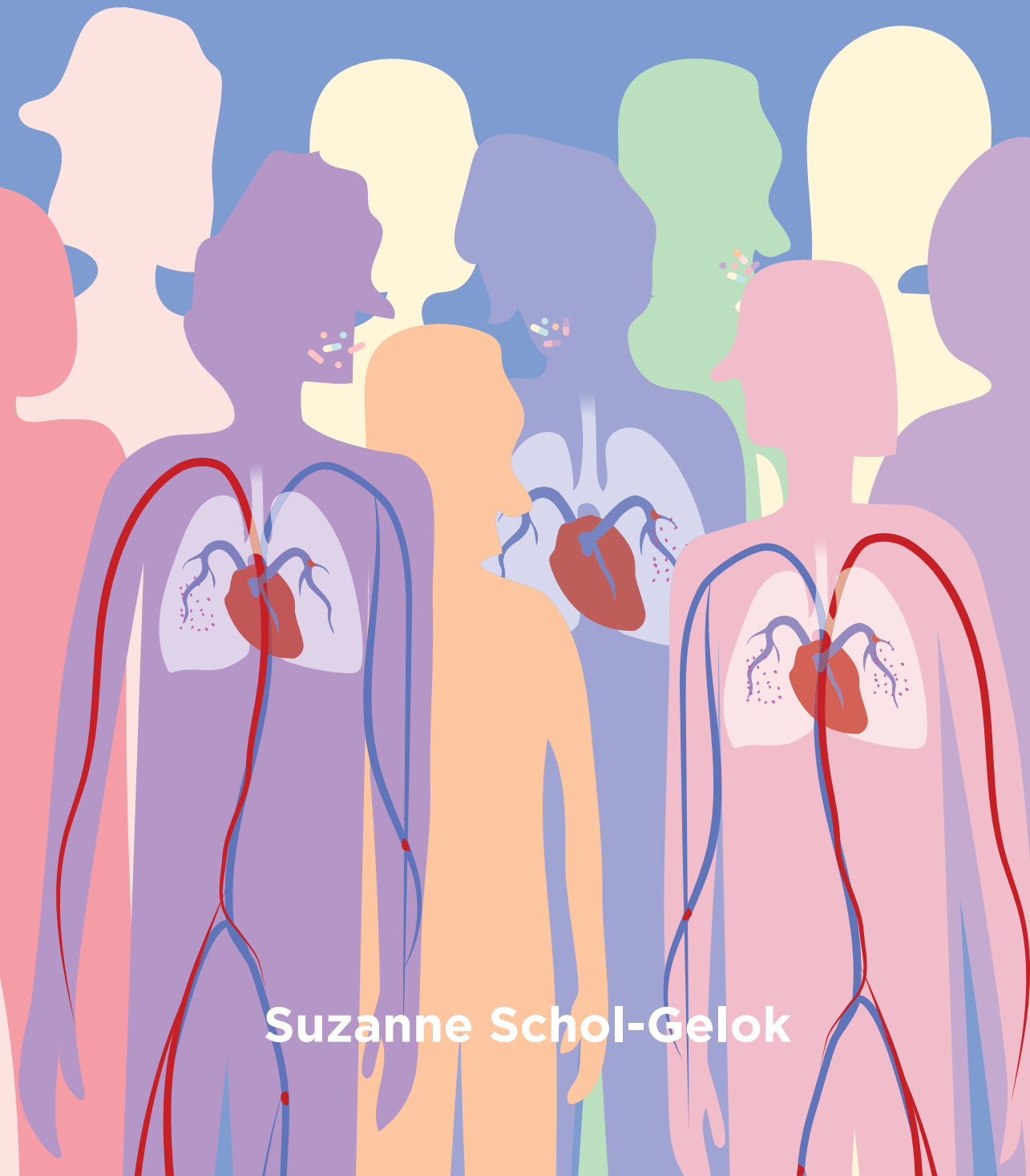
Influence of (Co-)Medication on Haemostatic Biomarkers



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Influence of (Co-)Medication on Haemostatic Biomarkers

De invloed van (co-)medicatie op biomarkers van de stolling

Proefschrift

ter verkrijging van de graad van doctor aan de
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Prof.dr. R.C.M.E. Engels

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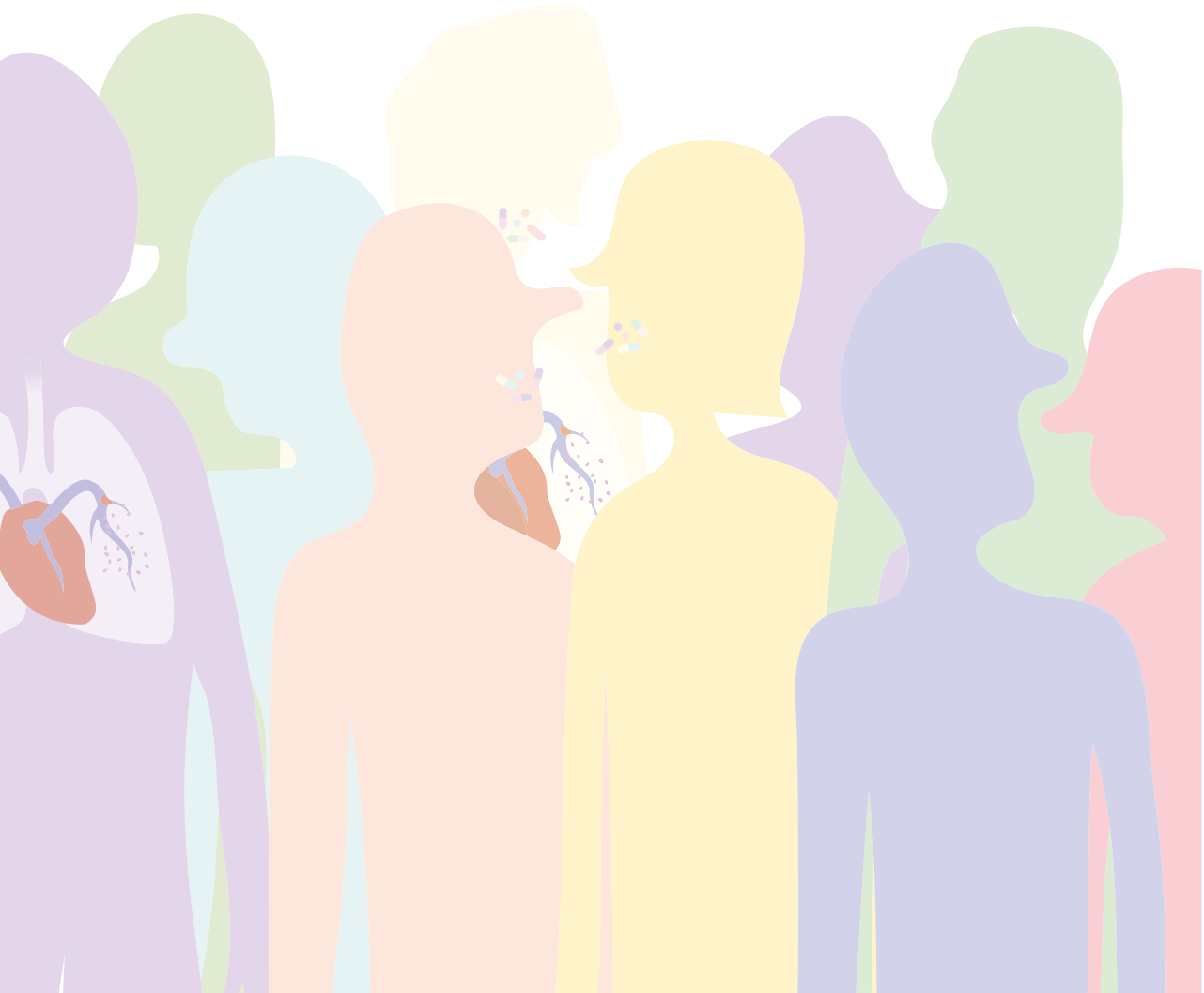
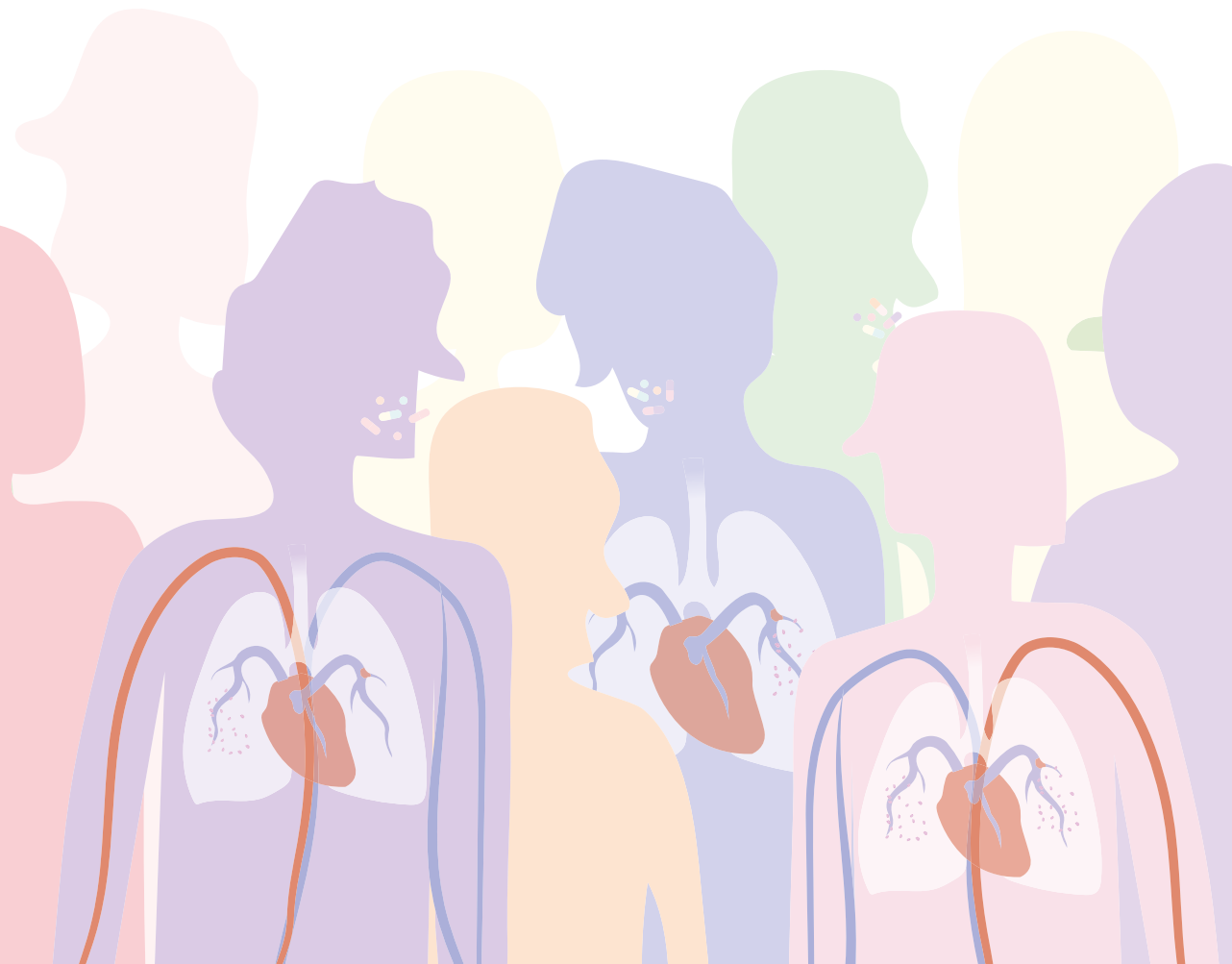
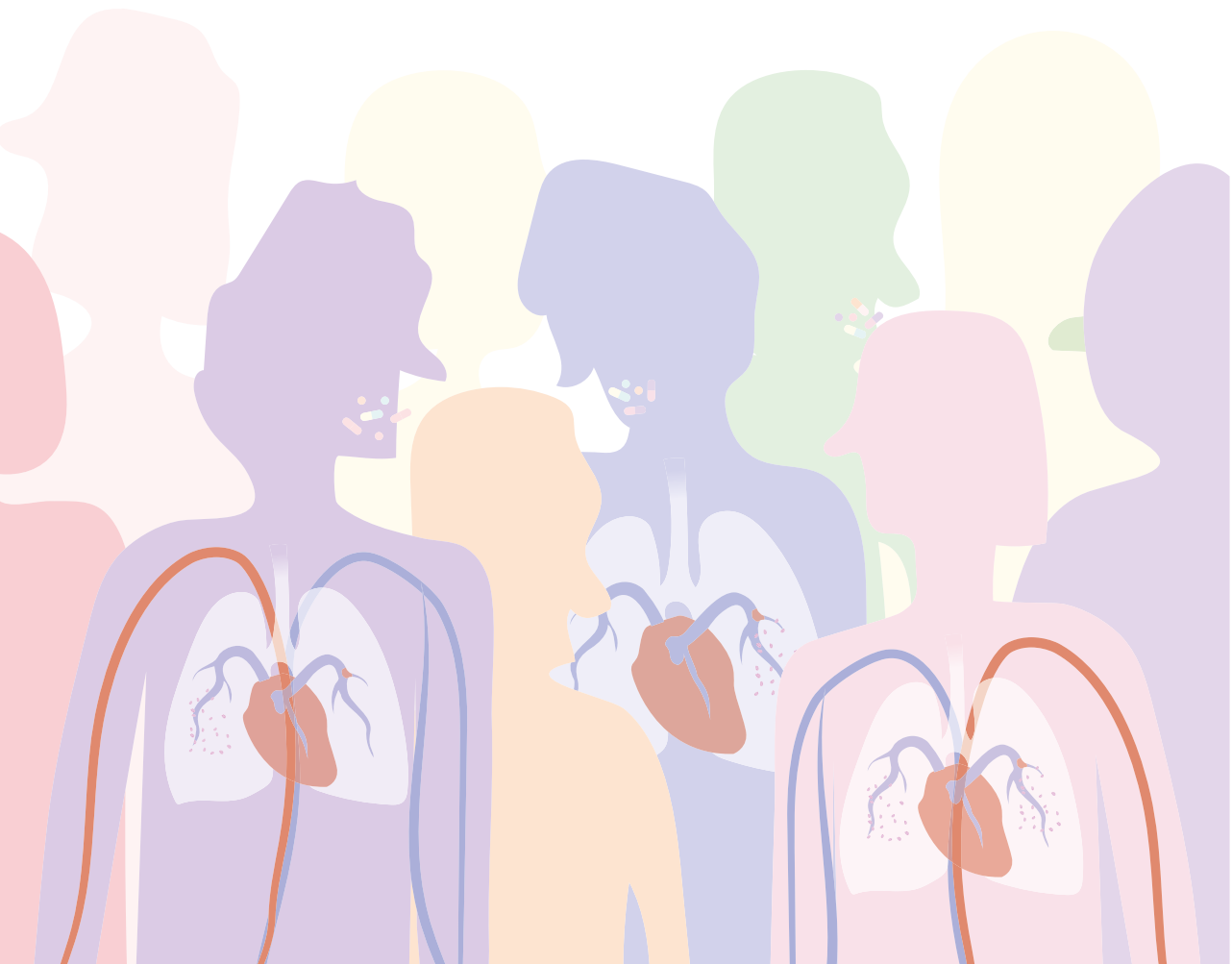


Introduction





1

General introduction and outline of the thesis

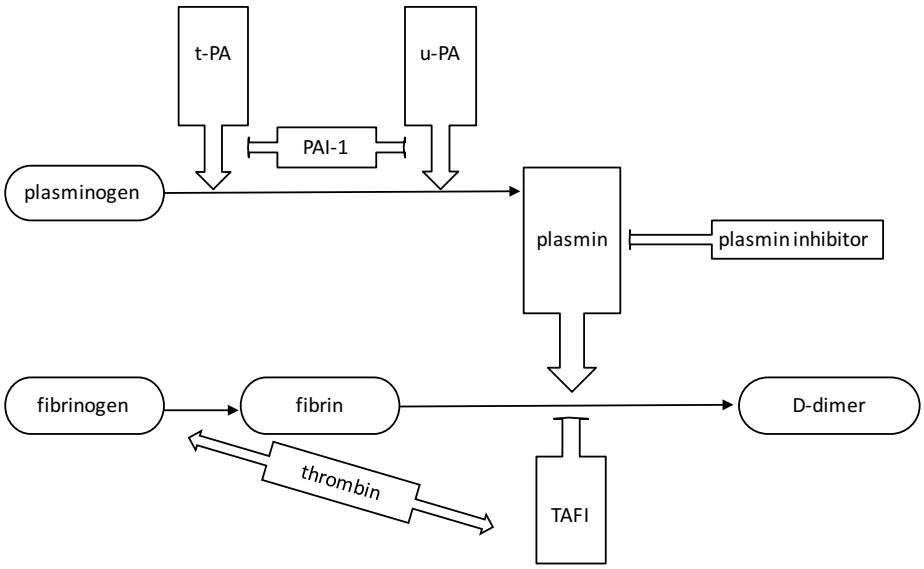
Venous thromboembolism

Venous thromboembolism (VTE) is a multifactorial disease with broadly two presenting entities: deep venous thrombosis (DVT) or pulmonary embolism (PE). A Belgian study found that 61% of the patients presenting with a confirmed DVT also had PE, and that 83% of the patients presenting with a confirmed PE also had a DVT.[1] In the pathogenesis of venous thromboembolism three main components have been identified and are known as the Virchow's triad, named after a nineteenth century German physician.[2] This triad consists of alterations in blood coagulation, diminished blood flow and damage of the vascular endothelium. Risk factors of VTE influencing one or more of these components include immobility, previous VTE, active infection or cancer, smoking, trauma, advanced age, pregnancy, venous insufficiency, antiphospholipid antibodies and certain genetic traits such as the factor V Leiden mutation.[3-5] The more risk factors, especially when targeting different Virchow's categories, the higher the risk of VTE.[6] Despite all known risk factors and availability of numerous anticoagulant drugs VTE is still a common health problem with an incidence of 1 per 1000 in adult populations.[7] In the past century, the one-month survival rate of patients diagnosed with VTE in Minnesota, US, was 94.5% for DVT and 67% for PE.[8,9] This makes the risk of early death 18-fold higher among PE patients compared with patients with DVT alone.[10] However, mortality rate after PE was lower in more recent studies performed in Europe, with a 3-month mortality rate of 8.2 % in the Dutch population, in-hospital case fatality rate of 10.1 % in an Italian study and one-month mortality rate of 4.9% in a Spanish cohort study.[11-13] It must be noticed that reported mortality rates include deaths from all causes, the proportion of PE-related deaths is much smaller with only 1.8% PE-related deaths reported in the Spanish cohort.[13]

Haemostatic system and fibrinolysis

The haemostatic system in general triggers formation of a clot in case of a trauma to prevent further bleeding, but inappropriate activation of the haemostatic system may lead to thrombosis.[6] During primary haemostasis, a soft aggregate platelet plug is formed. Secondary haemostasis is responsible to stabilize and strengthen this soft plug into a cross-linked fibrin clot.[14] Next, the fibrinolytic system plays an important role to dissolve this blood clot. First, the inactive proenzyme plasminogen is converted by tissue plasminogen activator (t-PA) or urokinase-type plasminogen activator (u-PA) to the active enzyme plasmin.[15,16] Plasminogen activator Inhibitor-1 (PAI-1) can regulate these converting enzymes. Second, thrombin converts fibrinogen into fibrin, but also activates thrombin-activatable fibrinolysis inhibitor (TAFI) which can inhibit fibrinolysis. Lastly, the activated plasmin degrades fibrin into fibrin degradation products, which is regulated by plasmin inhibitor (Figure 1).

Figure 1: Schematic overview of fibrinolysis



The inactive proenzyme plasminogen is converted by tissue plasminogen activator (t-PA) or urokinase-type plasminogen activator (u-PA) to the active enzyme plasmin. Plasminogen activator Inhibitor-1 (PAI-1) can regulate these converting enzymes. Thrombin converts fibrinogen into fibrin, and activates thrombin-activatable fibrinolysis inhibitor (TAFI) which can inhibit fibrinolysis. The activated plasmin degrades fibrin into fibrin degradation products (including D-dimers), which is regulated by plasmin inhibitor.
Arrows: positive influence; Blocked end: negative influence.

Haemostatic biomarkers

Biomarkers are measurable indicators of a specific biological state, particularly relevant to the presence of, or risk for a disease.[17,18] They can be used for screening, diagnosing or monitoring of the activity of a disease, to guide targeted therapy or to assess therapeutic response. A biological marker (biomarker) is defined by the Biomarkers Definitions Working Group as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention." [18] Within the haemostatic system blood levels of clotting factors, proteins involved in haemostasis and fibrinolysis and also coagulation times could be considered as biomarkers following this definition.[19,20] For interpretation and determination of the role and value of haemostatic biomarkers in VTE, it is important to know their function within the haemostatic system. The D-dimer, a fibrin degradation product, is probably the most well-known haemostatic biomarker. In clinical practice this D-dimer has a central role in the diagnostic work-up of VTE and could

also be used in the decision to discontinue anticoagulant therapy after a DVT or PE.[21,22] Other haemostatic biomarkers that might give more information about the fibrinolytic activity within patients include fibrinogen, plasminogen activator inhibitor-1 (PAI-1), tissue-type plasminogen activator (t-PA), TAFI and plasmin inhibitor. Plasma fibrinolytic potential could be used to evaluate fibrinolysis in general, by determining the clot lysis time.[23,24] A balance in the activity of all enzymes within the fibrinolytic system is crucial as the risk of VTE has been shown to be increased by hypofibrinolysis.[24,25] Patients with elevated blood levels of coagulation factor VIII and antithrombin deficiency are also considered to have a higher risk of VTE.[26,27] Although haemostatic biomarkers, especially D-dimer levels, are used in the diagnostic management of suspected VTE, they may be influenced by different factors like cancer, infection and co-medication. Therefore a very high D-dimer level in an individual patient might point to a thrombotic disease, but it needs to be confirmed by imaging tests.

Diagnostic strategies

Diagnosing VTE in clinical practice can be challenging. VTE can only be diagnosed using imaging tests, at present usually compression venous ultrasonography for diagnosing DVT and computed tomography pulmonary angiography (CTPA) for PE.[28,29] These imaging tests and in particular CTPA are associated with high healthcare costs, time consumption, radiation exposure, and risk of allergic reactions and contrast-induced nephropathy.[28,30] VTE guidelines therefore recommend combining clinical decision rules and measurement of D-dimer levels to identify patients in whom DVT or PE may be ruled out without performing imaging tests (with high level of evidence).[21,31] This integrated approach in a validated diagnostic algorithm helps to stratify patients into different risk categories leading to the most appropriate diagnostic management. The most famous and commonly used clinical decision rule in DVT and PE was introduced by Wells et al.[32,33] The Wells score for PE consists of seven different items and is sequential. When these clinical decision rules are used correctly, the physician can exclude VTE safely in patients which are considered unlikely to have PE after scoring the items in combination with a low D-dimer test result without performance of an imaging test. After correct application of the Wells algorithm, imaging tests are not needed in 32% of the patients initially suspected to have PE.[34] When CTPA was indicated following the algorithm, 20.4% of the patients were diagnosed to have pulmonary embolism. Unfortunately, in clinical practice adherence to this validated diagnostic strategies is variable, probably because of hectic emergency departments and the complexity of the algorithms.[35,36]

Medication and association to venous thromboembolism

Risk factors of VTE such as immobility, active infection, cancer, pregnancy, trauma, advanced age, antiphospholipid antibodies, obesity and genetic traits such as the factor V Leiden mutation all influence one or more of the three components described by Virchow.[5,6] Additionally, it has gradually become clear that many drugs can lower or increase the risk of VTE by different mechanisms influencing this triad of Virchow.[37]

Antiplatelet drugs, such as aspirin and clopidogrel, inhibit platelet aggregation and prevent thrombus formation. As expected by this mechanism, antiplatelet drugs reduce the risk of VTE and have been considered as secondary prevention in patients with VTE.[38] Also other groups of drugs, with a less obvious effect on the haemostatic system, can lower the risk of thrombosis. HMG-CoA reductase inhibitors, more commonly known as statins, lead to a lower risk of venous thrombosis as confirmed in a recent meta-analysis of intervention studies: the risk of a primary venous thrombosis was 15% lower in the statin-treated group.[39] This effect on incidence of thrombosis is probably due to inhibition of geranylgeranylation of the Rho/Rho kinase pathway as one of the key mechanisms of the anticoagulant effects. The antithrombotic action of statins is one of the so-called pleiotropic effects of this class of drugs.[40,41] It is unknown whether novel lipid lowering drugs, such as Proprotein Convertase Subtilisin/Kexin 9 (PCSK9) inhibitors have similar effects.[42]

That certain drugs can also increase the risk of VTE became strikingly obvious in the 1990s. Based on several case series describing an association between oral contraceptives and a higher risk of VTE, eventually a large case-control study was performed by the World Health organization (WHO). This study confirmed a two- to four-fold increase in the risk of VTE in oral contraceptive users, particularly in third generation contraceptives.[43] A riot started when both the German Federal Institute for drugs and medical services and the British government initially discouraged the use of third generation oral contraceptives because of this increased risk of VTE. The European Medicines Agency (EMA) and Food and Drug Administration (FDA) on the other hand had decided that these drugs should not be withdrawn. This resulted in many more studies, which were evaluated in a Cochrane review in 2014. The final conclusion was that oral contraceptive users indeed have a higher risk of VTE with third generation contraceptives, and that this increased risk is a slightly higher compared to the risk of VTE associated with the use of second generation contraceptives.[44] Glucocorticoids, another class of commonly prescribed drugs, are also well known for their increased risk of thrombosis as expected by their working mechanism leading to increased levels

of coagulation factors and fibrinogen.[45] Other less frequently prescribed drugs may also increase the risk of VTE as expected based on their mechanism of action. For example, anti-epidermal growth factor receptor (EGFR) agents, classified as either monoclonal antibodies (MoAbs) or tyrosine kinase inhibitors (TKIs) have both been associated with a significant increase in the risk of VTE.[46] The most difficult associations to detect are in the groups of drugs that unexpectedly increase the risk of VTE. Schizophrenic patients for example are at increased risk of developing VTE, because of many factors including the use of antipsychotics. In this specific population symptoms such as lethargy and impaired pain perception may result in different pain perception and pain expression. Therefore they unfortunately are also more likely to have a delay in the diagnosis of VTE.[47-49] Lists of drugs associated with a higher risk of arterial or venous thromboembolism have been published before.[50,51] An overview of drugs that are associated with a higher risk on VTE specifically is presented in Table 1.

Table 1: An overview of medication associated with a higher risk of venous thromboembolism

Oral and transdermal contraceptives	Monoclonal antibodies (MoAbs)
Hormone replacement therapy	Tyrosine kinase inhibitors (TKIs)
Thalidomide analogs	Antipsychotics
Testosterone	Antidepressants
Selective estrogen receptor modulators	Cisplatin
Glucocorticoids	

Based on spontaneous reporting from various resources to the pharmacovigilance databases such as the Netherlands Pharmacovigilance Centre of Lareb and the worldwide Vigilyze pharmacovigilance database maintained by the WHO collaborating centre for international drug monitoring, Reporting Odds Ratios (RORs) have been developed. These RORs have been developed as a hypothesis generating tool in the signal detection of an association between a certain drug and a side effect.[52] As shown by the publication of several case series about the association between oral contraceptives and the higher risk of VTE, it remains of main importance that physicians keep reporting unexpected cases of VTE that might be related to a certain drug to pharmacovigilance databases. This will increase our knowledge on the risk of thrombosis and possibly may prevent new events.

Aim and Outline of the Thesis

The goal of the studies described in this thesis is to improve diagnostic strategies and therapeutic management in VTE for specific patient groups. From the above we know that the diagnosis of venous thromboembolism may be ruled out with different decision making strategies. D-dimer levels can be used as haemostatic biomarkers and low D-dimer levels can support the clinician in deciding to not expose the patient to radiologic imaging. However, co-medication can influence the thrombotic risk and haemostatic biomarkers, and potentially could affect the diagnostic performance of clinical decision rules. With these considerations we have formulated the following aims of this thesis.

Aim 1. To present optimal diagnostic management of venous thromboembolism in different (sub)populations

In Chapter 2.1 we prospectively validated a simplified diagnostic algorithm (the YEARS algorithm) for suspected acute pulmonary embolism. Chapter 2.2 investigates if this YEARS algorithm could also be safely used in statin and antiplatelet users.

