Vermeer C. Effect of food composition on vitamin K absorption in human volunteers. Br J Nutr 1996; 76: 223–9.
11. Shearer MJ, McBurney A, Barkhan P. Studies on the absorption and metabolism of phylloquinone (vitamin K1) in man. Vitam Horm 1974; 32: 513–42.

12. Haroon Y, Bacon DS, Sadowski JA. Liquid-chromatographic determination of vitamin K1 in plasma, with fluorometric detection. Clin Chem 1986; 32: 1925–9.
13. Gunther KE, Conway G, Leibach L, Crowther MA. Low-dose oral vitamin K is safe and effective for outpatient management of patients with an INR>10. Thromb Res 2004; 113: 205–9.
14. Crowther MA, Julian J, McCarty D, Douketis J, Kovacs M, Biagoni L, Schnurr T, McGinnis J, Gent M, Hirsh J, Ginsberg J. Treatment of warfarin-associated coagulopathy with oral vitamin K: a randomised controlled trial. Lancet 2000; 4: 1551–3.
15. Baker P, Gleghorn A, Tripp T, Paddon K, Eagleton H, Keeling D. Reversal of asymptomatic over-anticoagulation by orally administered vitamin K. Br J Haematol 2006; 133: 331–6.
16. Weibert RT, Le DT, Kayser SR, Rapaport SI. Correction of excessive anticoagulation with low-dose oral vitamin K1. Ann Intern Med 1997; 15: 959–62.
17. Crowther MA, Donovan D, Harrison L, McGinnis J, Ginsberg J. Low-dose oral vitamin K reliably reverses over-anticoagulation due to warfarin. Thromb Haemost 1998; 79: 1116–18.
18. Lubetsky A, Yonath H, Olchovsky D, Loebstein R, Halkin H, Ezra D. Comparison of oral vs intravenous phytonadione (vitamin K1) in patients with excessive anticoagulation: a prospective randomized controlled study. Arch Intern Med 2003; 10: 2469–73.
19. Schurgers LJ, Shearer MJ, Hamulyak K, Stocklin E, Vermeer C. Effect of vitamin K intake on the stability of oral anticoagulant treatment: dose–response relationships in healthy subjects. Blood 2004; 1: 2682–9.

APPENDIX 1

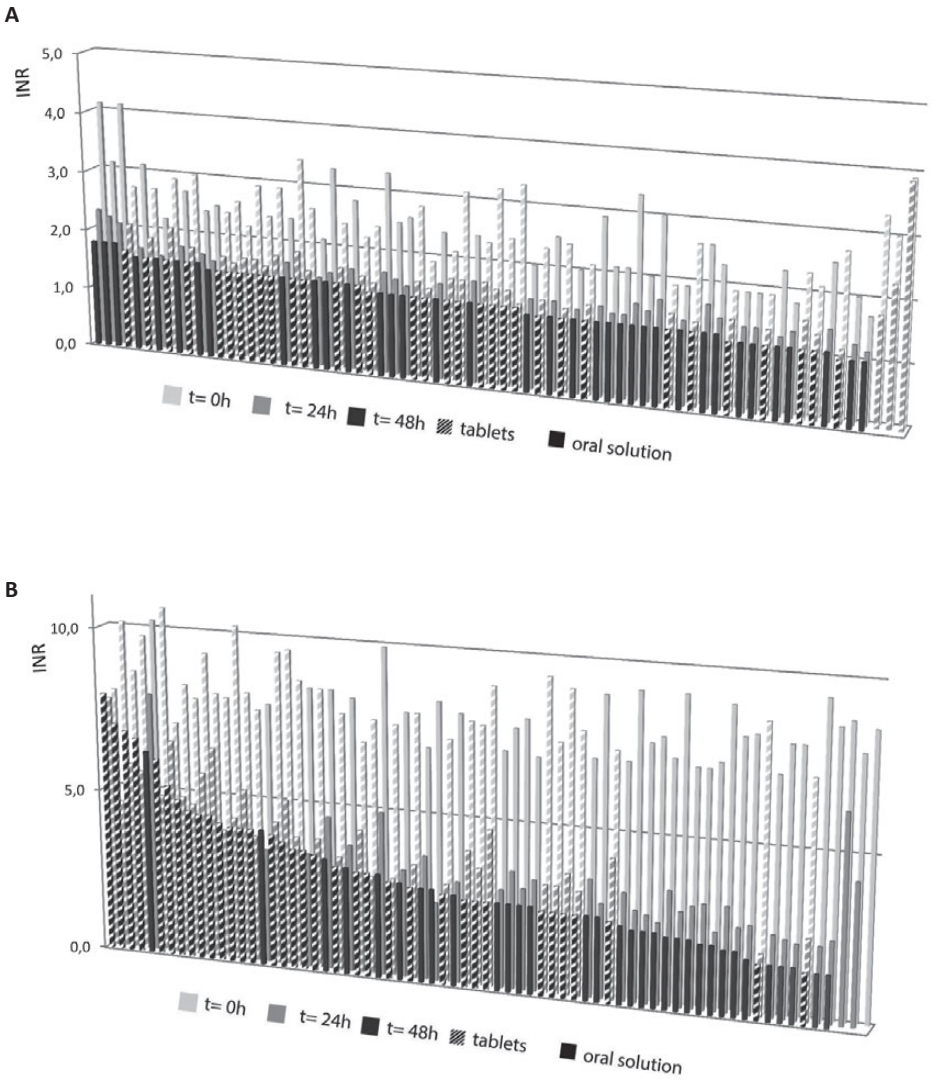
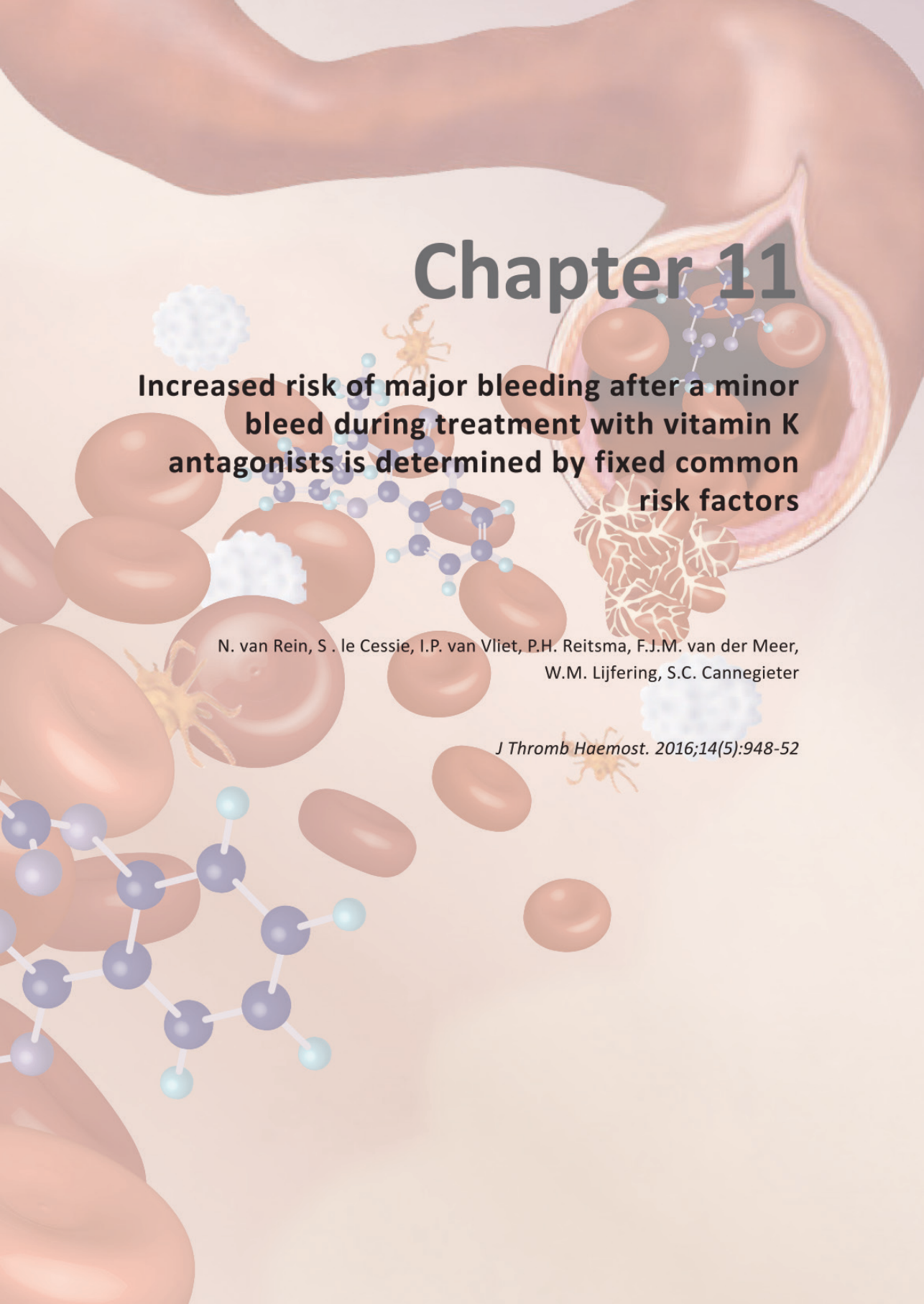


Figure 1. INRs of all patients sorted by INR at 48 h. (A) Invasive procedure study (B) High-INR study.



Chapter 11

Increased risk of major bleeding after a minor bleed during treatment with vitamin K antagonists is determined by fixed common risk factors

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ABSTRACT

Background: Patients who have a minor bleed during treatment with vitamin K antagonists (VKAs) have a 3-fold increased risk of subsequent major bleeding. The nature of the underlying risk factors is largely unknown.

Objectives: To indicate why patients with minor bleeds are at increased risk of subsequent major bleeds (e.g. are risk factors of a transient or a fixed nature).

Methods: Patients who started VKA treatment between 2003 and 2013 were included. Exposure was from the minor bleed until 3 months later. We used two analyses: a Cox model which we adjusted for several known risk factors, and a case-crossover (CCO) design, which corrects for all fixed risk factors (such as chronic diseases and genes) as patients are compared with themselves. The combination of both analyses gives insight into whether the association of minor with major bleeds is a result of fixed or transient risk factors.

Results: Out of 26 130 patients who were included and followed for '61 672 patient years', 7194 experienced a minor bleed and 913 a major bleed. The Cox model indicated that patients with minor bleeds had a 2.5-fold increased risk of experiencing subsequent major bleeding after adjustment for known risk factors, whereas the CCO gave risk estimates around unity (odds ratio, 0.9; 95% confidence interval, 0.5–1.5).

Conclusions: The combination of both analyses indicates that minor bleeds are markers for fixed and currently unknown risk factors for major bleeding events.

INTRODUCTION

Vitamin K antagonists (VKAs) are used to treat and prevent thromboembolism. Treatment with VKAs requires regular monitoring of the international normalized ratio (INR), which in the Netherlands is performed by anticoagulation clinics.[1] The most common side-effects are bleeding events, which, depending on the severity of the symptoms, are classified as major or minor.[2] Minor bleeds occur 15–20 times per 100 patients per year, whereas major bleeding events occur one to two times per 100 patients per year.[3]

A previous study showed that patients on VKAs with minor bleeding events have a 3.2-fold (95% confidence interval [CI] 1.3–8.0) increased risk of a major bleeding event in the following month; the underlying causal mechanism for this was not elucidated.[4] A minor bleed is most likely a marker for other risk factors present in an individual that will cause a major bleed. Nevertheless, after adjustment for known risk factors in the aforementioned study (i.e. age, over-anticoagulation, diabetes mellitus, diuretic use and malignancy), a 2.9-fold (95% confidence interval [CI], 1.1–7.2) increase was still found.[4] This remaining increased risk indicates that not all risk factors for major bleeds were adjusted for, possibly because not all are known. More knowledge on the nature of these yet unknown risk factors may provide new leads on risk factors for major bleeds and hence improve prediction of who is at high risk of major bleeding during VKA treatment.

The aim of this study was therefore to provide such leads on why patients with minor bleeds are at increased risk of subsequent major bleeds, such as whether risk factors are of a transient or a fixed nature. For this purpose, two analyses were carried out: a conventional follow-up analysis and a case-crossover analysis. The study was performed in a community-based cohort (n = 26 130) of patients on VKAs who were followed for, on average, 2 years, with a large number of minor (n = 7194) and major (n = 913) bleeding events.

METHODS

Study population and data collection

All patients who started treatment with VKAs at the Leiden Anticoagulation Clinic between January 2003 and December 2013 were included. Patients' characteristics and outcomes were extracted from the computerized records at the anticoagulation clinic. Baseline data consisted of sex, date of birth, indication for therapy with VKAs, starting date of VKA therapy, INR target range (2.5–3.5 or 3.0–4.0), type of VKA (acenocoumarol or phenprocoumon) and co-medication (non-steroidal anti-inflammatory drugs [NSAIDs], and antihypertensive, antidiabetic and antiplatelet medications). Blood was drawn to determine the INR at 1- to 6-week intervals. With every venepuncture, a

standard short history was taken regarding bleeds and changes in co-medication.[1] Additionally, the drug-dispensing pharmacy provided starting dates for usage of drugs that interact with VKAs.

Exposure and outcome

Non-traumatic bleeds were classified as minor or major. Bleeding events were categorized as major if they required hospitalization or blood transfusion, were symptomatic in a critical area or organ, or led to death. All other bleeds were classified as minor.[5]

Three exposure categories were defined: current exposure to minor bleeds, not exposed to minor bleeds and past exposure to minor bleeds (Fig. 1). The first category ('current exposure to minor bleeds') started at the time of the minor bleed. This exposure continued, dependent on the analysis, until 1, 2 or 3 months after the minor bleeding event occurred (see Fig. 1B–D). The use of three different time frames was chosen to study a potential transient component: risk estimates would dilute in the 3-month analysis compared with the 1-month analysis if underlying risk factor(s) were of a transient nature. The second exposure category 'not exposed to minor bleeds', was defined as the period until the first minor bleed occurred (and if no minor bleeding event occurred, until the end of follow-up; see Fig. 1A–D). The last exposure category ('past exposure to a minor bleed') was defined as the period where patients had experienced a previous minor bleed, but were not currently exposed to a minor bleed. If minor bleeding events are a marker for fixed risk factors for major bleeding events, it is to be expected that 'past exposure to minor bleeds' would also be associated with an increased risk of major bleeds.

Statistical analysis

Follow-up was from the start of treatment with VKAs until occurrence of a major bleeding event, death, a move to a municipality that was not covered by the Leiden Anticoagulation Clinic, end of treatment with VKAs or the end date of the study (31 December 2013). Incidence rates were calculated by dividing the number of major bleeds by the observation time. The 95% confidence intervals were based on a Poisson distribution.

A Cox proportional hazards model with time-dependent variables was used to estimate hazard ratios (HRs) and 95% CIs with 'not exposed to minor bleeds' as the reference category. Analyses were time-dependently adjusted for age, sex, INR target range, antidiabetic medication, antihypertensive medication, NSAIDs and antiplatelet drugs.

Second, a case-crossover analysis was performed where only patients with a major bleed were included. For all these patients, the presence or absence of a minor bleed was compared for the time period directly preceding the major bleed and 1 year before

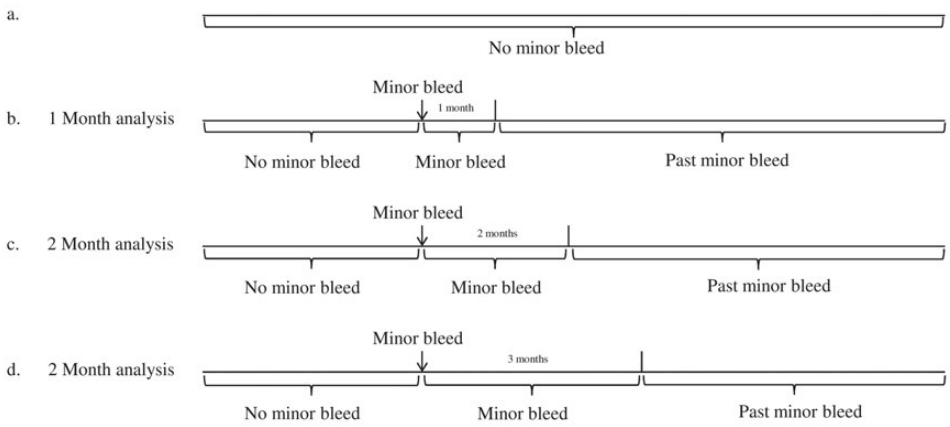


Figure 1. Exposure categories with three time frames (i.e. 1, 2 and 3 months) for the exposure to minor bleeds.

the major bleed (the reference date). An advantage of this analysis is that it corrects for all fixed risk factors, such as sex, chronic medication and socioeconomic status, as individuals are compared with themselves.[6] This analysis would therefore inform us about the extent to which such fixed risk factors are involved. Relative risk estimates (ORs) and 95% CIs were estimated by means of conditional logistic regression, which only takes discordant pairs into account (i.e. only pairs in which one patient is exposed during the index date and not during the reference date or vice versa).

A sensitivity analysis was performed, in which skin hemorrhages were excluded because these are often trauma inflicted and may not be a good marker for nontraumatic causes of bleeding.

All analyses were performed in R 2.15.2 (Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/).

RESULTS

The study cohort contained 26 130 patients. As patients could interrupt their treatment with VKA and start again later, these patients had 28 950 VKA treatment periods altogether (Table 1). The most common reasons for ending treatment were that the indicated period of treatment had passed (n = 6176, 21%) or that patients were cured of the disease for which they had received VKA treatment (n = 4755, 16%). The mean age at baseline was 70 years (standard deviation, 15) and 15 158 (52%) patients were male. Atrial fibrillation was the most common indication for treatment with VKAs (16 436 treatment periods, 57%) and the INR target range was 2.5–3.5 in 26 595 (92%) treatment periods.

Table 1. Baseline characteristics

	No minor bleeds	Minor bleeds
No. of patients	21 599	4531
No. of treatments	24 330 (100)	4620 (100)
Age	67 (16)	72 (12)
Male sex	12 764 (53)	2394 (52)
Vitamin K antagonist		
Phenprocoumon treatment	21 079 (87)	4263 (92)
Acenocoumarol treatment	3251 (13)	357 (8)
Indication		
Atrial fibrillation	13 206 (54)	3230 (70)
Venous thrombosis	6303 (26)	795 (17)
Acute coronary syndrome	11 185 (5)	398 (9)
Other	4509 (19)	496 (11)
Concomitant drugs, n (%)		
Anti-platelet drugs	1740 (7)	424 (9)
NSAIDs	1214 (5)	209 (5)
Diabetes mellitus, n (%)	2733 (11)	516 (11)
High blood pressure, n (%)	11 175 (46)	2664 (58)
INR target range, n (%)		
2.5-3.5	22 482 (92)	4113 (89)
3.0-4.0	1848 (8)	507 (11)

Continuous variables denoted as mean (standard deviation), categorical variables as number (percent)

The mean follow-up per treatment period was 2.1 years, resulting in a total follow-up of 61 672 person-years; 913 major bleeds and 7194 minor bleeds, of which 1537 were minor skin bleeds, occurred during the follow-up period (Appendix 1 Table 1). The incidence rates of minor and major bleeding (per 100 patient years) were 11.7 (95% CI, 11.4–11.9) and 1.5 (95% CI, 1.4–1.6), respectively.