Aim 2. To investigate the influence of co-medication on haemostatic biomarkers or VTE risk

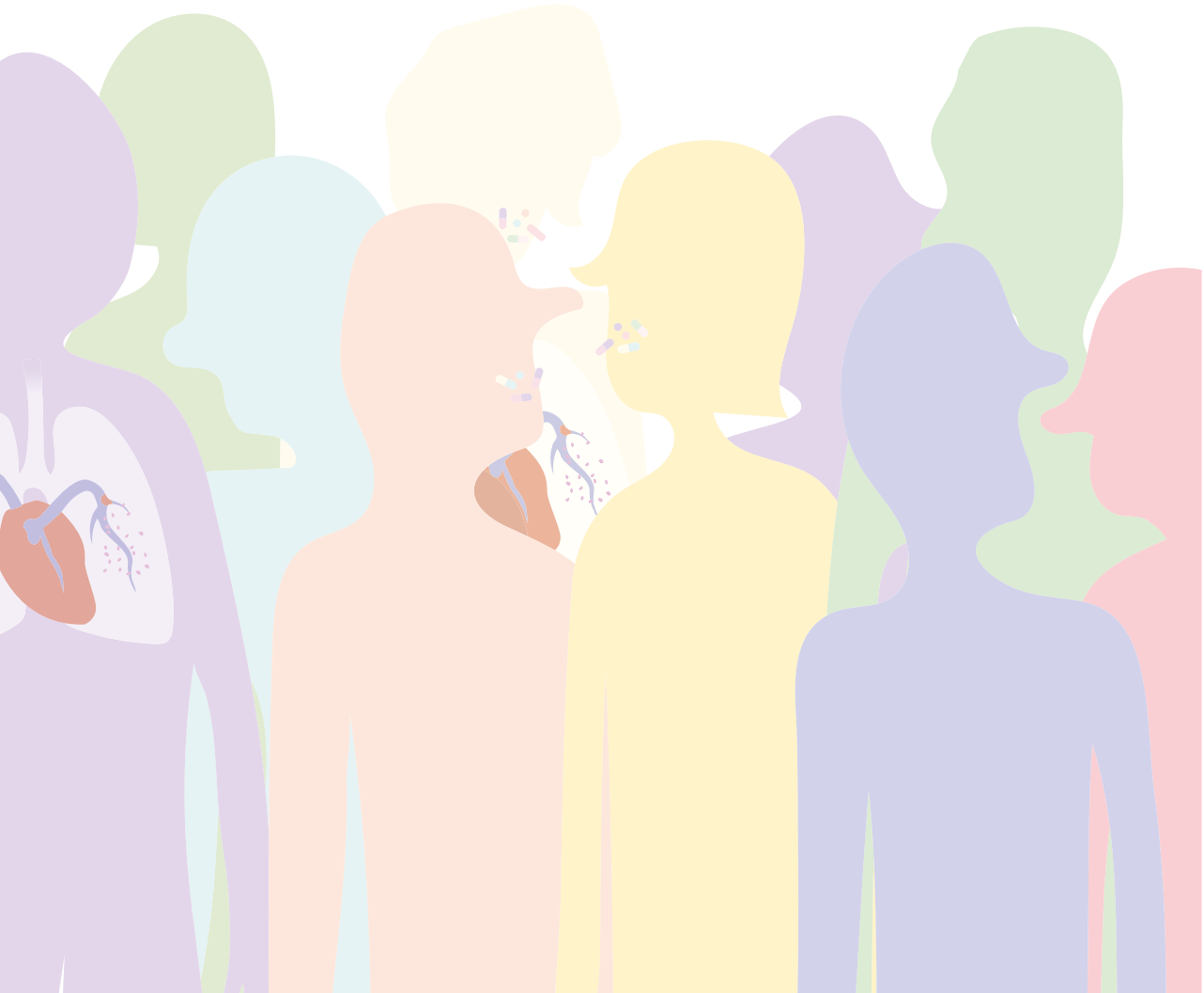
Chapters 3.1 and 3.2 provide an overview of the literature evaluating the effect of statins and antiplatelet drugs on D-dimer levels. In addition the effects of PCSK9 inhibitors on D-dimer and fibrinogen levels in patients with familial hypercholesterolemia were evaluated in Chapter 4. Chapter 5 explores the association between olanzapine and VTE. In Chapter 6 we describe the effect of rosuvastatin use on fibrinolysis in patients with previous VTE.

References

1. Ghaye B, Willems V, Nchimi A, et al. Relationship between the extent of deep venous thrombosis and the extent of acute pulmonary embolism as assessed by CT angiography. *Br J Radiol.* 2009;82(975):198-203.
2. Bagot CN, Arya R. Virchow and his triad: a question of attribution. *Br J Haematol.* 2008;143(2):180-190.
3. Lijfering WM, Rosendaal FR, Cannegieter SC. Risk factors for venous thrombosis - current understanding from an epidemiological point of view. *Br J Haematol.* 2010;149(6):824-833.
4. Goldhaber SZ. Risk factors for venous thromboembolism. *J Am Coll Cardiol.* 2010;56(1):1-7.
5. Anderson FA, Jr., Spencer FA. Risk factors for venous thromboembolism. *Circulation.* 2003;107(23 Suppl 1):I9-16.
6. Esmon CT. Basic mechanisms and pathogenesis of venous thrombosis. *Blood Rev.* 2009;23(5):225-229.
7. White RH. The epidemiology of venous thromboembolism. *Circulation.* 2003;107(23 Suppl 1):I4-8.
8. Heit JA, Spencer FA, White RH. The epidemiology of venous thromboembolism. *J Thromb Thrombolysis.* 2016;41(1):3-14.
9. Heit JA, Silverstein MD, Mohr DN, Petterson TM, O'Fallon WM, Melton LJ, 3rd. Predictors of survival after deep vein thrombosis and pulmonary embolism: a population-based, cohort study. *Arch Intern Med.* 1999;159(5):445-453.
10. Andresen MS, Sandven I, Brunborg C, et al. Mortality and recurrence after treatment of VTE: long term follow-up of patients with good life-expectancy. *Thromb Res.* 2011;127(6):540-546.
11. Nijkeuter M, Sohne M, Tick LW, et al. The natural course of hemodynamically stable pulmonary embolism: Clinical outcome and risk factors in a large prospective cohort study. *Chest.* 2007;131(2):517-523.
12. Dentali F, Ageno W, Pomero F, Fenoglio L, Squizzato A, Bonzini M. Time trends and case fatality rate of in-hospital treated pulmonary embolism during 11 years of observation in Northwestern Italy. *Thromb Haemost.* 2016;115(2):399-405.
13. Jimenez D, de Miguel-Diez J, Guijarro R, et al. Trends in the Management and Outcomes of Acute Pulmonary Embolism: Analysis From the RIETE Registry. *J Am Coll Cardiol.* 2016;67(2):162-170.
14. Boon GD. An overview of hemostasis. *Toxicol Pathol.* 1993;21(2):170-179.
15. Rijken DC, Lijnen HR. New insights into the molecular mechanisms of the fibrinolytic system. *J Thromb Haemost.* 2009;7(1):4-13.
16. Zorio E, Gilabert-Estelles J, Espana F, Ramon LA, Cosin R, Estelles A. Fibrinolysis: the key to new pathogenetic mechanisms. *Curr Med Chem.* 2008;15(9):923-929.
17. Rifai N, Gillette MA, Carr SA. Protein biomarker discovery and validation: the long and uncertain path to clinical utility. *Nat Biotechnol.* 2006;24(8):971-983.
18. Biomarkers Definitions Working G. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther.* 2001;69(3):89-95.
19. Pedersen A, Redfors P, Lundberg L, et al. Haemostatic biomarkers are associated with long-term recurrent vascular events after ischaemic stroke. *Thromb Haemost.* 2016;116(3):537-543.
20. Stern R, Chanoine F, Criswell K. Are coagulation times biomarkers? Data from a phase I study of the oral thrombin inhibitor LB-30057 (CI-1028). *J Clin Pharmacol.* 2003;43(2):118-121.
21. Konstantinides SV, Torbicki A, Agnelli G, et al. 2014 ESC guidelines on the diagnosis and management of acute pulmonary embolism. *Eur Heart J.* 2014;35(43):3033-3069, 3069a-3069k.

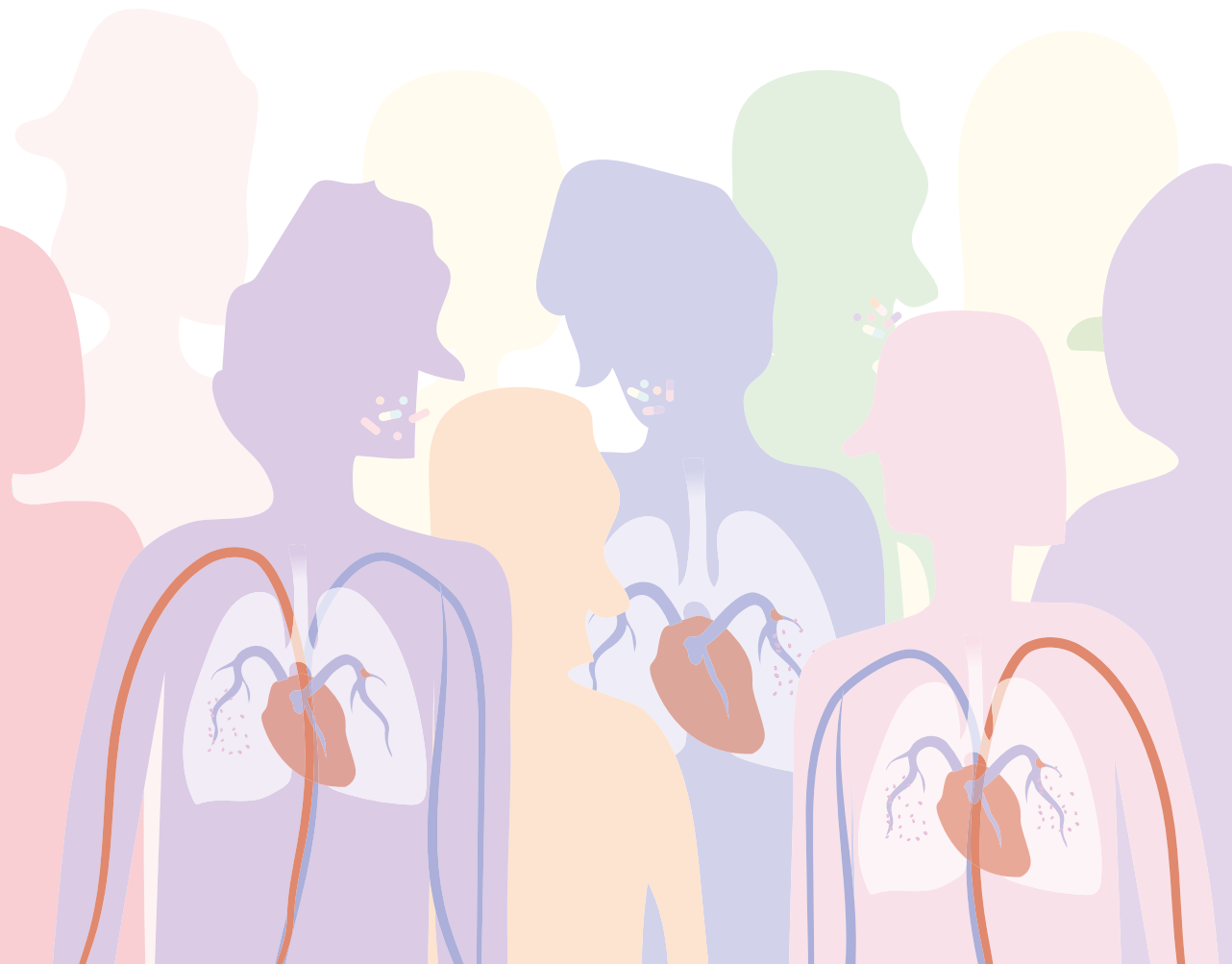
22. Rodger MA, Le Gal G, Anderson DR, et al. Validating the HERDOO2 rule to guide treatment duration for women with unprovoked venous thrombosis: multinational prospective cohort management study. *BMJ.* 2017;356:j1065.
23. Talens S, Malfliet JJ, Rudez G, et al. Biological variation in tPA-induced plasma clot lysis time. *Thromb Haemost.* 2012;108(4):640-646.
24. Lisman T, de Groot PG, Meijers JC, Rosendaal FR. Reduced plasma fibrinolytic potential is a risk factor for venous thrombosis. *Blood.* 2005;105(3):1102-1105.
25. Meltzer ME, Lisman T, Doggen CJ, de Groot PG, Rosendaal FR. Synergistic effects of hypofibrinolysis and genetic and acquired risk factors on the risk of a first venous thrombosis. *PLoS Med.* 2008;5(5):e97.
26. Kamphuisen PW, Eikenboom JC, Rosendaal FR, et al. High factor VIII antigen levels increase the risk of venous thrombosis but are not associated with polymorphisms in the von Willebrand factor and factor VIII gene. *Br J Haematol.* 2001;115(1):156-158.
27. Rietveld IM, Lijfering WM, le Cessie S, et al. High levels of coagulation factors and venous thrombosis risk: strongest association for factor VIII and von Willebrand factor. *J Thromb Haemost.* 2018.
28. Sarma A, Heilbrun ME, Conner KE, Stevens SM, Woller SC, Elliott CG. Radiation and chest CT scan examinations: what do we know? *Chest.* 2012;142(3):750-760.
29. Konstantinides SV. 2014 ESC Guidelines on the diagnosis and management of acute pulmonary embolism. *Eur Heart J.* 2014;35(45):3145-3146.
30. Kooiman J, Klok FA, Mos IC, et al. Incidence and predictors of contrast-induced nephropathy following CT-angiography for clinically suspected acute pulmonary embolism. *J Thromb Haemost.* 2010;8(2):409-411.
31. Raja AS, Greenberg JO, Qaseem A, et al. Evaluation of Patients With Suspected Acute Pulmonary Embolism: Best Practice Advice From the Clinical Guidelines Committee of the American College of Physicians. *Ann Intern Med.* 2015;163(9):701-711.
32. Wells PS, Anderson DR, Bormanis J, et al. Value of assessment of pretest probability of deep-vein thrombosis in clinical management. *Lancet.* 1997;350(9094):1795-1798.
33. Wells PS, Anderson DR, Rodger M, et al. Excluding pulmonary embolism at the bedside without diagnostic imaging: management of patients with suspected pulmonary embolism presenting to the emergency department by using a simple clinical model and d-dimer. *Ann Intern Med.* 2001;135(2):98-107.
34. van Belle A, Buller HR, Huisman MV, et al. Effectiveness of managing suspected pulmonary embolism using an algorithm combining clinical probability, D-dimer testing, and computed tomography. *JAMA.* 2006;295(2):172-179.
35. Newnham M, Stone H, Summerfield R, Mustfa N. Performance of algorithms and pre-test probability scores is often overlooked in the diagnosis of pulmonary embolism. *BMJ.* 2013;346:f1557.
36. Teismann NA, Cheung PT, Frazee B. Is the ordering of imaging for suspected venous thromboembolism consistent with D-dimer result? *Ann Emerg Med.* 2009;54(3):442-446.
37. Monie DD, DeLoughery EP. Pathogenesis of thrombosis: cellular and pharmacogenetic contributions. *Cardiovasc Diagn Ther.* 2017;7(Suppl 3):S291-S298.
38. Cohen AT, Imfeld S, Markham J, Granziera S. The use of aspirin for primary and secondary prevention in venous thromboembolism and other cardiovascular disorders. *Thromb Res.* 2015;135(2):217-225.
39. Kunutsor SK, Seidu S, Khunti K. Statins and primary prevention of venous thromboembolism: a systematic review and meta-analysis. *Lancet Haematol.* 2017;4(2):e83-e93.

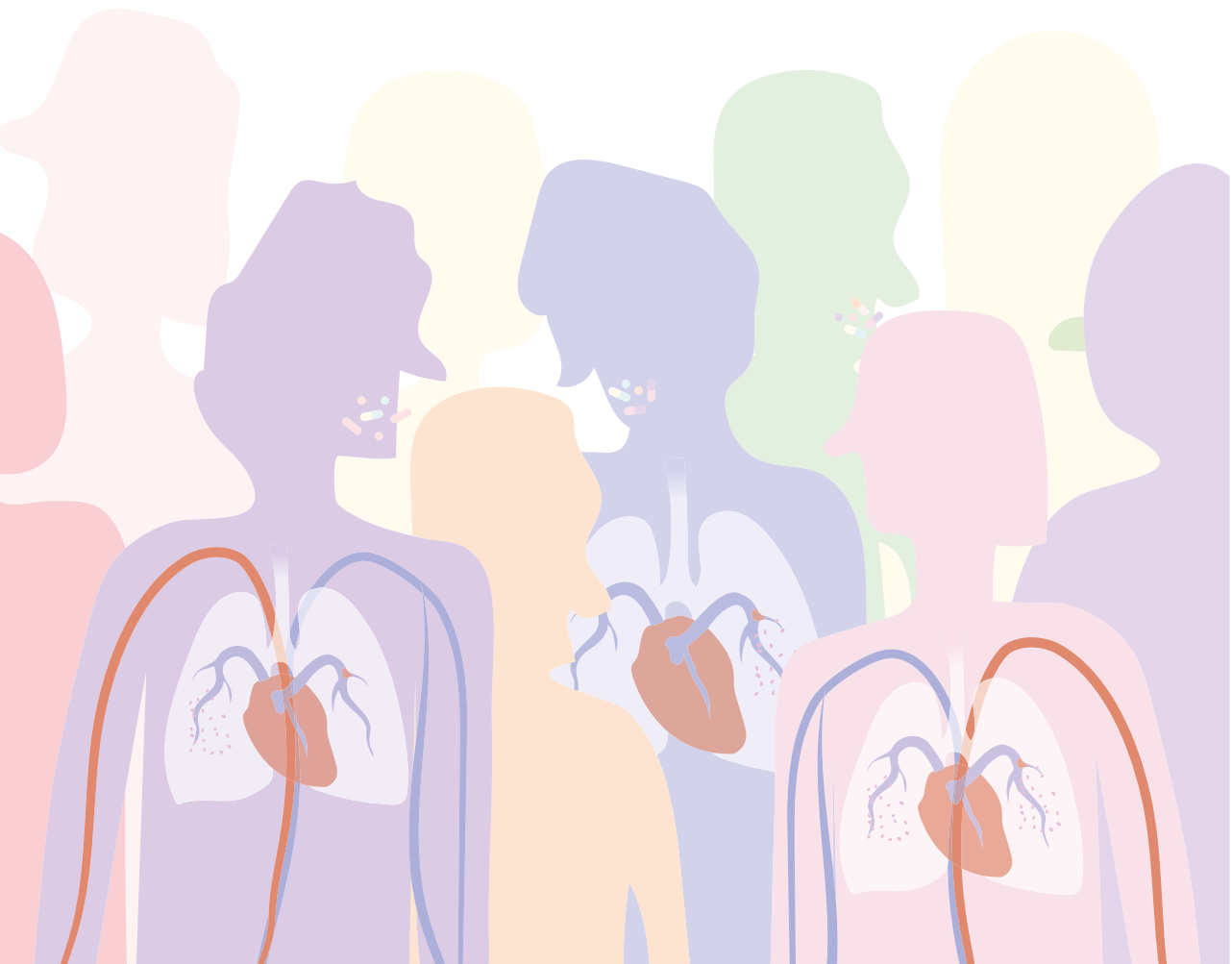
40. Violi F, Calvieri C, Ferro D, Pignatelli P. Statins as antithrombotic drugs. *Circulation.* 2013;127(2):251-257.
41. Oesterle A, Laufs U, Liao JK. Pleiotropic Effects of Statins on the Cardiovascular System. *Circ Res.* 2017;120(1):229-243.
42. Karagiannis AD, Liu M, Toth PP, et al. Pleiotropic Anti-atherosclerotic Effects of PCSK9 InhibitorsFrom Molecular Biology to Clinical Translation. *Curr Atheroscler Rep.* 2018;20(4):20.
43. Venous thromboembolic disease and combined oral contraceptives: results of international multicentre case-control study. World Health Organization Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. *Lancet.* 1995;346(8990):1575-1582.
44. de Bastos M, Stegeman BH, Rosendaal FR, et al. Combined oral contraceptives: venous thrombosis. *Cochrane Database Syst Rev.* 2014(3):CD010813.
45. Johannesdottir SA, Horvath-Puho E, Dekkers OM, et al. Use of glucocorticoids and risk of venous thromboembolism: a nationwide population-based case-control study. *JAMA Intern Med.* 2013;173(9):743-752.
46. Petrelli F, Cabiddu M, Borgonovo K, Barni S. Risk of venous and arterial thromboembolic events associated with anti-EGFR agents: a meta-analysis of randomized clinical trials. *Ann Oncol.* 2012;23(7):1672-1679.
47. Antioch I, Ciobica A, Paulet M, Bild V, Lefter R, Timofte D. Pain manifestations in schizophrenia - clinical and experimental aspects in human patients and animal models. *Psychiatr Danub.* 2015;27(2):142-152.
48. Urban-Kowalczyk M, Pigonska J, Smigielski J. Pain perception in schizophrenia: influence of neuropeptides, cognitive disorders, and negative symptoms. *Neuropsychiatr Dis Treat.* 2015;11:2023-2031.
49. Hu H-C, Chiu N-M. Delayed Diagnosis in an Elderly Schizophrenic Patient with Catatonic State and Pulmonary Embolism. *International Journal of Gerontology.* 2012;7(3):183-185.
50. Ramot Y, Nyska A, Spectre G. Drug-induced thrombosis: an update. *Drug Saf.* 2013;36(8):585-603.
51. Bauer KA, Lip GYH. Overview of the causes of venous thrombosis. In: Leung LLK, Mandel J, Finlay G, eds. Waltham, MA, USA (accessed on August 20, 2018): UpToDate.
52. Kabel JS, van Puijenbroek EP. [Side effects of tramadol: 12 years of experience in the Netherlands]. *Ned Tijdschr Geneeskd.* 2005;149(14):754-757.



Part 1

Diagnostic challenges in venous thromboembolism





2.1

Simplified diagnostic management of suspected pulmonary embolism (the YEARS study): a prospective, multicentre, cohort study

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Abstract

Background: Validated diagnostic algorithms in patients with suspected pulmonary embolism are often not used correctly or only benefit subgroups of patients, leading to overuse of computed tomography pulmonary angiography (CTPA). The YEARS clinical decision rule that incorporates differential D-dimer cutoff values at presentation, has been developed to be fast, to be compatible with clinical practice, and to reduce the number of CTPA investigations in all age groups. We aimed to prospectively evaluate this novel and simplified diagnostic algorithm for suspected acute pulmonary embolism.

Methods: We did a prospective, multicentre, cohort study in 12 hospitals in the Netherlands, including consecutive patients with suspected pulmonary embolism between Oct 5, 2013, to July 9, 2015. Patients were managed by simultaneous assessment of the YEARS clinical decision rule, consisting of three items (clinical signs of deep vein thrombosis, haemoptysis, and whether pulmonary embolism is the most likely diagnosis), and D-dimer concentrations. In patients without YEARS items and D-dimer less than 1000 ng/mL, or in patients with one or more YEARS items and D-dimer less than 500 ng/mL, pulmonary embolism was considered excluded. All other patients had CTPA. The primary outcome was the number of independently adjudicated events of venous thromboembolism during 3 months of follow-up after pulmonary embolism was excluded, and the secondary outcome was the number of required CTPA compared with the Wells' diagnostic algorithm. For the primary outcome regarding the safety of the diagnostic strategy, we used a per-protocol approach. For the secondary outcome regarding the efficiency of the diagnostic strategy, we used an intention-to-diagnose approach. This trial is registered with the Netherlands Trial Registry, number NTR4193.

Findings: 3616 consecutive patients with clinically suspected pulmonary embolism were screened, of whom 151 (4%) were excluded. The remaining 3465 patients were assessed of whom 456 (13%) were diagnosed with pulmonary embolism at baseline. Of the 2946 patients (85%) in whom pulmonary embolism was ruled out at baseline and remained untreated, 18 patients were diagnosed with symptomatic venous thromboembolism during 3-month follow-up (0·61%, 95% CI 0·36–0·96) of whom six had fatal pulmonary embolism (0·20%, 0·07–0·44). CTPA was not indicated in 1651 (48%) patients with the YEARS algorithm compared with 1174 (34%) patients, if Wells' rule and fixed D-dimer threshold of less than 500 ng/mL would have been applied, a difference of 14% (95% CI 12–16).

Interpretation: In our study pulmonary embolism was safely excluded by the YEARS diagnostic algorithm in patients with suspected pulmonary embolism. The main advantage of the YEARS algorithm in our patients is the absolute 14% decrease of CTPA examinations in all ages and across several relevant subgroups.

Introduction

The clinical diagnosis of pulmonary embolism is non-specific and should therefore be followed by objective testing. Because of its diagnostic accuracy and wide availability, multidetector row computed tomography pulmonary angiography (CTPA) is the imaging test of choice to confirm acute pulmonary embolism in most patients. Increasing use of CTPA with diminishing prevalence of pulmonary embolism—to even less than 10%[1]—has led to overdiagnosis of mostly subsegmental pulmonary embolism and unnecessary risks of radiation exposure and contrast medium induced nephropathy.[2-6] To avoid these problems, validated diagnostic algorithms for suspected acute pulmonary embolism, using sequential testing, have been introduced.[7] In these algorithms, a normal D-dimer test result in patients with low probability safely excludes pulmonary embolism.[8] Correct application of these algorithms obviates the need for CTPA in 20–30% of patients, with an overall 3-month diagnostic failure rate of less than 1·5% after initial negative ruling of the algorithm.[7-9] An age-adjusted D-dimer threshold (age × 10 ng/mL for patients aged >50 years) has been validated prospectively, reporting an absolute reduction of 11·6% (95% CI 10·5-12·9) in the need for CTPA.[10] Importantly, only patients aged 50 years or older, and foremost those older than 75 years benefit from this strategy whereas when considering the life-time attributable cancer risk, the exposure to unnecessary radiation is considered more relevant to younger individuals, particularly women.[1]

Despite firm evidence of its safety and efficiency, adherence to recommended diagnostic strategies in clinical practice is variable. This variation might be partly due to complexity of these strategies, and insufficient time at busy emergency departments, which hampers the use of sequential tests.[11–14] In daily practice, D-dimer testing is frequently ordered and known at a low clinical threshold or even before the clinical assessment.[15,16] Improved adherence to the algorithm, for instance by implementation of a clinical decision support system, has been shown to significantly decrease the mean number of diagnostic tests used along with— and more importantly—the number of diagnostic failures.[17,18]

On the basis of a post-hoc derivation and validation study,[19] three items of the original Wells' clinical decision rule—ie, clinical signs of deep vein thrombosis, haemoptysis, and whether pulmonary embolism is the most likely diagnosis—were the most predictive for pulmonary embolism. They allowed the use of a differential D-dimer threshold based on the presence of one of these items, without losing sensitivity. Hence, this algorithm—which we call YEARS—involves the simultaneous assessment of only the three abovementioned items and a D-dimer test threshold of 500 ng/mL in presence, and 1000 ng/mL in absence of one of the YEARS items. The YEARS algorithm was designed

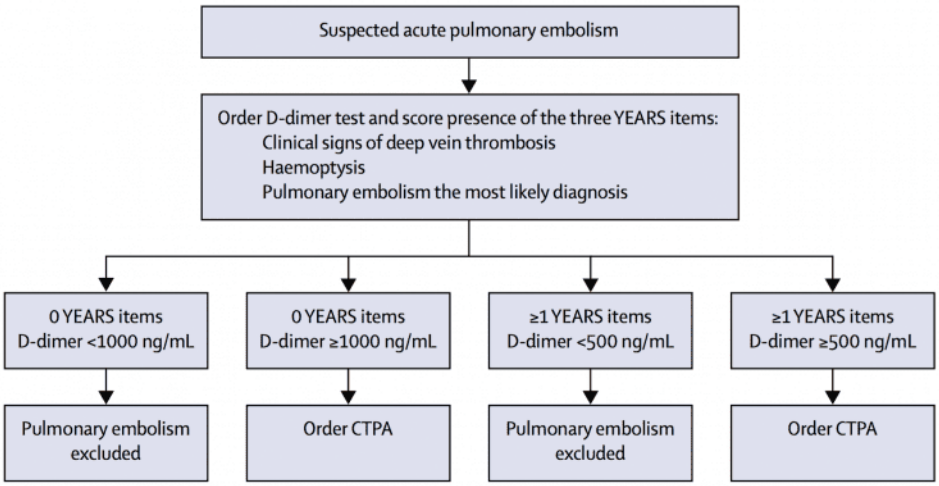
to be more easily applied in a busy clinical practice than currently used diagnostic strategies, and to further decrease the number of necessary CTPA examinations in patients of all ages. In this study, we aimed to prospectively evaluate this novel and simplified diagnostic algorithm for suspected acute pulmonary embolism.

Methods

Study design and patients

We did a prospective, multicentre, cohort outcome study evaluating the safety and efficiency of the YEARS algorithm in patients with suspected acute pulmonary embolism between Oct 5, 2013, and July 9, 2015 (Figure 1).[19] The algorithm was implemented as standard diagnostic strategy in 12 participating hospitals in the Netherlands. The full study protocol is available in the appendix.