In the Cox proportional hazards analysis, 'current exposure to minor bleeds' was associated with a 2.4- to 2.7-fold increased risk of major bleeding events in the subsequent 1–3 months (Table 2). Risk estimates attenuated slightly after adjustment for known risk factors for bleeding. An approximately 2-fold increased risk of major bleeding was found in patients with 'past minor bleeds' (Table 2). Exclusion of skin bleeds did not affect these relative risk estimates.

Results of the case-crossover study (that adjusts for all fixed risk factors) showed that minor bleeds were not associated with major bleeds. Relative risk estimates remained close to unity when considering exposure periods of 1–3 months (Table 3). After exclusion of skin bleeds, risk estimates became slightly lower in all analyses.

Table 2. Association of minor bleeding complications with major bleeding complications

	Patient-time (years)	No. of events	Events/100 patient-years	Hazard ratio* (95% CI)	Hazard ratio† (95% CI)
All minor bleeding complications					
No minor bleeds	50378	668	1.3	Reference	Reference
1 Month analysis					
Minor bleed	538	18	3.3	2.4 (1.5-3.9)	2.2 (1.4-3.5)
Past minor bleed	10 755	227	2.1	2.1 (1.8-2.4)	1.9 (1.6-2.2)
2 Month analysis					
Minor bleed	1017	35	3.4	2.6 (1.8-3.6)	2.3 (1.6-3.2)
Past minor bleed	10 272	210	2.0	2.0 (1.7-2.4)	1.8 (1.6-2.2)
3 Month analysis					
Minor bleed	1455	52	3.6	2.7 (2.1-2.9)	2.5 (1.9-3.3)
Past minor bleed	9838	193	2.0	1.9 (1.6-2.3)	1.8 (1.5-2.1)
Minor bleeding complications except skin bleeds					
No minor bleeds	52578	728	1.4	Reference	Reference
1 Month analysis					
Minor bleed	419	13	3.1	2.1 (1.2-3.7)	1.9 (1.1-3.4)
Past minor bleed	8675	172	2.0	1.8 (1.5-2.1)	1.6 (1.4-2.0)
2 Month analysis					
Minor bleed	788	26	3.3	2.3 (1.6-3.5)	2.1 (1.4-3.2)
Past minor bleed	5299	159	1.9	1.7 (1.4-2.1)	1.6 (1.3-1.9)
3 Month analysis					
Minor bleed	1123	38	3.4	2.5 (1.8-3.4)	2.3 (1.6-3.1)
Past minor bleed	7965	147	1.9	1.7 (1.4-2.0)	1.5 (1.3-1.9)

* Time dependent analysis. † Time dependent analysis adjusted for age, sex, INR target range, antidiabetic -, antihypertensive -, antiplatelet drugs and NSAIDs

DISCUSSION

This study showed that patients with minor bleeding had a 2.5-fold increased risk of a subsequent major bleed. The increased risk did not change substantially after adjusting for several known risk factors, similar to what has been shown in a previous study on this issue.[4] This suggests that the risk factors we and others adjusted for (age, sex, INR target range, antidiabetic medication, antihypertensive medication, NSAIDs and antiplatelet drugs) hardly affect the risk of major bleeding in patients who have had a prior minor bleed.

Table 3. Association of minor bleeding complications with major bleeding complications in all patients with a major bleeds in a case-crossover study

	Bleed+/ Reference-*	Bleed-/ Reference+*	Odds ratio (95% CI)
All minor bleeding complications			
1 Month analysis	7	8	0.9 (0.3-2.4)
2 Month analysis	15	19	0.8 (0.4-1.6)
3 Month analysis	24	27	0.9 (0.5-1.5)
Minor bleeding complications without skin bleeds			
1 Month analysis	4	6	0.7 (0.2-2.4)
2 Month analysis	9	15	0.6 (0.3-1.4)
3 Month analysis	16	22	0.7 (0.4-1.4)

* 'Bleed+': exposed to a minor bleed during the major bleed; 'Bleed-': not exposed to a minor bleed during the major bleed; 'Reference+': exposed to a minor bleed one year before the major bleed; 'Reference-': not exposed to a minor bleed one year before the major bleed. Reference category of the OR: Bleeds-/Reference+

The results were similar in the conventional analysis when considering different time frames (i.e. 1, 2 and 3 months). This suggests that risk factors for major bleeds have a fixed nature, as otherwise it would be expected that the association would attenuate over time. Moreover, the association between minor and major bleeds disappeared in the case-crossover analysis. As such an analysis adjusts for all fixed risk factors, this result also implies that minor bleeding events are markers of fixed risk factors for major bleeding. Identification of these fixed risk factors, of which already several can be excluded as mentioned above, may provide new leads on who is at high risk of major bleeding during anticoagulant treatment. Such new predictors are necessary, considering that current prediction models for major bleeds do not perform well and cannot distinguish between patients who are at high risk for bleeds and thromboembolism.[7]

Based on the literature, there are some candidates for these risk factors for major bleeding. One characteristic that indicates who is at risk of bleeding complications, which was not taken into account in this study, is renal insufficiency[8,9], as this variable was not available in our dataset. Other characteristics include coagulation-related indicators. One of them is ABO blood group, as it was previously shown that blood group O is associated with an increased risk of major bleeding events during VKA treatment.[10] This is biologically plausible because individuals with blood group O have lower plasma concentrations of both von Willebrand factor and factor VIII.[11] Another candidate could be vessel wall damage, as suggested by the increased risk of bleeding associated with a high concentration of soluble trombomodulin[12,13], which is

a marker for vessel damage. Other candidates are genetic variants (e.g. cytochrome P450 2C9 and vitamin K epoxide reductase complex 1), as these affect the required VKA dosage[14] and risk of bleeding.[15] Unfortunately, we have no information on these factors in our study.

A strength of this community-based cohort is that it contained over 900 major and 7000 minor bleeding events, which made the results robust. The incidence rates of major bleeding events in this study were similar to those found in other community-based studies of patients treated with VKAs[1,4,16], indicating generalizability. A limitation is that minor bleeds were mainly self-reported and could be trauma related, although this was not reported by the patient. We tried to overcome this limitation with the sensitivity analysis where skin hemorrhages were excluded, which did not change our major outcomes. Still, this analysis was based on the assumption that skin bleeds are more likely to be caused by trauma, which may not be true for all skin hemorrhages.

To conclude, this study showed that in VKA patients, minor bleeding events are markers for fixed and currently unknown risk factors for major bleeding events.

REFERENCES

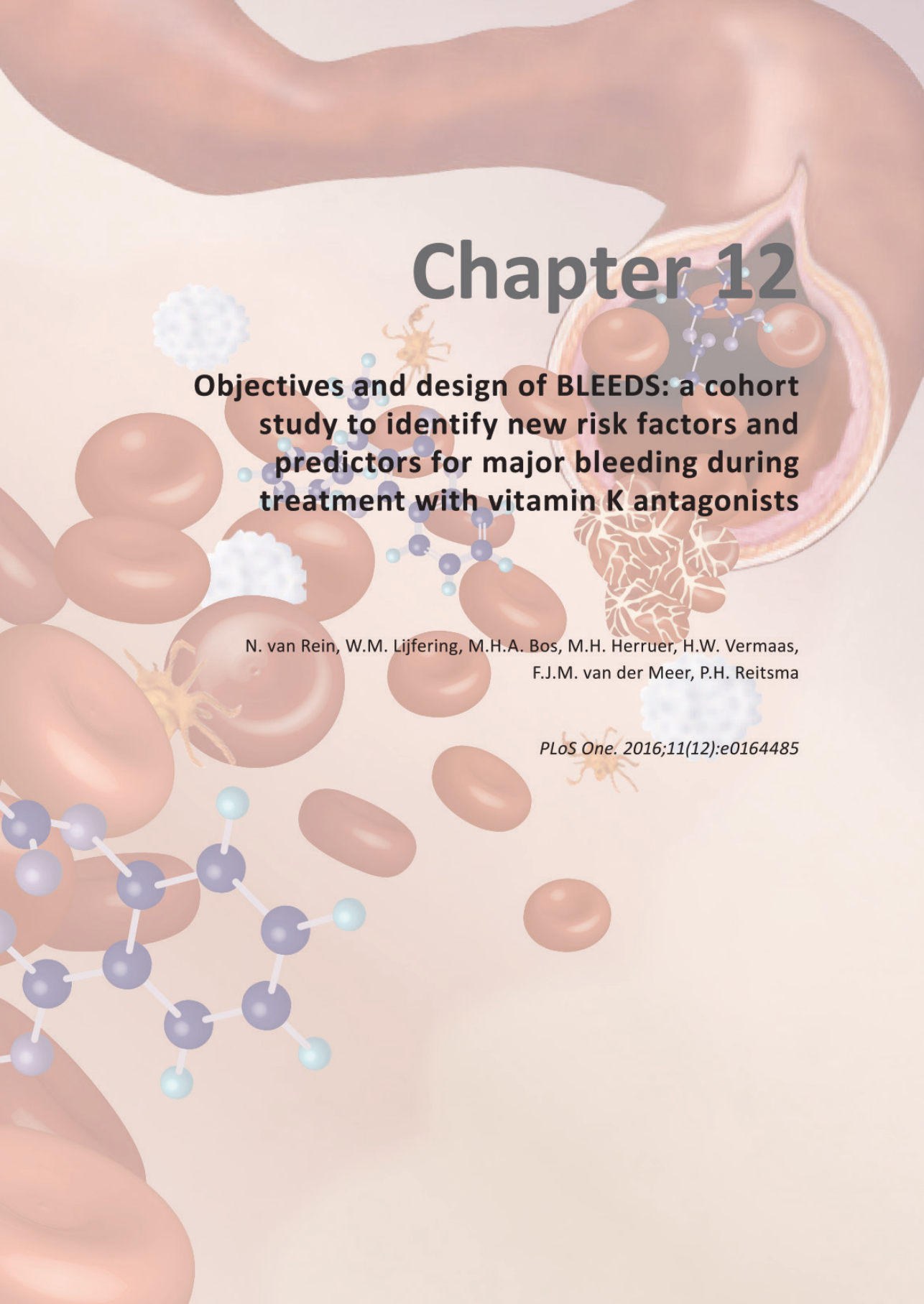
1. van der Meer FJ, Rosendaal FR, Vandenbroucke JP, Briet E. Bleeding complications in oral anticoagulant therapy. An analysis of risk factors. Arch Intern Med 1993; 153: 1557–62.
2. Ansell J, Hirsh J, Hylek E, Jacobson A, Crowther M, Palareti G. Pharmacology and management of the vitamin K antagonists: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). Chest 2008; 133: 160S–98S.
3. Levi M, Hovingh GK, Cannegieter SC, Vermeulen M, Buller HR, Rosendaal FR. Bleeding in patients receiving vitamin K antagonists who would have been excluded from trials on which the indication for anticoagulation was based. Blood 2008; 111: 4471–6.
4. Veeger NJ, Piersma-Wichers M, Meijer K, Hillege HL. Minor bleeds alert for subsequent major bleeding in patients using vitamin K antagonists. Br J Haematol 2011; 153: 508–14.
5. Schulman S, Kearon C. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. J Thromb Haemost 2005; 3: 692–4.
6. Maclure M. The case-crossover design: a method for studying transient effects on the risk of acute events. Am J Epidemiol 1991; 133: 144–53.
7. You JJ, Singer DE, Howard PA, Lane DA, Eckman MH, Fang MC, Hylek EM, Schulman S, Go AS, Hughes M, Spencer FA, Manning WJ, Halperin JL, Lip GY. Antithrombotic therapy for atrial fibrillation: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest 2012; 141: e531S–75S.
8. Bleeding during antithrombotic therapy in patients with atrial fibrillation. The Stroke Prevention in Atrial Fibrillation Investigators. Arch Intern Med 1996; 156: 409–16.

9. Fihn SD, McDonell M, Martin D, Henikoff J, Vermes D, Kent D, White RH. Risk factors for complications of chronic anticoagulation. A multicenter study. Warfarin Optimized Outpatient Follow-up Study Group. Ann Intern Med 1993; 118: 511–20.
10. Garcia AA, van der Heijden JF, Meijers JC, Rosendaal FR, Reitsma PH. The relationship between ABO blood group and the risk of bleeding during vitamin K antagonist treatment. J Thromb Haemost 2006; 4: 1418–20.
11. Jenkins PV, O'Donnell JS. ABO blood group determines plasma von Willebrand factor levels: a biologic function after all? Transfusion 2006; 46: 1836–44.
12. Lind M, Boman K, Johansson L, Nilsson TK, Ohlin AK, Birgander LS, Jansson JH. Thrombomodulin as a marker for bleeding complications during warfarin treatment. Arch Intern Med 2009; 169: 1210–5.
13. van der Heijden JF, Rekke B, Hutten BA, van der Meer FJ, Remkes MG, Vermeulen M, Buller HR, Reitsma PH. Non-fatal major bleeding during treatment with vitamin K antagonists: influence of soluble thrombomodulin and mutations in the propeptide of coagulation factor IX. J Thromb Haemost 2004; 2: 1104–9.
14. Cooper GM, Johnson JA, Langaee TY, Feng H, Stanaway IB, Schwarz UI, Ritchie MD, Stein CM, Roden DM, Smith JD, Veenstra DL, Rettie AE, Rieder MJ. A genome-wide scan for common genetic variants with a large influence on warfarin maintenance dose. Blood 2008; 112: 1022–7.
15. Reitsma PH, van der Heijden JF, Groot AP, Rosendaal FR, Buller HR. A C1173T dimorphism in the VKORC1 gene determines coumarin sensitivity and bleeding risk. PLoS Med 2005; 2: e312.
16. Palareti G, Leali N, Coccheri S, Poggi M, Manotti C, D'Angelo A, Pengo V, Erba N, Moia M, Ciavarella N, Devoto G, Berrettini M, Musolesi S. Bleeding complications of oral anticoagulant treatment: an inception-cohort, prospective collaborative study (ISCOAT). Italian Study on Complications of Oral Anticoagulant Therapy. Lancet 1996; 348: 423–8.

APPENDIX 1

Table 1. Type of bleeds

Bleeds, n (%)	Minor, n= 7194	Major, n= 913
Intracranial	-	224 (25)
Aneurysm	-	16 (2)
Joint, muscle	-	57 (6)
Gastro intestinal	713 (8)	410 (45)
Haematoma	1534 (17)	38 (2)
Epistaxis	1255 (14)	23 (3)
Intraocular	-	22 (2)
Conjuctiva bleed	1867 (20)	-
Haematuria	1348 (15)	55 (6)
Respiratory tract	167 (2)	34 (4)
Urogenital	163 (2)	12 (1)
Other	147 (2)	22 (2)



Chapter 12

Objectives and design of BLEEDS: a cohort study to identify new risk factors and predictors for major bleeding during treatment with vitamin K antagonists

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ABSTRACT

Background: Risk scores for patients who are at high risk for major bleeding complications during treatment with vitamin K antagonists (VKAs) do not perform that well. BLEEDS was initiated to search for new biomarkers that predict bleeding in these patients.

Objectives: To describe the outline and objectives of BLEEDS and to examine whether the study population is generalizable to other VKA treated populations.

Methods: A cohort was created consisting of all patients starting VKA treatment at three Dutch anticoagulation clinics between January-2012 and July-2014. We stored leftover plasma and DNA following analysis of the INR.

Results: Of 16 706 eligible patients, 16 570 (99%) were included in BLEEDS and plasma was stored from 13 779 patients (83%). Patients had a mean age of 70 years (SD 14), 8 713 were male (53%). The most common VKA indications were atrial fibrillation (10 876 patients, 66%) and venous thrombosis (3 920 patients, 24%). 326 Major bleeds occurred during 17 613 years of follow-up (incidence rate 1.85/100 person years, 95%CI 1.66–2.06). The risk for major bleeding was highest in the initial three months or VKA treatment and increased when the international normalized ratio increased. These results and characteristics are in concordance with results from other VKA treated populations.

Conclusion: BLEEDS is generalizable to other VKA treated populations and will permit innovative and unbiased research of biomarkers that may predict major bleeding during VKA treatment.

INTRODUCTION

Vitamin K antagonists (VKAs) are used to treat and prevent thromboembolic events.[1] Monitoring of VKA treatment is required because VKAs have a narrow therapeutic window and the dosage depends on inter-individual, but also intra-individual factors.[1] In the Netherlands, patients on VKA treatment are monitored by specialized anticoagulation clinics.[2] The clinics are regionally organized and all patients who live in a certain area are monitored by the same clinic.[2] At these clinics, the international normalized ratios (INRs) are measured on a regular basis, after which a specialized physician determines the VKA dosage and the time interval between INR measurements.[2]

Despite this monitoring system, the most common side effects of VKAs remain bleeding complications.[1] Bleeding complications are, depending on the severity, categorized as minor or major bleeding complications. Minor bleedings, such as skin bruises or nosebleeds, occur annually in 6-10% of patients on VKAs and major bleedings, including (fatal) intra-organ bleeds, occur in 1-3% of VKA treated patients per year.[2-4] Risk factors for major bleeding events have been identified and subsequent bleeding risk scores have been developed.[5-10] However, these risk scores do not accurately predict major bleeding (range of C statistics: 0.59-0.69).[11] Additional biomarkers and genetic variants potentially yield a better accuracy of predicting major bleeding, but information on such predictors is scarce. The goal of the Biomarkers in the Leiden Etiology and Epidemiology of bleeding in vitamin K antagonists Drug users Study (BLEEDS) is to identify novel biomarkers and genetic variants that predict patients at risk for major bleeding events during treatment with VKAs. Here, we delineate the outline of the study. In addition, we provide an overview on classical risk factors for major bleeding to ensure that our population is generalizable to other VKA treated populations.

METHODS

Study design

BLEEDS is a population based cohort study with longitudinal follow-up in 16 570 patients who started VKA treatment and were recruited from three anticoagulation clinics in the Netherlands.

Study population

Consecutive patients aged 18 years or older who started VKA treatment at one of the three participating anticoagulation clinics in the Netherlands (Leiden, The Hague and Hoofddorp) between January 2012 and July 2014 were eligible (Figure 1). These regional anticoagulation clinics monitor the VKA therapy of those patients living in well-defined geographical areas surrounding Leiden, The Hague and Hoofddorp.

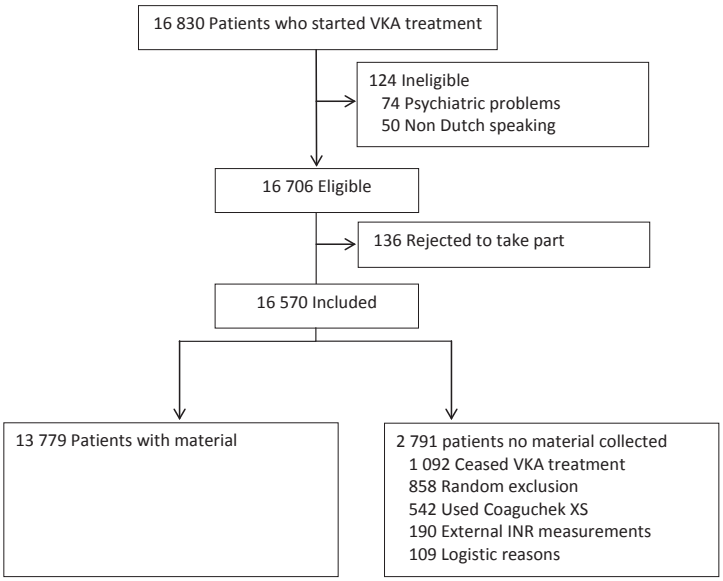

Figure 1. Flow chart of number of individuals included

Patients were included if the planned treatment duration was at least six weeks, and patients who did not speak Dutch (n= 50) or experienced psychiatric problems (n= 74) were excluded.

Considering an alpha value of 5%, statistical power of 80%, exposure prevalence of 10%, a relative risk of 1.8, an incidence rate of bleeding of 1.8 per year 100 patient years and a mean follow-up of one year, we estimated the necessary sample size at approximately 16 500 patients. Inclusion of patients took place by an opt-out procedure: all eligible patients received information regarding the study and were included if they did not decline to take part. The study was approved by the medical ethical committee of the Leiden University Medical Center. The included study population consisted of 16 706 eligible patients, of whom 136 opted out (< 1%), resulting in 16 570 included patients.

Baseline examination and surveillance

When enlisted by the anticoagulation clinic, several patient characteristics were registered, including date of birth, sex, co-medication, indication for VKA treatment, planned duration of VKA treatment and INR target range. To monitor the INR, appointments are made with a frequency of at least every six weeks. The time interval between these measurements depends on the stability of the INR. In case of an unstable INR, the INR will be reassessed more frequently. In case of a stable INR, INR measurements will be performed after a maximum period of six weeks.

To measure the INR, venous blood is drawn into vacuum tubes containing 0.1-volume 0.109 mol/L trisodium citrate as anticoagulant. Blood was centrifuged (10 minutes at 2800 G) within 4 hours of collection, upon which the INR was measured. Another second, less frequently performed method to measure the INR is by using a point-of-care device (CoaguChek XS). At each appointment, a standardized short questionnaire is taken (and electronically stored) by a nurse in order to document changes in co-medication, onset of co-morbidities, the occurrence of bleeding events, or scheduled invasive procedures (e.g. planned surgery or dental extractions).

Data collection

Patient characteristics were extracted from the computerized patient records of the anticoagulation clinics. Baseline characteristics included sex, age, indication for VKA treatment, type of VKA, INR target range, and co-medications. The study population included 16 185 patients, and because some patients stopped VKA treatment and started again, these patients represented a total of 16 570 treatment periods. There were 8 713 male patients (53%) and the mean age was 70 years (standard deviation [SD] 14; Table 1). The most common indications for VKA treatment were atrial fibrillation (10 876 treatment periods, 66%) and venous thrombosis (3 920 treatment periods, 24%). Phenprocoumon was used during 12 083 (73%) periods, and approximately half of the patients used antihypertensive medication (8 354 patients, 50%) or glucose lowering drugs (8 013 patients, 48%).

Material collection

For his study, we used patient's blood and plasma samples that were leftover following INR analyses. The sample collection started three weeks after initiations of VKA therapy and, if applicable, two weeks after termination of low-molecular-weight-heparin (LMWH) treatment. To guarantee the privacy of the patients, technicians who were not involved in the study recoded patient numbers to study numbers. After recoding, patient specific characteristics were concealed and samples were labelled according to study number. The 'key' linking patient to study numbers is maintained by a data manager who is not involved in the study. Per patient, a minimum volume of 2.0 ml plasma was collected, which resulted from blood samples of two to three subsequent visits to the anticoagulation clinic. Plasma samples were initially stored at -20˚C up to one week. The remaining white blood cells, also encoded with the corresponding study number, were stored for up to one week at 2-8˚C, after which DNA was isolated.[12] Plasma and DNA were both long-term stored at -80˚C.

Plasma and DNA was collected from 13 779 patients (83%). Material collection failed for 2 791 patients because they ceased VKA treatment early (1 092 patients), were randomly excluded due to a high workload at the anticoagulation clinic (858 patients),

the INR was established by the point-of-care device CoaguChek XS (542 patients), the INR measurement was performed externally (190 patients), and due to logistic complications (109 patients).

Table 1. Baseline characteristics

General characteristics	
Patients	16 185
Treatment periods	16 570
Men	8 713 (53)
Age	70 (14)
INR target range	
2.5-3.5	15 509 (93)
3.0-4.0	1 061 (7)
Treatment indication	
Atrial fibrillation	10 876 (66)
Venous thrombosis	3 920 (24)
Mechanical heart valves	435 (3)
Ischemic heart disease	519 (3)
Vascular disease	412 (3)
Postoperative	125 (1)
Other	348 (2)
Vitamin K antagonist	
Phenprocoumon	12 068 (73)
Acenocoumarol	4 481 (27)
Warfarin	18 (0)
Fluindione	3 (0)
Co-Medication	
Anti-platelet drugs	2 705 (16)
NSAIDs	1 004 (6)
Glucose lowering drugs	8 013 (48)
Anti-hypertensive drugs	8 354 (50)
Cholesterol lowering drugs	6 288 (38)
Digoxin	1 767 (11)
Anti-cancer drugs	339 (2)
Opioids	1 306 (8)
Methotrexate	155 (1)

Follow-up and outcome

The follow-up lasted from starting VKA therapy to either the termination of VKA treatment, migration to an area not covered by the three anticoagulation clinics, death, the occurrence of a major bleeding event, or end of the study (31st December 2014), whichever occurred first, resulting in a total follow-up of 17 613 years and a mean follow-up time of 13 months. Patients were followed according to the routine procedures of the anticoagulation clinic. During the appointments, major bleeding events were identified through short interviews that were part of the standard procedures of the anticoagulation clinic. If patients mentioned any bleeding event or hospitalization related to a bleeding event, information was obtained from the hospital, general practitioner or patient to classify the bleeding event as minor or major. Major bleeding events were defined according to the guidelines of the Federation of Dutch Anticoagulation clinics (FNT) by trained anticoagulation clinic physicians, who were not involved in the current study. Bleeding events were classified as major if these were fatal, lead to a blood transfusion or hospital admission, were an intracranial bleeding, objectively diagnosed joint bleed, or a bleeding event in a critical organ.[13] In total, 326 major bleeding events were identified during 17 613 years of follow-up.

RESULTS AND DISCUSSION

BLEEDS provides a large set of patient information and material that will be used to discover new information on risk factors for major bleeding events during VKA treatment. The large number of major bleeding events (n=326) provides a unique opportunity to perform subgroup analyses and study relatively rare risk factors.

Previously, three studies have been performed in which plasma and DNA were collected to discover new risk factors for major bleeding during VKA treatment.[14-16] However, in all of these studies, only a subgroup of the total VKA-treated patients was included. The first study excluded patients who died or became mentally disabled by the bleeding event and included only 22% of all patients who experienced a major bleeding complication in the final analyses.[16] This makes the results susceptible to survivor bias.[17] The second study's inclusion criterion was a time in therapeutic range (TTR) of 100%[15], while the third study included only 75% of the warfarin-treated patients. Furthermore, the patients were followed up to 5.5 years[14], which may have diluted the results. By including only subsets of patients on VKA therapy, results can be biased and cannot be extrapolated to all VKA-treated patients.[16,17] In BLEEDS, follow-up was short which creates the possibility to study risk factors that predict the short term risk for major bleeding. In addition, we have included 99% of the eligible patients treated with VKAs, thereby providing a strong case that our population represents real world patients. Further support for this stems from the observation that the

incidence rate of the major bleeding events (1.85 per 100 patient-years, 95% CI 1.66-2.06; see Table 2) compares well with major bleeding rates of other population-based studies.[2,18,19] The major bleeding complications observed in our study also concur with previous findings, given that intracranial bleedings were the most common fatal major bleeding complications (37, 54% of all major bleeding events), while the non-fatal bleedings mostly resulted from digestive (106, 41%) and intracranial (40, 15%; see Table 3) bleedings.[2,18,19]

To further analyze if BLEEDS agrees with other VKA treated populations in terms of predictors for bleeding, we decided to calculate the TTR by linear interpolation as described by Rosendaal *et al*.[20] Previous studies have shown that high TTRs are associated with lower bleeding rates as compared with low TTRs. The lowest TTR was observed shortly after the initiation of VKA treatment, which increased to approximately 80% and stabilized until the end of follow-up (Figure 2), which in agreement with previous findings. This pattern is as expected based on other population based studies, although it should be mentioned that a TTR of 80% is within the upper range of normal for Western European anticoagulation clinics that on average achieve a TTR of 70%.[21]

Table 2. Incidence rates of bleeding events stratified by clinical characteristics

	No. of events	Patient time (years)	Events/100 patient-years (95% CI)
Total	326	17 613	1.85 (1.66-2.06)
Sex			
Male	184	9 224	1.99 (1.72-2.30)
Female	142	8 387	1.69 (1.43-1.99)
INR target range			
2.5-3.5	306	16 454	1.86 (1.66-2.08)
3.0-4.0	20	1 157	1.73 (1.09-2.62)
Vitamin K antagonist			
Phenprocoumon	262	13 278	1.97 (1.75-2.22)
Acenocoumarol	64	4 333	1.48 (1.15-1.87)
Indication			
Atrial fibrillation	241	13 162	1.83 (1.61-2.07)
Venous thrombosis	53	2 702	1.96 (1.48-2.55)
Mechanical heart valves	4	351	1.14 (0.36-2.75)
Ischemic hearts disease	7	555	1.26 (0.55-2.50)
Vascular	9	433	2.08 (1.01-3.81)
Postoperative	3	105	2.86 (0.72-7.78)
Other	9	384	2.34 (1.14-4.30)

Table 3. Number of bleeding events stratified by area

	Fatal	Non-fatal
Total	68	260
Gastrointestinal	12	106
Intracranial	37	40
Muscle	0	13
Joint	0	17
Epistaxis	0	16
Respiratory	3	9
Urinary tract	1	16
Ocular	0	1
Skin	0	8
Circulatory	9	6
Retroperitoneal	0	1
Other	6	27

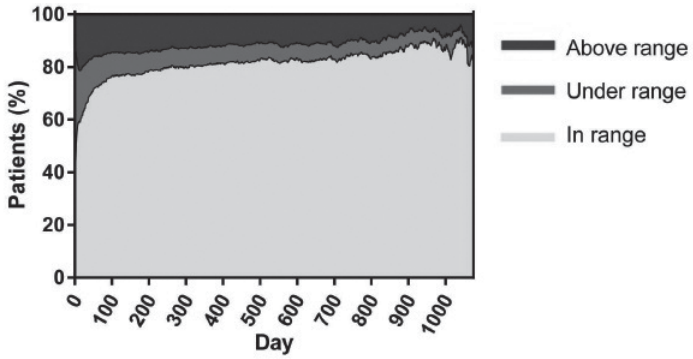

Figure 2. Time in therapeutic range per day after starting VKA treatment

Further TTR assessment revealed that a low TTR was dose-dependently associated with increased bleeding rates (see Table 4).[20,22,23] Additional analyses confirmed that, similar to other population based cohorts, in BLEEDS the bleeding rates i) increased with age (see Figure 3)[2,10,18,24-26], ii) were highest shortly after initiation of VKA treatment and became stable after four months of VKA treatment (Figure 4)[18,25,27,28], iii) and increased after a high INR (Figure 5).[18,26] Results of the subgroup analyses (atrial fibrillation patients, venous thrombosis patients, and patients with an INR target range between 2.5 and 3.5) showed similar results as compared with the main analyses (see Appendix 1).

Table 4. Association of TTR with bleeding events

	No. of events	Patient years	Events/100 patient-years (95% CI)
Time in range			
< 35%	63	448	13.02 (10.09-16.54)
> 35% and < 50%	33	1 145	2.88 (2.02-4.00)
> 50% and < 60%	35	1 538	2.28 (1.61-3.13)
> 60% and < 70%	48	2 212	2.17 (1.62-2.85)
> 70% and < 80%	40	2 821	1.42 (1.03-1.91)
> 80% and < 90%	37	3 059	1.21 (0.86-1.65)
> 90%	62	5 361	1.16 (0.89-1.47)

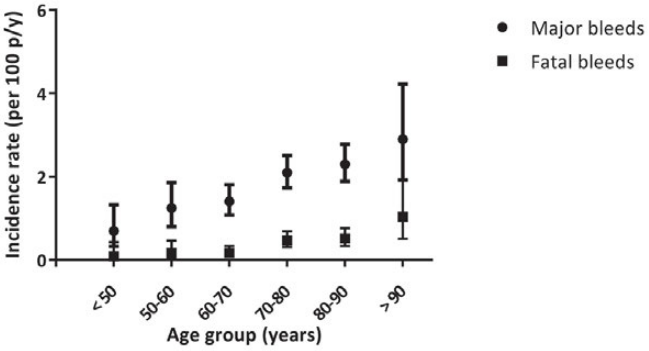

Figure 3. Incidence rates of bleeding events stratified by age

Two characteristics of the study deserve a further comment. Material (plasma and DNA) of all patients was collected from the third week after initiating VKA therapy and, if applicable, two weeks after termination of LMWH therapy. As such, we did not collect material of 32 patients who experienced a major bleeding complication before this third week. As a possible consequence, risk estimates may dilute because the patients with the strongest risk factors 'drop out' of the study. Second, the low INR target range is 2.5-3.5 in the Netherlands, which is 2.0-3.0 in other countries. This may result in slightly higher major bleeding rates in these patients.

In this study, 99% of the eligible patients were included which results in a unique, unselected study population. The number of major bleeding events is relatively high, which creates the possibility to perform subgroup analyses and study whether protein levels are dose dependently associated with major bleeding events. In addition, the availability of stored biological specimens (citrated plasma and DNA) will allow us to uncover new risk

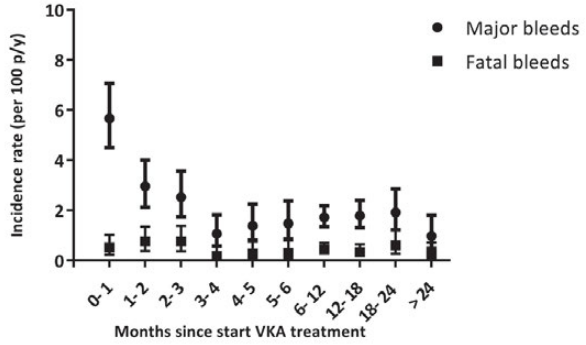

Figure 4. Incidence rates of bleeding events stratified by time since start of VKA treatment

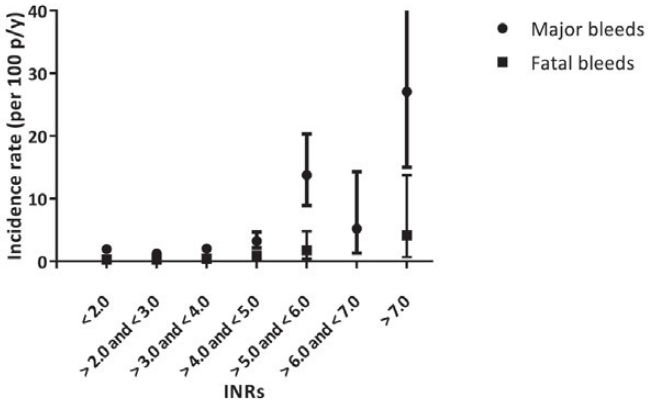

Figure 5. Incidence rates of bleeding events stratified by INR

factors for major bleeding complications during VKA treatment, with the goal of discovering new predictors for VKA-treated patients at high risk for major bleeding events. We would like to emphasize that results of BLEEDS with respect to classical risk factors for major bleeding are similar to other cohorts of patients who received VKAs for all long term indications, which indicates that this population is generalizable to other populations. In summary, the BLEEDS will permit innovative and unbiased research of multiple exposures for major bleeding events and will assist in the prevention of major bleeding events in patients treated with VKAs.

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REFERENCES

1. Ansell J, Hirsh J, Hylek E, Jacobson A, Crowther M, Palareti G. Pharmacology and management of the vitamin K antagonists: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest.* 6/2008 2008;133(6 Suppl):160S-198S.
2. van der Meer FJ, Rosendaal FR, Vandenbroucke JP, Briet E. Bleeding complications in oral anticoagulant therapy. An analysis of risk factors. *Arch.Intern.Med.* 7/12/1993 1993;153(13):1557-1562.
3. Linkins LA. Bleeding risks associated with vitamin K antagonists. *Blood Rev.* 5/2013 2013;27(3):111-118.
4. Schulman S, Beyth RJ, Kearon C, Levine MN. Hemorrhagic complications of anticoagulant and thrombolytic treatment: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest.* 6/2008 2008;133(6 Suppl):257S-298S.
5. Beyth RJ, Quinn LM, Landefeld CS. Prospective evaluation of an index for predicting the risk of major bleeding in outpatients treated with warfarin. *Am.J.Med.* 8/1998 1998;105(2):91-99.
6. Fang MC, Go AS, Chang Y, et al. A new risk scheme to predict warfarin-associated hemorrhage: The ATRIA (Anticoagulation and Risk Factors in Atrial Fibrillation) Study. *J.Am.Coll.Cardiol.* 7/19/2011 2011;58(4):395-401.
7. Gage BF, Yan Y, Milligan PE, et al. Clinical classification schemes for predicting hemorrhage: results from the National Registry of Atrial Fibrillation (NRAF). *Am.Heart J.* 3/2006 2006;151(3):713-719.
8. Kuijer PM, Hutten BA, Prins MH, Buller HR. Prediction of the risk of bleeding during anticoagulant treatment for venous thromboembolism. *Arch.Intern.Med.* 3/8/1999 1999;159(5):457-460.
9. Lip GY, Nieuwlaat R, Pisters R, Lane DA, Crijns HJ. Refining clinical risk stratification for predicting stroke and thromboembolism in atrial fibrillation using a novel risk factor-based approach: the euro heart survey on atrial fibrillation. *Chest.* 2/2010 2010;137(2):263-272.
10. Shireman TI, Howard PA, Kresowik TF, Ellerbeck EF. Combined anticoagulant-antiplatelet use and major bleeding events in elderly atrial fibrillation patients. *Stroke.* 10/2004 2004;35(10):2362-2367.
11. Zhu W, He W, Guo L, Wang X, Hong K. The HAS-BLED Score for Predicting Major Bleeding Risk in Anticoagulated Patients With Atrial Fibrillation: A Systematic Review and Meta-analysis. *Clin.Cardiol.* 9/2015 2015;38(9):555-561.
12. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 2/11/1988 1988;16(3):1215.
13. Trombosediensten FvN. *FNT- NORMEN.* 2015 2015.
14. Lind M, Boman K, Johansson L, et al. Thrombomodulin as a marker for bleeding complications during warfarin treatment. *Arch.Intern.Med.* 7/13/2009 2009;169(13):1210-1215.
15. Roldan V, Marin F, Muina B, et al. Plasma von Willebrand factor levels are an independent risk factor for adverse events including mortality and major bleeding in anticoagulated atrial fibrillation patients. *J.Am.Coll.Cardiol.* 6/21/2011 2011;57(25):2496-2504.