Figure 1: YEARS algorithm



CTPA=computed tomography pulmonary angiography

Consecutive outpatients and inpatients with clinically suspected acute (first or recurrent) pulmonary embolism were eligible for inclusion if they were aged 18 years or older. Exclusion criteria were treatment with therapeutic doses of anticoagulants initiated 24 hours or more before eligibility assessment, life expectancy less than 3 months or geographic inaccessibility precluding follow-up, pregnancy, or allergy to intravenous contrast agent. The protocol was centrally approved by the institutional review board

of the Leiden University Medical Center, Leiden, Netherlands, which waived the need for informed consent; this decision was endorsed by the local institutional review board of each participating centre.

Procedures

An attending physician who suspected acute pulmonary embolism assessed the patients, and then evaluated the YEARS score by assessing the presence or absence of each of the YEARS items—ie, symptomatic deep vein thrombosis, haemoptysis, and whether pulmonary embolism is the most likely diagnosis—(scored as yes or no) with the pretest probability dependent threshold of the D-dimer test (Figure 1). D-dimer concentrations were measured upon presentation of the patient, according to local practice, with automated well validated high-sensitive quantitative D-dimer assays (Vidas D-dimer Exclusion, Biomerieux, Marcy-L'Étoile, France; Tinaquant, Roche Diagnostica, Mannheim, Germany; STA-LIA, DiagnosticaStago, Asnieres, France; and Innovance, Siemens, Marburg, Germany). Our study reflected daily clinical practice in which D-dimer concentrations are often determined at presentation to the emergency ward. Physicians were not blinded for the D-dimer test result when they assigned the YEARS items.

In patients with no YEARS items and a D-dimer concentration less than 1000 ng/mL, pulmonary embolism was considered excluded and further testing was withheld. In patients with one or more YEARS items and a D-dimer concentration less than 500 ng/mL, pulmonary embolism was also considered excluded and further testing was withheld. All other patients—ie, either with no YEARS item and a D-dimer concentration of 1000 ng/mL or more, or with one or more items and a concentration of 500 ng/mL or more—were referred for CTPA to show or exclude the diagnosis of pulmonary embolism. The appendix shows the full CTPA scan protocol. Patients in whom pulmonary embolism was ruled out were left untreated and followed up for 3 months. They were instructed to return to the hospital in the event of symptoms of venous thromboembolism, after which objective diagnostic tests were done to confirm or refute the disease. Follow-up consisted of a scheduled outpatient visit or telephone interview after 3 months. At this visit, information about complaints suggestive of venous thromboembolism was obtained. Patients in whom acute pulmonary embolism was confirmed at baseline were treated with anticoagulants according to international guidelines.

Outcomes

The primary outcome was the 3-month incidence of symptomatic venous thromboembolism in the overall population and in patients managed with and without

CTPA separately. The diagnosis of pulmonary embolism or deep vein thrombosis was based on predefined criteria (appendix). In case of clinically suspected pulmonary embolism or deep vein thrombosis, objective diagnostic tests were required, including CTPA for suspected pulmonary embolism and compression ultrasonography for suspected deep vein thromboembolism. In case of death, information was obtained from the hospital records. Deaths were classified as caused by pulmonary embolism if it was confirmed by autopsy, was shown by objective testing before death, or could not be confidently excluded as a cause of death. An independent adjudication committee assessed and adjudicated all suspected venous thromboembolism and deaths during follow-up.

The secondary outcome was the proportion of required CTPA examinations to complete the YEARS algorithm at baseline, as compared post hoc with the theoretical proportion of CTPA examinations that would have been required if the algorithm, using the two-level Wells' rule outcome and fixed D-dimer threshold of less than 500 ng/mL, would have been applied in the study population and to historical data.[20] Finally, we compared the efficiency to the scenario in which the age-adjusted D-dimer concentration would have been applied (calculated by age × 10 μg/L in patients >50 years). This comparison was done post hoc because the final evidence supporting this approach was not available at the moment of drafting of the protocol.[10] The Wells' rule was calculated by an independent researcher (TvdH) based on the YEARS criteria entered in the case record form and information from the medical charts.

Statistical analysis

On the basis of derivation cohort of the YEARS algorithm, we expected a failure rate of 1·2% in patients managed without CTPA.[19] The sample size was based on this assumption, with the aim to keep the upper limit of the 95% CI of this point estimate below 2·7%.[21] This number reflects the 3-month incidence of venous thromboembolism after normal conventional pulmonary angiography. Any venous thromboembolism incidence with a complete confidence interval below this safety threshold was considered to be safe. We calculated that we needed to include 1333 patients managed without CTPA, with a two-sided α of 5% and a β of 80%. Because 44% of patients in the combined YEARS derivation and validation cohort could have been managed without CTPA and accounting for up to 7·5% loss to follow-up, a total of 3260 patients with suspected pulmonary embolism would be required.[19] For the primary outcome regarding the safety of the diagnostic strategy, we used a per-protocol approach. For the secondary outcome regarding the efficiency of the diagnostic strategy, we used an intention-to-diagnose approach. The difference between approaches was how to report the number of CTPA that were done but not indicated by

the strategy. By using this approach, pulmonary embolism diagnosed at presentation on a CTPA that was not indicated was considered as failures of the diagnostic strategy.

For the secondary outcome analysis, we determined the absolute difference in the number of required CTPA examinations between the different clinical scenarios. Finally, we reported outcomes of not predefined post-hoc analyses for relevant subgroups: patients with malignancy, patients 50 years or older, patients with a history of venous thromboembolism, and inpatients and patients with complaints for more than 7 days. All descriptive parameters and exact 95% CIs around the observed incidences were calculated. All analyses were done with SPSS (version 23).

This study is registered with the Netherlands Trial Register, number NTR4193.

Role of the funding source

This study was an academically sponsored trial. The steering committee, consisting of the authors, had final responsibility for the study design, oversight, and data verification and analyses. The sponsor was not involved in the study. All members of the steering committee contributed to the interpretation of the results, approved the final version of the manuscript, and vouch for the accuracy and completeness of the data reported. The final decision to submit the manuscript was made by the corresponding author on behalf of all coauthors.

Appendix

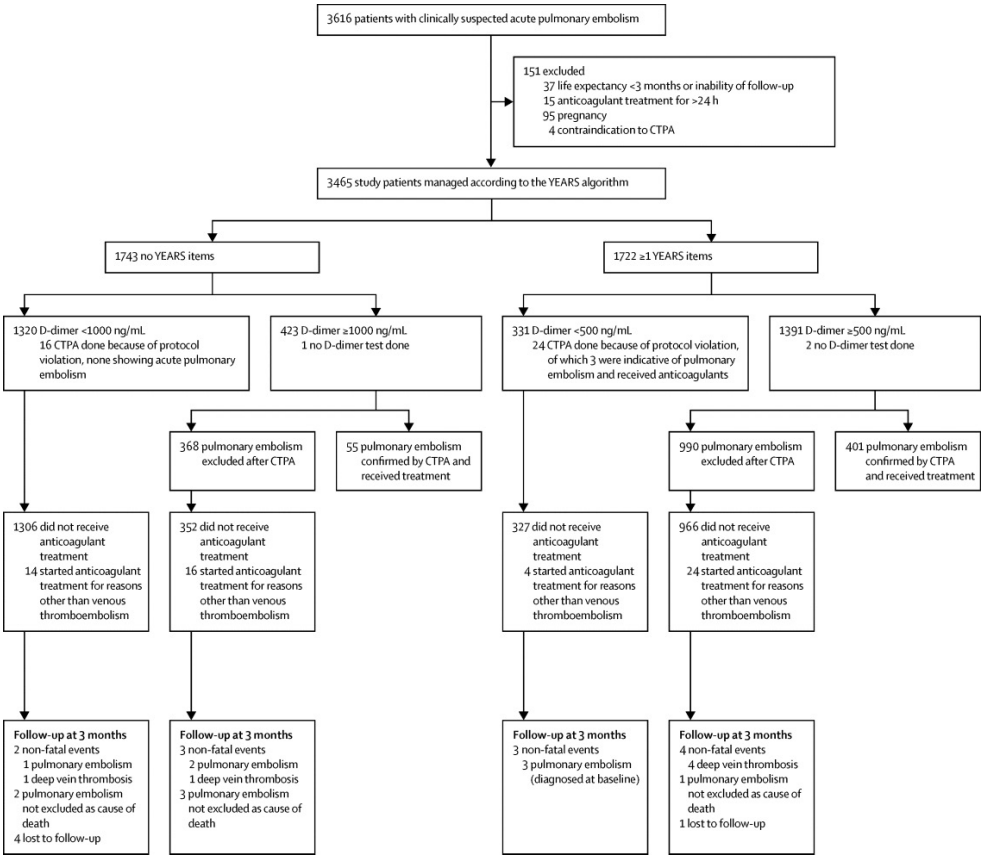
see online for appendix
https://www.thelancet.com/cms/10.1016/S0140-6736(17)30885-1/attachment/b47fbcc6-a3a0-4608-beb4-8ab8261ab559/mmc1.pdf

Results

From Oct 5, 2013, to July 9, 2015, 3616 consecutive patients with clinically suspected pulmonary embolism were screened in the 12 participating hospitals, of whom 151 (4·2%) were excluded (Figure 2).

Table 1 summarises the baseline characteristics. Overall, pulmonary embolism was detected in 456 (13%) of 3465 patients: in 55 (3·2%) of 1743 patients with none of the YEARS items and 401 (23%) of 1722 patients with one or more YEARS items.

Figure 2: Flowchart of study patients



CTPA= computed tomography pulmonary angiography

Table 1: Baseline characteristics of 3465 included patients with suspected pulmonary embolism

Mean age, years (SD)	53 (18)
Female, n (%)	2154 (62)
Duration of complaints, days (median and IQR)	3 (1-8)
COPD with treatment, n (%)	423 (12)
Heart failure with treatment, n (%)	137 (4.0)
Estrogen use, n (% of women)	337 (16)
Immobilization or surgery in the previous 4 weeks	407 (12)
Outpatient, n (%)	2996 (86)
Heart rate greater than 100/min, n (%)	683 (20)
Previous history of PE or DVT, n (%)	359 (10)
Malignancy, n (%)	336 (9.7)

Data are mean (SD), n (%), or median (IQR). COPD=chronic obstructive pulmonary disease.

According to the intention-to-diagnose approach, of the 2946 (85%) patients in whom pulmonary embolism was ruled out at baseline, who remained untreated, and completed the follow-up period, 18 patients were diagnosed with symptomatic venous thromboembolism during 3-month follow-up, with an incidence of 0·61% (95% CI 0·36-0·96). The incidence of fatal pulmonary embolism was 0·20% (six patients, 95% CI 0·07-0·44; Table 2). In a worst case scenario, accounting the five patients who were lost to follow-up (four patients had pulmonary embolism excluded without CTPA and one patient had a negative CTPA) as recurrent venous thromboembolism, the 3-month incidence would have been 0·78% (23 of 2951 patients, 95% CI 0·49-1·2). For the per-protocol approach, the failure rate of the diagnostic algorithm was 0·51% (15 of 2943 patients, 95% CI 0·31-0·84) with a 0·20% 3-month risk of fatal pulmonary embolism (six of 2943, 0·08-0·46).

Table 2: Primary outcomes of venous thromboembolism events during 3-month follow-up

Category	Patients (n)	Total venous thromboembolism, (n [%, 95% CI])	Fatal pulmonary embolism* (n [%, 95% CI])
Completed algorithm	2944	18 (0.61%) [0.36-0.96]	6 (0.20%) [0.07-0.44]
Patients managed without CTPA	1629	7 (0.43%) [0.17-0.88]	2 (0.12%) [0.01-0.44]
Patients managed with CTPA	1315	11 (0.84%) [0.47-1.5]	4 (0.30%) [0.12-0.78]

*Patients in whom pulmonary embolism was excluded by either a low YEARS score or CT scanning were left untreated. CTPA=computed tomography pulmonary angiography. *Patients who remained untreated and were not lost to follow-up*

In the intention-to-diagnose approach, CTPA was not done in 1611 (46%) patients and it was not indicated in 1651 (48%) patients following the per-protocol approach. If the standard diagnostic algorithm using Wells' rule and D-dimer with fixed threshold of <500 ng/mL would have been applied, 1174 (34%) patients could have been managed without CTPA at baseline, for an absolute difference of 13% (difference in intention-to-diagnose approach 437 CTPA examinations, 95% CI 10–15%) and 14% (difference in per-protocol approach 477 CTPA examinations, 12–16%) in favour of the YEARS algorithm.

If Wells' rule and the age-adjusted D-dimer threshold would have been applied, 1348 (39%) patients could have been managed without CTPA at baseline, an absolute difference

of 8·7% (difference in per-protocol approach CTPA examinations 303, 95% CI 6·4-11%) and of 7·6% (difference in intention-to-diagnose approach CTPA examinations 263, 95% CI 5·3-9·9%).

In the subgroups of patients younger than 50 years and 50 years and older, a 14% absolute reduction in the number of required CTPA examinations was observed when the YEARS algorithm was applied compared with the standard diagnostic algorithm, with failure rates of 0·11% (one of 894, 95% CI 0·02-0·63) and 0·81% (six of 740, 0·37-1·8), respectively. Table 3 summarises the results for the other subgroups.

Figure 2 shows the management of all 3465 included patients. Of the 1651 patients who should have been managed without CTPA, the protocol was violated in 40 patients. CTPA showed pulmonary embolism in three patients who were treated with anticoagulants. These observations were considered diagnostic failures and are included in the primary outcome. Furthermore, 18 (1·1%) of 1651 patients were treated with oral anticoagulants for other reasons (ie, eight atrial fibrillation, one superficial thrombophlebitis, and nine other reasons including idiopathic pulmonary hypertension and peripheral arterial disease) and four (0·24%) of 1651 patients were lost to follow-up. Four of the remaining 1589 patients returned with symptomatic events of venous thromboembolism (Table 4). The 3-month incidence of venous thromboembolism in patients who did not have CTPA according to the YEARS algorithm was 0·43% (seven of 1629, 95% CI 0·17-0·88) and of fatal pulmonary embolism was 0·12% (two of 1629, 0·01-0·44; Table 2). Seven other patients (0·43%) died of non-venous-thromboembolism-related causes.

Of the 1358 patients in whom CTPA ruled out pulmonary embolism, 40 patients (2·95%) were treated with anticoagulants for other reasons (ie, 20 atrial fibrillation, three superficial thrombophlebitis, one splanchnic vein thrombosis, one thrombus in the left ventricle, one high-dose thrombosis prophylaxis, one suspected but later ruled out pulmonary vein thrombosis, one vena cava superior syndrome due to mediastinal mass, and 12 other reasons including idiopathic pulmonary hypertension and peripheral arterial disease) and one patient (0·07%) was lost to follow-up. Of the 1317 remaining patients, 11 patients returned with symptomatic events of venous thromboembolism (Table 5). The 3-month incidence of venous thromboembolism was 0·84% (11 of 1317, 95% CI 0·47-1·5) and incidence of fatal pulmonary embolism was 0·30% (four of 1317, 0·12–0·78; Table 2). 85 other patients (6·5%) died of non-venous-thromboembolism-related causes.

Table 3: Primary endpoint and efficacy in subgroups of the total study population

	Patients	PE at baseline	Managed without CTPA	Risk of VTE during 3-months follow-up						Efficiency compared with Wells' rule in combination with a D-dimer threshold of <500 ng/mL		
				incidence in patients managed without CTPA		incidence in patients managed with CTPA		Overall incidence after pulmonary embolism was excluded at baseline		Managed without CTPA(n)	Difference with YEARS algorithm	
				events/ patients	%(95%CI)	events/ patients	%(95%CI)	events/ patients	%(95%CI)		n/N	%(95%CI)
Malignancy	336	57 17%	62	2/61	3.2 (0.90-11)	5/209	2.4 (1.0-5.5)	7/270	2.6 (1.3-5.3)	37	25/336	7.4 (5.0-11)
No malignancy	3129	399 13%	1590	5/1573	0.32 (0.14-0.74)	6/1106	0.54 (0.25-1.2)	11/2679	0.41 (0.23-0.73)	1137	453/3129	15 (13-16)
Age < 50 years	1448	126 8.7%	900	1/894	0.11 (0.02-0.63)	1/415	0.24 (0.04-1.4)	2/1309	0.15 (0.04-0.56)	704	196/1448	14 (12-15)
Age ≥ 50 years	2017	330 16%	752	6/740	0.81 (0.37-1.8)	10/900	1.1 (0.6-2.0)	16/1640	0.98 (0.6-1.6)	470	282/2017	14 (13-16)
No history of VTE	3106	349 11%	1529	6/1517	0.40 (0.18-0.86)	10/1191	0.84 (0.46-1.5)	16/2708	0.59 (0.36-0.96)	1120	409/3106	13 (12-14)
History of VTE	359	107 30%	123	1/117	0.85 (0.15-4.7)	1/124	0.81 (0.14-4.6)	2/241	0.83 (0.23-3.0)	54	69/359	19 (15-24)
Inpatient	469	66 14%	200	1/195	0.51 (0.09-2.9)	3/197	1.5%(0.52-4.4)	4/392	1.0 (0.40-2.6)	135	65/469	14 (11-17)
Outpatient	2996	390 13%	1452	6/1439	0.42 (0.19-0.91)	8/1118	0.72 (0.36-1.4)	14/2557	0.55 (0.33-0.92)	1039	413/2996	14 (13-15)
Complaints ≤7 days	2599	362 14%	1266	7/1253	0.56 (0.27-1.2)	9/940	0.96 (0.50-1.8)	16/2195	0.73 (0.46-1.2)	901	365/2599	14 (13-15)
Complaints >7 days	866	94 11%	386	0/381	0 (0-1.0)	2/375	0.53 (0.15-1.9)	2/756	0.26 (0.07-0.96)	273	113/866	13 (11-15)

Data are n or n (%), unless otherwise specified. PE=pulmonary embolism. CTPA=computed tomography pulmonary angiography. VTE=venous thromboembolism

Table 4: Diagnostic failures in patients who were managed without CTPA at baseline

	Sex	Age (years)	Years score	Wells score*	D-dimer concentration (ng/mL)	Interval (days)	Outcome	Circumstances of outcome event	Adjudicated as
Patient 1	Female	59	0	0	609	54	Death	Developed cardiac arrest during admission for acute severe pancreatitis. Known with myotonic dystrophy type 1 with severe cardiomyopathy and arrhythmias. ICD was earlier deactivated after regular unjustified defibrillations. Resuscitation was unsuccessful	Pulmonary embolism not excluded as cause of death
Patient 2	Male	78	0	1	898	11	Death	Diagnosed with end-stage metastasized oropharyngeal carcinoma. Found deceased in nursing home	Pulmonary embolism not excluded as cause of death
Patient 3	Female	89	0	1.5	610	18	Pulmonary embolism	Subsegmental PE diagnosed on CTPA during admission for pneumonia and acute heart failure related to severe aortic valve stenosis and mitral valve insufficiency. Patient died seven days after treatment was voluntarily withheld	Non-fatal pulmonary embolism
Patient 4	Male	52	0	1	560	49	Deep vein thrombosis	DVT 14 days after surgery for glioblastoma multiforme	Deep vein thrombosis
Patient 5	Female	21	2	5.5	380	0	Pulmonary embolism	CTPA performed due to protocol violation at baseline	Non-fatal pulmonary embolism
Patient 6	Male	58	1	3	420	0	Pulmonary embolism	CTPA performed due to protocol violation at baseline	Non-fatal pulmonary embolism
Patient 7	Female	71	1	6	410	0	Pulmonary embolism	CTPA performed due to protocol violation at baseline	Non-fatal pulmonary embolism

*CTPA=computed tomography pulmonary angiography. *Calculated post hoc.*

Table 5: Diagnostic failures in patients who were managed with CTPA at baseline

	Sex	Age (years)	Years score	Wells score*	D-dimer concentration (ng/mL)	Interval (days)	Outcome	Circumstances of outcome event	Adjudicated as
Patient 1	Male	50	0	1.5	1070	34	Deep vein thrombosis	Vena cava superior syndrome caused by thrombosis at the site of pacemaker leads	Thrombosis of the vena cava superior
Patient 2	Female	73	0	3	1480	69	Death	Died in hospital under the clinical diagnosis of a pneumonia and acute heart failure	PE not excluded as cause of death
Patient 3	Female	79	0	3	2400	26	Pulmonary embolism	Initiation of anticoagulation because of suspected pulmonary embolism without CTPA confirmation after hospital admission because of heart failure and COPD exacerbation	Non-fatal PE
Patient 4	Female	82	0	0	2550	Unknown	Death	Died in nursing home after hospital admission because of acute heart failure and exacerbation of COPD	PE not excluded as cause of death
Patient 5	Female	57	0	1	4170	12	Pulmonary embolism	Known with a recurrent sarcoma of the uterus. Subsegmental pulmonary embolism diagnosed postoperatively. Died 33 days after diagnosis of pulmonary embolism during palliative care in a hospice	Non-fatal PE
Patient 6	Female	70	0	1	2400	17	Death	Died after sudden collapse followed by unsuccessful resuscitation 1 day after surgery for gastric carcinoma	PE not excluded as cause of death
Patient 7	Female	73	1	5.5	2500	6	Deep vein thrombosis	Known with leukemia. Developed thrombosis of the brachial vein after superficial thrombophlebitis related to an intravenous catheter	DVT
Patient 8	Male	84	1	4	5000	32	Deep vein thrombosis	Known with metastasized prostate cancer. Developed DVT after immobilization during admission at the hospital	DVT
Patient 9	Female	66	1	7	1325	43	Death	Known with lung cancer for which curative treatment. Post-radiation stenosis of the trachea for which a stent placed. Died at home after sudden hemoptysis	PE not excluded as cause of death
Patient 10	Male	70	1	3	5000	68	Deep vein thrombosis	Subclavian vein thrombus associated with intravenous catheter	DVT
Patient 11	Female	48	1	3	747	78	Deep vein thrombosis	Developed deep vein thrombosis and was diagnosed with antiphospholipid syndrome	DVT

*CTPA=computed tomography pulmonary angiography. COPD=chronic obstructive pulmonary disease. *Calculated post hoc.*

Discussion

Our study showed that the YEARS algorithm safely excluded acute pulmonary embolism. An absolute 14% decrease in the need for CTPA was achieved, compared with the standard algorithm. The 3-month incidence of venous thromboembolism in patients who did not undergo CTPA was in line with that observed in studies using algorithms with sequential diagnostic testing and traditional two-level Wells' score, and a fixed cutoff concentration of D-dimer of 500 ng/mL: 0·43% (95% CI 0·17-0·88) in our study versus 0·34% (0·036-0·96) reported by a meta-analysis.[20] Moreover, the risk of recurrent venous thromboembolism in patients with a normal CTPA was comparable to the risk observed in previous studies using standard algorithms: 0·84% (95% CI 0·47-1·5) versus 1·2% (0·8-1·8).[22] Additionally, fatal pulmonary embolism occurred in 0·30% (95% CI 0·12-0·78) of patients in our study compared with 0·6% (0·4-1·1) in another study using standard algorithms.[22]

The advantage of the YEARS algorithm over existing algorithms is the large reduction in the need for CTPA, which reduces radiation exposure and overdiagnosis,[1–4,23] and is achieved by using variable D-dimer thresholds depending on the clinical probability. This study is the first prospective outcome study that validated a D-dimer threshold of 1000 ng/mL in patients with a low clinical probability.

While our study was ongoing, another strategy to reduce the number of CTPA has been validated in a prospective outcome study: the age-adjusted D-dimer threshold.[10] If this strategy would have been applied to our study population, the YEARS algorithm would have led to an absolute reduction of 8·7% (95% CI 6·4-11) of CTPA. The main reason for this difference is the applicability of the YEARS algorithm to patients with suspected acute pulmonary embolism in all ages, and not only in patients older than 50 years. In patients younger than 50 years, the YEARS algorithm leads to a 14% absolute reduction of CTPA. Of note, reducing the number of CTPA is very relevant for young patients, particularly women, in whom concerns have been raised about long-term effects of radiation on the risk of breast cancer.

Methodological strengths of the study include the large number of consecutive patients, the near complete follow-up, and the independent adjudication of endpoints. Furthermore, by studying a real-world cohort of patients in daily practice, we expect that the YEARS algorithm can be easily implemented outside the participating study sites, and that our data for safety and efficiency are representative for non-trial conditions. Additionally, our results are in line with the numbers reported in the initial derivation

and retrospective validation study of our algorithm.[19] Of note, although haemodynamic instability was not a formal exclusion criterion of this study, we have described a cohort of only haemodynamically stable patients.

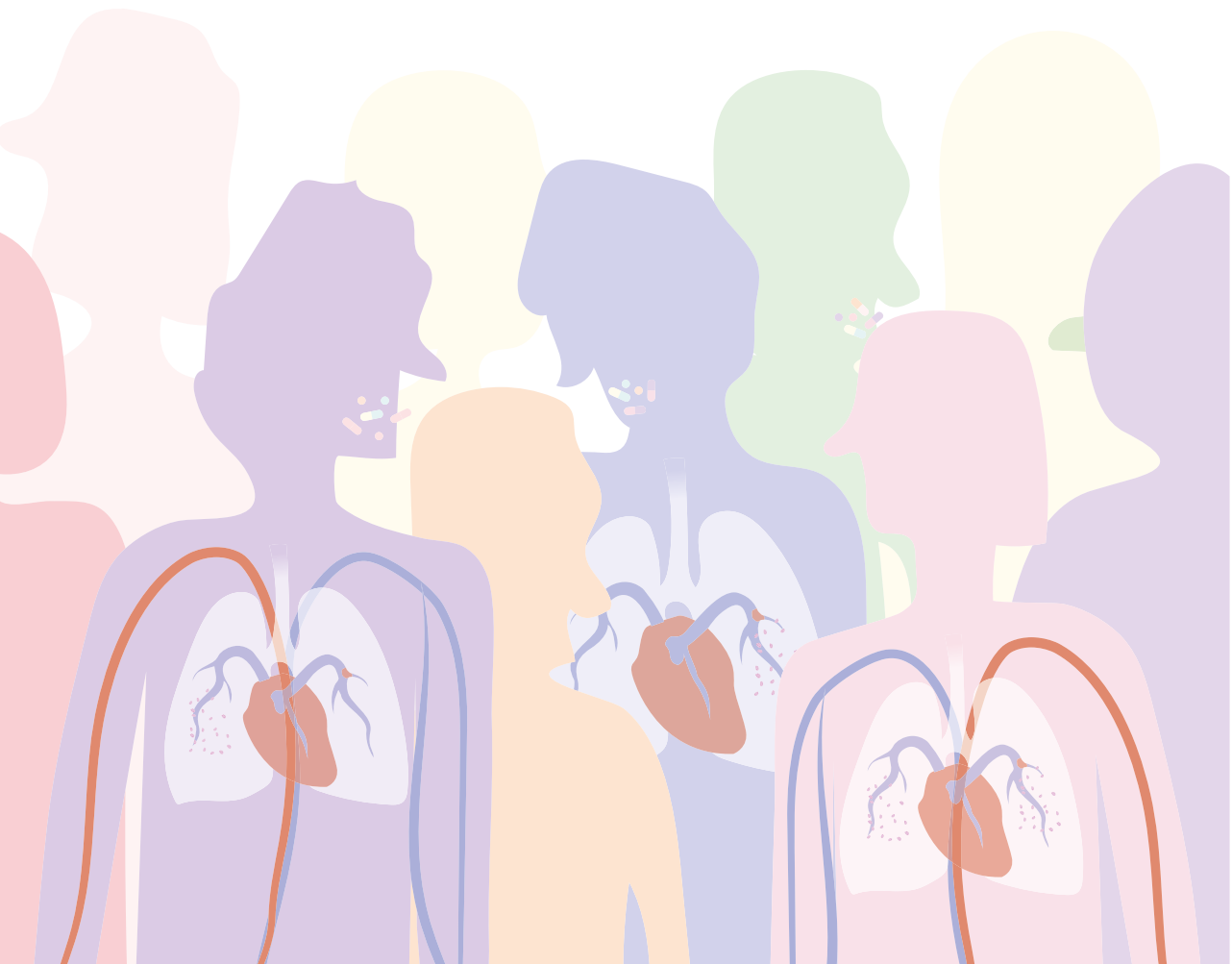
Limitations of our the study are the absence of a control group because we did not do a randomised study and could therefore not directly compare the risk of venous thromboembolism with a control group that would have been managed with traditional algorithms. However, the low observed 3-month risk of venous thromboembolism and near complete follow-up strongly support the chosen study design. Moreover, although an independent committee evaluated and adjudicated all endpoints, autopsy was hardly scarcely done. As a consequence, it was difficult to exclude pulmonary embolism as a possible cause of death in six patients during follow-up. These patients already had or developed extensive comorbidity, or went into the final stage of a terminal illness during the follow-up period, with most of them dying in an outpatient setting. Even so, although pulmonary embolism was conservatively adjudicated as the cause of death in these patients, the recurrence rate observed in our study remained well below the safety threshold, reinforcing the validity of our findings. Furthermore, the prevalence of pulmonary embolism was higher than observed in large cohorts in North America, but lower than observed in previous studies in Europe. The study patients were relatively young, but identical to those in an earlier large diagnostic management study by our group.[7] The results of the subgroup analyses, however, confirm the validity of applying the YEARS algorithm in a patient cohort with higher pulmonary embolism prevalence of up to 30% and provide evidence of the generalisability of our findings. Lastly, there were 43 violations of the study protocol, with a D-dimer test not done in three patients and a non-indicated CTPA done in 40 patients, of which three confirmed the presence of acute pulmonary embolism. This number is comparable to that in the Christopher study, in which two of 25 unjustified CTPA examinations revealed pulmonary embolism.[7] Finally, because of the small number of patients with cancer included in our study, the safety of this algorithm for patients with suspected pulmonary embolism in the presence of cancer remains to be determined.

In conclusion, the YEARS diagnostic algorithm safely ruled out acute pulmonary embolism in patients presenting with clinically suspected pulmonary embolism, with a low risk for venous thromboembolism during a 3-month follow-up. The main advantage of the YEARS algorithm is the absolute 14% decrease in the number of CTPA examinations that is applicable to all ages and was shown consistently across subgroups.

References

1. Wiener RS, Schwartz LM, Woloshin S. Time trends in pulmonary embolism in the United States: evidence of overdiagnosis. *Arch Intern Med.* 2011; 171: 831-837.
2. Sarma A, Heilbrun ME, Conner KE, et al. Radiation and chest CT scan examinations: what do we know? *Chest* 2012; 142:750-760.
3. Brenner DJ, Hall EJ. Computed tomography--an increasing source of radiation exposure. N Engl J Med 2007; 357: 2277-2284.
4. O'Neill J, Murchison JT, Wright L, et al. Effect of the introduction of helical CT on radiation dose in the investigation of pulmonary embolism. *Br J Radiol* 2005; 78: 46-50.
5. Kooiman J, Klok FA, Mos IC, et al. Incidence and predictors of contrast-induced nephropathy following CT-angiography for clinically suspected acute pulmonary embolism. *J Thromb Haemost* 2010; 8: 409-411.
6. Schuur JD, Carney DP, Lyn ET, et al. A top-five list for emergency medicine: a pilot project to improve the value of emergency care. *JAMA Intern Med* 2014; 174: 509-515.
7. Christopher Study Investigators. Effectiveness of Managing Suspected Pulmonary Embolism Using an Algorithm Combining Clinical Probability, D-Dimer Testing, and Computed Tomography. *JAMA* 2006; 295: 172-179.
8. van Es N, van der Hulle T, van Es J, et al. Wells Rule and d-Dimer Testing to Rule Out Pulmonary Embolism: A Systematic Review and Individual-Patient Data Meta-analysis. *Ann Intern Med* 2016; doi:10.7326/M16-0031.
9. Douma RA, Mos IC, Erkens PM, et al. Performance of 4 clinical decision rules in the diagnostic management of acute pulmonary embolism: a prospective cohort study. *Ann Intern Med* 2011; 154: 709-718.
10. Righini M, van Es J, den Exter PL, et al. Age-adjusted D-dimer cutoff levels to rule out pulmonary embolism: the ADJUST-PE study. *JAMA* 2014; 311: 1117-1124.
11. Roy PM, Meyer G, Vielle B, et al. Appropriateness of diagnostic management and outcomes of suspected pulmonary embolism. *Ann Intern Med.* 2006; 144: 157-164.
12. Newnham M, Stone H, Summerfield R, et al. Performance of algorithms and pre-test probability scores is often overlooked in the diagnosis of pulmonary embolism. *BMJ* 2013; 346: f1557.
13. Teismann NA, Cheung PT, Frazee B. Is the ordering of imaging for suspected venous thromboembolism consistent with D-dimer result? *Ann Emerg Med* 2009; 54: 442-446.
14. Adams DM, Stevens SM, Woller SC, et al. Adherence to PIOPED II investigators' recommendations for computed tomography pulmonary angiography. *Am J Med* 2013; 126: 36-42.
15. Jones P, Elangbam B, Williams NR. Inappropriate use and interpretation of D-dimer testing in the emergency department: an unexpected adverse effect of meeting the "4-h target". Emerg Med J 2010; 27: 43-47.
16. Gibson NS, Sohne M, Gerdes VE, Nijkeuter M, Buller HR. The importance of clinical probability assessment in interpreting a normal d-dimer in patients with suspected pulmonary embolism. Chest 2008: 134: 789-793.
17. Roy PM, Durieux P, Gillaizeau F, et al. A computerized handheld decision-support system to improve pulmonary embolism diagnosis: a randomized trial. *Ann Intern Med* 2009; 151: 677-686.
18. Jiménez D, Resano S, Otero R, et al. Computerised clinical decision support for suspected PE. *Thorax* 2015; 70: 909-911.

19. van Es J, Beenen LFM, Douma RA, et al. A simple decision rule including D-dimer to reduce the need for computed tomography scanning in patients with suspected pulmonary embolism. *J Thromb Haemost* 2015; 138: 1428–1435.
20. Pasha SM, Klok FA, Snoep JD, et al. Safety of excluding acute pulmonary embolism based on an unlikely clinical probability by the Wells rule and normal D-dimer concentration: a meta-analysis. *Thromb Res.* 2010; 125: e123-e127.
21. van Beek EJ, Brouwerst EM, Song B, et al. Clinical validity of a normal pulmonary angiogram in patients with suspected pulmonary embolism-a critical review. *Clin Radiol* 2001; 56: 838-842.
22. Mos IC, Klok FA, Kroft LJ, et al. Safety of ruling out acute pulmonary embolism by normal computed tomography pulmonary angiography in patients with an indication for computed tomography: systematic review and meta-analysis. *J Thromb Haemost* 2009; 7: 1491-1498.
23. Wiener RS, Schwartz LM, Woloshin S. When a test is too good: how CT pulmonary angiograms find pulmonary emboli that do not need to be found. *BMJ* 2013; 347: f3368.



2.2

Clinical effects of antiplatelet drugs and statins on D-dimer levels

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Abstract

Background: Acute pulmonary embolism may be ruled out by combining non-high clinical probability and a normal D-dimer level. Both antiplatelet drugs and HMG-CoA reductase inhibitors (statins) have been associated with effects on thrombus formation, potentially influencing D-dimer levels in this setting, leading to a higher rate of false-negative tests. Therefore we determined whether D-dimer levels in patients with suspected pulmonary embolism are affected by concomitant use of antiplatelet drugs and/or statins and evaluated whether the effect of antiplatelet drugs or statins might affect diagnostic accuracy.

Materials and methods: We performed a post-hoc analysis in the YEARS diagnostic study, comparing age- and sex-adjusted D-dimer levels among users of antiplatelet drugs, statins and nonusers. We then reclassified patients within the YEARS algorithm by developing a model in which we adjusted D-dimer cut-offs for statin use and evaluated diagnostic accuracy.

Results: We included 156 statins users, 147 antiplatelet drugs users and 726 nonusers of either drugs, all with suspected pulmonary embolism**.** Use of antiplatelet drugs did not have a significant effect, whereas statin use was associated with 15% decrease in D-dimer levels (95% CI, -28% to -0.6%). An algorithm with lower D-dimer thresholds in statin users yielded lower specificity (0.42 compared to 0.33) with no difference in false negative tests.

Conclusions: We conclude that use of statins but not of antiplatelet agents is associated with a modest decrease in D-dimer levels. Adjusting D-dimer cut-offs for statin use did however not result in a safer diagnostic strategy in our cohort.

Introduction

D-dimer levels have a central role in the diagnostic workup of venous thromboembolism (VTE). Guidelines recommend combining clinical decision rules and a D-dimer test to identify patients in whom pulmonary embolism (PE) or deep venous thrombosis may be ruled out without performing imaging tests (high level of evidence).[1,2] Recently, the YEARS algorithm, incorporating a variable D-dimer cut-off dependent on the pretest probability based on three clinical variables (Figure 1), has been proven to be safe and compatible with clinical practice.[3]

D-dimers are fibrin degradation fragments, generated after fibrinolysis of a blood clot by the sequential action of thrombin, factor XIIIa and plasmin.[4] The D-dimer level in blood can be influenced by many factors such as age, active malignancy, infection, pregnancy or use of anticoagulants.[5-8] Other drugs that affect thrombus formation and therefore may influence D-dimer levels as well are antiplatelet drugs and HMG-CoA reductase inhibitors, more commonly known as statins. Antiplatelet drugs can delay thrombin generation and in general inhibit blood coagulation.[9] Statins inhibit a variety of platelet factors and decrease tissue factor activity.[10,11] Through the latter mechanism, a smaller amount of factor X is activated and generation of thrombin is depleted. These effects might explain that statins may exert cardiovascular protective effects that are independent of LDL-cholesterol lowering, the so-called pleiotropic effects.[12] Indeed, most clinical studies on this subject have found that statin therapy, especially treatment with lipophilic statins (simvastatin, atorvastatin or fluvastatin), does lower D-dimer levels whereas antiplatelet therapy does not seem to have an effect.[13] Although most studies are retrospective and susceptible to bias due to other factors influencing D-dimer level.[13-19] Studies on the effect of statins and antiplatelet therapy on the sensitivity of D-dimer used as diagnostic test in patients with suspected PE are lacking.

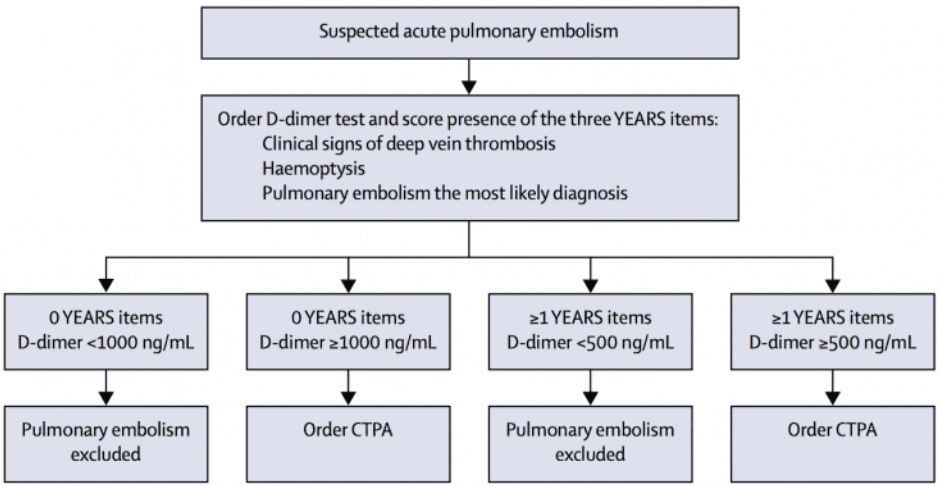
To test the hypothesis that D-dimer cut-offs may need to be adjusted in statin and/or antiplatelet therapy users, we performed a post hoc analysis of the YEARS diagnostic study.[3] D-dimer levels were compared among users and nonusers of antiplatelet drug and/or statin. Further, we evaluated the rate of false negative D-dimer tests among users of statins, as well as the sensitivity and specificity of D-dimer thresholds adjusted to statin use.

Methods

Study design

This was a post-hoc analysis within the YEARS study, of which the design was previously described in detail (Netherlands Trial Registry number NTR4193).[3] Briefly, 3465 patients with suspected acute PE were included between 5 October 2013, and 9 July 2015 in a prospective multicentre cohort outcome study evaluating the safety and accuracy of the YEARS diagnostic algorithm. In this algorithm, patients were managed by combining simultaneous assessment of a clinical decision rule consisting of 3 items (clinical signs of deep vein thrombosis (DVT), haemoptysis, PE most likely diagnosis) and a D-dimer test. In patients without YEARS items and D-dimer <1000 ng/mL, or ≥1 YEARS items and D-dimer <500 ng/mL, PE was considered excluded without further imaging. All other patients underwent computed-tomography pulmonaryangiography (CTPA; Figure 1).

Figure 1: YEARS algorithm



CTPA=computed tomography pulmonary angiography

D-dimer levels were measured upon presentation of the patient, using automated high-sensitive quantitative D-dimer assays (according to local practice Vidas D-dimer Exclusion®, Biomerieux, Marcy-L'Étoile, France; STA-LIA® DiagnosticaStago, Asnieres, France; Innovance®, Siemens, Marburg, Germany). At study inclusion, the following baseline characteristics were assessed: age, sex, body mass index (BMI), smoking status, malignancy, hypertension, CRP level, previous VTE and present use of antiplatelet drugs and statins.

Follow-up consisted of a scheduled outpatient visit or telephone interview after three months. At this visit, information was obtained on complaints suggestive of VTE. In case of clinically suspected VTE during follow-up, objective diagnostic tests were required, including CTPA for suspected PE and compression ultrasonography for suspected deep vein thrombosis.

Patients

Hospitalized patients as well as patient visiting the emergency department with clinically suspected acute (first or recurrent) PE were eligible for inclusion in the YEARS study if they were 18 years of age or older. Exclusion criteria were: treatment with therapeutic doses of anticoagulants initiated ≥24 hours prior to eligibility assessment, life expectancy less than three months, geographic inaccessibility precluding follow-up, pregnancy or allergy to intravenous contrast agent. In two of the participating hospitals - the Erasmus University Medical Center and the Leiden University Medical Center - we also obtained data about use of antiplatelet drugs and statins. For that reason, we restricted the present post-hoc analysis to patients evaluated in the latter two hospitals.

Study aim and endpoints

The primary aim of this study was to assess the effect of antiplatelet drugs and statins on D-dimer levels in patients suspected of PE. The secondary aim of this study was to evaluate if the effect of antiplatelet drugs or statins might affect diagnostic accuracy.
The primary endpoint of this study was the difference in D-dimer levels among users and nonusers of antiplatelet drugs and statins. The secondary endpoints of this study were the proportion of patients categorized differently in the YEARS algorithm and the change in diagnostic accuracy and diagnostic failure rate (VTE related death, VTE or lost to follow-up) when the effect of statins on D-dimer levels is taken into account. Reporting of this study conforms to the STROBE statement and the broader EQUATOR guidelines.[20]

Statistical analysis

D-dimer levels were log-transformed to normalize the distribution. Multiple linear regression analyses were performed with adjustment for age, sex, current smoking, use of antiplatelet drugs, use of statins, current smoking and history of VTE according to different stepwise regression models. The contribution of these variables to the changes in D-dimer levels was determined by comparing the explained variance from the different multivariable linear regression analyses. Due to missing data in 397 (43%) patients, we could not adjust for BMI. The variables that explained most of the change in D-dimer were then identified. We calculated change in D-dimer levels for each drug exposure by exponentiation of the correlation coefficient and their corresponding 95% confidence intervals (95%CI). A two-sided p-value of 0.05 was considered to indicate statistical

significance. All statistical analyses were carried out using 'IBM SPSS Statistics for Windows, version 21 (IBM Corp., Armonk, N.Y., USA)'.

For the secondary outcome analysis, we tested the amount of patients that should have been managed differently in the YEARS algorithm when the effect of antiplatelet drugs and/or statins on D-dimer levels was taken into account. Therefore we developed a model to reclassify antiplatelet drugs and/or statin users by adjusting the D-dimer cut-offs. The cut-offs were adjusted based on the expected change in D-dimer levels, as derived from the regression analysis, and were inserted post-hoc in the YEARS algorithm. Further we evaluated diagnostic accuracy by comparing the diagnostic failure rate (VTE related death, VTE or lost to follow-up) with and without this reclassification of antiplatelet drugs and/or statin users in the YEARS algorithm.

Results

Within the YEARS study, 925 patients were included in this post-hoc analysis. Mean age of these patients was 54 years (standard deviation (SD) 17) and mean BMI was 27 kg/m2 (SD 5.7), 369 of them (39.9%) were male and 178 (19.2%) current smokers. Following the YEARS algorithm, 395 (42.7%) patients scored no YEARS items, 479 (51.8%) scored one, 48 (5.2%) scored two and 3 (0.3%) patients scored three items. PE was diagnosed by CTPA at baseline in 16.1% of the patients not using an antiplatelet drug or a statin, compared to 17.1% of the patients using either drug. Among the group of patients in whom PE was excluded using the YEARS algorithm, only two developed a PE during follow-up and in one patient PE could not be excluded as cause of death. None of these patients used antiplatelet drugs or statins. In total five patients were lost to follow-up, four of them did not use either drug.

The mean D-dimer level was 1666 ng/mL (SD 1642 ng/mL) and the median 985 ng/mL (interquartile range 479-1203 ng/mL). Of all patients, 22% used an antiplatelet agent and/or statin. More than half of them (n=104) were using both drugs, 43 patients were only using antiplatelet drugs and 52 patients only statins. In 72% of the statin users a lipophilic statin (simvastatin, atorvastatin or fluvastatin) had been prescribed. Median D-dimer values were 912 ng/mL (interquartile range 573-1941 ng/mL) in lipophilic statin users compared to 1050 ng/mL (558-1540 ng/mL) in hydrophilic statin (rosuvastatin and pravastatin) users, but this difference was non-significant. Compared to nonusers, antiplatelet drug and/or statin users were older, had a higher BMI, smoked less, and the proportion of male gender, previous VTE and presence of hypertension was higher (Table 1). There was no difference in the median level of CRP or presence of malignancy between users and nonusers of antiplatelet drug and/or statins.

Table 1: General characteristics of patients

	No use of statins and antiplatelet drugs (n=726)	Only statins (n=52)	Only antiplatelet drugs (n=43)	Statins and antiplatelet drugs (n=104)	Statins and/or antiplatelet drugs^ς (n=199)	p-value*
Male (%)	271 (37.3)	21 (40.4)	21 (48.8)	56 (53.8)	98 (49.2)	0.002
Age	48.7 (17.2)	61.7 (12.0)	69.6 (14.7)	67.5 (11.9)	66.4 (12.9)	<0.001
Body mass index (kg m^{-2})	26.0 (5.4)	30.1 (6.5)	26.5 (5.9)	28.8 (5.6)	28.6 (6.0)	<0.001
CRP (mg/L)	9 (3-32)	10 (3-26)	7 (3-29)	7 (3-19)	7 (3-22)	0.89
Hypertension	108 (14.9)	33 (63.5)	19 (45.2)	67 (65.0)	119 (60.4)	<0.001
Current smoker	154 (21.9)	6 (11.8)	6 (14.0)	12 (11.7)	24 (12.2)	0.003
Prior VTE	71 (9.8)	11 (21.2)	8 (18.6)	11 (10.6)	30 (15.1)	0.03
Malignancy	104 (14.3)	6 (11.5)	8 (18.6)	11 (10.6)	25 (12.6)	0.52
PE at CTPA	117(16.1)	13 (25.0)	10 (23.3)	11 (10.6)	34 (17.1)	0.45
Death of any reason during follow-up	44(6.1)	1 (1.9)	2 (4.7)	2 (1.9)	5 (2.5)	NA
PE during follow-up	2(0.3)	0 (0)	0 (0)	0 (0)	0 (0)	NA
Lost to follow up	1 (0.1)	0 (0)	0 (0)	0 (0)	1 (0.5)	NA

Data are shown as mean values (SD); numbers (percentages) or median (interquartile ranges)
Due to missing data, n=529 for body mass index; n=897 for CRP; n=920 for hypertension; n= 901 for smoking; n= 924 for prior VTE; n=924 for malignancy
^antiplatelet drugs: acetylsalicylic acid n= 136; clopidogrel n=6; other n=3; unknown n=2;
ςstatins: simvastatin n=73; rosuvastatin n=22; atorvastatin n= 36; pravastatin n=19; fluvastatin n=3; unknown n=3
**P-value between groups no use vs use of statins and/or antiplatelet drugs; Pearson chi-square test for categorical variables, Mann Whitney U test for continuous variables*
CTPA: computed-tomography pulmonary-angiography; VTE: venous thromboembolism; PE: pulmonary embolism

The effect of antiplatelet drugs and statins on D-dimer levels

We estimated the proportion of the variation (R^2 value) and change in plasma D-dimer levels explained by age, sex, current smoking, use of antiplatelet drugs, use of statins, history of VTE in different linear regression models (Table 2). The change in plasma levels of D-dimer was explained for 18.3% by age, sex, use of antiplatelet drugs and use of statins. Notably, age explained most of the proportion of variation (17.8%) and current smoking added a significant 0.8% of the explained variance to this model.

When adjusted for age, sex and use of statins, use of antiplatelet drugs resulted in a decrease of 7.6% (95%CI, -25% to 13%)). In this similar regression model, use of statins resulted in a 12% (95%CI, -27% to 7.5%)) decrease of D-dimer values. Both these correlations were not significant. Yet, when only adjusted for age and sex, use of statins was significantly correlated and was associated with a reduction of D-dimer values by 15% (95%CI, -28% to -0.6%). A subgroup regression analysis with inclusion of only lipophilic statin users and non statin users and adjustment for age and sex did not result in a significant correlation (12.5% decrease, 95% CI, -27% to 5%).

Table 2: Effect on D-dimers according to the different linear regression models

Independent factors used in linear regression model	**Proportion of variance explained (%)***	**Adjusted % change in D-dimer in users for statins (95% CI)****	**Adjusted % change in D-dimer for antiplatelet drug users (95% CI)****
Age	17.8	Not applicable	Not applicable
Age and use of statins	18.2	-15% (-28%; -1.4%)^	Not applicable
Age, sex, use of statins	18.2	-15% (-28%; -0.6%)^	Not applicable
Age, sex, use of lipophilic statins	18.6	-12.5% (-27%; 5%)	Not applicable
Age, sex, use of statins, use of antiplatelet drugs	18.3	-12% (-27%; 7.5%)	-7.6% (-25%;13%)
Age, sex, use of statins, use of antiplatelet drugs, current smoker	19.1	-11% (-27%; 8.0%)	-7.6% (-25%;13%)
Age, sex, use of statins, use of antiplatelet drugs, current smoker, history of VTE	19.3	-11% (-27%; 7.6%)	-7.0% (-24%;14%)

** R^2 value*

*** adjusted change in D-dimer values calculated by exponentiation of the correlation coefficient and their corresponding 95% confidence intervals (95% CI)*

^ significant p-value <0.05

Model for reclassifying statin users within the YEARS algorithm

Since antiplatelet drugs did not show any significant effect on D-dimers we only calculated adjusted D-dimer cut-offs for statin users. This calculation was based on the results of the linear regression model containing age, sex and use of statins. Adhering to this model, we adjusted the D-dimer cut-off to be 15% lower for patients using statins. This resulted in an adjusted cut-off determined at 850 ng/mL instead of 1000 ng/mL for patients having no YEARS criteria. For patients having one or more YEARS criteria the adjusted cut-off was determined at 425 ng/mL instead of 500 ng/mL.

Performance of YEARS algorithm after reclassification of statin users

In the original YEARS algorithm, we found that 56 statin users (35.9%) were managed without CTPA. None of these patients was lost to follow-up, no VTE or VTErelated death was detected. When we tested the algorithm using the adjusted cut-offs, thus incorporating the effects of statins on D-dimer levels, we found that 12 patients were reclassified to the group needing a CTPA to rule out PE. In these patients, we did not detect any additional diagnostic failures during follow-up, in one patient CTPA was conducted for other reasons, showing no PE (Table 3). After reclassification of these 12 patients, specificity decreased from 0.42 to 0.33, while sensitivity did not change.

Table 3: Characteristics of statin users reclassified according to lower adjusted D-dimer level cut-offs

Patient	Sex (M/F)	Age	YEARS criteria (0-3)	D-dimer (ng/mL)	Use of antiplatelet drugs (yes/no)	Diagnostic failures^ (yes/no)	Death during follow-up or lost to follow-up (yes/no)
1	M	79	1	442	yes	no	no
2	F	70	1	450	no	no	no
3	M	76	1	468	yes	no	no
4	M	62	1	470	yes	no	no
5	M	72	1	480	no	no	no
6	M	62	1	492	no	no	no
7	M	67	0	860	Yes	no	no
8	F	54	0	890	no	no	no
9	M	77	0	910	yes	no	no
10	F	74	0	913	no	no	no
11	M	85	0	930	yes	no	no
12	M	75	0	990	yes	no*	no

Reclassification of statin users having no YEARS and D-dimer ≥ 850 and <1000 ng/mL or statin users having YEARS ≥ 1 and D-dimer ≥425 and <500ng/mL

^ Diagnostic failures are defined by venous thromboembolism during follow-up, VTE related death during follow-up or lost to follow-up

** CTPA (computed-tomography pulmonary-angiography) conducted during follow-up showing no pulmonary embolism*

Discussion

Our study results showed that in our population of patients suspected of having a PE, statins decreased D-dimer levels but antiplatelet drugs did not. Adjusting D-dimer cut-offs for statin use did however not result in a safer diagnostic strategy. Based on our study, there is no need for adjusting D-dimer cut-off values for statin users. Moreover, age explained the largest proportion of the D-dimer variance, underlining that age has a higher impact on D-dimer levels than antiplatelet drugs and statins.

Nowadays diagnostic algorithms for detecting PE are sensitive, but overall 3-month diagnostic failure rate is still not reduced to zero.[21-23] This is to our best knowledge the first study to test the effect of statins and antiplatelet drugs on the sensitivity of D-dimer tests for the diagnosis of acute VTE. The reported effect of statins on D-dimer levels differs between previous studies. Both the results of a review and a meta-analysis suggested a decrease in plasma D-dimer levels with the use of statins.[13,15] One small intervention study in patients with hypercholesterolemia for example did not show a significant change in D-dimer levels after start of treatment with statins whereas another study including type 2 diabetes patients found a significant reduction of approximately 8% in D-dimer levels.[18,24] The studies included in this review and meta-analysis concerned different patient populations, not taking into account concurrent antiplatelet therapy.[25,26] This is dissimilar to our study, in which we analysed the effect of antiplatelet drugs and statins separately. Our study results are best compared with those of a study by Adams et al. comparing haemostatic factor levels between statin users and nonusers.[14] In a cohort of 6814 healthy men and women age 45-84 years, participants using statins had a 9% lower D-dimer level after adjusting for age, race/ethnicity, education, income, hormone replacement therapy and major cardiovascular risk factors. The main difference with our study is that this was a cohort study in a healthy population, whereas in our study, D-dimer levels were determined in the clinical setting of suspected PE. Another difference is that we could not adjust for all cardiovascular risk factors (including BMI and diabetes status), race/ethnicity, education, income and hormone replacement therapy, as these data were not fully collected at baseline. Notably, in our subgroup analysis with inclusion of only lipophilic statin users and nonusers of statins we could not detect a significant correlation with D-dimer levels, however we might have been underpowered.

The evidence for the absence of effect of antiplatelet drugs on D-dimers is compellingly illustrated in two studies in healthy male volunteers using acetylsalicylic acid.[16,27] One of these studies was a randomized placebo controlled trial in 30 healthy volunteers.

Also, Kamath et al showed that D-dimer levels in patients with atrial fibrillation treated with aspirin were not lower than in those given no treatment.[17] A study in patients on peritoneal dialysis likewise found no effect on D-dimer levels in those using low-dose aspirin for 8 weeks.[28]

A methodological strength of this post hoc analysis in the YEARS population is that we studied a real-world cohort of patients with a near complete follow-up. Also, by using the different linear regression models, the change in proportion of the variation explained by each variable and in particular statins could be extracted. Having analysed this population in daily practice using a clinical approach, we expect that results can be easily interpreted and translated to clinical care.