16. van der Heijden JF, Rekke B, Hutten BA, et al. Non-fatal major bleeding during treatment with vitamin K antagonists: influence of soluble thrombomodulin and mutations in the propeptide of coagulation factor IX. *J.Thromb.Haemost.* 7/2004 2004;2(7):1104-1109.
17. van Rein N, Cannegieter SC, Rosendaal FR, Reitsma PH, Lijfering WM. Suspected survivor bias in case-control studies: stratify on survival time and use a negative control. *J.Clin.Epidemiol.* 2/2014 2014;67(2):232-235.
18. Palareti G, Leali N, Coccheri S, et al. Bleeding complications of oral anticoagulant treatment: an inception-cohort, prospective collaborative study (ISCOAT). Italian Study on Complications of Oral Anticoagulant Therapy. *Lancet.* 8/17/1996 1996;348(9025):423-428.
19. Go AS, Hylek EM, Chang Y, et al. Anticoagulation therapy for stroke prevention in atrial fibrillation: how well do randomized trials translate into clinical practice? *JAMA.* 11/26/2003 2003;290(20):2685-2692.
20. Rosendaal FR, Cannegieter SC, van der Meer FJ, Briet E. A method to determine the optimal intensity of oral anticoagulant therapy. *Thromb.Haemost.* 3/1/1993 1993;69(3):236-239.
21. Singer DE, Hellkamp AS, Piccini JP, et al. Impact of global geographic region on time in therapeutic range on warfarin anticoagulant therapy: data from the ROCKET AF clinical trial. *J.Am.Heart Assoc.* 2/2013 2013;2(1):e000067.
22. Connolly SJ, Pogue J, Eikelboom J, et al. Benefit of oral anticoagulant over antiplatelet therapy in atrial fibrillation depends on the quality of international normalized ratio control achieved by centers and countries as measured by time in therapeutic range. *Circulation.* 11/11/2008 2008;118(20):2029-2037.
23. White HD, Gruber M, Feyzi J, et al. Comparison of outcomes among patients randomized to warfarin therapy according to anticoagulant control: results from SPORTIF III and V. *Arch. Intern.Med.* 2/12/2007 2007;167(3):239-245.
24. Fang MC, Chang Y, Hylek EM, et al. Advanced age, anticoagulation intensity, and risk for intracranial hemorrhage among patients taking warfarin for atrial fibrillation. *Ann.Intern. Med.* 11/16/2004 2004;141(10):745-752.
25. Landefeld CS, Goldman L. Major bleeding in outpatients treated with warfarin: incidence and prediction by factors known at the start of outpatient therapy. *Am.J.Med.* 8/1989 1989;87(2):144-152.
26. Cannegieter SC, Rosendaal FR, Wintzen AR, van der Meer FJ, Vandenbroucke JP, Briet E. Optimal oral anticoagulant therapy in patients with mechanical heart valves. *N.Engl.J.Med.* 7/6/1995 1995;333(1):11-17.
27. Fihn SD, McDonell M, Martin D, et al. Risk factors for complications of chronic anticoagulation. A multicenter study. Warfarin Optimized Outpatient Follow-up Study Group. *Ann.Intern.Med.* 4/1/1993 1993;118(7):511-520.
28. Petitti DB, Strom BL, Melmon KL. Duration of warfarin anticoagulant therapy and the probabilities of recurrent thromboembolism and hemorrhage. *Am.J.Med.* 8/1986 1986;81(2):255-259.

APPENDIX 1

Table 1. Incidence rates of bleeding events stratified by clinical characteristics (atrial fibrillation patients, venous thrombosis patients, patients with a low target range)

	No. of events	Patient time (years)	Events/100 patient-years (95% CI)	AF patients Events/100 patient-years (95% CI)	VT patients Events/100 patient-years (95% CI)	Low target range Events/100 patient-years (95% CI)
Total	326	17 613	1.85 (1.66-2.06)	1.83 (1.61-2.07)	1.96 (1.48-2.55)	1.86 (1.66-2.08)
Sex						
Male	184	9 224	1.99 (1.72-2.30)	1.99 (1.68-2.35)	2.12 (1.45-3.01)	2.02 (1.73-2.34)
Female	142	8 387	1.69 (1.43-1.99)	1.66 (1.37-2.00)	1.80 (1.18-2.63)	1.69 (1.43-2.00)
INR target range						
2.5-3.5	306	16 454	1.86 (1.66-2.08)	1.83 (1.61-2.08)	1.98 (1.50-2.57)	NA
3.0-4.0	20	1 157	1.73 (1.09-2.62)	1.70 (0.08-8.36)	*	NA
Vitamin K antagonist						
Phenprocoumon	262	13 278	1.97 (1.75-2.22)	1.92 (1.67-2.21)	2.10 (1.54-2.80)	1.96 (1.73-2.22)
Acenocoumarol	64	4 333	1.48 (1.15-1.87)	1.53 (1.14-2.02)	1.54 (0.78-2.74)	1.53 (1.18-1.96)
Indication						
Atrial fibrillation	241	13 162	1.83 (1.61-2.07)	NA	NA	1.83 (1.61-2.08)
Venous thrombosis	53	2 702	1.96 (1.48-2.55)	NA	NA	1.98 (1.50-2.57)
Mechanical heart valves	4	351	1.14 (0.36-2.75)	NA	NA	1.53 (0.49-3.68)
Ischemic heart disease	7	555	1.26 (0.55-2.50)	NA	NA	1.13 (0.19-3.73)
Vascular	9	433	2.08 (1.01-3.81)	NA	NA	*
Postoperative	3	105	2.86 (0.72-7.78)	NA	NA	3.13 (0.79-8.51)
Other	9	384	2.34 (1.14-4.30)	NA	NA	2.90 (0.92-6.99)

* No events occurred in this subgroup

Table 2. Association of TTR with bleeding events stratified by clinical characteristics (atrial fibrillation patients, venous thrombosis patients, patients with a low target range)

			Events/100 patient-years (95% CI)			
			All	**AF patients**	**VT patients**	**Low target range**
Time in range						
< 35%	63	448	13.02 (10.09-16.54)	14.33 (10.65-18.89)	16.92 (8.90-29.41)	14.59 (11.26-18.62)
> 35% and < 50%	33	1 145	2.88 (2.02-4.00)	3.38 (2.27-4.85)	0.59 (0.03-2.90)	3.06 (2.12-4.30)
> 50% and < 60%	35	1 538	2.28 (1.61-3.13)	2.33 (1.55-3.36)	2.54 (1.03-5.29)	2.35 (1.64-3.26)
> 60% and < 70%	48	2 212	2.17 (1.62-2.85)	0.84 (0.58-1.17)	0.98 (0.40-2.04)	2.16 (1.59-2.88)
> 70% and < 80%	40	2 821	1.42 (1.03-1.91)	0.65 (0.45-0.91)	1.35 (0.73-2.30)	1.36 (0.97-1.86)
> 80% and < 90%	37	3 059	1.21 (0.86-1.65)	0.55 (0.38-0.78)	0.43 (0.16-0.96)	1.18 (0.83-1.62)
> 90%	62	5 361	1.16 (0.89-1.47)	0.42 (0.31-0.55)	0.51 (0.27-0.88)	1.21 (0.93-1.54)

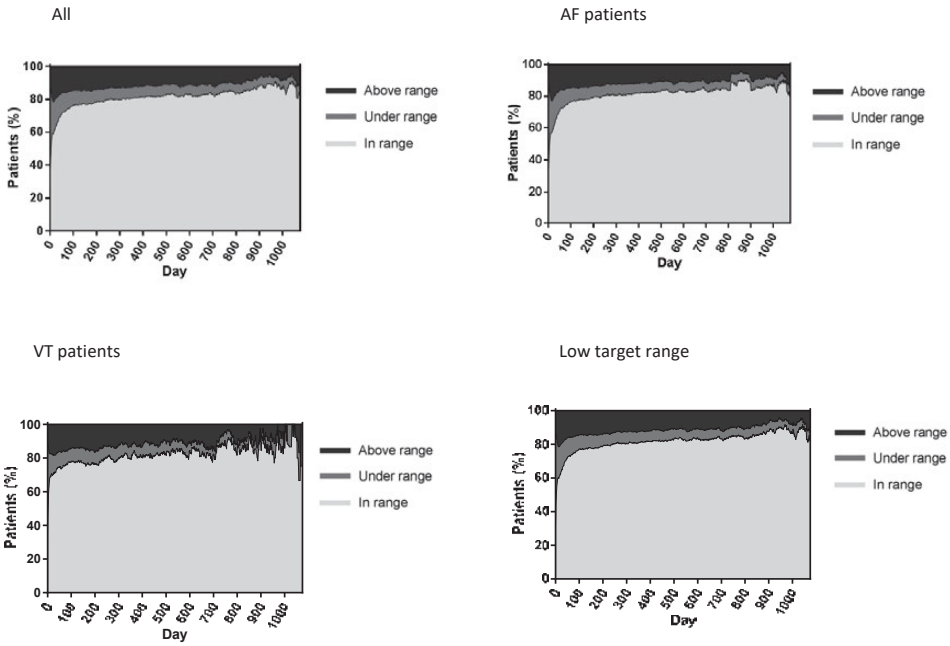


Figure 1. Time in therapeutic range per day after starting VKA treatment presented per sub group (atrial fibrillation patients, venous thrombosis patients, patients with a low target range)

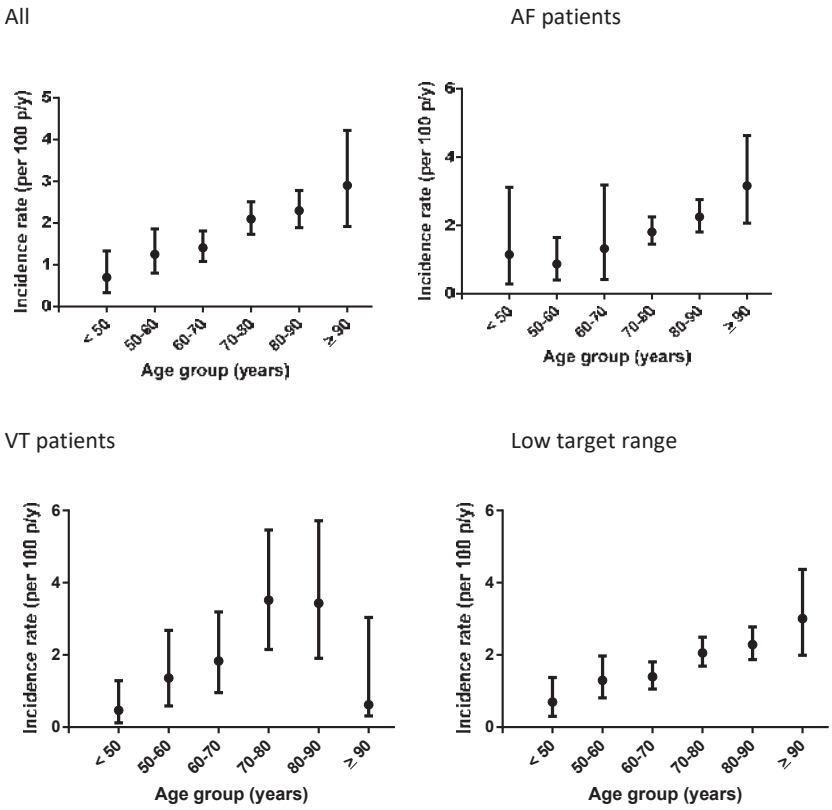
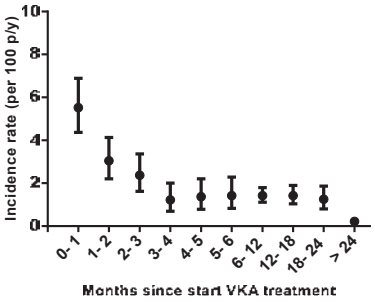
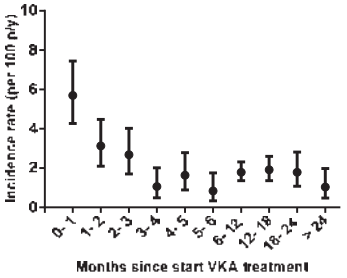


Figure 2. Incidence rates of bleeding events stratified by age presented per sub group (atrial fibrillation patients, venous thrombosis patients, patients with a low target range)

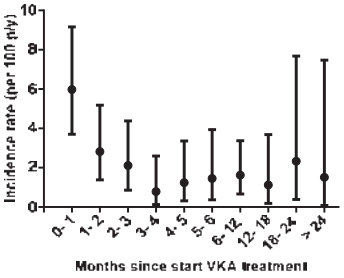
All



AF patients



VT patients



Low target range

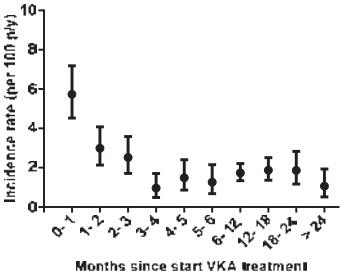
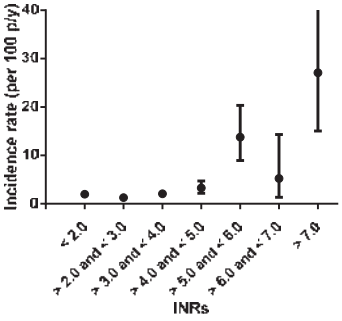
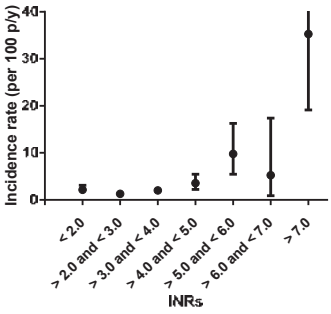


Figure 3. Incidence rates of bleeding events stratified by time since start of VKA treatment presented per sub group (atrial fibrillation patients, venous thrombosis patients, patients with a low target range)

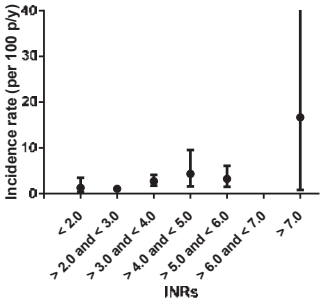
All



AF patients



VT patients



Low target range

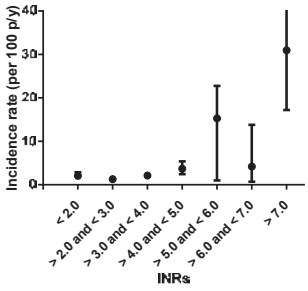
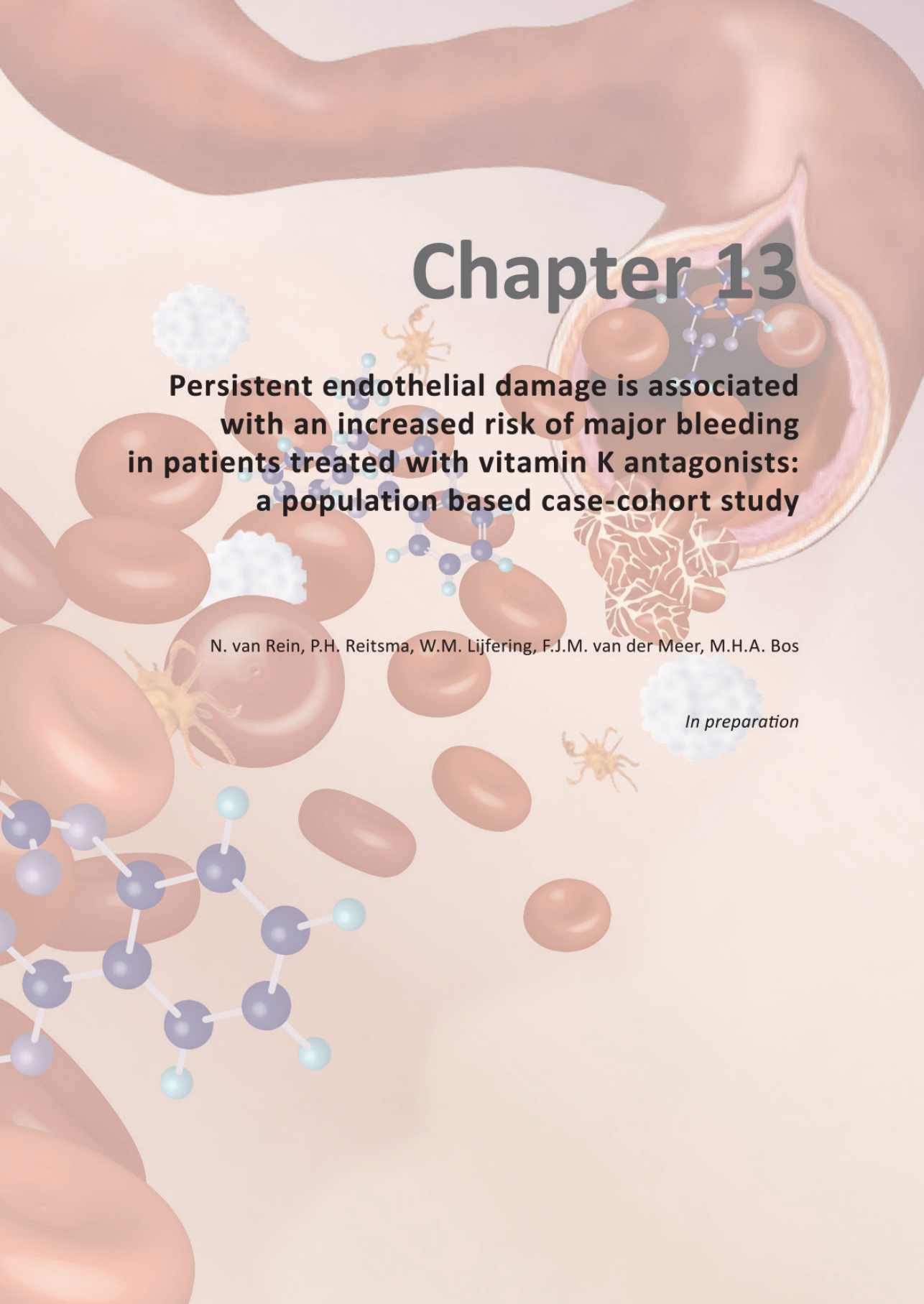


Figure 4. Incidence rates of bleeding events stratified by INR, presented per sub group (atrial fibrillation patients, venous thrombosis patients, patients with a low target range)



Chapter 13

Persistent endothelial damage is associated with an increased risk of major bleeding in patients treated with vitamin K antagonists: a population based case-cohort study

N. van Rein, P.H. Reitsma, W.M. Lijfering, F.J.M. van der Meer, M.H.A. Bos

In preparation

ABSTRACT

Background: Major bleeding is a serious side effect of anticoagulant treatment. Existing prediction scores for major bleeds in anticoagulated patients perform moderately well, necessitating the need for better predictors for major bleeding. Here we assess whether markers of chronic endothelial damage, i.e. soluble thrombomodulin (sTM), or acute endothelial damage, i.e. von Willebrand propeptide (VWFpp), are associated with an increased risk for major bleeding in VKA patients.