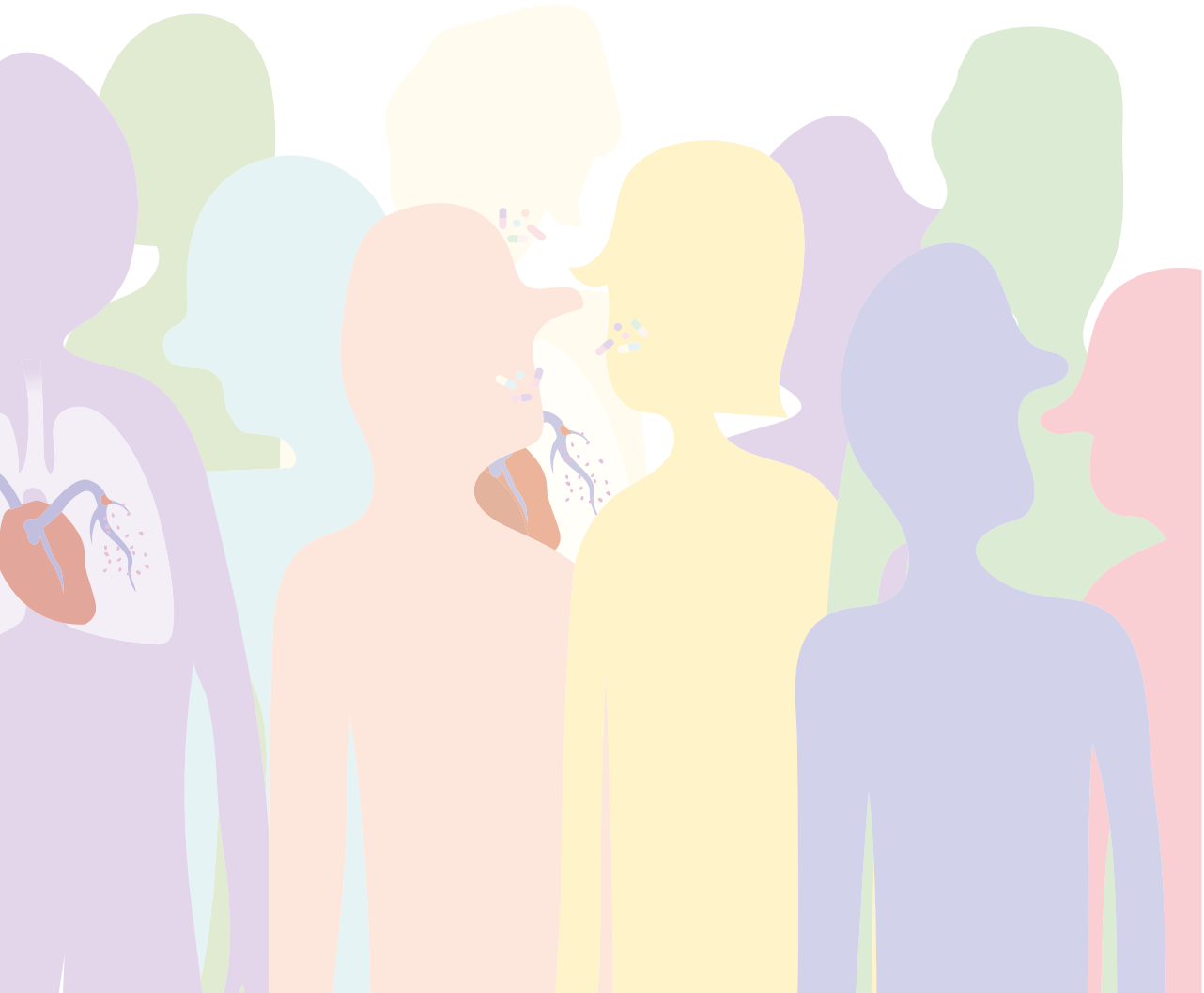
Limitations of our study include that this reports on a post-hoc finding of a large outcome study, in which extensive data on baseline medication use was only available for two of the participating centers. For this reason, the secondary endpoints of our study are likely underpowered and we cannot rule out a relevant effect of statin use in clinical practice with sufficient certainty. Nevertheless, our study offers currently the best available evidence evaluating the diagnostic accuracy of a clinical diagnostic algorithm on PE in statin users explicitly. Also, we expect that a separate prospective study designed and powered to address these endpoints specifically will probably not be performed in future because of lack of financial incentives. Another limitation of our study is that CTPA results of the reclassified statin users were not available and the change in D-dimer in statin users was used for modification of the D-dimer cut-off in the same population. Also, the adjusted cut-offs were calculated post hoc and management decisions were based on the original cut-offs. Therefore, extrapolation of our findings to other populations or healthcare settings warrants caution. Further, we used different quantitative D-dimer assays and were unable to stratify the results by assay, as patient-level information on the assay used was not available. However, all were contemporary, well-validated high sensitive quantitative D-dimer assays with similar fixed cut-off levels.[29]

Based on our results, we conclude that use of statins is associated with a 15 % decrease in D-dimer levels. In our cohort this was not associated with an increase in false negative test results, compared to nonusers. Nevertheless further validation in a larger clinical cohort would be needed, however we consider it unlikely that a prospective study designed and powered to address all endpoints separately will be performed in future.

References

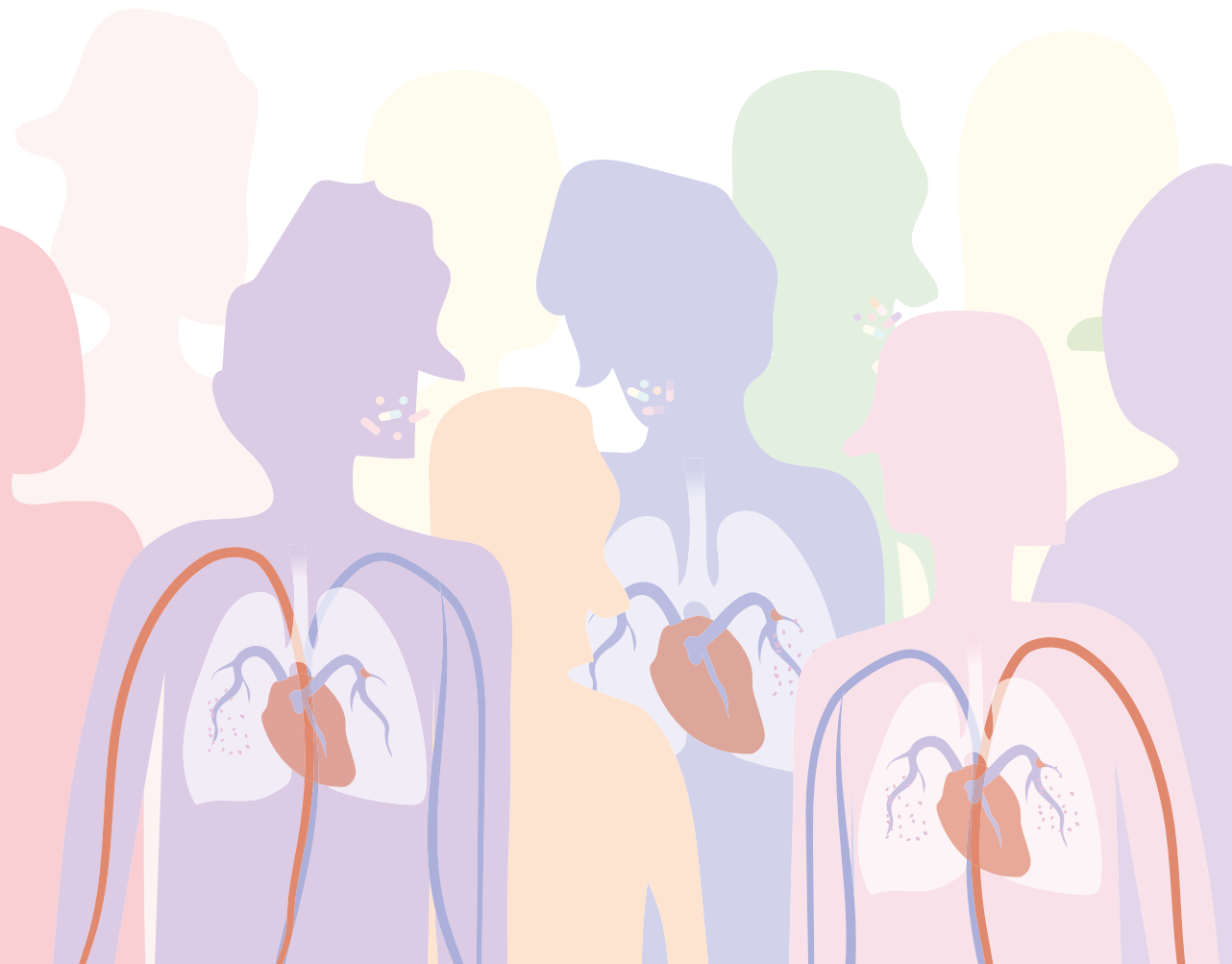
1. Konstantinides SV, Torbicki A, Agnelli G, et al. 2014 ESC guidelines on the diagnosis and management of acute pulmonary embolism. *Eur Heart J.* 2014;35(43):3033-3069, 3069a-3069k.
2. Raja AS, Greenberg JO, Qaseem A, et al. Evaluation of Patients With Suspected Acute Pulmonary Embolism: Best Practice Advice From the Clinical Guidelines Committee of the American College of Physicians. *Ann Intern Med.* 2015;163(9):701-711.
3. van der Hulle T, Cheung WY, Kooij S, et al. Simplified diagnostic management of suspected pulmonary embolism (the YEARS study): a prospective, multicentre, cohort study. *Lancet.* 2017;390(10091):289-297.
4. Adam SS, Key NS, Greenberg CS. D-dimer antigen: current concepts and future prospects. *Blood.* 2009;113(13):2878-2887.
5. Douma RA, van Sluis GL, Kamphuisen PW, et al. Clinical decision rule and D-dimer have lower clinical utility to exclude pulmonary embolism in cancer patients. Explanations and potential ameliorations. *Thromb Haemost.* 2010;104(4):831-836.
6. Harb TS, Zareba W, Moss AJ, et al. Association between inflammatory markers, hemostatic, and lipid factors in postinfarction patients. *Am J Cardiol.* 2003;91(9):1120-1123.
7. Chabloz P, Reber G, Boehlen F, Hohlfeld P, de Moerloose P. TAFI antigen and D-dimer levels during normal pregnancy and at delivery. *Br J Haematol.* 2001;115(1):150-152.
8. Crop MJ, Siemes C, Berendes P, van der Straaten F, Willemsen S, Levin MD. Influence of C-reactive protein levels and age on the value of D-dimer in diagnosing pulmonary embolism. *Eur J Haematol.* 2014;92(2):147-155.
9. Szczeklik A, Krzanowski M, Gora P, Radwan J. Antiplatelet drugs and generation of thrombin in clotting blood. *Blood.* 1992;80(8):2006-2011.
10. Owens AP, 3rd, Mackman N. The antithrombotic effects of statins. *Annu Rev Med.* 2014;65:433-445.
11. Violi F, Calvieri C, Ferro D, Pignatelli P. Statins as antithrombotic drugs. *Circulation.* 2013;127(2):251-257.
12. Oesterle A, Laufs U, Liao JK. Pleiotropic Effects of Statins on the Cardiovascular System. *Circ Res.* 2017;120(1):229-243.
13. Sahebkar A, Serban C, Mikhailidis DP, et al. Association between statin use and plasma d-dimer levels: A systematic review and meta-analysis of randomised controlled trials. *Thromb Haemost.* 2015;114(3):546-557.
14. 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.
15. Squizzato A, Romualdi E, Ageno W. Why should statins prevent venous thromboembolism? A systematic literature search and a call for action. *J Thromb Haemost.* 2006;4(9):1925-1927.
16. Derhaschnig U, Schweeger-Exeli I, Marsik C, Cardona F, Minuz P, Jilma B. Effects of aspirin and NO-aspirin (NCX 4016) on platelet function and coagulation in human endotoxemia. *Platelets.* 2010;21(5):320-328.
17. Kamath S, Blann AD, Chin BSP, et al. A study of platelet activation in atrial fibrillation and the effects of antithrombotic therapy. *Eur Heart J.* 2002;23(22):1788-1795.
18. Van De Ree MA, De Maat MP, Kluft C, Meinders AE, Princen HM, Huisman MV. Decrease of hemostatic cardiovascular risk factors by aggressive vs. conventional atorvastatin treatment in patients with Type 2 diabetes mellitus. *J Thromb Haemost.* 2003;1(8):1753-1757.

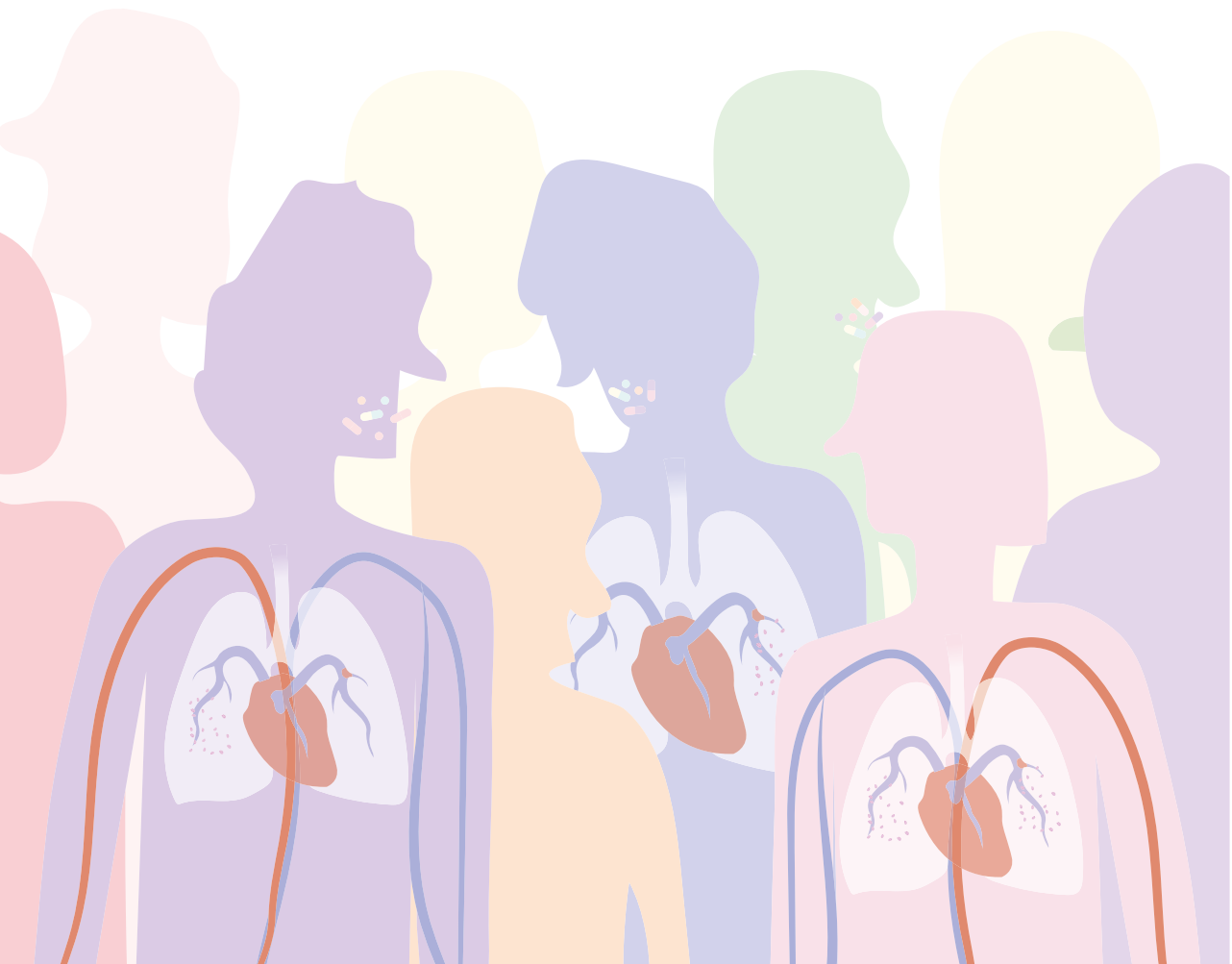
19. Wada H, Mori Y, Kaneko T, et al. Hypercoagulable state in patients with hypercholesterolemia: Effects of pravastatin. *CLIN THER*. 1992;14(6):829-834.
20. Simera I, Moher D, Hoey J, Schulz KF, Altman DG. A catalogue of reporting guidelines for health research. *Eur J Clin Invest*. 2010;40(1):35-53.
21. van Belle A, Buller HR, Huisman MV, et al. Effectiveness of managing suspected pulmonary embolism using an algorithm combining clinical probability, D-dimer testing, and computed tomography. *JAMA*. 2006;295(2):172-179.
22. Righini M, Van Es J, Den Exter PL, et al. Age-adjusted D-dimer cutoff levels to rule out pulmonary embolism: the ADJUST-PE study. *JAMA*. 2014;311(11):1117-1124.
23. Douma RA, Mos IC, Erkens PM, et al. Performance of 4 clinical decision rules in the diagnostic management of acute pulmonary embolism: a prospective cohort study. *Ann Intern Med*. 2011;154(11):709-718.
24. Bolaman Z, Kadikoylu G, Özgel N, Yenisey C. Effects of atorvastatin on coagulation parameters and homocysteine in patients with primary hypercholesterolemia. *J Natl Med Assoc*. 2006;98(8):1273-1277.
25. Dangas G, Badimon JJ, Smith DA, et al. Pravastatin therapy in hyperlipidemia: effects on thrombus formation and the systemic hemostatic profile. *J Am Coll Cardiol*. 1999;33(5):1294-1304.
26. Walter T, Szabo S, Suselbeck T, et al. Effect of atorvastatin on haemostasis, fibrinolysis and inflammation in normocholesterolaemic patients with coronary artery disease: a post hoc analysis of data from a prospective, randomized, double-blind study. *Clin Drug Investig*. 2010;30(7):453-460.
27. Kim KM, Kim H, Chi HS, Park JS, Kim SB. Comparison of antiplatelet potency of sarpogrelate, aspirin, and beraprost in healthy volunteers according to in-vitro closure time. *Blood Coagul Fibrinolysis*. 2010;21(3):262-265.
28. Kim SB, Lee SK, Min WK, Chi HS, Park JS. Lack of effects of low-dose aspirin on high sensitivity C-reactive protein, hemostatic factors, and troponin T in CAPD patients. *Peritoneal Dial Int*. 2002;22(6):721-723.
29. Oude Elferink RF, Loot AE, Van De Klashorst CG, Hulsebos-Huygen M, Piersma-Wichers M, Oudega R. Clinical evaluation of eight different D-dimer tests for the exclusion of deep venous thrombosis in primary care patients. *Scand J Clin Lab Invest*. 2015;75(3):230-238.



Part 2

Influence of (co-)medication on haemostatic biomarkers





3.1

A revised systematic review and meta-analysis on the effect of statins on D-dimer levels

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Abstract

Background: D-dimers are generated during endogenous fibrinolysis of a blood clot and have a central role in diagnostic algorithms to rule out venous thromboembolism. HMG-CoA reductase inhibitors, more commonly called statins, are known to have effects independent of LDL-cholesterol lowering, including antithrombotic properties. An effect of statins on D-dimer levels has been reported in a prior systematic review and meta-analysis but methodological shortcomings might have led to an overestimated effect. To re-evaluate the association between statins and D-dimer levels we systematically reviewed all published articles on the influence of statins on D-dimer levels and conducted a novel meta-analysis (PROSPERO registration number CRD42017058932).

Materials and methods: We electronically searched EMBASE, Medline Epub, Cochrane, Web of Science and Google Scholar (100 top relevance) (date of last search 5 October 2017). We included randomized controlled trials, cohort studies, and cross-sectional studies. Two reviewers independently screened all articles retrieved and extracted data on study and patient characteristics, study quality and D-dimer levels.

Results: Study-level meta-analysis involving 18,052 study participants showed lower D-dimer levels in those receiving statin treatment than controls (SMD: -0.165, 95% CI -0.234; -0.096, p= <0.001). Sensitivity analyses and additional analyses on treatment duration (<12 weeks vs ≥ 12 weeks) and type of statin (lipophilic or hydrophilic) did not modify this overall result.

Conclusion: This meta-analysis suggests an association between use of statins and reduction of D-dimer levels, independent of treatment duration and type of statin used. This effect is small but robust, and should be interpreted with caution.

Introduction

In case of a thromboembolism, D-dimers are generated in the blood clot during fibrinolysis by the sequential action of thrombin, activated factor XIII and plasmin.[1,2] Age, active malignancy, infection, pregnancy and use of anticoagulants are well known to have an influence on D-dimer levels.[3-6] Use of medication with an effect on thrombus formation, such as HMG-CoA reductase inhibitors, more commonly known as statins, may influence D-dimer levels as well. These antithrombotic properties are part of what has been referred to as the cholesterol-independent or "pleiotropic" effects of statins, explaining why the benefits observed with statins appear to exceed what might be expected from changes in cholesterol levels alone.[7-9] In line with these antithrombotic effects, statin treatment might lead to a 15% lower risk of primary venous thrombosis as confirmed in a recent meta-analysis of intervention studies.[7]

In clinical practice, D-dimer levels have a central role in diagnostic algorithms to rule out venous thromboembolism (VTE).[10,11] Several studies have addressed the effect of statins on D-dimer levels, with some of them being evaluated in a systematic review and meta-analysis by Sahebkar et al.[12] This meta-analysis included nine randomized controlled trials, and reported a significant reduction of 0.988 μg/ml (95%CI: -1.590 to -0.385, p=0.001) in D-dimer levels in statin users. However, this estimate is inappropriate since the used Cohen's d effect size should be dimensionless while 0.988 μg/ml suggests a tremendous clinical impact of statin use on D-dimer levels. Triggered by this inaccuracy, we further elucidated the used methods and results and found several important shortcomings. Our main concerns next to misuse of Cohen's d are incorrect extraction of data from original studies and unreported assumptions.

Because the research question is of high importance though, we decided to conduct a novel systematic review and meta-analysis on the effect of statins on D-dimer levels, including recent studies.

Methods

Protocol, registration

This study was registered on 10 March 2017 in the PROSPERO international prospective register of systematic reviews (CRD42017058932) and designed according to the guidelines of the 2009 Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement.[13]

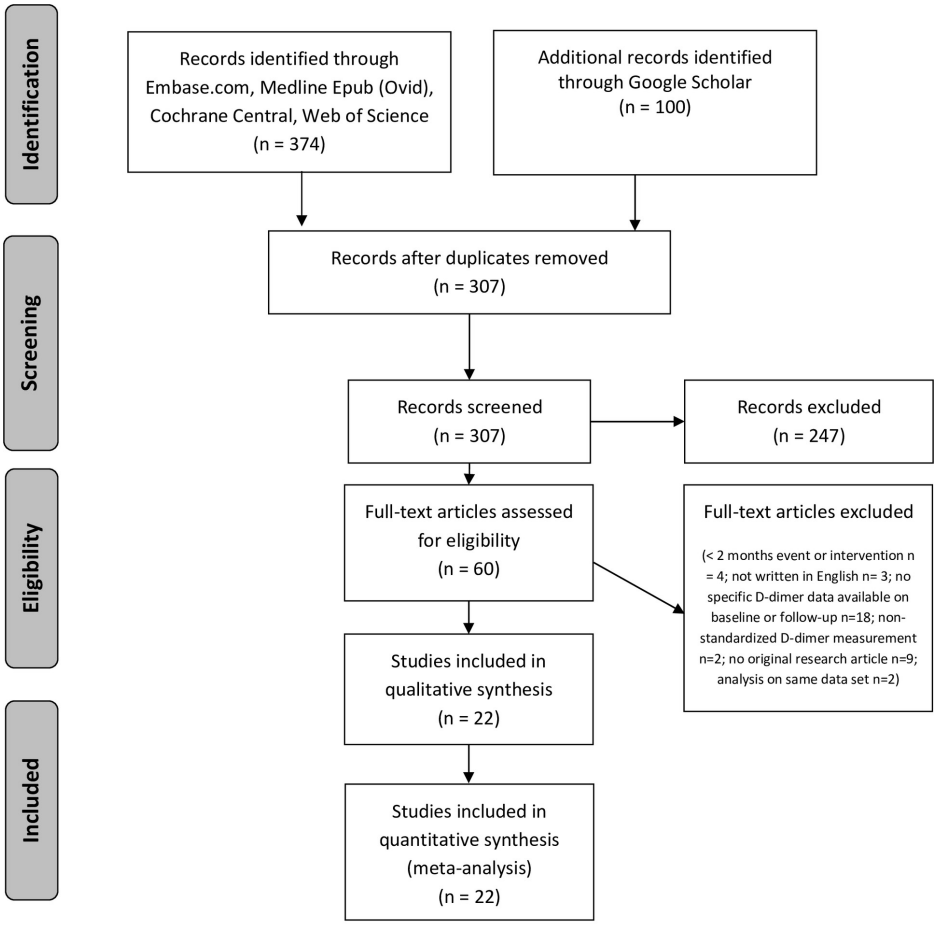
Search methods for identification of studies

Together with a biomedical information specialist, SS-G electronically searched the following databases: EMBASE (Ovid SP); MEDLINE Epub (Ovid SP); Cochrane Central Register of Controlled Trials (CENTRAL); Web of Science and Google Scholar (100 top relevance) (date of last search 5 October 2017). We used search terms as reported in Methods S1, page 107, in summary: D-dimer OR D-dimers AND statin OR statins OR hydroxymethylglutaryl reductase OR HMG CoA reductase in combination with individual drug names of statins. To improve sensitivity we also combined these search terms with the wild-card term "*" and the accessory MeSH terms.

Data collection and extraction process

Two authors (SS-G and FM) independently screened titles and abstracts retrieved by the electronic survey, and disagreement in selection was resolved by discussion. After consensus was reached, the two reviewers independently selected eligible articles based on the results in full text. Selection of articles was discussed in detail, and in case of disagreement, a third author (TvG) was consulted for final decision. We present a flow diagram to show the decision-making process for including studies in the review (Figure 1).[13] The first reviewer (SS-G) extracted the following data: first author's name, year of publication, study design, country where the study was performed, D-dimer assay used, use of co-medication, number of participants, time of exposure, statin regimen, D-dimer levels with its variation and the conclusions of the individual studies on the effect of statins on the D-dimer levels. Also all QUADAS-2 items were assessed. If results could not be extracted from original articles (table or well described in the text), authors were requested repeatedly to send their original data. All D-dimer levels were converted to μg/mL. If multiple D-dimer levels were available, we chose to report those values close to 6 month follow-up. All results after extraction were double-checked and confirmed by the second reviewer (FM).

Figure 1: Flow Diagram on decision-making process for including studies following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement



Selection of studies

We included randomized controlled trials, cohort studies and cross-sectional studies conducted in humans, in which D-dimers levels were described or reported and results could be compared among users or nonusers of statins. For both randomized controlled trials and cohort studies, we defined that statins should be used for at least 7 days in order to achieve a pharmacodynamically relevant effect.[14,15] Also, to prevent interference of the effect of anticoagulant drugs on D-dimer levels, we excluded randomized controlled trials or cohorts primary conducted among patients treated with anticoagulant drugs at baseline or during follow-up. Studies in which any medical intervention or cardiovascular event within 2 months between baseline and follow-up measurement of D-dimer levels

was part of the inclusion criteria, were also excluded to reduce confounding effects on D-dimer levels. Since different D-dimer tests are used in clinical practice, we decided to include only standardized enzyme-linked immunoassays or latex (semi) quantitative tests.[16] Studies without availability of full-text that were also not available after repeated requests to the (corresponding) authors, or articles not written in English language were excluded, because quality of these articles could not be assessed.

Risk of bias in individual studies and across studies

The data extraction form incorporated a quality assessment section comprising items from Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2)[17]. Following this revised tool, we omitted and added signalling questions and two independent reviewers (SS and FM) applied the QUADAS-2 score in a small number of studies. After refinement of the tool (as described in detail in Methods S3, page 112) with review-specific signalling questions and appropriate items, grouped into three domains (patient selection, index test and flow and timing) also scoring conflicts of interest, we applied this tool for all studies. We evaluated the influence of each study on the overall effect size by removing one study each time and repeating the analysis, a so-called leave-one-out method sensitivity analysis.[18] We also performed a subanalysis including only studies with low risk patient selection bias and low concern about applicability according to the scoring of these QUADAS-2 items and performed a separate subanalysis only including controlled trials. To detect potential publication bias, we visually inspected the distribution of the studies within a funnel plot and also created a funnel plot taking into account the Trim and Fill adjustment of Duval and Tweedie.[19] Also, Begg's rank correlation and Egger test were used to detect publication bias.[20,21] Furthermore, as another marker of publication bias, we estimated the number of missing studies we would need to retrieve and impute in the meta-analysis to make the p-value nonsignificant using the "fail-safe N" method.[22]

Quantitative data-synthesis

The meta-analysis was conducted using Comprehensive Meta-analysis (version 3; Biostat). In studies in which participants were exposed to different statin regimens, the different statin exposed groups were analysed separately and values were compared to the control group in case of (randomized) controlled studies. When medians and interquartile ranges (IQR) were reported, we estimated the average standard deviation (SD) using the following formula: SD = ((75th percentile-25th percentile)/1.35) and in case of reporting medians and full range, we estimated the average SD using the following formula: SD = ((75th percentile-25th percentile)/5.16).[23] If not reported, the mean difference was estimated using the following formula: SD = square root [(SDpre-treatment)2 + (SDpost-treatment)2 - (2R × SDpre-treatment × SDpost-treatment)], assuming a correlation

coefficient (R) = 0.5. Net changes in measurements (change scores) were calculated for controlled trials, as follows: (value at end of follow-up in the treatment group – value at baseline in the treatment group) – (value at end of follow-up in the control group – value at baseline in the control group). If percentage change of D-dimer levels was reported, we estimated mean or median D-dimer post-treatment levels by multiplying reported mean or median pre-treatment D-dimer levels with 1+ percentage change and assumed that the post-treatment SD was equal to reported SD before treatment. For crossover studies, we used the reported results of delta mean change and its corresponding SD to prevent artificial widening of confidence intervals of the pooled treatment effect.[24] For cohorts, we calculated change scores by (value at end of follow-up in the treatment group – value at baseline in the treatment group) assuming that in a fictional control group D-dimer would not change during follow-up. For results on cross-sectional studies we measured change scores by (value in the statin users group – value in the nonexposed group). When the authors adjusted D-dimer levels for other confounding factors, we used the adjusted D-dimer levels for analysis. We expressed effect sizes as a standardized mean difference (SMD) with its corresponding 95% confidence intervals (CIs) using the dimensionless Cohen's d as the summary statistic.[25] To compensate for heterogeneity including study design, population characteristics, statin dose, and treatment duration, we used a random-effects model. Post-hoc subanalyses were performed to assess the potential effects of treatment duration of statin therapy (<12 weeks vs ≥ 12 weeks) and type of statin (lipophilic or hydrophilic). Simvastatin, atorvastatin and fluvastatin were classified as lipophilic statins and pravastatin and rosuvastatin as hydrophilic statins.[15]

Results

Study selection and evaluation of bias of individual studies

In total, we screened 307 studies, of which 60 were assessed for eligibility reading full-text, and finally 22 studies were included in this review (Figure 1).[26-47] Reasons for exclusion were an event or intervention < 2 months (n = 4), not written in English (n= 3), no specific D-dimer data available on baseline or follow-up (n=18), nonstandardized D-dimer measurement (n=2), no original research article (n=9) and repeated analysis on same data set (n=2). We included 7 controlled trials, 11 cohort studies and 4 cross-sectional studies. Taken together, this analysis included 22 control groups and 27 statin exposed groups with a total number of 18,052 study participants (Table 1). The included studies were performed among different study populations. Six studies were performed in subjects with dyslipidaemia, 6 studies in patients with proven cardiovascular disease, 4 studies in HIV infected patients, 2 in patients with type 2 diabetes mellitus, one in

healthy subjects, one in patients diagnosed with lupus, one in COPD patients and one in heart transplant patients. Of all 27 statin exposed groups, 17 groups were defined as lipophilic type statin users and 7 as hydrophilic type statin users, while the other 3 groups comprised of lipophilic type as well as hydrophilic type statin users. Of the 23 statin exposed groups in which we could assess treatment duration, 19 groups were exposed to statins for 12 weeks or longer.

The risk of bias regarding patient selection was regarded low for only 6 of the 22 included studies and for 8 studies we had concerns about applicability of the results based on the specific characteristics of the statin exposed groups and control groups included in these studies. (Figure 2, Table 2). For four studies the D-dimer test was not clearly described, and we assumed a standardized test.[34,37,38,47]

Meta-analysis

Study-level meta-analysis involving 18,052 study participants showed significantly lower D-dimer levels in those receiving statin treatment compared to controls (SMD: -0.165, 95% CI -0.234; -0.096, p= <0.001) (Figure 3). The estimated effect sizes were similar in sensitivity analyses that omitted any single study (Figure 4). The 6 studies with low risk of patient selection (SMD: -0.099, 95%CI -0.140; -0.058, p <0.001) and the 16 studies with low risk of limited patient applicability (SMD: -0.216, 95%CI -0.334; -0.099, p <0.001) also resulted in lower D-dimer values after statin treatment. A separate meta-analysis of the 7 controlled trials did not show a different effect on D-dimer levels (SMD:-0.096, 95%CI -0.138; -0.055, p<0.001). Furthermore, treatment duration (<12 weeks vs ≥ 12 weeks) did not influence the effect on D-dimer levels in statin users (p = 0.887) (Figure 5) and type of statin (lipophilic or hydrophilic) also did not modify this overall result (p=0.167) (Figure 6).

Table 1: Characteristics of included studies for meta-analysis on the effect of statins on plasma D-dimer level

	Location	Population	D-dimer assay	Information about use of co-medication	Age (years)	Time of exposure	Regimen (daily dose)	Participants (number)	D-dimer (µg/mL) before exposure	D-dimer (µg/mL) after exposure	Conclusion	Details
Controlled trials												
Chang, 2002	South-Korea	Hemodialysis patients with hypercholesterolemia5	ELISA Asserachrome D-Di (Diagnostica Stago, Asnieres-sur-seine, France)	Exclusion cholesterol modifying or oxidation medication	63 (11) 60 (12)	8 wk	simvastatin (20mg) No simvastatin	28 30	1.05 (0.90) 1.12 (1.01)	0.99 (0.83) 1.09 (0.97)	No effect	Open RCT
Eckhard, 2014	USA	Non hypercholesterolemic HIV infected	LPIA (Diagnostica-Stago, Parsippany, NJ)	On antiretroviral therapy ASA, steroids, NSAIDs, antihypertensive medication	45.6 (41.1-51.4) 46.9 (39.2-53.6)	24 wk	rosuvastatin (10mg) placebo	67 69	0.19(0.13-0.33) 0.18 (0.09, 0.29)	baseline+ 6.9% (43.8 to -35.0) baseline +21.9% (-9.1 to 73.3)	No effect	double-blind RCT
Kinlay, 2009	USA	acute coronary syndromes	Not reported	ASA, heparin, nitrates, and β-blockers	64 (12)	16 wk	atorvastatin (80 mg) placebo	387	Overall 0.3447 (0.0708 to 5.351)	baseline +0.0108 µg/mL (-93,2 to 145) baseline +0.0244 µg/mL (-0.1097 to 0.1234)	No effect	double-blind RCT
Nixon, 2016^	USA	HIV infected	ELISA (Diagnostica Stago, Asnières sur Seine, France)	On antiretroviral therapy Exclusion of immunosuppressant users	48 (41-55)	20 wk	a)atorvastatin (10-20 mg) a)placebo b)placebo b)atorvastatin (10-20mg)	37 37 36 36	0.1870 (0.1209-0.3196) 0.1998 (0.1319-0.3383) 0.1785 (0.1256-0.2545) 0.1727 (0.1212-0.3039)	0.219 (0.1352-0.3177) 0.2127 (0.1467-0.3393) 0.1804 (0.1316-0.2250) 0.1755 (0.1113-0.2387)	No difference	double-blind RCT with cross-over design with 4 wk wash-out period
Sommeijer, 2004	The Netherlands	type 2 diabetes mellitus	LPIA (BioMérieux, Durham, NC)op	Antihypertensive medication, ASA	Overall: 59 (54-64) median (IQR)	8 wk	pravastatin (40 mg) no pravastatin	50 50	-	Between pravastatin and no pravastatin group change: -0.02 (-0.09 to 0.05)	No effect	Open RCT met cross-over design.

Table 1: Continued

	Location	Population	D-dimer assay	Information about use of co-medication	Age (years)	Time of exposure	Regimen (daily dose)	Participants (number)	D-dimer (µg/mL) before exposure	D-dimer (µg/mL) after exposure	Conclusion	Details
Tonkin, 2015	Australia	acute coronary syndrome	LPIA (Architect c8000, Abbott Diagnostics)	ASA	62 (55-67) 63 (56-68)	12 mnth	pravastatin (40mg) placebo	3941 3922	0.172 (0.112-0.269) 0.173 (0.112-0.276)	0.166 (0.108-0.263) 0.178 (0.115-0.284)	Significant reduction	double-blind RCT
Van de Ree, 2003	The Netherlands	Type 2 diabetes mellitus	ELISA (Dade-Behring, Marburg, Germany)	-	59.7 (7.6) 60.3 (7.8) 58.6 (7.5)	30 wk	a)atorvastatin (10mg) b)atorvastatin (40-80mg) placebo	69 66 61	0.115 (0.086-0.160) 0.137(0.104-0.186) 0.123 (0.101-0.151)	Baseline -7.4% baseline -8.5% Baseline +1.9%	Significant reduction in both atorvastatin groups	double-blind RCT
Cohort studies												
Bolaman, 2006	Turkey	Primary hypercholesterolemia	ELISA (not otherwise specified)	-	55 (10)	24 wk	atorvastatin (10mg – 20mg)	44	0.195(0.073)	0.197 (0.085)	No effect	
Calza, 2017	Italy	HIV-1 infected	ELISA (Medical Systems, Genova, Italy)	On antiretroviral therapy Exclusion of steroid, androgen, estrogen, growth hormone, antihypertensive medication, thyroid preparation and acid-reducing agent users	46.8 (40.6-55.9)	6 mnth	rosuvastatin (10mg)	57	0.345 (0.166-0.445)	0.275 (0.149-0.381)	Significant reduction	
Costejon, 2017	Spain	Females with stable systemic lupus erythematosus	Not reported	Antimalarials and immunosuppressant	47 (23-80)	8 wk	Atorvastatin (20mg)	37	0.49 (0.46)	0.51 (0.39)	No effect	
Hölschermann, 2000	Germany	heart transplant recipients receiving oral immunosuppression	ELISA (Asserachrom; Boehringer Mannheim Diagnostics, Mannheim, Germany)	immunosuppresssants	48 (12) (mean (SD))	7 d or 1 mnth	simvastatin (10mg)	15	0.695 (total range 0.160-1.580)	0.490 (total range 0.160-1.470)	Significant reduction	

Table 1: Continued

	Location	Population	D-dimer assay	Information about use of co-medication	Age (years)	Time of exposure	Regimen (daily dose)	Participants (number)	D-dimer (µg/mL) before exposure	D-dimer (µg/mL) after exposure	Conclusion	Details
Joukhadar, 2001	Austria	hypercholesterolemia	ELISA (Diagnostica Stago, Asnières, France)	Exclusion of hypolipemic, anticoagulant, antiinflammatory or antihypertensive medication users	55 (9)	3 mnth	a)atorvastatin (10mg)	24	0.42 (0.53)	0.35 (0.34)	No effect	
					52 (9)		b)pravastatin (40mg)	24	0.29 (0.15)	0.29(0.16)		
					55 (8)		c)simvastatin (40mg)	27	0.35 (0.25)	0.33(0.17)		
								75	0.35(0.34)	0.33(0.23)		
Lin, 2000	Taiwan	hypercholesterolemia	LPIA (Diagnostica Stago, France)	Antihypertensive medication, hormone replacement	59.8 (7.1)	8 wk	Pooled data fluvastatin (40mg)	23	0.38 (0.31)	0.28 (0.19)	Significant reduction	Exclusion of familial hypercholesterolemia
Lin, 2006	Taiwan	hyperlipidemia	LPIA (Diagnostica Stago, France)	-	58.5 (9.7)	16 wk	simvastatin (20mg-40mg)	22	0.33 (0.17)	0.29 (0.14)	No effect	
Seljeflot, 2002	Norway	dyslipidemia and history of angina pectoris	ELISA in plasma and serum (Asserachrom D-di; Stago Diagnostica, Asniere, France)	Antihypertensive medication, warfarin, ASA, nitrates	Not reported	12 mnth	a)atorvastatin (20-40mg)	28	0.493 (0.296-0.767)	0.416 (0.269-0.749)	No effect	Both serum and plasma D-dimers reported. In this review plasma D-dimer (mostly used assay) reported
							b)simvastatin (20-40mg)	30	0.384(0.218-0.657)	0.385(0.221-0.541)		
Trifiletti, 2003	Italy	hypercholesterolemia	ELISA (Asserachrom; Diagnostica Stago)	Exclusion of ASA users	55 (3)	6 mnth	atorvastatin (20mg)	32	0.248 (0.055)	0.229 (0.042)	No effect	
Wada, 1992	Japan	hypercholesterolemia	Frelisa D-dimer (Agen, Brisbane, Australia)	-	55.2 (14.6)	> 3 mnth	pravastatin (10mg)	48	0.11 (0.06)	0.056 (0.039)	Significant reduction	
Weiss, 2016	France	HIV-1–infected receiving cART	ELISA (Asserachrom DDi)	c-ART	47 (41-54)	12 wk	rosuvastatin (20 mg)	43	0.194 (0.147-0.279)	Baseline +3.7% (-18.2 to +23.3)	No effect	

Table 1: Continued

	Location	Population	D-dimer assay	Information about use of co-medication	Age (years)	Time of exposure	Regimen (daily dose)	Participants (number)	D-dimer (µg/mL) before exposure	D-dimer (µg/mL) after exposure	Conclusion	Details
Cross-sectional studies												
Adams, 2013	USA	Caucasian, African-American, Hispanic and Chinese, free of cardiovascular diseases or active cancer	LPIA(Liatest D-DI; Diagnostica Stago, Parsippany, NJ)	-	65.9 (8.7) 61.5 (10.3)	-	Statin users Nonusers	1001 5786	Not reported	0.21 0.23	Significant reduction	adjustment for age, sex, education, individual income, and cardiovascular risk factors
Kaba, 2004	USA	≥2 months post myocardial infarction	ELISA (American Diagnostica, Greenwich, CT, USA)	ASA, antihypertensive medication , oral anticoagulants	60 (12) 58 (11)	-	Statin users Nonusers	644 401	0.487 (0.434) 0.731 (1.2)	-	Significant reduction	
Vidula, 2010	USA	peripheral artery disease	ELISA (Asserachrom D-Di kit; Diagnostica Stago, Asnières-sur-Seine, France)	-	72.1 (7.9) 73.0 (8.9)		Statin users Nonusers	242 337	1.1 (1.4) 0.97 (1.4)	-	No effect	
Walter, 2010	Germany	undergoing elective coronary angiography	ELISA (Asserachrom D-Di, Stago, Asnières, France)	Antihypertensive medication, oral antihyperglycaemic drugs, ASA, clopidogrel	60.6 (10.4) 62.4 (9.0)	-	Atorvastatin (10-40mg) Nonusers	54 54	0.466 (0.173) 0.454 (0.182)	-	No effect	Matching based on cholesterol levels

Data are reported as means (SD) or medians (75th percentile to 25th percentile) unless stated otherwise
^original data on effects on D-dimers received and reported
Used abbreviations: NA: not available; mg: miligram; d: days; wk: weeks; mnth: months; ELISA: Enzyme-Linked Immuno Sorbent Assay; LPIA: latex-enhanced photometric immunoassays; ASA: acetylsalicylic acid; RCT: randomized controlled trial

Figure 2: Graphical display for QUADAS-2 results of the 22 studies included

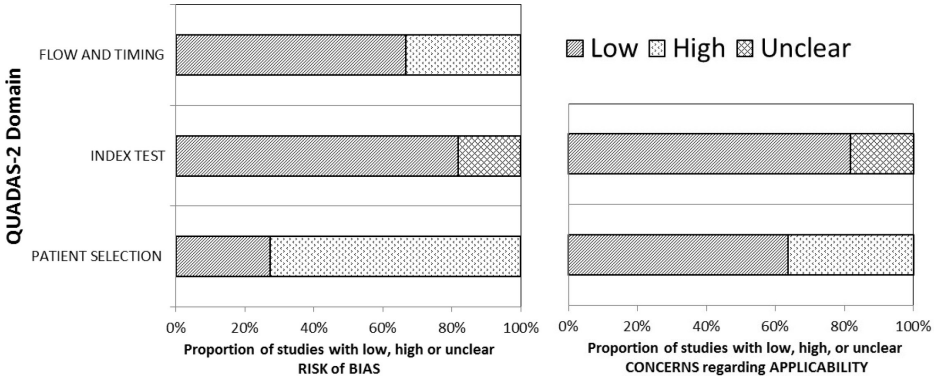


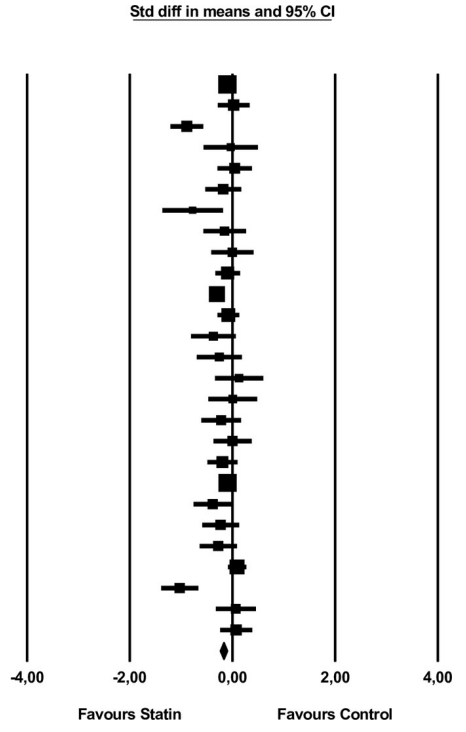
Table 2: Tabular presentation of QUADAS-2 results

	RISK OF BIAS				APPLICABILITY CONCERNS	
	PATIENT SELECTION	**INDEX TEST**	**FLOW AND TIMING**	**CONFLICTS OF INTEREST**	**PATIENT SELECTION**	**INDEX TEST**
Controlled trials						
Chang, 2002	☺	☺	☺	☹	☹	☺
Eckhard, 2014	☺	☺	☺	☹	☺	☺
Kinlay, 2009	☺	?	☺	☹	☺	?
Nixon, 2016	☹	☺	☺	☹	☺	☺
Sommeijer, 2004	☺	☺	☺	☹	☺	☺
Tonkin, 2015	☺	☺	☹	☹	☹	☺
Van de Ree, 2003	☺	☺	☺	☹	☺	☺
Cohort studies						
Bolaman, 2006	☹	?	☺	?	☺	?
Calza, 2017	☹	☺	☺	☺	☺	☺
Costejon, 2017	☹	?	☺	☺	☹	?
Hölschermann, 2000	☹	☺	☺	☺	☹	☺
Joukhadar, 2001	☹	☺	☹	?	☺	☺
Lin, 2000	☹	☺	☹	?	☺	☺
Lin, 2006	☹	☺	☹	☹	☺	☺
Seljeflot, 2002	☹	☺	☹	☹	☹	☺
Trifiletti, 2003	☹	☺	☺	?	☺	☺
Wada, 1992	☹	?	☺	☺	☺	?
Weiss, 2016	☹	☺	☹	☺	☺	☺
Crosssectional studies						
Adams, 2013	☹	☺	NA	☺	☺	☺
Kaba, 2004	☹	☺	NA	☺	☹	☺
Vidula, 2010	☹	☺	NA	☺	☹	☺
Walter, 2010	☹	☺	NA	☺	☹	☺

☺*Low Risk* ☹*High Risk* *? Unclear Risk* *NA Not Applicable*

Figure 3: Forest plot for the effect of statin therapy on plasma D-dimer concentrations.

Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Adams et al., 2013	-0,097	0,012	0,000	-0,121	-0,073	-7,991	0,000
Bolaman et al., 2006	0,025	0,151	0,023	-0,270	0,321	0,166	0,868
Calza et al., 2017	-0,887	0,156	0,024	-1,193	-0,580	-5,673	0,000
Chang et al., 2002	-0,032	0,263	0,069	-0,547	0,483	-0,122	0,903
Costejon et al., 2017	0,047	0,164	0,027	-0,276	0,369	0,283	0,777
Eckhard et al., 2014	-0,178	0,172	0,030	-0,514	0,159	-1,033	0,301
Hölschermann et al., 2000	-0,773	0,294	0,087	-1,350	-0,196	-2,627	0,009
Joukhadar et al., 2001a	-0,151	0,205	0,042	-0,553	0,252	-0,733	0,463
Joukhadar et al., 2001b	0,000	0,204	0,042	-0,400	0,400	0,000	1,000
Joukhadar et al., 2001c	-0,090	0,116	0,013	-0,317	0,136	-0,782	0,434
Kaba et al., 2004	-0,300	0,064	0,004	-0,425	-0,174	-4,686	0,000
Kinlay et al., 2009	-0,078	0,102	0,010	-0,277	0,121	-0,766	0,444
Lin et al., 2000	-0,369	0,216	0,046	-0,792	0,053	-1,714	0,087
Lin et al., 2006	-0,255	0,217	0,047	-0,679	0,170	-1,175	0,240
Nixon et al., 2016a	0,132	0,233	0,054	-0,324	0,588	0,567	0,571
Nixon et al., 2016b	0,009	0,236	0,056	-0,453	0,471	0,037	0,971
Seljeflot et al., 2002a	-0,219	0,191	0,037	-0,593	0,156	-1,143	0,253
Seljeflot et al., 2002b	0,003	0,183	0,033	-0,354	0,361	0,019	0,985
Sommeijer et al., 2004	-0,193	0,143	0,020	-0,473	0,087	-1,351	0,177
Tonkin et al., 2015	-0,092	0,023	0,001	-0,136	-0,048	-4,078	0,000
Trifiletti et al., 2003	-0,382	0,183	0,034	-0,740	-0,023	-2,084	0,037
van de Ree et al., 2003a	-0,228	0,176	0,031	-0,574	0,117	-1,295	0,195
van de Ree et al., 2003b	-0,274	0,178	0,032	-0,624	0,076	-1,536	0,125
Vidula et al., 2010	0,093	0,084	0,007	-0,072	0,258	1,101	0,271
Wada et al., 1992	-1,024	0,178	0,032	-1,373	-0,675	-5,746	0,000
Walter et al., 2010	0,068	0,193	0,037	-0,310	0,445	0,351	0,726
Weiss et al., 2016	0,074	0,153	0,023	-0,226	0,373	0,482	0,630
	-0,165	0,035	0,001	-0,234	-0,096	-4,676	0,000



Effect sizes were expressed as as standardized mean difference (SMD) with its corresponding 95% confidence intervals (CI) using Cohen's d as the summary statistic. A random-effect model was used for performance of the meta-analysis.

Figure 4: Leave-one-out sensitivity analysis of the effect of statin therapy on D-dimer

Study name	Point	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Adams et al., 2013	-0,182	0,048	0,002	-0,275	-0,089	-3,824	0,000
Bolaman et al., 2006	-0,173	0,036	0,001	-0,243	-0,102	-4,774	0,000
Calza et al., 2017	-0,135	0,032	0,001	-0,197	-0,073	-4,258	0,000
Chang et al., 2002	-0,168	0,036	0,001	-0,238	-0,097	-4,683	0,000
Costejon et al., 2017	-0,172	0,036	0,001	-0,243	-0,102	-4,785	0,000
Eckhard et al., 2014	-0,165	0,036	0,001	-0,236	-0,094	-4,576	0,000
Hölschermann et al., 2000	-0,157	0,035	0,001	-0,225	-0,088	-4,485	0,000
Joukhadar et al., 2001a	-0,166	0,036	0,001	-0,236	-0,095	-4,613	0,000
Joukhadar et al., 2001b	-0,169	0,036	0,001	-0,240	-0,099	-4,716	0,000
Joukhadar et al., 2001c	-0,170	0,037	0,001	-0,241	-0,098	-4,635	0,000
Kaba et al., 2004	-0,153	0,036	0,001	-0,223	-0,082	-4,226	0,000
Kinlay et al., 2009	-0,171	0,037	0,001	-0,243	-0,099	-4,650	0,000
Lin et al., 2000	-0,161	0,036	0,001	-0,231	-0,091	-4,503	0,000
Lin et al., 2006	-0,163	0,036	0,001	-0,234	-0,093	-4,560	0,000
Nixon et al., 2016a	-0,171	0,036	0,001	-0,241	-0,101	-4,788	0,000
Nixon et al., 2016b	-0,169	0,036	0,001	-0,239	-0,099	-4,710	0,000
Seljeflot et al., 2002a	-0,164	0,036	0,001	-0,235	-0,094	-4,561	0,000
Seljeflot et al., 2002b	-0,170	0,036	0,001	-0,241	-0,100	-4,729	0,000
Sommeijer et al., 2004	-0,164	0,036	0,001	-0,235	-0,093	-4,535	0,000
Tonkin et al., 2015	-0,181	0,046	0,002	-0,271	-0,091	-3,949	0,000
Trifiletti et al., 2003	-0,159	0,036	0,001	-0,229	-0,089	-4,452	0,000
van de Ree et al., 2003a	-0,164	0,036	0,001	-0,234	-0,093	-4,541	0,000
van de Ree et al., 2003b	-0,162	0,036	0,001	-0,233	-0,092	-4,512	0,000
Vidula et al., 2010	-0,183	0,036	0,001	-0,254	-0,112	-5,048	0,000
Wada et al., 1992	-0,136	0,031	0,001	-0,197	-0,074	-4,338	0,000
Walter et al., 2010	-0,171	0,036	0,001	-0,242	-0,101	-4,777	0,000
Weiss et al., 2016	-0,174	0,036	0,001	-0,245	-0,103	-4,831	0,000
	-0,165	0,035	0,001	-0,234	-0,096	-4,676	0,000

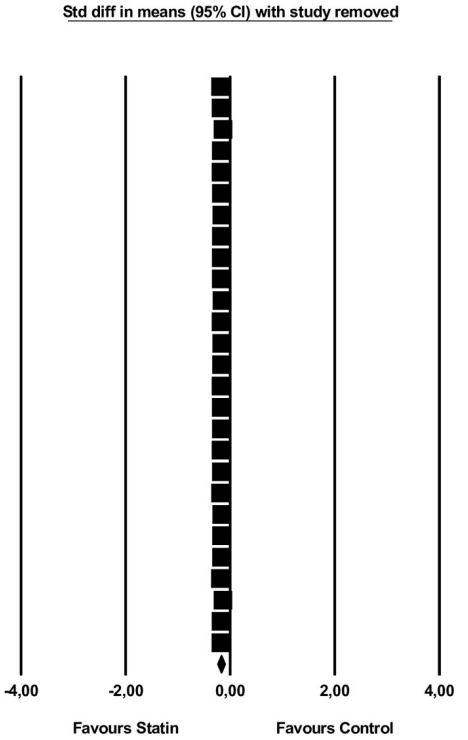
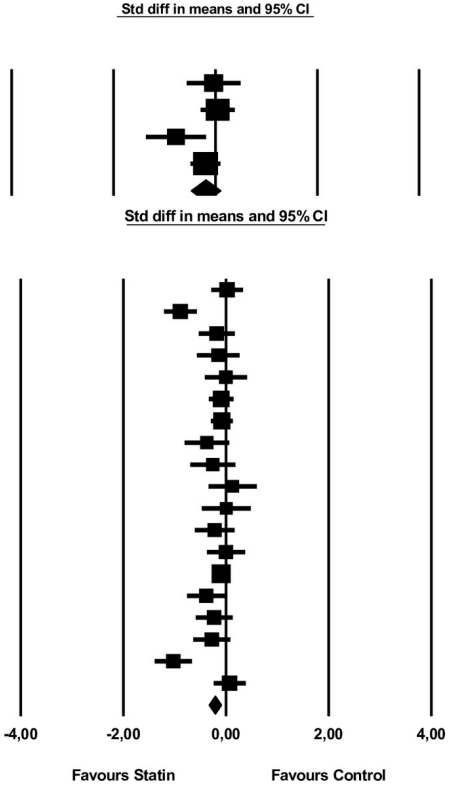


Figure 5: Forest plot of the effect of statin therapy on D-dimer with treatment durations of <12 weeks (above) and >12 weeks (below).

Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Chang et al., 2002	-0,032	0,263	0,069	-0,547	0,483	-0,122	0,903
Costejon et al., 2017	0,047	0,164	0,027	-0,276	0,369	0,283	0,777
Hölschermann et al., 2000	-0,773	0,294	0,087	-1,350	-0,196	-2,627	0,009
Sommeijer et al., 2004	-0,193	0,143	0,020	-0,473	0,087	-1,351	0,177
	-0.185	0.146	0.021	-0.471	0.101	-1.265	0.206

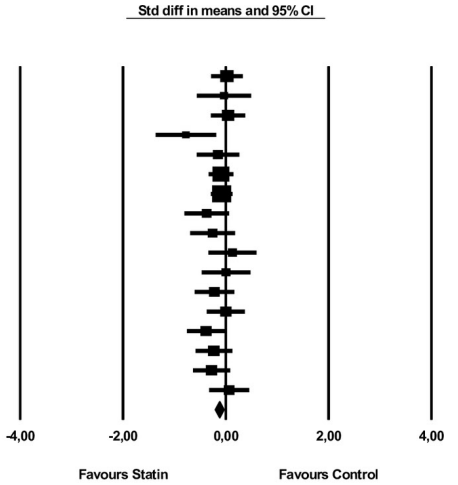
Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Bolaman et al., 2006	0,025	0,151	0,023	-0,270	0,321	0,166	0,868
Calza et al., 2017	-0,887	0,156	0,024	-1,193	-0,580	-5,673	0,000
Eckhard et al., 2014	-0,178	0,172	0,030	-0,514	0,159	-1,033	0,301
Joukhadar et al., 2001a	-0,151	0,205	0,042	-0,553	0,252	-0,733	0,463
Joukhadar et al., 2001b	0,000	0,204	0,042	-0,400	0,400	0,000	1,000
Joukhadar et al., 2001c	-0,090	0,116	0,013	-0,317	0,136	-0,782	0,434
Kinlay et al., 2009	-0,078	0,102	0,010	-0,277	0,121	-0,766	0,444
Lin et al., 2000	-0,369	0,216	0,046	-0,792	0,053	-1,714	0,087
Lin et al., 2006	-0,255	0,217	0,047	-0,679	0,170	-1,175	0,240
Nixon et al., 2016a	0,132	0,233	0,054	-0,324	0,588	0,567	0,571
Nixon et al., 2016b	0,009	0,236	0,056	-0,453	0,471	0,037	0,971
Seljeflot et al., 2002a	-0,219	0,191	0,037	-0,593	0,156	-1,143	0,253
Seljeflot et al., 2002b	0,003	0,183	0,033	-0,354	0,361	0,019	0,985
Tonkin et al., 2015	-0,092	0,023	0,001	-0,136	-0,048	-4,078	0,000
Trifiletti et al., 2003	-0,382	0,183	0,034	-0,740	-0,023	-2,084	0,037
van de Ree et al., 2003a	-0,228	0,176	0,031	-0,574	0,117	-1,295	0,195
van de Ree et al., 2003b	-0,274	0,178	0,032	-0,624	0,076	-1,536	0,125
Wada et al., 1992	-1,024	0,178	0,032	-1,373	-0,675	-5,746	0,000
Weiss et al., 2016	0,074	0,153	0,023	-0,226	0,373	0,482	0,630
	-0,207	0,060	0,004	-0,324	-0,090	-3,459	0,001



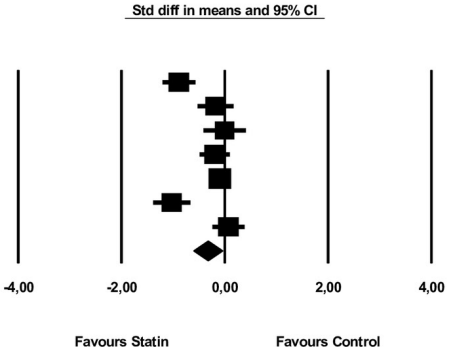
Effect sizes were expressed as as standardized mean difference (SMD) with its corresponding 95% confidence intervals (CI) using Cohen's d as the summary statistic. A random-effect model was used for performance of the meta-analysis.