Methods: Plasma was collected from a cohort of 16570 patients starting VKA treatment between January 2012 and December 2014. Patients were followed until a major bleed, the end of VKA treatment, death, or December 31st 2014, whichever came first. From the cohort, we assembled a case-cohort study that included all 326 cases with a major bleeding and a random sample of 652 patients at baseline (subcohort). Plasma sTM and VWFpp levels were measured by ELISA and stratified by the 25th, 50th, 70th, and 85th percentiles. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated by means of weighted Cox regression and adjusted for age, sex, diabetes mellitus and hypertension.

Results: Plasma was available from 263 cases and 578 subcohort patients. Adjusted HRs increased dose dependently with increasing sTM levels, from 1.30 (95%CI 0.82-2.06) in the 25th to 50th percentile to 1.77 (95%CI 1.09-2.87) above the 85th percentile. Adjusted VWFpp levels were not associated with an increased risk for major bleeding.

Conclusion: Increased sTM levels are associated with major bleeding during VKA treatment, suggesting a role for chronic endothelial injury markers as predictors of major bleeding.

INTRODUCTION

Vitamin K antagonists (VKAs) are used to treat and prevent thromboembolism. Treatment with VKAs requires regular monitoring of the international normalized ratio (INR), which in the Netherlands is performed by anticoagulation clinics.[1] Despite frequent monitoring, the risk of major bleeding associated with VKA therapy remains substantial: approximately 1–3% of VKA-treated patients suffer from major bleeding complications each year.[1-3]

These major bleeding complications could be prevented by ceasing or adjusting the treatment for patients at high risk for major bleeding. Studies have been performed to identify patients at risk for major bleeding, with patient characteristics, co-medication, and co-morbidity emerging as potential risk factors. These risk factors have been combined in five different prediction models, which each performs modestly well.[4] For instance, The HAS-BLED score is considered to perform best in predicting bleeding risk, but has a c-statistic of only 0.65 (95% CI, 0.61-0.69).[4] Other prediction scores, like CHADS2 or CHA2DS2-VASc, poorly predict major bleeding risk as indicated by c-statistics of 0.55 (95% CI, 0.49-0.61) and 0.56 (95% CI, 0.53-0.59), respectively.[4] The present rather low capability to predict major bleeding events in anticoagulated patients therefore asks for an enhanced understanding of risk factors for major bleeding, which may lead to new and better predictors for major bleeding.

To reach this goal, we designed a case–cohort study in 16 570 patients who recently started with VKA treatment, of whom 326 experienced a major bleeding event.[5] Here we describe whether a marker of chronic endothelial damage, i.e. soluble thrombomodulin (sTM)[6-9], or of acute endothelial damage, i.e. propeptide of von Willebrand factor (VWFpp)[10,11], are associated with major bleeding during VKA treatment.

METHODS

Study Population

This population-based study enrolled VKA-treated patients in the “Biomarkers in the Leiden Etiology and Epidemiology of bleeding in vitamin K antagonists Drug users Study” (BLEEDS). The design is described in detail elsewhere.[5] In brief, between January 2012 and December 2014, 16 570 consecutive patients aged 18 years or older who started treatment with VKAs were included from three anticoagulation clinics in the Netherlands. Patients were followed according to the routine procedures of the anticoagulation clinics, and the INR was monitored with a frequency of at least once every six weeks. Patient characteristics were extracted from the computerized patient records of the anticoagulation clinics. Baseline characteristics included sex, age, indication for VKA treatment, type of VKA, INR target range, and co-medications. The BLEEDS was approved by the medical ethics committee of the Leiden University Medical Center.

Case identification

Major bleeding events were defined according to the guidelines of the Federation of Dutch Anticoagulation clinics (FNT) by trained anticoagulation clinic physicians who were not involved in the current study.[5] Bleeding events were classified as major if these were fatal, an intracranial bleeding, an objectively diagnosed joint bleed, a bleeding event in a critical organ, or lead to a blood transfusion or hospital admission.[12]

Blood sampling and laboratory procedures

We stored patient's blood and plasma samples that were leftover following INR analyses. The sample collection started three weeks after initiation of VKA therapy and, if applicable, two weeks after termination of low-molecular-weight-heparin (LMWH) treatment. To guarantee the privacy of the patients, technicians who were not involved in the study recoded patient numbers to study numbers. After recoding, patient specific characteristics were concealed and samples were labeled according to study number. The key linking patient to study numbers was maintained by a data manager who is not involved in the study.

Material was collected from 13 779 patients (83%). Material collection failed for 2791 patients because of logistic complications (1699 patients) or because they ceased VKA treatment prior to blood sampling (1092 patients).[5]

Plasma levels of sTM (R&D Systems, Minneapolis, US) and VWFpp[13] were assessed employing enzyme-linked immunosorbent assays. The assays were performed by a laboratory technician who was unaware of the case status of the samples.

Case-cohort study

A case-cohort study was performed in which we included all patients with a major bleeding (cases) and included a random subcohort from the BLEEDS cohort that was sampled at baseline as described by Prentice.[14] All 326 cases were sampled with plasma available from 263 cases. For the subcohort, a random sample of 4% within the whole cohort (652 patients) was selected, with plasma available from 538 subcohort patients. Due to the case-cohort design in which every person in the cohort, including the cases, had the same probability of being selected to the subcohort, six cases were also included in the subcohort.

Statistical analysis

For each VKA treatment, person time was calculated from the start of VKA therapy until the bleeding event, death, moving to a city that was not covered by the participating anticoagulation clinics, end of treatment, or end of the study period (31st of December 2014), whichever occurred first.

To account for the oversampling of cases, we estimated hazard ratios (HRs) and 95% confidence intervals (CIs) by means of weighted Cox proportional hazards models as described by Prentice [14]. The proportional hazards assumption was tested based on Schoenfeld residuals. Levels of sTM and VWFpp were stratified by the 25th, 50th, 70th, and 85th percentiles, with the 25th percentile acting as the reference category. HRs were adjusted for the confounding factors age, sex, diabetes, and hypertension.

An additional analysis was performed in which events were restricted to the first six and twelve months of treatment as we expected that effects would dilute when time between the measurement and event elapsed.

All analyses were performed with R version 2.15.2 (R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/).

Table 1. Baseline characteristics

	Whole Cohort	Subcohort	Cases
General characteristics			
Patients	16 185	538	263
Treatment periods	16 570	538	263
Men	8 713 (53)	293 (54)	147 (56)
Age	70 (14)	70 (12)	75 (11)
INR target range			
2.5-3.5	15 509 (93)	508 (94)	247 (94)
3.0-4.0	1 061 (7)	30 (6)	16 (6)
Treatment indication			
Atrial fibrillation	10 876 (66)	374 (70)	200 (76)
Venous thrombosis	3 920 (24)	112 (21)	40 (15)
Mechanical heart valves	435 (3)	17 (3)	3 (1)
Ischemic heart disease	519 (3)	17 (3)	7 (3)
Vascular disease	412 (3)	6 (1)	5 (2)
Postoperative	125 (1)	3 (1)	1 (0)
Other	348 (2)	11 (2)	7 (3)
Vitamin K antagonist			
Phenprocoumon	12 068 (73)	390 (72)	208 (79)
Acenocoumarol	4 481 (27)	148 (28)	54 (21)
Warfarin	18 (0)	0	1 (0)
Fluindione	3 (0)	0	0

RESULTS

We included 263 cases and 538 subcohort patients in the study (Table 1). Cases were on average 75 years old (SD 11 years), 147 (56%) were male, and the indication atrial fibrillation occurred most frequently (200 patients, 76%). Venous thrombosis patients were less prevalent among cases (40, 15%) than subcohort patients (112, 21%). The average age of the subcohort was 70 years (SD 12 years), 293 (54%) were male, and the indication atrial fibrillation was most frequent (374 patients, 70%). Phenprocoumon was used by 208 cases (79%) versus 390 subcohort patients (70%). Acenocoumarol was used by 148 cases (28%) and 54 subcohort patients (21%) 1 case used warfarin (0%).

The plasma levels of sTM and VWFpp were assessed for all subcohort patients and for 263 and 262 cases, respectively; one sample failed for technical reasons. The lower limit of detection was 5 ng/ml for the plasma sTM levels and 4 ml/dL for the VWFpp plasma levels. The highest plasma level measured was 53.5 ng/ml for sTM and 4.2 U/ml for VWFpp (Figure 1).

Plasma levels of sTM $\geq$ 8.9 ng/ml (85th percentile) were associated with a 1.77-fold increased risk for major bleeding (95%CI 1.09 to 2.87), which remained similar after adjustment for confounding (Table 2). There was no significant difference between the results of sTM of the whole follow-up as compared with the first six and twelve months of follow-up.

Increased plasma levels of VWFpp were also associated with an increased risk for major bleeding (VWFpp $\geq$ 1.6 U/ml, 85th percentile, HR 1.45, 95%CI 0.88 to 2.39) in a crude analysis, but were around unity after adjustment for confounding (Table 2). When the

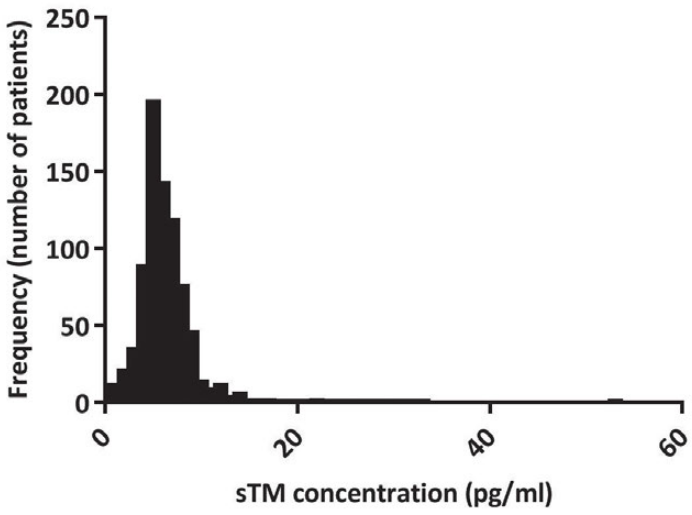
Table 2. Plasma levels of sTM and VWFpp in association with major bleeding during VKA treatment

	Cases n (%)	HR (95% CI)	HR[a] (95% CI)
Total	263 (100)		
sTM			
< 25 (<5.3 ng/ml)	57 (22)	reference	reference
25-50 (≥5.3 and <6.3 ng/ml)	63 (24)	1.15 (0.74-1.79)	1.30 (0.82-2.06)
50-70 (≥6.3 and <7.5 ng/ml)	46 (17)	1.18 (0.73-1.91)	1.27 (0.77-2.09)
70-85 (≥7.5 and <8.9 ng/ml)	44 (17)	1.51 (0.92-2.49)	1.63 (0.96-2.75)
> 85 (≥8.9 ng/ml)	53 (20)	1.77 (1.09-2.87)	1.79 (1.08- 2.97)
VWFpp			
< 25 (<0.7 U/ml)	53 (20)	reference	reference
25-50 (≥0.7 and <1.0 U/ml)	55 (21)	1.08 (0.65- 1.71)	1.04 (0.65-1.68)
50-70 (≥1.0 and <1.3 U/ml)	61 (23)	1.26 (0.79-1.99)	1.05 (0.64-1.71)
70-85 (≥1.3 and <1.6 U/ml)	47 (18)	1.39 (0.85-2.27)	1.15 (0.68-1.95)
> 85 (≥1.6 U/ml)	46 (18)	1.45 (0.88-2.39)	1.17 (0.69-1.99)

[a]Adjusted for age, sex, diabetes, and hypertension

analyses were restricted to the first six and twelve months of VKA treatment, increased VWFpp levels were associated with a non-significant 1.44- and 1.30-fold increased risk of major bleeding as compared with patients with low VWFpp levels (Table 3).

A



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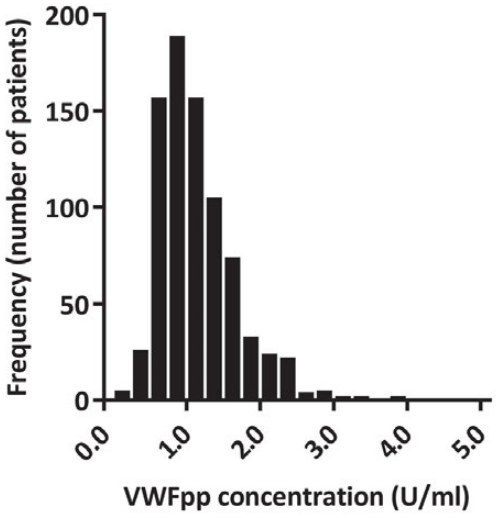


Figure 1. Distribution of concentrations of sTM (A) and VWFpp (B)

DISCUSSION

Using a unique case-cohort comprising 263 cases that experienced major bleeding complications during VKA therapy, we showed that levels of VWFpp were associated with major bleeding, but that the association disappeared after adjustment for confounding factors (i.e. age, sex, diabetes, and hypertension). Levels of sTM were dose-dependently associated with major bleeding during VKA treatment before and after adjusting for confounding. The results on sTM are consistent with previous findings,[7-9] which supports the premise that sTM is a marker of endothelial damage[15,16] and that endothelial damage leads to major bleeding. On the contrary, while VWFpp is also a marker of endothelial damage[10] and VWFpp plasma levels were not - strongly - associated with major bleeding after adjustment for confounding factors. A possible explanation for this discrepancy is that VWFpp is a marker of acute endothelial damage[10] while sTM is a marker for chronic endothelial damage.[15] In addition, the analyses on sTM restricted to cases with a major bleeding during the first six and twelve months gave similar results as the analyses with all cases. This also indicates that the association of sTM with major bleeding is as strong for the near- as the distant future, which could make it a good predictor for patients at risk for major bleeding.

This population based cohort study contained data from over 16 000 individuals with over 300 major bleeding events, which makes the results robust and allowed us to perform multiple subgroup analyses. A notable result was that venous thrombosis patients were less prevalent among cases than subcohort patients. This difference is due to the shorter duration of VKA treatment of venous thrombosis patients and therefore a shorter period to develop a major bleeding than patients with a chronic indication (e.g. atrial fibrillation).

A limitation of the study is that plasma was not collected from all patients who participated in the study as some patients experienced a major bleeding before plasma was collected. As a possible consequence, risk estimates may dilute because the patients with the strongest risk factors 'dropped out' of the study. Another limitation is that while the sTM concentrations measured were in the same range as those observed in other studies,[7,9] the median upper quartile sTM levels varied from 4-8 ng/ml (this study and [7]) to 61 ng/ml.[9] This is likely due to the different assays used in the studies and impede a direct comparison of sTM levels between studies.

Overall, this study showed that VWFpp levels were not associated with major bleeding during VKA treatment. On the contrary, sTM levels were associated with major bleeding, which indicates that chronic endothelial damage plays a role in major bleeding during VKA treatment.

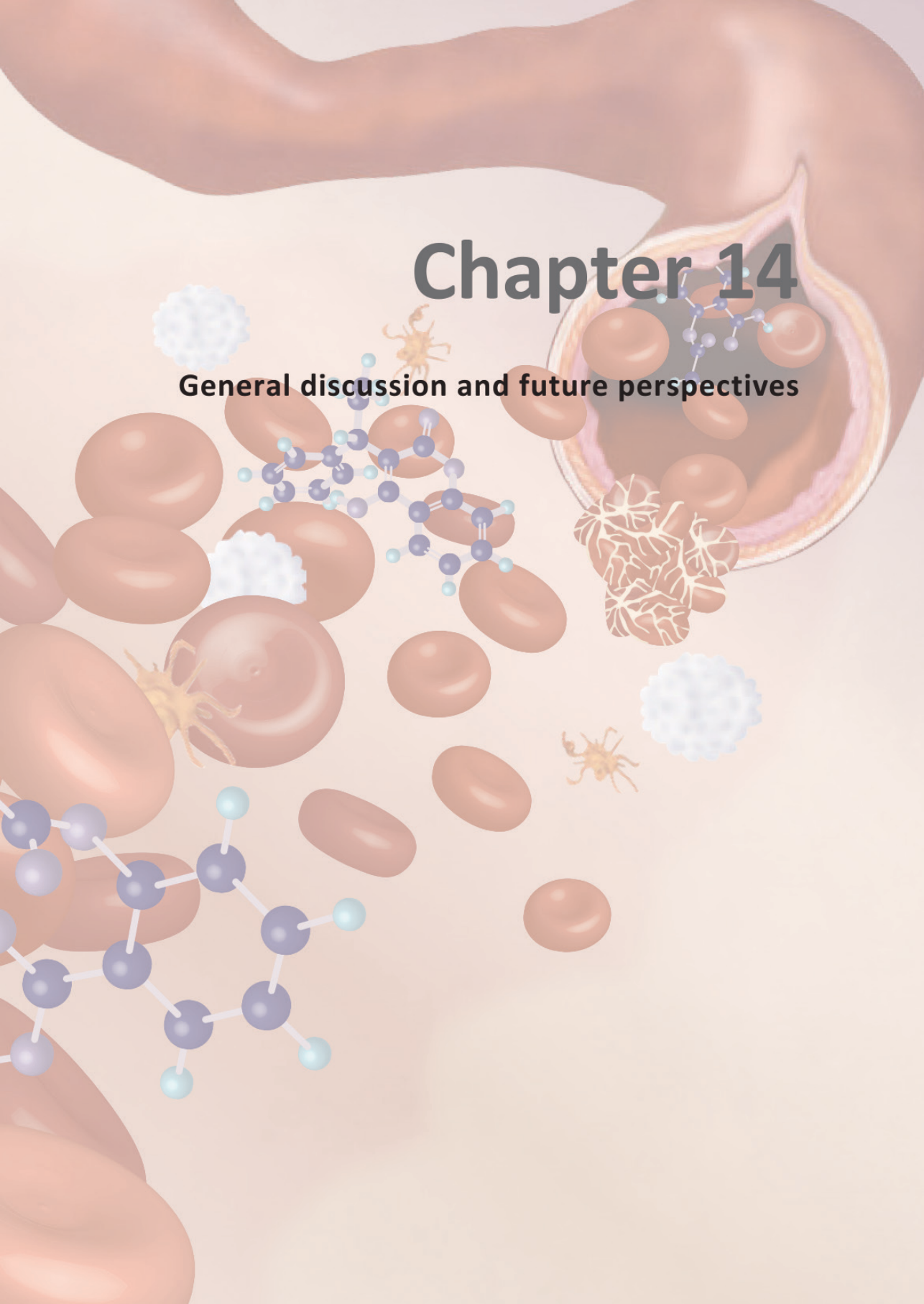
Table 3. Risk of major bleeding during VKA treatment at 6- and 12-month follow-up, stratified according to sTM and VWFpp plasma levels

	Cases n (%)	HR (95% CI)	HR[c] (95% CI)
6 month follow-up[a]	145 (100)		
sTM			
< 25 (<5.3 ng/ml)	33 (23)	reference	reference
25-50 (≥5.3 and <6.3 ng/ml)	29 (20)	0.88 (0.50-1.53)	0.94 (0.53-1.66)
50-70 (≥6.3 and <7.5 ng/ml)	25 (17)	0.97 (0.54-1.73)	1.01 (0.56-1.82)
70-85 (≥7.5 and <8.9 ng/ml)	25 (17)	1.25 (0.69-2.25)	1.29 (0.70-2.37)
> 85 (≥8.9 ng/ml)	33 (23)	1.61 (0.92-2.80)	1.59 (0.90-2.81)
VWFpp			
< 25 (<0.7 U/ml)	27 (19)	reference	reference
25-50 (≥0.7 and <1.0 U/ml)	30 (21)	1.12 (0.63-1.98)	1.09 (0.60-1.96)
50-70 (≥1.0 and <1.3 U/ml)	35 (24	1.57 (0.90-2.76)	1.32 (0.73-2.37)
70-85 (≥1.3 and <1.6 U/ml)	26 (18)	1.61 (0.88-2.94)	1.30 (0.69-2.46)
> 85 (≥1.6 U/ml)	27 (19)	1.74 (0.95-3.18)	1.44 (0.76-2.71)
12 month follow-up[b]	206 (100)		
sTM			
< 25 (<5.3 ng/ml)	40 (19)	reference	reference
25-50 (≥5.3 and <6.3 ng/ml)	47 (23)	1.18 (0.73-1.93)	1.32 (0.79-2.18)
50-70 (≥6.3 and <7.5 ng/ml)	38 (18)	1.29 (0.77-2.16)	1.35 (0.79-2.30)
70-85 (≥7.5 and <8.9 ng/ml)	37 (18)	1.61 (0.95-2.74)	1.69 (0.97-2.94)
>85 (≥ 8.9 ng/ml)	44 (21)	1.90 (1.40-3.18)	1.90 (1.11-3.23)
VWFpp			
< 25 (<0.7 U/ml)	39 (19)	reference	reference
25-50 (≥0.7 and <1.0 U/ml)	47 (23)	1.21 (0.74-1.98)	1.17 (0.70-1.94)
50-70 (≥1.0 and <1.3 U/ml)	46 (22)	1.33 (0.81-2.20)	1.13 (0.67-1.90)
70-85 (≥1.3 and <1.6 U/ml)	39 (19)	1.59 (0.94-2.70)	1.33 (0.76-2.32)
> 85 (≥1.6 U/ml)	35 (17)	1.58 (0.92-2.70)	1.30 (0.74-2.30)

Analysis of the risk for major bleedings that had occurred during the first [a]six or [b]twelve months of VKA therapy. [c]Adjusted for age, sex, diabetes, and hypertension

REFERENCES

1. van der Meer FJ, Rosendaal FR, Vandenbroucke JP, Briet E. Bleeding complications in oral anticoagulant therapy. An analysis of risk factors. *Arch.Intern.Med.* 7/12/1993 1993;153(13):1557-1562.
2. Linkins LA. Bleeding risks associated with vitamin K antagonists. *Blood reviews.* May 2013;27(3):111-118.
3. Levine MN, Raskob G, Beyth RJ, Kearon C, Schulman S. Hemorrhagic complications of anticoagulant treatment: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest.* Sep 2004;126(3 Suppl):287S-310S.
4. Zhu W, He W, Guo L, Wang X, Hong K. The HAS-BLED Score for Predicting Major Bleeding Risk in Anticoagulated Patients With Atrial Fibrillation: A Systematic Review and Meta-analysis. *Clin.Cardiol.* 9/2015 2015;38(9):555-561.
5. van Rein N, Lijfering WM, Bos MH, et al. Objectives and design of BLEEDS: a cohort study to identify new risk factors and predictors for major bleeding during treatment with vitamin K antagonists. *Plos One.* 2016.
6. Takano S, Kimura S, Ohdama S, Aoki N. Plasma thrombomodulin in health and diseases. *Blood.* Nov 15 1990;76(10):2024-2029.
7. van der Heijden JF, Rekke B, Hutten BA, et al. Non-fatal major bleeding during treatment with vitamin K antagonists: influence of soluble thrombomodulin and mutations in the propeptide of coagulation factor IX. *Journal of thrombosis and haemostasis : JTH.* Jul 2004;2(7):1104-1109.
8. Lind M, Boman K, Johansson L, et al. Thrombomodulin as a marker for bleeding complications during warfarin treatment. *Archives of internal medicine.* Jul 13 2009;169(13):1210-1215.
9. Jansson JH, Boman K, Brannstrom M, Nilsson TK. High concentration of thrombomodulin in plasma is associated with hemorrhage: a prospective study in patients receiving long-term anticoagulant treatment. *Circulation.* Nov 4 1997;96(9):2938-2943.
10. Haberichter SL. VWF propeptide in defining VWD subtypes. *Blood.* May 7 2015;125(19):2882-2883.
11. De Jong A, Eikenboom J. Developments in the diagnostic procedures for von Willebrand disease. *Journal of thrombosis and haemostasis : JTH.* Mar 2016;14(3):449-460.
12. Trombosediensten FvN. *FNT- NORMEN.* 2015 2015.
13. Borchiellini A, Fijnvandraat K, ten Cate JW, et al. Quantitative analysis of von Willebrand factor propeptide release in vivo: effect of experimental endotoxemia and administration of 1-deamino-8-D-arginine vasopressin in humans. *Blood.* Oct 15 1996;88(8):2951-2958.
14. Prentice R. A case-cohort design for epidemiologic cohort studies and disease prevention trials. *Biometrika.* 1986;73:1-11.
15. Ishii H, Uchiyama H, Kazama M. Soluble thrombomodulin antigen in conditioned medium is increased by damage of endothelial cells. *Thrombosis and haemostasis.* May 6 1991;65(5):618-623.
16. Seigneur M, Dufourcq P, Conri C, et al. Plasma thrombomodulin: new approach of endothelium damage. *International angiology : a journal of the International Union of Angiology.* Dec 1993;12(4):355-359.



Chapter 14

General discussion and future perspectives

Pharmacoepidemiology is the study of utilization and effects of drugs in large numbers of people. It provides estimates of the probability of beneficial effects and the probability of adverse events. The first part of this thesis focusses on methodological aspects of pharmacoepidemiology research. The second part of this thesis describes interventions to reduce rates of major bleeding during treatment with antithrombotic drugs. New insights arise from this thesis, which will be discussed here. In addition, recommendations will be given for future research.

PHARMACOEPIDEMIOLOGY

Causality inferred from pharmacoepidemiologic statin studies

The many beneficial effects that are attributed to statins may not be causal but due to biases. In **Chapters 2** and **Chapter 3**, we set out to examine whether one beneficial effect (on bleeding risk in this case) of statin usage is due to three sorts of bias: survivor bias, prevalent user bias and healthy user bias. In the case-control study described in **Chapter 2**, we first determined if statin use was associated with a lower probability of major bleeding, and indeed we initially documented a protective association (odds ratio [OR]: 0.56; 95% confidence interval [CI]: 0.29 to 1.08). This protective association could, however, be fully attributed to survivor bias. This type of bias can occur when exposed cases are less likely to get included in a study as compared with other participants. Survivor bias became evident by stratifying on time between the onset of the major bleeding and the lapsed time before inclusion in the study, and repeated for a negative control: an exposure which is not related with death but is related with bleeding (i.e. blood group non-O). In **Chapter 3** we showed that a 'new user design' (i.e. a comparison of patients who started using statins during follow-up with non-users) produces more reliable risk estimates than a 'current user design' (i.e. statin users at baseline compared with non-users) for statin studies. Furthermore, it became apparent that in the elderly, beneficial associations can be attributed to a healthy user bias. Both Chapters showed that results from the straightforward analysis indicated that statins reduce the risk of bleeding complications, whereas sensitivity analyses indicated that these associations were likely due to bias.

Survivor bias in case-control studies

One question that arose from **Chapter 2** was whether survivor bias should always be suspected in case-control studies that showed positive effects of statin usage. In this context it is interesting to note results from a meta-analysis on the effect of statins on venous thrombosis.[1] In this meta-analysis the authors report the lowest relative risk of venous thrombosis in studies that had enrolled individuals on statins who had survived their event until inclusion (relative risk estimates between 0.20 and 0.60). All other

studies reported relative risk estimates that were close to unity (between 0.74 and 1.02).[1] These results suggest that the studies with the lowest relative risks may also have suffered from survivor bias, supporting the conclusions in **Chapter 2**, that this type of bias may be common in case-control studies on effects of statins. This is a major concern, as causality is often inferred from such pharamacoepidemiological studies. For this reason, this thesis advocates checking for survivor bias in case-control studies by performing a sensitivity analysis where results are stratified by time between event and inclusion. Of note, such a sensitivity analysis only gives useful insight when patients were included gradually over a time period after the event. Often, cases are included immediately after the event, and as a result, the proposed sensitivity analysis will not add information on differential inclusion of (un)exposed cases.

Ideally one would be able to know in advance whether a combination of an exposure and outcome is susceptible to survivor bias. To get more insight in survivor bias Hu et al. used a cohort and studied whether survivor bias would occur when time between the outcome (pancreatic cancer) and 'simulated inclusion' elapsed. Patients were considered able to participate in the study when they were alive.[2] They showed that the association between the exposures body mass index and waist circumference and the outcome pancreatic cancer underestimates the risk when time between the event and inclusion elapsed. However, a downside of such a method is that it assumes that all patients will actually participate in a study, which is usually not the case. Another method to study issues regarding participation in a case-control study is by comparing included patients in case-control studies with groups of patients in registries, as patient populations from registries are usually unselected.

A second recommendation from this thesis is to use negative- or positive controls in (pharmaco)epidemiological studies. Positive and negative controls (i.e. performing an analysis under conditions in which a null result [negative control] or a positive result [positive control]) is expected have been standard practise in experimental research for a long time. In (pharmaco)epidemiology, additional sensitivity analyses with a positive and/or negative control will provide additional information that may strengthen the conclusions. For statins, a negative control could be fibrate usage, as these drugs are prescribed for similar indications as statins, but have different pharmacodynamics.[3] If protective results for fibrates are similar to results regarding statins, or even stronger, this may indicate that protective associations are due to bias, and not due to the statins.

Prevalent- and healthy user bias in cohort studies

The analysis of the cohort study described in **Chapter 3** suffered from prevalent user bias. In addition, results from our examination indicated that a 'new user' design may not overcome all biases, as elderly patients who initiate a statin may be healthier than elderly patients who did not initiate a statin.

Whether these biases affect results from other cohort studies on effects of statins is not known. A meta-analysis showed that studies where prevalent statins users were compared with non-users gave overoptimistic results.[4] The bias that occurs from using current users instead of new users was also described in relation to the protective effects on cardiovascular disease attributed to hormone replacement therapy.[5] Results of these studies suggest that a 'new user' design is always required when studying causal effects of drugs.

The past few years it was advocated that all pharmacoepidemiological studies should be following a 'new user' design. However, a disadvantage of such a design is that the power of the study is lower as compared with a current user design and that a 'new user' design may not be preferable for all research questions. For example, one may expect that treatments that are initiated and mainly continued by health-conscious people (e.g. statins, hormone replacement therapy, vitamins) combined with an outcome that relates to life style (e.g. cardiovascular disease) are more susceptible to bias due to a current user design. On the other hand, treatments that are not likely to be ceased (e.g. painkillers for chronic pain) and outcomes scarcely related to life style (breast cancer) may be less susceptible.[6] In addition, exposures with a lasting effect may not suffer from current user bias (oral contraceptives on breast cancer). In contrast, the same exposure with a temporary and current effect can (oral contraceptives on venous thrombosis). These examples illustrate that a 'new user' design may not be necessary for al research questions.[6]

Causality in studies on vitamins

Vitamins are known for their wide range of beneficial effects from observational studies, such as cardiovascular disease, cancer, and venous thrombosis.[7] However, previous reports did not take confounding fully into account. The aim in **Chapter 4** was to determine whether vitamin supplementation decreases the risk of venous thrombosis using a case-control study. The MEGA case-control study provided an excellent opportunity to study this because the study has two controls groups (random-digit dialing (RDD) controls or patients' partners), which enabled us to correct for both measured and unmeasured confounding factors. The comparison with RDD controls was corrected for measured confounding. The comparison with partners enabled to also correct for unmeasured confounding as partners tend to have the same life-style. We found that vitamin use led to a 37% lower risk of venous thrombosis than no vitamin use (OR: 0.63, 95% CI 0.57 to 0.70) when comparing patients with RDD controls. Adjustment for confounding factors did not change the estimate (OR 0.68, 95% CI 0.61 to 0.77). However, when patients were compared with partner controls, ORs attenuated to unity. Therefore, the initial protective risk estimates that were found were due to improper control selection. These results reinforce that adjusting

for lifestyle-dependent confounding might not be sufficient when studying health-conscious exposures. In addition, the control group must be selected with care, which may also be true for other studies on health-conscious exposures such as sports, food patterns and alcohol intake.

Control selection in case-control studies

Chapter 5 focused on differences in risk estimates when using RDD controls or partner controls in case-control studies. The motivation to perform this study was that patients were less willing to participate in the trial described in **Chapter 10** if they had a relative (e.g. partner, child) who was also ill. We therefore hypothesized that partner controls are less willing to participate in a study if they, next to their partner (i.e. the case), are seriously ill. If true, differences in participation rates can lead to discrepancies between effect estimates (ORs) when exposures that impair quality of life are studied and partner- and RDD controls are used. The results of this study showed that decreased quality of life due to exposure to a chronic condition was associated with higher ORs when using partner controls as compared with RDD controls. The results were confirmed by an analysis with a negative control that showed that two exposures not associated with changes in quality of life (genetic variants), gave similar risk estimates for both control groups.

Unfortunately, we were not able to study the reason for the differences between the risk estimates. This was because data on reasons not to participate were not available. In addition, literature that describes differences between participation rates of partner- and RDD controls is not available. However, RDD control groups have been compared with other population based controls. One study found that RDD controls, as compared with controls who were recruited by mail and visited at home, were subject to more screening tests and had a slightly higher prevalence of health outcomes such as a high cholesterol.[8] These differences may indicate that ill subjects recruited by RDD may participate more frequently as compared with other control groups. This difference in participation rate may explain the differences in risk estimates found between the partner- and RDD controls. An alternate explanation for the different risk estimates may be that the burden of care for the patient by a very ill partner (control) is high and may result in declining to participate in a study, as was also experienced during participant selection of **Chapter 10**. These lower participation rates would result in higher risk estimates when using partner controls instead of RDD controls.

A limitation of this study is that it is not possible to study the cause of the difference in risk estimates. Therefore, a recommendation cannot be given on the most appropriate control group when studying research questions with exposures that impair the quality of life considerably. Future research is needed to determine what the source of these discrepancies is and show what control group is most appropriate under these circumstances.

BLEEDING DURING ANTICOAGULANT TREATMENT

Pharmacological interventions

The second part of this thesis looks into major bleeding during treatment with oral anticoagulants. Patients have been treated with vitamin K antagonists for over half a century[9], but we still cannot predict very well who is at high risk of major bleeding and should receive no or alternative anticoagulant treatment. This urgently calls for optimization of treatment, interventions to ease medication intake, and a better ability to identify patients at risk of major bleeding complications.

Optimization of treatment

Triple therapy

Atrial fibrillation patients often have coexisting cardiovascular diseases that may require concurrent treatment with platelet inhibitors. Some patients receive triple therapy (VKA, aspirin, clopidogrel), which increases the risk of major bleeding four-fold as compared with patients who receive VKA monotherapy.[10] In previous studies it was not clear what the major bleeding rates were for different groups of patients and thus what the clinical impact of this four-fold increased risk was. Results from **Chapter 6** showed that patients who receive triple therapy have very high rates of major bleeding (5.8 to 50.0 per 100 patient-years). These high rates of major bleeding raise the question whether concomitant use of three anticoagulants is recommended. Such recommendations could not be given based on this study for two reasons. First, risk factors for ischemic events and major bleeding overlap[11], making it hard to distinguish which patients are at high risk for major bleeding, but not at risk for ischemic events, and vice versa. Second, due to confounding by indication, this non-randomized study does not permit evaluation of the effectiveness of combinations of antithrombotic drugs (i.e., medication could have been indicated due to high risk of thromboembolic outcomes). Still, two groups that stood out were patients over 90 years of age (incidence rate of major bleeding 50.0 per 100 patient-years) and those with a CHA2DS2-VASc of 7 to 9 (incidence rate 20.0 per 100 patient-years). These high rates of major bleeding suggest that triple therapy is contra-indicated in these patients.

Low-Molecular-Weight-Heparins

The Low-Molecular-Weight-Heparins (LMWHs) currently on the market are considered members of a class of drugs with similar anticoagulant properties. However, pharmacodynamics and pharmacokinetics between LMWHs differ.[12] These differences could result in different bleeding risks for single LMWH agents. **Chapter 7** and **Chapter 8** suggest that, in venous thrombosis patients, nadroparin was associated with the lowest bleeding risk of all LMWHs. In addition, results suggest that twice daily dosing of nadroparin is associated with a higher bleeding risk as compared with once daily

dosing. Results from the network meta-analysis (**Chapter 8**) also show that once daily nadroparin is not associated with an increased risk of venous thrombosis as compared with other LMWHs. Based on these results, the most optimal LMWH strategy in patients with acute venous thrombosis appears to be treatment with nadroparin once daily. Still, small numbers of events and considerable risks of bias prevented us from concluding whether one LMWH should be preferred over the other.

One way to further explore whether a single LMWH agent and dosing schedule has benefits over other LMWHs and dosing schedules is by conducting a randomized controlled trial. However, such a trial would have to be very large (for example for a trial with two LMWHs, with a risk of 5% of recurrent venous thrombosis during LMWH treatment, one would need 1600 patients to find a relative risk of 0.6 with sufficient power) and will therefore be expensive and time consuming. An alternative for such trials could be a pragmatic trial, which is a trial conducted in routine clinical practice.[13] In such a trial, hospitals or general physicians are asked to participate and patients will be randomized to one of the available LMWHs by the prescription system when the physician prescribes the LMWH. As there is currently no preference for a particular LMWH, it may not even be necessary to obtain written informed consent for such a study. Outcomes can be collected through registries. Such pragmatic trials may not only provide an adequate way to study differences between LMWH agents, but also for other classes of drugs. It is often assumed that drugs from the same class do have similar effects on the studies outcomes. However, pharmacodynamics can differ and pharmacokinetics do usually differ between agents from a drug class, which may result in differences in effectiveness and rates of side effects. Pragmatic trials may provide a feasible way to study these differences in the population that actually receives the drugs.

Practical interventions

Multidose drug dispensing

Adherence can be challenging for polypharmacy patients. Research shows that adherence decreases with the number of prescribed medications and increasing regimen complexity.[14,15] An intervention that may improve medications adherence is multidose drug dispensing (MDD). MDD is a dosing aid that provides patients with disposable bags containing all drugs intended for one dosing moment. The effect of MDD was only examined in one cross sectional study by Kwint *et al.* where MDD was associated with a better self-reported medication adherence.[16] **Chapter 9** shows that in non-compliant patients, MDD improves medication adherence within one month, whereas instructing patients is associated with equal improvement of drug adherence over a four month period. This result suggests that medication adherence improves faster by MDD than by instructing patients. In addition, MDD contains all medications,

whereas the instructions in this study were limited to VKAs. Currently, this and the study from Kwint *et al.*[16] are the only studies on adherence improvement of MDD, and more research is necessary to confirm these results. A relating question is which patients would benefit from MDD. Currently, patients are using MDD on request of the patient or when a relative or health care professional of the patient initiates MDD. By identifying patients in need of MDD proactively, it may be possible to prevent medication intake errors and subsequent failure of therapy or side effects.

Vitamin K tablets

In **Chapter 10** we compared a soluble vitamin K1 formulation with tablets with respect to the bioavailability and effects on the international normalized ratio (INR) in three randomized controlled trials. Results show that the bioavailability from the vitamin K1 solution is higher than that from the tablets. Still, the tablets were as effective and safe as the vitamin K1 solution with regard to the ability to decrease the INR in patients on phenprocoumon. This indicates that pharmacies can safely provide tablets instead of the solution.

This was the first study to report the effect of 5 mg vitamin K1 in patients using phenprocoumon. Patients who participated in the study with a scheduled invasive procedure all had an INR low enough for the procedure, indicating that 5 mg of vitamin K1 is an appropriate dosage for lowering the INR for invasive procedures in phenprocoumon users. In the study involving patients with an INR between 7.0 and 11.0, 11% of all patients had an INR < 2.0 after 48 h. Future research may be required to determine the best vitamin K1 dosage to decrease the INR in phenprocoumon users with a high INR. Such data is also necessary for acenocoumarol and warfarin users.

In our study, fewer 'late low INRs' were found than in the literature on warfarin.[17-20] The difference between our and the earlier findings may be explained by the longer half-life of phenprocoumon than of warfarin. When phenprocoumon is restarted on the third day, blood concentrations of phenprocoumon may be higher than those of warfarin, which may result in faster-rising INRs.

One other notable result was that two patients had no INR decrease after ingesting vitamin K1. Some physicians or dentists do not measure the INR before performing an invasive procedure. These results indicate that it may be necessary to measure the INR before starting procedures where it is clinically relevant that the INR is low.

Prediction of major bleeding complications

Chapter 11 describes why patients on vitamin K antagonists with minor bleeds are at increased risk of subsequent major bleeding (e.g. are risk factors of a transient or fixed nature). Two analyses were performed: a Cox proportional hazards model which was adjusted for known risk factors and a case-crossover design which corrects for fixed risk factors because patients are compared with themselves. The Cox model showed that

the risk of major bleeds was 2.5-fold increased in patients with a preceding minor bleed. The case-crossover analysis gave risk estimates around unity (OR 0.9, 95% CI 0.5 to 1.5). The combination of both analyses indicated that minor bleeds are markers for fixed and currently unknown risk factors for major bleeds. Identification of these risk factors may provide new predictors for whom is at risk of major bleeding complications during treatment with vitamin K antagonists, especially considering that current prediction models for major bleeding complications do not distinguish well between patients who are at moderate and high risk of major bleeding complications.[21]

Biomarkers and genetics may be a source for new risk factors for major bleeding during VKA treatment. These may be discovered in the Biomarkers in the Leiden Etiology and Epidemiology of bleeding in vitamin K antagonists Drug users Study (BLEEDS) which is described in **Chapter 12**. Patients starting VKA treatment were included in the BLEEDS, with an inclusion rate of 99%, resulting in 16 570 patients, who experienced 326 major bleeding complications during 17 613 patient years of follow-up. The characteristics of the study showed that the population is in concordance with previously published cohorts with regard to bleeding complications during treatment with VKAs. This is the first population based study with a high number of bleeding complications that will provide the possibility to perform subgroup analyses and provide information on the currently treated population with vitamin K antagonists.

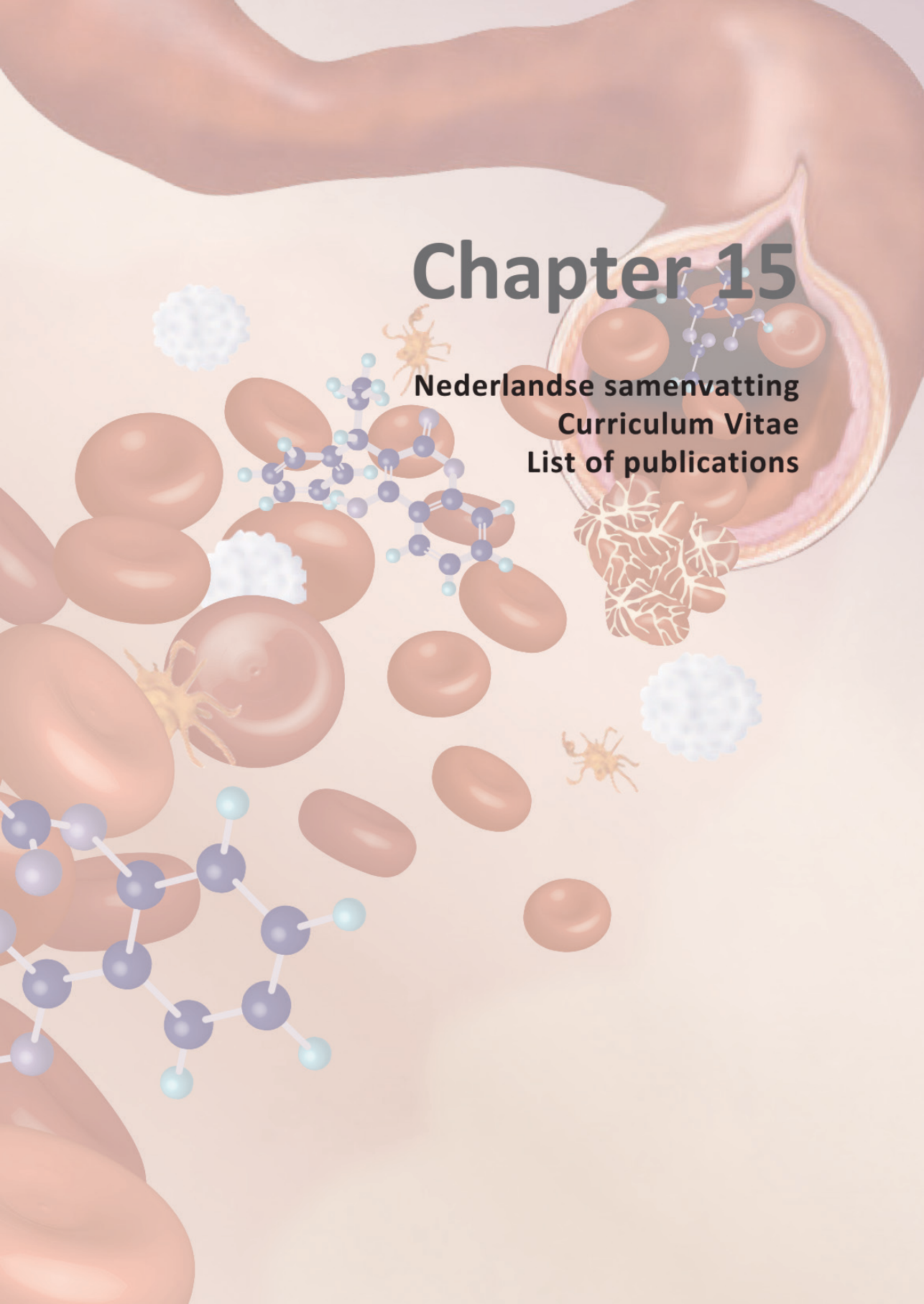
Chapter 13 shows the first results from the BLEEDS, where elevated levels of soluble thrombomodulin - a biomarker for chronic endothelial damage - were associated with major bleeding during VKA treatment. These results do cohere with those from two other studies.[22,23] Results also showed that another potential biomarker, von Willebrand factor propeptide, which reflects acute endothelial damage, was not associated with major bleeding during VKA treatment. These results were corroborated by the results from **Chapter 11**, which indicated that fixed risk factors are associated with major bleeding. Future research needs to clarify what these risk factors are and subsequently improve prediction models to ensure that patients receive their optimal personalized treatment.

REFERENCES

1. Pai M, Evans NS, Shah SJ, Green D, Cook D, Crowther MA. Statins in the prevention of venous thromboembolism: a meta-analysis of observational studies. *Thrombosis research.* Nov 2011;128(5):422-430.
2. Hu ZH, Connett JE, Yuan JM, Anderson KE. Role of survivor bias in pancreatic cancer case-control studies. *Annals of epidemiology.* Jan 2016;26(1):50-56.
3. *Informatorium Medicamentorium.* Den Haag: KNMP WINAp; 2016.

4. Danaei G, Tavakkoli M, Hernan MA. Bias in observational studies of prevalent users: lessons for comparative effectiveness research from a meta-analysis of statins. *American journal of epidemiology.* Feb 15 2012;175(4):250-262.
5. Hernan MA, Alonso A, Logan R, et al. Observational studies analyzed like randomized experiments: an application to postmenopausal hormone therapy and coronary heart disease. *Epidemiology.* Nov 2008;19(6):766-779.
6. Vandenbroucke J, Pearce N. Point: incident exposures, prevalent exposures, and causal inference: does limiting studies to persons who are followed from first exposure onward damage epidemiology? *American journal of epidemiology.* Nov 15 2015;182(10):826-833.
7. Myung SK, Ju W, Cho B, et al. Efficacy of vitamin and antioxidant supplements in prevention of cardiovascular disease: systematic review and meta-analysis of randomised controlled trials. *Bmj.* 2013;346:f10.
8. Olson SH, Kelsey JL, Pearson TA, Levin B. Evaluation of random digit dialing as a method of control selection in case-control studies. *American journal of epidemiology.* Jan 15 1992;135(2):210-222.
9. Griminger P. Vitamin K antagonists: the first 50 years. *The Journal of nutrition.* Jul 1987;117(7):1325-1329.
10. Hansen ML, Sorensen R, Clausen MT, et al. Risk of bleeding with single, dual, or triple therapy with warfarin, aspirin, and clopidogrel in patients with atrial fibrillation. *Archives of internal medicine.* Sep 13 2010;170(16):1433-1441.
11. Lip GY, Lane DA, Buller H, Apostolakis S. Development of a novel composite stroke and bleeding risk score in patients with atrial fibrillation: the AMADEUS Study. *Chest.* Dec 2013;144(6):1839-1847.
12. White RH, Ginsberg JS. Low-molecular-weight heparins: are they all the same? *British journal of haematology.* Apr 2003;121(1):12-20.
13. Staa TP, Goldacre B, Gulliford M, et al. Pragmatic randomised trials using routine electronic health records: putting them to the test. *Bmj.* 2012;344:e55.
14. Sabate E. Adherence to long-term therapies: evidence for action. *World Health Organisation (WHO). 2003. http://www.who.int/chp/knowledge/publications/adherence_report/en/. Accessed 22 January 2017.* 2003.
15. Kardas P, Lewek P, Matyjaszczyk M. Determinants of patient adherence: a review of systematic reviews. *Frontiers in pharmacology.* 2013;4:91.
16. Kwint HF, Stolk G, Faber A, Gussekloo J, Bouvy ML. Medication adherence and knowledge of older patients with and without multidose drug dispensing. *Age and ageing.* Sep 2013;42(5):620-626.
17. Gunther KE, Conway G, Leibach L, Crowther MA. Low-dose oral vitamin K is safe and effective for outpatient management of patients with an INR>10. *Thrombosis research.* 2004;113(3-4):205-209.

18. Crowther MA, Julian J, McCarty D, et al. Treatment of warfarin-associated coagulopathy with oral vitamin K: a randomised controlled trial. *Lancet.* Nov 4 2000;356(9241):1551-1553.
19. Weibert RT, Le DT, Kayser SR, Rapaport SI. Correction of excessive anticoagulation with low-dose oral vitamin K1. *Annals of internal medicine.* Jun 15 1997;126(12):959-962.
20. Crowther MA, Donovan D, Harrison L, McGinnis J, Ginsberg J. Low-dose oral vitamin K reliably reverses over-anticoagulation due to warfarin. *Thrombosis and haemostasis.* Jun 1998;79(6):1116-1118.
21. Zhu W, He W, Guo L, Wang X, Hong K. The HAS-BLED Score for Predicting Major Bleeding Risk in Anticoagulated Patients With Atrial Fibrillation: A Systematic Review and Meta-analysis. *Clinical cardiology.* Sep 2015;38(9):555-561.
22. Lind M, Boman K, Johansson L, et al. Thrombomodulin as a marker for bleeding complications during warfarin treatment. *Archives of internal medicine.* Jul 13 2009;169(13):1210-1215.
23. van der Heijden JF, Rekke B, Hutten BA, et al. Non-fatal major bleeding during treatment with vitamin K antagonists: influence of soluble thrombomodulin and mutations in the propeptide of coagulation factor IX. *Journal of thrombosis and haemostasis : JTH.* Jul 2004;2(7):1104-1109.



Chapter 15

Nederlandse samenvatting
Curriculum Vitae
List of publications

NEDERLANDSE SAMENVATTING

Dit proefschrift bestaat uit twee delen. Het eerste deel van het proefschrift gaat over de methodologie van geneesmiddelonderzoek (farmacoepidemiologie). Het tweede deel bevat informatie over bloedingen tijdens de behandeling met bloedverdunners.

Deel I: farmacoepidemiologie

Epidemiologie is het vakgebied dat patronen, oorzaken en effecten van interventies, bijvoorbeeld het starten van een geneesmiddel, bestudeert in groepen mensen. Farmacoepidemiologie is een deelgebied dat zich specifiek richt op geneesmiddelen (**hoofdstuk 1**). In **hoofdstuk 2 tot en met 5** worden verscheidene facetten beschreven waaraan moet worden gedacht wanneer epidemiologische studies worden uitgevoerd. Zo wordt in **hoofdstuk 2** een case-control studie beschreven waarbij patiënten met een ernstige bloeding in de voorgeschiedenis de cases zijn en patiënten die geen ernstige bloeding hebben gehad als controles fungeren. Cases gebruikten minder vaak statines (cholesterolverlagers) dan controles, waardoor kon worden geconcludeerd dat statines beschermen tegen ernstige bloedingen. Het beschermende effect uit de gebruikelijke analyse werd echter veroorzaakt door een systematische fout (bias): de aan statine blootgestelde cases namen minder vaak deel aan de studie dan de statine gebruikende controles. Deze bias kan in andere case-control studies worden opgemerkt door te stratificeren voor de tijd tussen de bestudeerde uitkomst, in dit geval een bloeding, en deelname aan de studie.

In hoofdstuk 3 wordt een cohortstudie beschreven waarin wederom het effect van statines op bloedingen werd bestudeerd. De resultaten van de standaardanalyse suggereren ook dat statines beschermen tegen bloedingen. Vervolgens zijn de statinegebruikers ingedeeld naar startdatum van statinegebruik, waarbij onderscheid werd gemaakt tussen patiënten die al statines gebruikten toen de studie begon en patiënten die met statines zijn begonnen tijdens de studie. Uit deze analyse bleek dat de gevonden beschermende effecten werden veroorzaakt door de groep patiënten die aan het begin van de studie al statines gebruikten. Literatuur beschrijft echter alleen dat patiënten die tijdens de studie starten met statines moeten worden meegenomen in de analyse. Toen de al gestarte groep uit de analyse werd gehaald, werden geen beschermende effecten meer waargenomen. De in **hoofdstuk 2** en **hoofdstuk 3** gevonden systematische fouten geven een mogelijke verklaring voor de beschermende effecten van statines die in andere studies worden gevonden tegen een groot scala aan ziektes zoals depressie, ziekte van Parkinson en botbreuken. Toekomstige studies over effecten van statines kunnen daarom de bovenstaande analyses meenemen als sensitiviteitsanalyse om te voorkomen dat gevonden beschermende effecten van statines kunnen worden verklaard door bias.

In het verlengde van de voorgaande hoofdstukken is in **hoofdstuk 4** en **hoofdstuk 5** gekeken naar bias die wordt veroorzaakt door verkeerde selectie van controle personen in case-control studies. In **hoofdstuk 4** wordt beschreven dat je voor het bestuderen van effecten van vitamines, dat wil zeggen leefstijlgerelateerde interventies, beter controles kunnen worden geselecteerd die een vergelijkbare levensstijl hebben als de cases (i.c. partners). Partnercontroles zijn niet altijd de beste optie. Zo laat **hoofdstuk 5** zien dat bias werd veroorzaakt doordat partnercontroles juist minder vaak meedoen dan controles die random werden gebeld indien de partners van de cases lijden aan een ernstige ziekte (bijvoorbeeld hartfalen of verlamming).

Deel II: bloedingen tijdens de behandeling met bloedverdunners

In **hoofdstuk 1** wordt ook de geschiedenis en farmacoepidemiologie van vitamine K-antagonisten beschreven. Vitamine K-antagonisten zijn bloedverdunners die voor het eerst in 1948 werden gebruikt als rattengif. Gedurende de jaren 50 werden deze bloedverdunners geregistreerd als geneesmiddel om trombose te voorkomen. Vanwege de grote variabiliteit van het antistollend effect tussen en binnen patiënten wordt de behandeling in Nederland gemonitord door trombosediensten. Bij de trombosedienst wordt minstens één keer per zes weken bloed afgenomen van alle patiënten die vitamine K-antagonisten gebruiken. In het bloed wordt gemeten of de mate van stolling (international normalized ratio, of INR) in het bloed goed is. Bij gezonde personen is de INR 1,0 en indien vitamine K-antagonisten worden geslikt moet de INR vaak tussen de 2,5 en 3,5 zijn. Nadat de INR door de trombosedienst is bepaald krijgen patiënten de volgende dag een kalender met daarop aangegeven hoeveel pillen dagelijks tot de volgende afspraak moeten worden ingenomen.

Ondanks het constant monitoren van de behandeling blijven de meest voorkomende bijwerkingen bloedingen. De kunst bij deze geneesmiddelen is dan ook om een goede balans tussen de effectiviteit (voorkomen trombose) en bijwerkingen (bloedingen) te vinden. De bloedingen waarnaar, net als in dit proefschrift, voornamelijk onderzoek wordt gedaan zijn ernstige bloedingen. Dit zijn bloedingen die leiden tot bloedtransfusies, opnames in het ziekenhuis of het overlijden van patiënten. Deze komen bij 1-2% van de patiënten per jaar voor. Verscheidene risicofactoren voor bloedingen zijn bekend, maar we zijn nog niet goed in staat om patiënten met een hoog bloedingsrisico te onderscheiden van de patiënten die dit niet hebben. Het tweede deel van het proefschrift gaat in op risicofactoren voor het krijgen van deze bloedingen en op interventies die kunnen zorgen dat het aantal bloedingen wordt verminderd.

Een eerste groep patiënten die is bestudeerd zijn patiënten die naast de vitamine K-antagonisten nog twee andere bloedverdunners (clopidogrel en aspirine) krijgen (**hoofdstuk 6**). De literatuur laat zien dat deze patiënten een bijna viervoudig verhoogd bloedingsrisico hebben ten opzichte van patiënten die één bloedverdunner innemen.

Er zijn echter groepen die een laag (basis)risico voor bloedingen hebben, waardoor een viermaal verhoogd risico geen hoog bloedingsrisico hoeft te zijn (bijvoorbeeld voor jonge patiënten, dit zijn patiënten van 50 tot 60 jaar). Anderzijds zijn er ook groepen die een hoog basisrisico voor bloedingen hebben, bijvoorbeeld oude patiënten (boven de 90 jaar), waardoor het bloedingsrisico bij drie bloedverdunners erg hoog zou moeten zijn. **Hoofdstuk 6** laat zien dat eigenlijk alle groepen, inclusief de groepen patiënten met laag basisrisico, die drie bloedverdunners tegelijkertijd gebruiken een hoog risico op ernstige bloedingen hebben. Groepen die een bijzonder hoog bloedingsrisico hebben zijn patiënten met een CHA_2DS_2-VASc score boven de 6 en patiënten boven de 90 jaar (20% en 50% per jaar respectievelijk).

In **hoofdstuk 7** en **hoofdstuk 8** wordt onderzocht of bloedverdunners uit de groep LMWHs (low molecular weight heparines) verschillende bloedingsrisico's geven. Er wordt meestal van uitgegaan dat geneesmiddelen in dezelfde groep eenzelfde patroon van werkzaamheid en bijwerkingen hebben, hoewel de geneesmiddelen verschillen in bepaalde opzichten. **Hoofdstuk 7** laat zien dat eenmaal daags doseren van de LMWH nadroparine minder bloedingen geeft dan tweemaal daags doseren. Dit resultaat komt overeen met de huidige literatuur. Bij een verlaagd bloedingsrisico bij eenmaal daags doseren lijkt het logisch dat het tromboserisico toeneemt. Dit bleek echter niet uit **hoofdstuk 8**. Een kanttekening bij beide studies is dat de aantallen te klein waren om definitieve uitspraken te doen over de vraag welke LMWH en welk doseringsregime het beste is voor de patiënten.

Een andere manier om bloedingen te voorkomen is door het innemen van medicatie makkelijker en overzichtelijker te maken. Een hulpmiddel om dit te doen zijn 'baxterrollen'. Dit zijn rollen bestaande uit zakjes met alle medicatie die op één moment moet worden ingenomen in één zakje (dus een zakje voor maandagochtend 8 uur, een zakje voor maandagochtend 10 uur, enzovoorts). Hoewel dit hulpmiddel veel wordt ingezet om inname van medicatie te verbeteren, is er weinig onderzoek gedaan naar de vraag of het gebruik van baxterrollen de therapietrouw van patiënten verbetert. In **hoofdstuk 9** wordt de therapietrouw van patiënten met een baxterrol vergeleken met de therapietrouw van patiënten die door de medewerkers van de trombosedienst worden gebeld indien bloedverdunners niet goed werden ingenomen. Uit het onderzoek bleek dat op korte termijn (binnen een maand) de therapietrouw van patiënten met baxterrol verbetert ten opzichte van de groep die alleen werd gebeld. Op lange termijn (vier maanden) lijkt de therapietrouw in de groep die werd gebeld over inname van de bloedverdunners door een trombosedienstmedewerker echter overeenkomstig te zijn met die van de groep die bloedverdunners via de baxterrol kreeg.

In **hoofdstuk 10** is onderzocht of er verschil is in de werkzaamheid van fytomenadion (vitamine K) drank en fytomenadion tabletten. Fytomenadion is het antidotum tegen vitamine K-antagonisten en wordt gebruikt indien patiënten een te hoge INR of een

bloeding hebben, of voor het tegengaan van de bloedverdunnende werking (couperen) van vitamine K-antagonisten voorafgaand aan een operatie. Fytomenadion was altijd in de vorm van een drank op de markt, waarbij orale spuitjes met de goede dosering fytomenadion werden klaargemaakt door de apotheek. Dit leidde bij patiënten soms tot verwarring: 'Moet ik dit spuitje oraal innemen of moet ik het inspuiten?'. Sinds enkele jaren zijn ook tabletten op de markt, maar hiervan was niet onderzocht of deze even effectief zijn als de drank.

Hoofdstuk 10 omvat drie studies waarin wordt onderzocht of de drank en tabletten in gelijke mate aankomen in het bloed en of ze even goed werken als antidotum. De resultaten van de eerste studie laten zien dat twee keer zo veel fytomenadion in het bloed aankomt na inname van de drank als na inname van de tabletten. In de twee andere studies is onderzocht of dit ook leidt tot verschillen in het couperend effect van het antidotum in patiënten die fenprocoumon gebruiken (een langwerkende vitamine K-antagonist). In de tweede studie is onderzocht of er verschillen tussen de tablet en drank zijn bij patiënten die een geplande operatie hebben. Bij deze groep patiënten bleken de drank en tabletten even effectief te zijn in het couperen van fenprocoumon. In de derde studie werd gekeken naar de effecten bij patiënten met een te hoge INR. In deze studie bleek dat de drank iets sneller werkte, maar dat na inname van de drank ook meer mensen doorschoten naar een te lage INR, waardoor de tabletten en drank even effectief bleken. Uit deze studie is geconcludeerd dat de drank en tabletten even effectief zijn.

In de slothoofdstukken van het proefschrift wordt getracht om nieuwe risicofactoren voor het krijgen van ernstige bloedingen tijdens de behandeling met vitamine K-antagonisten te identificeren. In **hoofdstuk 11** wordt gekeken naar welk type risicofactoren (kortdurende of chronische) moet worden gezocht. De resultaten van dit hoofdstuk laten zien dat risicofactoren voor ernstige bloedingen voornamelijk chronisch zijn.

Hoofdstuk 12 beschrijft de Biomarkers in the Leiden Etiology and Epidemiology of bleeding in vitamin K-antagonists Drug users Study (BLEEDS). Deze studie is opgezet om risicofactoren voor het krijgen van ernstige bloedingen tijdens de behandeling met vitamine K-antagonisten te identificeren. Het eerste wat is gecontroleerd, is of de populatie van BLEEDS overeenkomt met eerder beschreven populaties die vitamine K-antagonisten kregen en waarin ernstige bloedingen zijn onderzocht. Uit de resultaten blijkt dat de populatie van de BLEEDS qua karakteristieken overeenkomt met eerder onderzochte populaties, wat indiceert dat resultaten kunnen worden doorgetrokken naar andere populaties. Bij BLEEDS is van een grote groep patiënten (13.779) materiaal verzameld. Dit schept de mogelijkheid veel verschillende groepen met elkaar te vergelijken, waarbij bijvoorbeeld het al dan niet hebben van een bepaalde genetische variant of het hebben van een bepaalde waarde van een eiwit het optreden van

bloedingen voorspelt. **Hoofdstuk 13** is de eerste studie die is uitgevoerd in de BLEEDS waarbij wordt onderzocht of markers van vaatwandschade indiceren wie een hoog bloedingsrisico hebben. Het achterliggende idee is dat patiënten met een beschadigde vaatwand eerder een bloeding krijgen dan patiënten die minder schade aan de vaatwand hebben. Uit de studie bleek dat patiënten met hoge concentraties van markers van chronische vaatwandschade een verhoogd bloedingsrisico op ernstige bloedingen te hebben. Markers die samenhangen met kortdurende vaatwandschade bleken niet samen te hangen met een verhoogd bloedingsrisico. De resultaten uit dit hoofdstuk werden dan ook weer bevestigd door **hoofdstuk 11**, waarin werd gevonden dat chronische risicofactoren vooral voorspellen wie een verhoogd bloedingsrisico hebben.

Conclusies en toekomstperspectief

Dit proefschrift heeft inzicht gegeven in bias die ervoor kan zorgen dat effecten van geneesmiddelen (statines en vitamines) positiever lijken dan ze zijn. Omdat observationeel onderzoek een groot deel van de uitgevoerde onderzoeken behelst, is het relevant om genoeg epidemiologisch-methodologisch onderzoek uit te voeren om in de toekomst (nog) beter in te kunnen schatten waar de valkuilen zitten in observationele studies.

Verder zijn in dit proefschrift een aantal interventies beschreven om het aantal bloedingen tijdens de behandeling met bloedverdunners te verminderen. Resultaten laten zien dat couperen van fenprocoumon met fytomenadion tabletten veilig kan worden gedaan, het gebruik van baxterrollen therapietrouw op korte termijn verbetert en het kiezen van een specifieke LMWH die eenmaal daags wordt gedoseerd wellicht kan leiden tot minder bloedingen. Daarnaast is onderzoek gedaan naar groepen die een hoog risico hebben op ernstige bloedingen. Enkele risicogroepen die naar voren kwamen zijn patiënten boven de 90 jaar met drie bloedverdunners en patiënten met chronische vaatwandschade.

Toekomstig onderzoek dient zich te richten op andere chronische risicofactoren voor het krijgen van bloedingen tijdens de behandeling met bloedverdunners, omdat huidige risicoscores niet goed identificeren welke patiënt een hoog of laag bloedingsrisico heeft. Om een optimale klinische beslissing te maken is het wenselijk om naast het hoge bloedingsrisico ook te weten wie een laag of hoog tromboserisico heeft. Hiervoor hebben we op dit moment nog geen goede risicoscores. Bij het verbeteren van deze voorspellingen kan het BLEEDS-cohort wellicht een belangrijke rol spelen.

CURRICULUM VITAE

Nienke van Rein werd op 5 juni 1987 geboren te Veendam. In 2005 behaalde zij haar atheneum diploma aan de Winkler Prins te Veendam, waarna zij farmacie ging studeren aan de Rijksuniversiteit Groningen. Tijdens haar studie raakte ze geïnteresseerd in epidemiologie, waarna ze in 2009 begon te werken op de afdeling Farmacoepidemiology en Farmacoeconomie aan de Rijksuniversiteit Groningen onder begeleiding van prof. dr. L.T.W. de Jong-van den Berg. Later dat jaar vertrok ze naar Hanoi, Vietnam om onderzoek te doen naar de behandeling van tuberculose.

Na het behalen van haar apothekersexamen in 2011 is ze begonnen als PhD-student op de afdeling Trombose en Hemostase in het Leids Universitair Medisch Centrum onder begeleiding van prof. dr. P.H. Reitsma, dr. W.M. Lijfering, dr. F.J.M. van der Meer en prof. dr. S.C. Cannegieter. Tijdens haar promotietraject heeft ze de opleiding tot epidemioloog afgerond en heeft ze onderzoek uitgevoerd aan Aarhus University Hospital onder begeleiding van prof. dr. H.T. Sørensen en dr. U. Heide-Jørgensen. Daarnaast heeft ze op nationale en internationale congressen gepresenteerd en verscheidene abstractprijzen en posterawards gewonnen van de Nederlandse Vereniging van Trombose en Hemostase en International Society of Thrombosis and Haemostasis.

In 2015 is ze begonnen als projectapotheker op de afdeling Klinische Farmacie en Toxicologie in het Leids Universitair Medisch Centrum. Een jaar later is ze op dezelfde afdeling gestart met haar opleiding tot ziekenhuisapotheker, waarna ze in 2017 als penningmeester is toegetreden tot het bestuur van de Jong Nederlandse Vereniging van Ziekenhuisapothekers.

LIST OF PUBLICATIONS

Ravera S, van Rein N, de Gier JJ, de Jong-van den Berg LT. Road traffic accidents and psychotropic medication use in The Netherlands: a case-control study. Br J Clin Pharmacol. 2011;72(3):505-13

Nijenhuis CM, ter Horst PG, van Rein N, Wilffert B, de Jong-van den Berg LT. Disturbed development of the enteric nervous system after in utero exposure of selective serotonin re-uptake inhibitors and tricyclic antidepressants. Part 2: Testing the hypotheses. Br J Clin Pharmacol. 2012;73(1):126-34

Crijns HJ, van Rein N, Gispen-de Wied CC, Straus SM, de Jong-van den Berg LT. Prescriptive contraceptive use among isotretinoin users in the Netherlands in comparison with non-users: a drug utilisation study. Pharmacoepidemiol Drug Saf. 2012;21(10):1060-6

Ravera S, van Rein N, de Gier JJ, de Jong-van den Berg LT. A comparison of pharmacoepidemiological study designs in medication use and traffic safety research. Eur J Epidemiol. 2012;27(6):473-81

Vu DH, van Rein N, Cobelens FG, Nguyen TT, Le VH, Brouwers JR. Suspected tuberculosis case detection and referral in private pharmacies in Viet Nam. Int J Tuberc Lung Dis. 2012;16(12):1625-9

van Rein N, Cannegieter SC, Rosendaal FR, Reitsma PH, Lijfering WM. Suspected survivor bias in case-control studies: stratify on survival time and use a negative control. J Clin Epidemiol. 2014;67(2):232-5

Kooiman J, van Rein N, Spaans B, van Beers KA, Bank JR, van de Peppel WR, del Sol AI, Cannegieter SC, Rabelink TJ, Lip GY, Klok FA, Huisman MV. Efficacy and safety of vitamin K-antagonists (VKA) for atrial fibrillation in non-dialysis dependent chronic kidney disease. PLoS One. 2014;9(5):e94420

van Rein N, Gebuis EP, Lijfering WM, Groeneveld JJ, van der Horst FA, le Cessie S, Rosendaal FR, van der Meer FJ. Vitamin K1 in oral solution or tablets: a crossover trial and two randomized controlled trials to compare effects. J Thromb Haemost. 2014;12(12):2017-23

Vučković BA, van Rein N, Cannegieter SC, Rosendaal FR, Lijfering WM. Vitamin supplementation on the risk of venous thrombosis: results from the MEGA case-control study. Am J Clin Nutr. 2015;101(3):606-12

van Rein N, Florack M, Guchelaar HJ, Zwaveling J. Beperking spillage dure geneesmiddelen is lastig. Pharmaceutisch Weekblad. 2015;150(48)

Biedermann JS, van Rein N, van den Besselaar AM, Buhre PN, de Maat MP, van der Meer FJ, Leebeek FW, Kruip MJ. Impact of point-of-care international normalized ratio monitoring on quality of treatment with vitamin K antagonists in non-self-monitoring patients: a cohort study. J Thromb Haemost. 2016;14(4):695-703

van Rein N, le Cessie S, van Vliet IP, Reitsma PH, van der Meer FJ, Lijfering WM, Cannegieter SC. Increased risk of major bleeding after a minor bleed during treatment with vitamin K antagonists is determined by fixed common risk factors. J Thromb Haemost. 2016;14(5):948-52

van Rein N, Cannegieter SC, le Cessie S, Rosendaal FR, Reitsma PH, van der Meer FJ, Lijfering WM. Statins and Risk of Bleeding: An Analysis to Evaluate Possible Bias Due to Prevalent Users and Healthy User Aspects. Am J Epidemiol. 2016;183(10):930-6

van Rein N, Biedermann JS, Bonafacio SM, Kruip MJ, van der Meer FJ, Lijfering WM. Statin use decreases coagulation in users of vitamin K antagonists. Eur J Clin Pharmacol. 2016;72(12):1441-1447

van Rein N, Lijfering WM, Bos MH, Herruer MH, Vermaas HW, van der Meer FJ, Reitsma PH. Objectives and Design of BLEEDS: A Cohort Study to Identify New Risk Factors and Predictors for Major Bleeding during Treatment with Vitamin K Antagonists. PLoS One. 2016;11(12):e0164485

van Rein N, Muller AHG, Ng S, van Herrewegen F, van Wijk MP, Schimmel KJM. Nadeel van acetem is dat het kunststoffen kan aantasen. Pharmaceutisch Weekblad 2017;152(4):18-19.

van Rein N, Biedermann JS, van der Meer FJ, Cannegieter SC, Wiersma N, Vermaas HW, Reitsma PH, Kruip MJ, Lijfering WM. Major bleeding risks of different Low-Molecular-Weight-Heparin agents: a cohort study in 12 934 patients treated for acute venous thrombosis. *Accepted. J Thromb Haemost*

Van Rein N, de Geus KS, Cannegieter SC, Reitsma PH, van der Meer FJ, Lijfering WM. Multi-dose drug dispensing as a tool to improve medication adherence: a study in patients using vitamin K antagonists. *Submitted*

van Adrichem RA, Tolen NJ, Goldman LA, Boef ACG, van Rein N, Schipper IB, Nelissen RG, Driessen MJ, Vischjager M, Cannegieter SC. Prevention of venous thrombosis after an anterior cruciate ligament reconstruction: compression stockings with and without low-molecular-weight-heparin. *Submitted*

van Rein N, Heide-Jørgensen U, Lijfering WM, Dekkers OM, Sørensen HT, Cannegieter SC. Major Bleeding Rates are High in Atrial Fibrillation Patients on Triple Antithrombotic Therapy: Results from a nationwide Danish cohort study. *Submitted*

Kort D, van Rein N, van der Meer FJ, Vermaas HW, Wiersma N, Cannegieter SC, Lijfering WM. Relationship between neighbourhood socioeconomic status and venous thrombosis: Results from a population-based study. *Submitted*

van Rein N, Lijfering WM, Rosendaal FR, Reitsma PH, Cannegieter SC. Discrepancies between risk estimates when using different control groups in a case-control study. *In preparation*

van Rein N, de Geus KS, Stegeman BH, van der Hulle T, Reitsma PH, Lijfering WM, Dekkers OM. Low-molecular-weight-heparin therapy after acute venous thrombosis: systematic review and network meta-analysis. *In preparation*

van Rein N, Reitsma PH, Lijfering WM, van der Meer FJ, Bos MH. Persistent endothelial damage is associated with an increased risk of major bleeding in patients treated with vitamin K antagonists: a population based case-cohort study. *In preparation*

Lijfering WM, van Rein N, Teichert M, Rosendaal FR, van der Meer FJ, Huisman MV, Cannegieter SC. Persistence to oral anticoagulant treatment in the Netherlands: a surveillance study. *In preparation*