Figure 6: Forest plot for the post-hoc analysis on the effect of type of statin therapy on plasma D-dimer concentrations with lipophilic statins above and hydrophilic statins below.

Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Bolaman et al., 2006	0,025	0,151	0,023	-0,270	0,321	0,166	0,868
Chang et al., 2002	-0,032	0,263	0,069	-0,547	0,483	-0,122	0,903
Costejon et al., 2017	0,047	0,164	0,027	-0,276	0,369	0,283	0,777
Hölschermann et al., 2000	-0,773	0,294	0,087	-1,350	-0,196	-2,627	0,009
Joukhadar et al., 2001a	-0,151	0,205	0,042	-0,553	0,252	-0,733	0,463
Joukhadar et al., 2001c	-0,090	0,116	0,013	-0,317	0,136	-0,782	0,434
Kinlay et al., 2009	-0,078	0,102	0,010	-0,277	0,121	-0,766	0,444
Lin et al., 2000	-0,369	0,216	0,046	-0,792	0,053	-1,714	0,087
Lin et al., 2006	-0,255	0,217	0,047	-0,679	0,170	-1,175	0,240
Nixon et al., 2016a	0,132	0,233	0,054	-0,324	0,588	0,567	0,571
Nixon et al., 2016b	0,009	0,236	0,056	-0,453	0,471	0,037	0,971
Seljeflot et al., 2002a	-0,219	0,191	0,037	-0,593	0,156	-1,143	0,253
Seljeflot et al., 2002b	0,003	0,183	0,033	-0,354	0,361	0,019	0,985
Trifiletti et al., 2003	-0,382	0,183	0,034	-0,740	-0,023	-2,084	0,037
van de Ree et al., 2003a	-0,228	0,176	0,031	-0,574	0,117	-1,295	0,195
van de Ree et al., 2003b	-0,274	0,178	0,032	-0,624	0,076	-1,536	0,125
Walter et al., 2010	0,068	0,193	0,037	-0,310	0,445	0,351	0,726
	-0,119	0,042	0,002	-0,202	-0,036	-2,817	0,005



Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Calza et al., 2017	-0,887	0,156	0,024	-1,193	-0,580	-5,673	0,000
Eckhard et al., 2014	-0,178	0,172	0,030	-0,514	0,159	-1,033	0,301
Joukhadar et al., 2001b	0,000	0,204	0,042	-0,400	0,400	0,000	1,000
Sommeijer et al., 2004	-0,193	0,143	0,020	-0,473	0,087	-1,351	0,177
Tonkin et al., 2015	-0,092	0,023	0,001	-0,136	-0,048	-4,078	0,000
Wada et al., 1992	-1,024	0,178	0,032	-1,373	-0,675	-5,746	0,000
Weiss et al., 2016	0,074	0,153	0,023	-0,226	0,373	0,482	0,630
	-0,321	0,140	0,020	-0,596	-0,047	-2,294	0,022

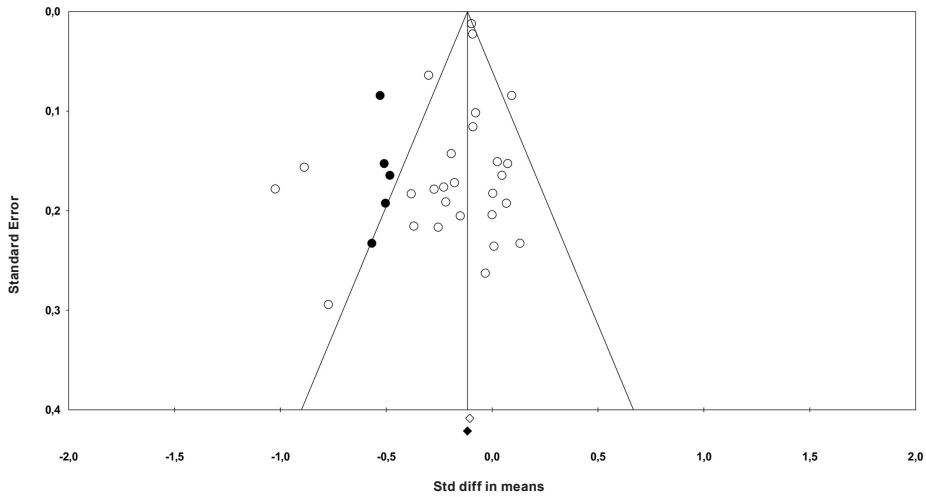


Effect sizes were expressed as as standardized mean difference (SMD) with its corresponding 95% confidence intervals (CI) using Cohen's d as the summary statistic. A random-effect model was used for performance of the meta-analysis.

Publication bias

A visual inspection of the funnel plot showed asymmetry, suggesting potential publication bias. Using the 'trim and fill' method with five potentially missing studies imputed, the effect size was estimated to an adjusted SMD with a larger effect (-0.224, 95% CI -0.295; -0.153) than the unadjusted SMD (Figure 7). Begg's rank correlation (Kendall's Tau with continuity correction = -0.160, Z = 1.167, two-tailed p = 0.243) and Egger test (intercept -0.611, 95% CI -1.447; 0.226, two tailed p =0.145) were both nonsignificant. Following the "fail-safe N" method, we would need to retrieve and impute 422 missing studies in the meta-analysis to make the p-value nonsignificant.

Figure 7: Funnel plot representing publication bias within literature analysed with Duval and Tweedie's Trim and Fill method about the effect of statin therapy on D-dimer levels.



Observed studies are shown as open circles, imputed studies are shown as filled circles.

Discussion

In this meta-analysis, for which we included randomized controlled trials, cohort and cross-sectional studies conducted in humans, we found that statin treatment is associated with lower D-dimer levels. This effect is small but robust and not driven by any single study. Results from post-hoc subanalyses on treatment duration and type of statin therapy were not different from this overall effect.

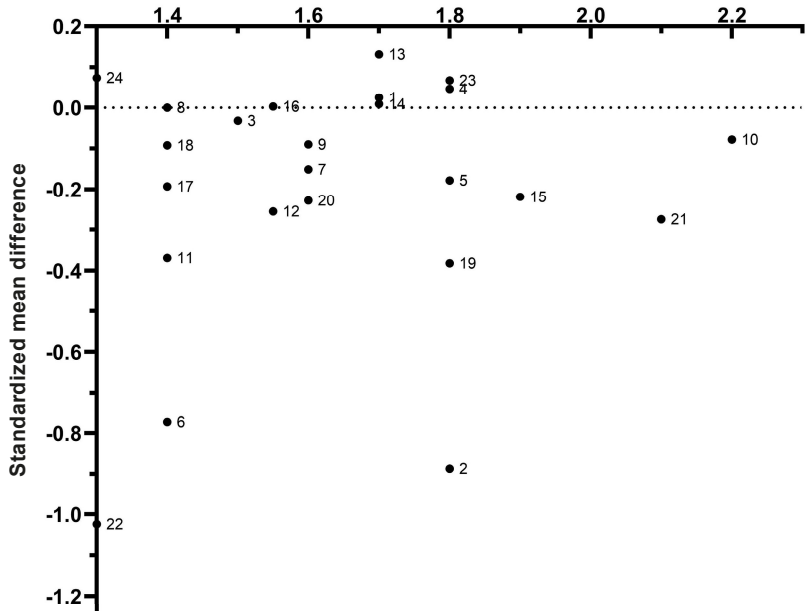
Our findings are important in further understanding the pleiotropic antithrombotic effects of statins. Statins have been shown to significantly lower the risk of primary VTE and therefore might have a role in the prevention of VTEs.[7,48] Several mechanisms have been described to explain these antithrombotic properties. Statins inhibit platelet activation within hours after intake by upregulation of the nitric oxide synthase and downregulation of phospholipase A2–mediated thromboxane A2 formation and probably also by reduced exposure of platelet-derived microparticles and glycoprotein IIIa, a receptor for fibrinogen and von Willebrand factor.[49-51] Also important, statins interfere directly with the clotting system. In vitro, two lipophilic types of statins decreased tissue factor activity in a dose-dependent manner.[52] As a result, a smaller amount of factor X is activated and generation of thrombin is diminished.[8,53,54] Other ways through which statins interfere with the clotting system are inhibition of isoprenoid intermediates, which indirectly activates the protein C pathway and lowering of the oxidized LDL-induced tissue factor expression. Inhibition of geranylgeranylation of the Rho/Rho kinase pathway is one of the key mechanisms of these anticoagulant effects.[8,55] By inhibition of this pathway, resulting in a shift in the fibrinolytic balance towards increased fibrinolytic activity is suggested by inhibition of the expression of plasminogen activator inhibitor-1 and upregulation of tissue-type plasminogen activator.[56,57]

These mechanisms might consequently result in lower D-dimer levels in statin users. This decrease of D-dimer levels may theoretically be stronger for lipophilic than for hydrophilic type of statin users. Lipophilic type of statins can enter cells in any organ and also penetrate cell membranes. In contrast, cellular uptake of hydrophilic type of statins is dependent on the presence of a specific carrier-mediated mechanism, which is only present in hepatocytes but not in extrahepatic cells.[58] Furthermore, tissue factor activity could in vitro only be decreased by lipophilic type of statins and not by pravastatin, a hydrophilic type of statin.[52] Clinical relevant difference of pleiotropic effects in general between lipophilic and hydrophilic type of statins is however controversial.[9] In our subanalyses on type of statin therapy, for both lipophilic and hydrophilic type of statin users D-dimer levels were significantly lower. This effect was not significantly different among these groups. Probably the clinical anticoagulant effect in vivo is independent on the mechanism of uptake.

The question of a possible dose-effect of statins in lowering D-dimer levels is also relevant, yet hard to answer because of difference in statin types and dosages that were applied in the included studies. Still, we applied a posthoc analysis, utilizing the previously developed concept of a 'statin correction factor', while adjusting for differences in the potency of statin type/dosage on LDL-lowering.[59] Following this concept, we

visually inspected the relation of the SMD in D-dimer levels against the statin correction factor, and found no clear dose-effect relation (Figure 8). An explanation for this lack of dose-effect on D-dimer levels could be that other mechanisms are involved in the anticoagulant effect of statins compared to the cholesterol dependent effects. The dose-effect relation of statins on D-dimers levels might therefore be independent of the potency of lowering LDL-cholesterol levels.

Figure 8: Dose-effect relation of statins in lowering D-dimer levels



Data of studies in which type of statin and dose was reported were plotted and labeled by a number: 1.Bolaman et al., 2006; 2.Calza et al., 2017; 3.Chang et al., 2002; 4.Costejon et al., 2014; 5.Eckhard et al., 2014; 6.Hölschermann et al., 2000; 7.Joukhadar et al., 2001a; 8.Joukhadar et al., 2001b; 9.Joukhadar et al., 2001c; 10.Kinlay et al., 2009; 11.Lin et al., 2000; 12.Lin et al., 2006;13.Nixon et al., 2016a; 14.Nixon et al., 2016b; 15.Seljeflot et al., 2002a; 16.Seljeflot et al., 2002b; 17.Sommeijer et al., 2004; 18.Tonkin et al., 2015; 19.Trifletti et al., 2003; 20.van de Ree et al., 2003a; 21.van de Ree et al., 2003b; 22.Wada et al., 1992; 23.Walter et al., 2010; 24.Weiss et al., 2016

Considering lower D-dimer levels in statin users, the performance of the diagnostic algorithms used for patients with suspected pulmonary embolism or deep vein thrombosis could be different for statin users. In these algorithms, a normal D-dimer level in combination with a low clinical probability of thrombosis safely excludes VTE.[60,61] Most D-dimer cut-offs in these diagnostic algorithms range between 0.5 to 1.0 µg/ml, depending on the clinical rule applied.[61,62] These cut-off levels have high sensitivity rates and therefore a false negative test in statin users is unlikely to occur.

In a recent retrospective post-hoc analysis, adjusting D-dimer cut-offs for statin users did not result in a safer diagnostic strategy.[63] However, further validation in a larger prospective cohort is needed.

It is important to note that there are main differences between our methodology and the systematic review and meta-analysis by Sahebkar et al.[12] First, in both studies effect sizes are expressed as standardized mean difference (SMD) using Cohen's d. However, Cohen's d is a dimensionless quantity, calculated as the ratio of the difference between the means of two samples and their pooled standard deviation. Thus, Cohen's d can be interpreted as a standardized difference.[64] Cohen's d was developed to compare effects across studies (even) when outcome variables vary, and results could be interpreted by referring to benchmarks with small (Cohen's d = 0.2), medium (0.5) and large (0.8) effect sizes.[25,64] Effect sizes should also be set in clinical perspective, incorporating that small effects could have large implications in clinical settings. In the article by Sahebkar et al. therefore, the overall effect of statins on the plasma D-dimer levels could have been interpreted as a large effect (d = -0.988), but not as a reduction of D-dimer levels by 0.988 µg/mL (which would be an extremely large effect). Second, in the meta-analysis by Sahebkar et al. we found inconsistencies in data extracted from the incorporated studies (Table 3). In seven of the nine studies differences in mean (standard deviation [SD]) D-dimer levels were reported incorrectly in Table 1 of their meta-analysis.[12] For example, in both studies of Sommeijer et al. and Walter et al., D-dimer values after treatment were reported as D-dimer changes.[26,31] Third, in our meta-analysis we explained essential assumptions with respect to the interpretation of the original data. In the meta-analysis by Sahebkar et al. on the other side, it remains unclear how exactly means or SDs were estimated if not reported in the study manuscripts. Because of concerns on the validity of the reported D-dimer results, due to inconsistent calculation of D-dimer changes, results of sensitivity analyses and unstandardized D-dimer measurement, one could argue about inclusion of the studies of Dangas et al., Min et al. and Undas et al..[65-67] In our meta-analysis we excluded these three studies.

Table 3: Tabular comparison of values imputed for meta-analysis by Sahebkar et al. and our extracted values

Study	**Chang et al.**				**Dangas et al.**				**Eckard et al.**				**Kinlay et al.**				**Min et al.**			
Treatment arm	simvastatin		control		pravastatin		placebo		rosuvastatin		placebo		atorvastatin		placebo		atorvastatin		placebo	
1: values from Sahebkar et al. 2: our verified values	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
number of participants^	*31*	28	*31*	30	26	26	36	36	*72*	67	*75*	69	*197*	n/387	*198*	n/387	43	43	46	46
D-dimer levels (μg/mL) at baseline																				
mean		1.05		1.12		0.38		0.365		0.19		0.18						0.6		0.5
SD		0.90		1.01		*0.05*		0.033		0.15		0.15						0.1		0.1
D-dimer levels (μg/mL) after treatment																				
mean		0.99		1.09		0.34		0.33		0.20		0.22						0.2		0.4
SD		0.83		0.97		0.05		0.03		0.15		0.15						0.1		0.1
difference in D-dimer levels (μg/mL)																				
mean	-0.06	-0.06	-0.03	-0.03	*0.05*	-0.05	*0.03*	-0.04	*0.07*	0.01	*0.22*	0.04	*10.80*	0.01	*24.40*	0.02	-0.4	-0.4	*0.3*	-0.1
SD	0.87	0.87	0.99	0.99	*0.3*	0.05	*0.13*	0.03	*13.13*	0.15	*13.73*	0.15	*39.70*	0.18	*38.85*	0.17	0.1	0.1	0.1	0.1
Our assumptions	SD of mean difference calculated*				Mean difference and SD of mean difference adopted from table (minus and plus signs were switched, because D-dimer levels were lower in the pravastatin group)				Medians are interpreted as mean values; SD calculated[1]; Medians after treatment calculated[2]; SD after treatment is equal to baseline SD				Proportion of participants in atorvastatin and placebo are equal (n=193 vs n=194). Medians are interpreted as mean values; SD calculated[1]				SD of mean difference calculated*			
Probable explanation of discrepencies	Number of participants changed due to loss to follow-up				SD of mean difference calculated*, SD reported in table not adopted				Number of participants changed due to loss to follow-up; percentage change is reported as mean difference; SD calculated using percentage change values in formula[3]				Total number of participants changed due to loss to follow-up; values are not recalculated from ng/mL to μg/mL; SD calculated using formula[3]				Mean difference in placebo group incorrectly subtracted			

Table 3: Continued

Study	Sommeijer et al.				Undas et al.				Van de Ree et al.						Walter et al.			
Treatment arm	pravastatin		control		simvastatin		control		atorvastatin 10mg		atorvastatin 80mg		placebo		atorvastatin		control	
1: values from Sahebkar et al. 2: our verified values	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
number of participants^	50	50	50	50	28	28	28	28	69	69	66	66	61	61	54	54	54	54
D-dimer levels (µg/mL) at baseline																		
mean						4.21		4.25		0.12		0.14		0.12				
SD						0.53		0.57		0.05		0.06		0.04				
D-dimer levels (µg/mL) after treatment																		
mean						3.54		4.21		0.11		0.13		0.13		0.47		0.45
SD						0.38		0.60		0.05		0.06		0.04		0.17		0.18
difference in D-dimer levels (µg/mL)																		
mean	*0.27*	-0.02[4]	*0.26*		-0.67	-0.67	-0.04	-0.04	-0.01	-0.01	*not reported*	-0.01	0.002	0.002	*0.47*	0.01	*0.45*	0.00
SD	*0.04*	0.1[4]	*0.03*		0.47	0.47	0.59	0.59	*0.01*	0.05	*not reported*	0.06	*0.01*	0.04	*0.17*	0.18	*0.18*	0.18
Our assumptions	Because of cross-sectional design, delta mean change and estimated delta mean change SD[1] as reported used				D-dmax interpreted as D-dimer values; SD of mean difference calculated*				Medians are interpreted as mean values; SD calculated[1]; Medians after treatment calculated[2]; SD after treatment is equal to baseline SD						Baseline mean values in atorvastatin group are equal to values after treatment in control group; SD in atorvastatin group is the same before and after treatment; values in control group do not change during follow-up			
Probable explanation of discrepencies	Values after treatment reported as difference; SD calculated[3]				No discrepancies				Results of atorvastatin 10mg and atorvastatin 80mg are not reported separately; SD of mean difference calculated using baseline values in formula[3]						Values after treatment reported as mean difference; study interpreted as randomized double-blind parallel-group trial			

Abbreviations: Standard deviation (SD), Interquartile range (IQR)
Values in 'italic' are deviating from our verified values
Mean differences were calculated using the following formula, unless stated otherwise: (measure at end of follow-up in the treatment group – measure at baseline in the treatment group) AND (measure at end of follow-up in the control group – measure at baseline in the control group)
^Number of participants are based on included participants for analysis of D-dimer levels on baseline and after treatment
**SD of mean difference was calculated using the following formula: SD = square root [(SDpre-treatment)2 + (SDpost-treatment)2 - (2R × SDpre-treatment × SDpost-treatment)], assuming a correlation coefficient (R) = 0.5*
[1]SD calculated using the following formula: (75% IQR - 25% IQR)/1.35, assuming that the IQR comprises of 1,35 SD approximately
*[2]Median D-dimer levels after treatment calculated using following formula: baseline median *(1+ (percentage change/100))*
[3]SD calculated using the following formula: (upper range - lower range)/6, incorrectly assuming that the range comprises of 6 SD approximately
[4] delta mean change and SD of delta mean change

The results of our meta-analysis should of course also be interpreted with caution. In this meta-analysis we did not only include randomized controlled trials, but also cohort and crosssectional studies. In the two latter types of studies, we scored the risk of bias to be high and heterogeneity between-individual studies will be higher. The meta-analysis was not limited to randomized controlled trials only, because we would then have ignored a large number of observational evidence.[68] It is however important to note that within the group of cross-sectional studies there are some differences in the retrieved data. The study of Adams adjusted results of D-dimer levels in statin users and nonusers for the following potential confounding factors: age, sex, education, individual income, race, smoking status, current alcohol use, body mass index, diabetes status, hypertension, use of acetylsalicylic acid and hormone therapy use among women.[46] On the other hand, Walter et al. matched users of atorvastatin with controls according to their total cholesterol levels and Kaba et al. and Vidula et al. did not adjust D-dimer levels for any confounding factors.[28,31,35] However, age and sex, two of the most influencing confounding factors, were not significantly different among statin users and nonusers in these studies. Also, duration of statin treatment was not assessed in these cross-sectional data. The described between-study heterogeneity is unlikely to have had a large impact on the results of our meta-analysis. In the subanalyses of the 6 controlled trials with low risk of patient selection and the 16 studies with low risk of limited patient applicability, change in D-dimer levels was not significantly different from the overall effect with all studies included. Also, a separate subanalysis only including the controlled trials did not differ from these results and resulted in lower D-dimer values after statin treatment. Moreover, the post-hoc analyses on treatment duration and statin type did not show a difference. Another concern might be that the included studies were heterogeneous in the characteristics of study participants. Studies were performed in patients with proven cardiovascular disease, HIV infection, type 2 diabetes mellitus, lupus, COPD and in heart transplant patients. All these conditions could have influenced D-dimer levels. By running our meta-analysis with a random-effects model we assumed the studies to be heterogeneous and our sensitivity analysis was robust. Furthermore, we could not fully exclude that publication bias has had an effect on the results of the meta-analysis. The adjusted effect size using the trim and fill method though was even larger than what we had observed, indicating that the effect size of reduction of D-dimer levels in statin users is more likely to be an underestimation rather than non-significant. Also Begg's rank correlation and Egger test were nonsignificant, indicating no publication bias and many missing studies (n=422) would be needed and imputed in our meta-analysis to come to a nonsignificant effect.

In conclusion, in this meta-analysis use of statins was associated with a reduction of D-dimer levels, independent of treatment duration and type of statin used. This antithrombotic effect is part of the "pleiotropic" effects of statins, and contributes to the benefits of statins on cardiovascular outcomes. The reduction of D-dimer levels in statin users may affect the performance of diagnostic algorithms on suspected VTE in this specific patient group, and prospective studies investigating the impact of statin use on these diagnostic algorithms are recommended.

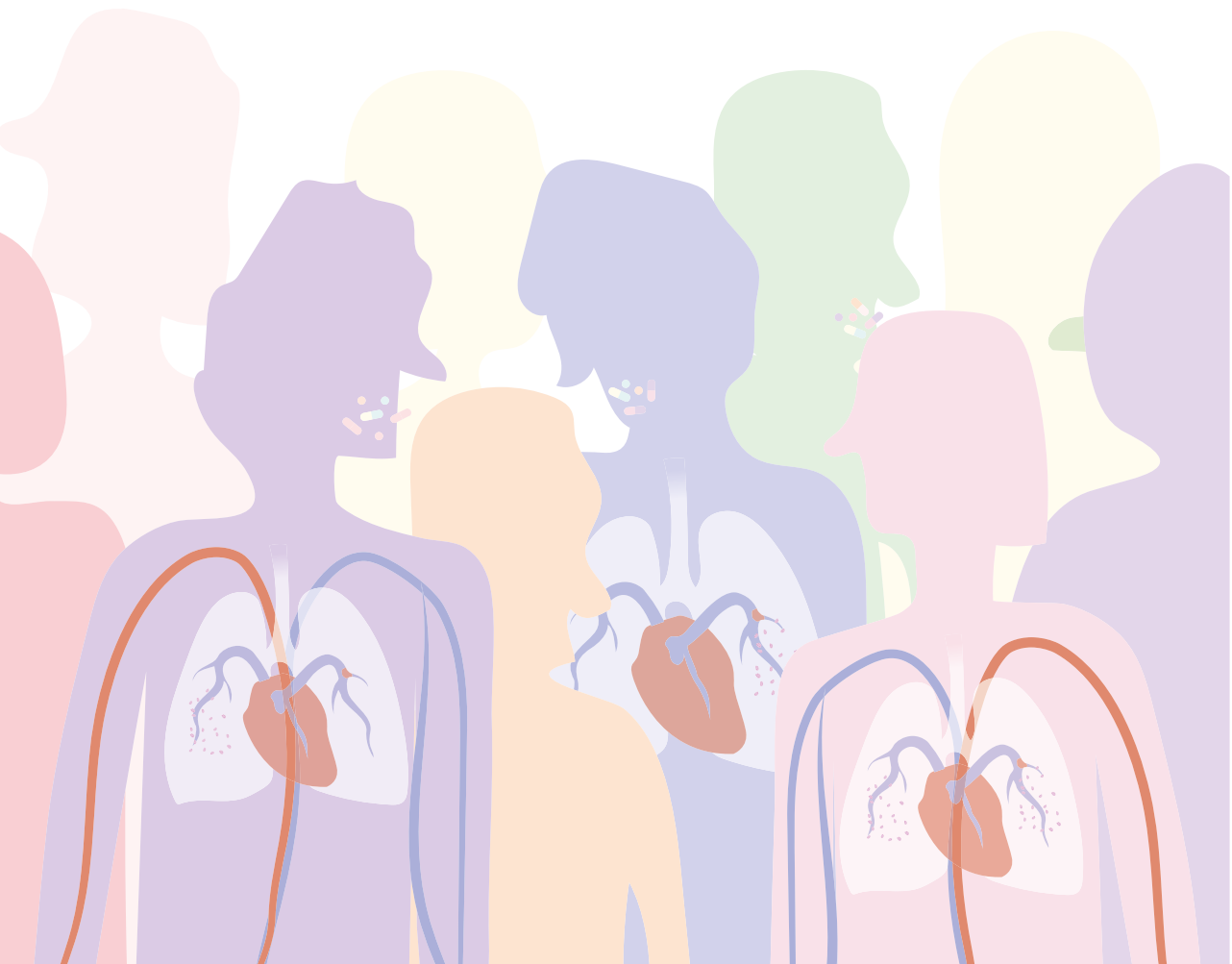
References

1. Adam SS, Key NS, Greenberg CS. D-dimer antigen: current concepts and future prospects. *Blood.* 2009;113(13):2878-2887.
2. Weitz JI, Fredenburgh JC, Eikelboom JW. A Test in Context: D-Dimer. *J Am Coll Cardiol.* 2017;70(19):2411-2420.
3. Douma RA, van Sluis GL, Kamphuisen PW, et al. Clinical decision rule and D-dimer have lower clinical utility to exclude pulmonary embolism in cancer patients. Explanations and potential ameliorations. *Thromb Haemost.* 2010;104(4):831-836.
4. Harb TS, Zareba W, Moss AJ, et al. Association between inflammatory markers, hemostatic, and lipid factors in postinfarction patients. *Am J Cardiol.* 2003;91(9):1120-1123.
5. Chabloz P, Reber G, Boehlen F, Hohlfeld P, de Moerloose P. TAFI antigen and D-dimer levels during normal pregnancy and at delivery. *Br J Haematol.* 2001;115(1):150-152.
6. Crop MJ, Siemes C, Berendes P, van der Straaten F, Willemsen S, Levin MD. Influence of C-reactive protein levels and age on the value of D-dimer in diagnosing pulmonary embolism. *Eur J Haematol.* 2014;92(2):147-155.
7. Kunutsor SK, Seidu S, Khunti K. Statins and primary prevention of venous thromboembolism: a systematic review and meta-analysis. *Lancet Haematol.* 2017;4(2):e83-e93.
8. Violi F, Calvieri C, Ferro D, Pignatelli P. Statins as antithrombotic drugs. *Circulation.* 2013;127(2):251-257.
9. Bonetti PO, Lerman LO, Napoli C, Lerman A. Statin effects beyond lipid lowering--are they clinically relevant? *Eur Heart J.* 2003;24(3):225-248.
10. Konstantinides SV, Torbicki A, Agnelli G, et al. 2014 ESC guidelines on the diagnosis and management of acute pulmonary embolism. *Eur Heart J.* 2014;35(43):3033-3069, 3069a-3069k.
11. Raja AS, Greenberg JO, Qaseem A, et al. Evaluation of Patients With Suspected Acute Pulmonary Embolism: Best Practice Advice From the Clinical Guidelines Committee of the American College of Physicians. *Ann Intern Med.* 2015;163(9):701-711.
12. Sahebkar A, Serban C, Mikhailidis DP, et al. Association between statin use and plasma d-dimer levels: A systematic review and meta-analysis of randomised controlled trials. *Thromb Haemost.* 2015;114(3):546-557.
13. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med.* 2009;151(4):264-269, W264.
14. Cilla DD, Jr., Whitfield LR, Gibson DM, Sedman AJ, Posvar EL. Multiple-dose pharmacokinetics, pharmacodynamics, and safety of atorvastatin, an inhibitor of HMG-CoA reductase, in healthy subjects. *Clin Pharmacol Ther.* 1996;60(6):687-695.
15. Schachter M. Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. *Fundam Clin Pharmacol.* 2005;19(1):117-125.
16. Konstantinides SV. 2014 ESC Guidelines on the diagnosis and management of acute pulmonary embolism. *Eur Heart J.* 2014;35(45):3145-3146.
17. Whiting PF, Rutjes AW, Westwood ME, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med.* 2011;155(8):529-536.
18. Cooper H, Hedges LV. *The Handbook of Research Synthesis.* Russell Sage Foundation; 1994.
19. Duval S, Tweedie R. Trim and fill: A simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics.* 2000;56(2):455-463.
20. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ.* 1997;315(7109):629-634.

21. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics.* 1994;50(4):1088-1101.
22. Rosenthal R. The file drawer problem and tolerance for null results. *Psychological Bulletin.* 1979;86(3):638-641.
23. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. *BMC Med Res Methodol.* 2014;14:135.
24. Nolan SJ, Hambleton I, Dwan K. The Use and Reporting of the Cross-Over Study Design in Clinical Trials and Systematic Reviews: A Systematic Assessment. *PLoS One.* 2016;11(7):e0159014.
25. Lakens D. Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. *Front Psychol.* 2013;4:863.
26. Sommeijer DW, MacGillavry MR, Meijers JCM, Van Zanten AP, Reitsma PH, Ten Cate H. Anti-Inflammatory and Anticoagulant Effects of Pravastatin in Patients with Type 2 Diabetes. *Diabetes Care.* 2004;27(2):468-473.
27. Tonkin AM, Blankenberg S, Kirby A, et al. Biomarkers in stable coronary heart disease, their modulation and cardiovascular risk: The LIPID biomarker study. *Int J Cardiol.* 2015;201:499-507.
28. Vidula H, Tian L, Liu K, et al. Comparison of Effects of Statin Use on Mortality in Patients With Peripheral Arterial Disease With Versus Without Elevated C-Reactive Protein and D-Dimer Levels. *Am J Cardiol.* 2010;105(9):1348-1352.
29. Van De Ree MA, De Maat MP, Kluft C, Meinders AE, Princen HM, Huisman MV. Decrease of hemostatic cardiovascular risk factors by aggressive vs. conventional atorvastatin treatment in patients with Type 2 diabetes mellitus. *J Thromb Haemost.* 2003;1(8):1753-1757.
30. Eckard AR, Jiang Y, Debanne SM, Funderburg NT, McComsey GA. Effect of 24 weeks of statin therapy on systemic and vascular inflammation in HIV-infected subjects receiving antiretroviral therapy. *J Infect Dis.* 2014;209(8):1156-1164.
31. Walter T, Szabo S, Suselbeck T, et al. Effect of atorvastatin on haemostasis, fibrinolysis and inflammation in normocholesterolaemic patients with coronary artery disease: A post hoc analysis of data from a prospective, randomized, double-blind study. *Clin Drug Invest.* 2010;30(7):453-460.
32. Lin TH, Huang CH, Voon WC, et al. The effect of fluvastatin on fibrinolytic factors in patients with hypercholesterolemia. *Kaohsiung J Med Sci.* 2000;16(12):600-606.
33. Nixon DE, Bosch RJ, Chan ES, et al. Effects of atorvastatin on biomarkers of immune activation, inflammation, and lipids in virologically suppressed, human immunodeficiency virus-1-infected individuals with low-density lipoprotein cholesterol <130 mg/dL (AIDS Clinical Trials Group Study A5275). *J Clin Lipidology.* 2016.
34. Bolaman Z, Kadikoylu G, Özgel N, Yenisey C. Effects of atorvastatin on coagulation parameters and homocysteine in patients with primary hypercholesterolemia. *J Natl Med Assoc.* 2006;98(8):1273-1277.
35. Kaba NK, Francis CW, Moss AJ, et al. Effects of lipids and lipid-lowering therapy on hemostatic factors in patients with myocardial infarction. *J Thromb Haemost.* 2004;2(5):718-725.
36. Chang JW, Yang WS, Min WK, Lee SK, Park JS, Kim SB. Effects of simvastatin on high-sensitivity C-reactive protein and serum albumin in hemodialysis patients. *Am J Kidney Dis.* 2002;39(6):1213-1217.
37. Kinlay S, Schwartz GG, Olsson AG, et al. Endogenous tissue plasminogen activator and risk of recurrent cardiac events after an acute coronary syndrome in the MIRACL study. *Atherosclerosis.* 2009;206(2):551-555.

38. Wada H, Mori Y, Kaneko T, et al. Hypercoagulable state in patients with hypercholesterolemia: Effects of pravastatin. *CLIN THER.* 1992;14(6):829-834.
39. Seljeflot I, Tonstad S, Hjermann I, Arnesen H. Improved fibrinolysis after 1-year treatment with HMG CoA reductase inhibitors in patients with coronary heart disease. *Thromb Res.* 2002;105(4):285-290.
40. Trifiletti A, Lasco A, Scamardi R, et al. Long-term hemostatic effects of cholesterol-lowering therapy with atorvastatin. *Pathophysiol Haemost Thromb.* 2003;33(2):84-87.
41. Lin TH, Voon WC, Yen HW, et al. Randomized comparative study of the effects of treatment with once-daily, niacin extended-release/lovastatin and with simvastatin on lipid profile and fibrinolytic parameters in Taiwan. *Kaohsiung J Med Sci.* 2006;22(6):257-265.
42. Weiss L, Chevalier MF, Assoumou L, et al. Rosuvastatin is effective to decrease CD8 T-cell activation only in HIV-infected patients with high residual T-cell activation under antiretroviral therapy. *J Acquired Immune Defic Syndr.* 2016;71(4):390-398.
43. Calza L, Colangeli V, Magistrelli E, et al. Significant Decrease in Plasma Levels of D-Dimer, Interleukin-8, and Interleukin-12 After a 12-Month Treatment with Rosuvastatin in HIV-Infected Patients Under Antiretroviral Therapy. *AIDS Res Hum Retroviruses.* 2017;33(2):126-132.
44. Joukhadar C, Klein N, Prinz M, et al. Similar effects of atorvastatin, simvastatin and pravastatin on thrombogenic and inflammatory parameters in patients with hypercholesterolemia. *Thromb Haemost.* 2001;85(1):47-51.
45. Hölschermann H, Hilgendorff A, Kemkes-Matthes B, et al. Simvastatin attenuates vascular hypercoagulability in cardiac transplant recipients. *Transplantation.* 2000;69(9):1830-1836.
46. 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.
47. Castejon R, Castañeda A, Sollet A, et al. Short-term atorvastatin therapy improves arterial stiffness of middle-aged systemic lupus erythematosus patients with pathological pulse wave velocity. *Lupus.* 2017;26(4):355-364.
48. Glynn RJ, Danielson E, Fonseca FA, et al. A randomized trial of rosuvastatin in the prevention of venous thromboembolism. *N Engl J Med.* 2009;360(18):1851-1861.
49. Pignatelli P, Carnevale R, Pastori D, et al. Immediate antioxidant and antiplatelet effect of atorvastatin via inhibition of Nox2. *Circulation.* 2012;126(1):92-103.
50. Tannous M, Cheung R, Vignini A, Mutus B. Atorvastatin increases ecNOS levels in human platelets of hyperlipidemic subjects. *Thromb Haemost.* 1999;82(5):1390-1394.
51. Sommeijer DW, Joop K, Leyte A, Reitsma PH, ten Cate H. Pravastatin reduces fibrinogen receptor gpIIIa on platelet-derived microparticles in patients with type 2 diabetes. *J Thromb Haemost.* 2005;3(6):1168-1171.
52. Colli S, Eligini S, Lalli M, Camera M, Paoletti R, Tremoli E. Vastatins inhibit tissue factor in cultured human macrophages. A novel mechanism of protection against atherothrombosis. *Arterioscler Thromb Vasc Biol.* 1997;17(2):265-272.
53. Owens AP, 3rd, Mackman N. The antithrombotic effects of statins. *Annu Rev Med.* 2014;65:433-445.
54. Undas A, Brummel-Ziedins KE, Mann KG. Anticoagulant effects of statins and their clinical implications. *Thromb Haemost.* 2014;111(3):392-400.
55. Oesterle A, Laufs U, Liao JK. Pleiotropic Effects of Statins on the Cardiovascular System. *Circ Res.* 2017;120(1):229-243.
56. Bourcier T, Libby P. HMG CoA reductase inhibitors reduce plasminogen activator inhibitor-1 expression by human vascular smooth muscle and endothelial cells. *Arterioscler Thromb Vasc Biol.* 2000;20(2):556-562.

57. Essig M, Nguyen G, Prie D, Escoubet B, Sraer JD, Friedlander G. 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors increase fibrinolytic activity in rat aortic endothelial cells. Role of geranylgeranylation and Rho proteins. *Circ Res.* 1998;83(7):683-690.
58. van Vliet AK, van Thiel GC, Huisman RH, Moshage H, Yap SH, Cohen LH. Different effects of 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors on sterol synthesis in various human cell types. *Biochim Biophys Acta.* 1995;1254(1):105-111.
59. Law MR, Wald NJ, Rudnicka AR. Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis. *BMJ.* 2003;326(7404):1423.
60. Douma RA, Mos IC, Erkens PM, et al. Performance of 4 clinical decision rules in the diagnostic management of acute pulmonary embolism: a prospective cohort study. *Ann Intern Med.* 2011;154(11):709-718.
61. van der Hulle T, Cheung WY, Kooij S, et al. Simplified diagnostic management of suspected pulmonary embolism (the YEARS study): a prospective, multicentre, cohort study. *Lancet.* 2017;390(10091):289-297.
62. van Es N, Kraaijpoel N, Klok FA, et al. The original and simplified Wells rules and age-adjusted D-dimer testing to rule out pulmonary embolism: an individual patient data meta-analysis. *J Thromb Haemost.* 2017;15(4):678-684.
63. Schol-Gelok S, van der Hulle T, Biedermann JS, et al. Clinical effects of antiplatelet drugs and statins on D-dimer levels. *Eur J Clin Invest.* 2018;48(7):e12944.
64. Cohen J. *Statistical Power Analysis for the Behavioral Sciences.* Lawrence Erlbaum Associates; 1988.
65. Dangas G, Badimon JJ, Smith DA, et al. Pravastatin therapy in hyperlipidemia: effects on thrombus formation and the systemic hemostatic profile. *J Am Coll Cardiol.* 1999;33(5):1294-1304.
66. Min L, Shao S, Wu X, et al. Anti-inflammatory and anti-thrombogenic effects of atorvastatin in acute ischemic stroke. *Neural Regen Res.* 2013;8(23):2144-2154.
67. Undas A, Kaczmarek P, Sladek K, et al. Fibrin clot properties are altered in patients with chronic obstructive pulmonary disease: Beneficial effects of simvastatin treatment. *Thromb Haemost.* 2009;102(6):1176-1182.
68. Sutton AJ, Abrams KR. Bayesian methods in meta-analysis and evidence synthesis. *Stat Methods Med Res.* 2001;10(4):277-303.



3.2

Effect of antiplatelet drugs on D-dimer levels: a systematic review and meta-analysis

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Abstract

Aims: D-dimer is a product of fibrinolysis. In clinical practice, D-dimer levels are commonly used to rule out venous thromboembolism. Antiplatelet drugs may influence D-dimer levels, potentially affecting the accuracy of this diagnostic tool. To evaluate the effect of antiplatelet drugs on D-dimer levels, we performed a systematic review and meta-analysis of all published articles on this topic (PROSPERO registration number CRD42017058932).

Methods and Results: We electronically searched EMBASE, Medline Epub, Cochrane, Web of Science and Google Scholar (100 top relevance) (last search on 5 October, 2017). We included randomized controlled trials, cohort studies and cross-sectional studies conducted in humans, with a drug exposure time of at least 7 days. Two reviewers independently selected eligible articles and extracted the data. Five controlled trials, 7 cohort studies and 5 cross-sectional studies were finally included. Meta-analysis involving all 1117 participants showed no change in dimer levels (standardized mean difference: -0.015, 95% confidence interval, -0.182; 0.151, p= 0.855).

Conclusions: In conclusion, antiplatelet drugs do not seem to influence D-dimer levels.

Introduction

D-dimer is a fragment of cross-linked fibrin. Fibrin is generated by cleavage of fibrinogen by thrombin. Fibrin fibers then aggregate to form a clot or "thrombus". To provide further stability, fibrin becomes cross-linked through the action of factor XIIIa.[1] Classically, thrombi are classified as white or red based on their composition. White thrombi are platelet-rich and develop in area of high shear stress, mainly the arteries. By contrast, red thrombi form in low-pressure systems, such as the venous system, and are made of fibrin and erythrocytes.[2]

The fibrin clot is then lysed by plasmin, and soluble fibrin degradation products, including D-dimers, are released.[1] The delicate balance between these processes, coagulation and fibrinolysis, is essential to achieve adequate hemostasis.

In case of a thromboembolic event, the D-dimer level reflects fibrin turn over. In clinical practice, D-dimer measurement is commonly used in the initial evaluation of suspected deep vein thrombosis and/or pulmonary embolism. In the appropriate setting, D-dimer concentration below a certain cut-off level can safely rule out the diagnosis of venous thromboembolism (VTE).[3, 4]

Antiplatelet drugs inhibit platelet function through different pathways. Beside their antithrombotic effect, they also have anti-inflammatory properties.[5] Furthermore, aspirin has been shown to impair thrombin generation, possibly by acetylation of prothrombin.[6] In addition, there is evidence that use of aspirin is associated with a reduction in postoperative VTE risk.[7]

Based on these observations, antiplatelet therapy could reduce D-dimer levels, and this effect may compromise the accuracy of diagnostic algorithms for VTE. Actually, the presence and amplitude of this effect remains unclear. Therefore, we systematically reviewed all published articles on this topic and conducted a meta-analysis.

Methods

Protocol

Our study was registered on the 10 March, 2017 in the PROSPERO International prospective register of systematic reviews (registration number CRD42017058932). It has been designed according to the 2009 Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement. [8]

Search strategy

Together with a biomedical information specialist, we searched several databases: EMBASE (Ovid SP), MEDLINE Epub (Ovid SP), Cochrane Central Register of Controlled Trials (CENTRAL), Web of Science ,and Google Scholar (100 top relevance). The last search was performed on the 5 October, 2017. In summary, the following search terms were used: D-dimer OR D-dimers AND antiplatelet OR antiplatelets OR platelet aggregation inhibitors OR platelet antagonist, in combination with individual drug names. To improve our search, we added the wild card term "*" and the accessory MeSH terms. A full list of search terms is reported in Methods S2, page 109.

Selection process and data extraction

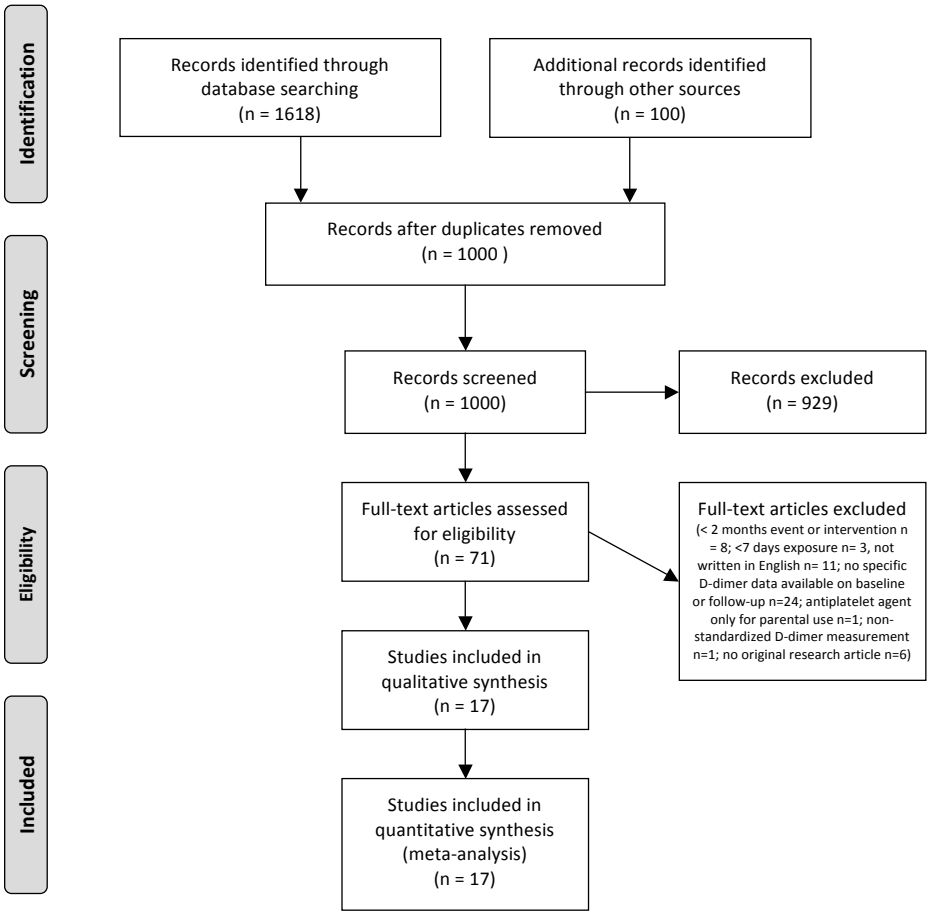
Two reviewers (FM and SS-G) independently selected eligible articles, based on titles and abstracts. In case of disagreement, discussion followed until consensus was reached. Thereafter, a further selection was performed, based on reading and discussion of full text. In case of disagreement, a third author (TvG) was consulted for final decision. The whole selection process is shown in Figure 1.[8]

We included controlled trials, cohort studies and crosssectional studies conducted in humans, in which D-dimer levels were reported in users and nonusers of antiplatelet drugs, in order to compare these 2 groups. When drug exposure time was reported, a treatment period of at least 7 days was required. This arbitrary period was chosen to avoid including studies in which the anti-platelet drug was not yet in steady-state, for both the pharmacokinetic and the pharmacodynamic effect.

Only full-text articles written in English were included. Studies conducted in patients on anticoagulant drugs were excluded.[9] Also, we excluded studies in which any cardiovascular event (ie, stroke) or intervention (ie, coronary angioplasty) occurred within 2 months from D-dimer measurement.

The first reviewer (FM) extracted the following data: first author's name, year of publication, study design, country, D-dimer assay, use of comedication (when reported), number of participants, time of exposure, antiplatelet regimen, D-dimer levels (on antiplatelet therapy and without it) and D-dimer variation. In one case, D-dimer measurements were not reported and were extracted from a graph. D-dimer levels were then converted to μg/mL. All results were doublechecked and confirmed by the second reviewer (SS-G).

Figure 1: PRISMA 2009 flow diagram



2.4 Risk of bias assessment

Each study was then evaluated according to the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2),[10] to assess risk of bias and conflicts of interest. We partially modified the original form *ad hoc*, by selecting the appropriate items and omitting irrelevant questions. The revised tool is described in detail in Methods S3, page 112. The reviewers (SS-G and FM) independently applied the modified QUADAS-2 score to all studies and discussed each evaluation to reach consensus. Furthermore, we visualized the distribution of the studies within a funnel plot, to detect potential publication bias. Therefore, we used the "trim and fill" adjustment method,[11] Egger test[12] and Begg's rank correlation.[13]

2.6 Quantative data-synthesis

The meta-analysis was conducted using Comprehensive Meta-analysis (version 3; Biostat).

When median and interquartile range (IQR) were reported, we estimated the average SD using the following formula: SD = [(IQR75-IQR25)/1.35]. In case of reporting median and full range, we estimated the average SD using the following formula: SD = [(IQR75-IQR25)/2.58]. Standard error of mean (SEM) was converted to SD by the formula SEM x √n. If 95% confidence interval (CI) was reported, we calculated the SD by estimating the SE as follows: [(95% CI upper limit – 95%CI lower limit) / (2x1.96)].[14]

For controlled trials, D-dimer change was calculated as follows: [(value at end of follow-up in the treatment group – value at baseline in the treatment group) – (value at end of follow-up in the control group – value at baseline in the control group)]. If mean fold change was reported, we estimated mean D-dimer after treatment by multiplying mean D-dimer before treatment with 1+ percentage change, and assumed that SD before treatment was equal to SD after treatment. For cohort studies, we calculated D-dimer variation by (value at end of follow-up in the treatment group – value at baseline in the treatment group), assuming that in a fictional control group D-dimer would not change during follow-up. For cross-sectional studies, we measured D-dimer difference by (value in antiplatelet users – value in nonusers).

Effect sizes were calculated using the dimensionless Cohen's d as the summary statistic,[15, 16] and expressed as standardized mean difference (SMD) with its corresponding 95% confidence intervals (CI).

We evaluated the influence of each study on the overall effect size by removing one study each time and repeating the analysis, a so-called leave-one-out method sensitivity analysis.[17]

Post-hoc subanalyses were performed to assess the potential effects of study design and underlying conditions (cardiovascular diseases, atrial fibrillation, chronic hemodialysis and HIV infection).

Results

Study selection

Based on title and abstract, we initially screened 1000 articles. Of these, 71 studies were assessed for eligibility by reading full text. After this evaluation, 17 studies were selected for review and meta-analysis (Figure 1). Reasons for exclusion were: no D-dimer values available at baseline or during follow-up (n=24), not written in English (n=11), event or intervention within 2 months from measurement (n=8), no original research article (n=6), drug exposure < 7 days (n=3), antiplatelet agent for parental use (n=1), nonstandardized D-dimer measurement (n=1).

These 17 studies consisted of 5 controlled trials,[18, 19, 20, 21, 22] 7 cohort studies[23, 24, 25, 26, 27, 28, 29] and 5 crosssectional studies[30, 31, 32, 33, 34] (Table 1), for a total of 1117 participants. This heterogeneous group included subjects affected by cardiovascular diseases (8 studies) and atrial fibrillation (4 studies), patients on dialysis (2 studies), healthy volunteers (2 studies) and HIV infected patients (1 study).

3.2 Meta-analysis and evaluation of bias

Meta-analysis involving all 1117 participants showed no reduction of D-dimer levels in patients on antiplatelet treatment, compared to controls (SMD: -0.015, 95% CI -0.182; 0.151, p= 0.855, Figure 2). The estimated effect sizes were similar in sensitivity analyses that omitted any single study (Figure 3). This effect did not change among the different study designs and underlying conditions (p=0.900 and p=0.584 respectively).

According to the QUADAS-2 score the risk of bias for most studies was high, as well as the rate of reported or potential conflicts of interest, as shown in Figure 4a,4b.
A visual inspection of the funnel plot showed asymmetry, suggesting potential publication bias. Using the "trim and fill" method by Duval and Tweedie, with 8 potentially missing studies imputed, the effect size was estimated to an adjusted SMD with still a non-significant effect (0.143, 95% CI -0.014; p= 0.300) (Figure 5). Egger regression test (intercept -3.246, 95% CI -6.408; -0.084, two tailed p = 0.045) was significant. However, Begg's rank correlation (Kendall's Tau with continuity correction = -0.124, Z = 0.785, twotailed p = 0.432) was nonsignificant.

Table 1: Characteristics of the selected studies

	Location	Population	Co-medication	Test	Age (years)*	Exposure	Dose (daily)	Participants (n)	D-dimer before exposure (µg/ml)	D-dimer after exposure (µg/ml)	Conclusion	Details
Controlled trials												
Derhaschnig, 2010	Austria	healthy volunteers (male)	none	ELISA Boerhinger Mannheim FRG	19-35 (range)	7 d	NO-ASA 800 mg	10	0.1 (0.05-0.2)	0.1 (0.1-0.2)	no effect	RCT
							ASA 425 mg	10	0.2 (0.01-0.2)	0.1 (0.06-0.2)		
							placebo	10	0.2 (0.02-0.3)**	0.2 (0.04-0.3)**		
Kim SB, 2002	Korea	peritoneal dialysis	exclusion: statins	ELISA assera-chrom D-Di, Diagno-stica Stago	56 ±12	8 w	ASA 200 mg	43	1.07 ±0.51	0.84 ±0.53	no effect	
							no ASA	41	1.01 ±0.60^	0.98 ±0.61^		
Kim KM, Kim HW, 2009	Korea	peritoneal dialysis	exclusion: statins	ELISA assera-chrom D-Di, Diagno-stica Stago	54	8 w	beraprost sodium 120 µg	45	0.87 ±1.12	0.62 ±0.53	significant reduction	
							no beraprost	49	1.15 ±1.60^	1.18 ±1.71^		
O'Brien, 2016	US	HIV	exclusion: NSAID	ELISA Diagnostica Stago	49	12 w	ASA 300 mg	38	0.16 ±0.07	1.08 (0.97-1.19)	no effect	RCT
							ASA 100 mg	38	0.16 ±0.08	0.99 (0.89-1.10)		
							Placebo	36	0.17 ±0.1 ^	1.02 (0.91-1.13) ""		
Trellopoulos, 2014	Greece	elective endovascular aneurysm repair	statins	ELISA Vidas D-Dimer Biomerieux	72.6	unknown	anti-platelet (aspirin, clopidogrel or dual therapy)	23	1.1 (0-2.66)	1.9 (0.8-3)	no effect	data from graph
							not on anti-platelet	12	1.4 (0.16-2.63) **	1.36 (0.5-2.24) **		

Table 1: Continued

	Location	Population	Co-medication	Test	Age (years)*	Exposure	Dose (daily)	Participants (n)	D-dimer before exposure (µg/ml)	D-dimer after exposure (µg/ml)	Conclusion	Details
Cohort studies												
Aliberti, 1997	Italy	aorto, femoral or carotid athero-sclerosis	none (2 w wash out)	LPIA Baxter-Dade	69.9 ±8.9	30 d	picotamide 900 mg	28	0.53 ± 0.09^	0.51 ± 0.71^	no effect	
Eritsland, 1992	Norway	coronary artery bypass grafting		ELISA assera-chrom D-Di, Diagno-stica Stago	57	9 m	ASA 300 mg	30	0.315 (0.09-2.35) ^^^	0.273 (0.12-1.4)^^^ ^^^	no effect	
Kamath, 2002 (American College of Cardiology)	UK	atrial fibrillation	exclusion: NSAID, corticosteroids, hormone replacement	ELISA Techno-lone, Wien, Austria	71 ±8	6 w	ASA 75 mg & clopidogrel 75 mg	32	1.1 (0.595-1.6)^^	0.835 (0.538-1.9)^^	no effect	recent stroke or MI excluded
Kim KM, Kim H, 2009	Korea	healthy volunteers	none	ELISA assera-chrom D-Di, Diagno-stica Stago	31.9 ±4.7	14 d	ASA 100 mg	20	0.28 ±0.11	0.28 ±0.15	no effect	open-label
							sarpogrelate 300 mg	20	0.25 ±0.09	0.28 ±0.15		
							beraprost sodium 120 µg	20	0.29 ±0.13^	0.27 ±0.14^		
Lip, 1996	UK	atrial fibrillation		ELISA (AGEN)	71.3 ±7.3	6 w	ASA 300 mg	26	0.186 (0.123 – 0.437) ^^	0.211 (0.113-0.363) ^^	no effect	
Panchenko, 1997	Russia	claudicatio intermittens (male)	none (3 months wash out)	ELISA Boehringer Mannheim	57 ±1.5	6 m	indobufen 400 mg	29	0.834 ±0.183	0.833 ±0.194	no effect	randomized open-label
							pentoxi-fylline 600 mg	29	0.612 ±0.129”	0.62 ±0.116”		

Table 1: Continued

	Location	Population	Co-medication	Test	Age (years)*	Exposure	Dose (daily)	Participants (n)	D-dimer before exposure (µg/ml)	D-dimer after exposure (µg/ml)	Conclusion	Details
Park, 2017	Korea	atrial fibrillation	all on ASA statins anti-hypertensive drugs	LPIA STA-Liatest D-DI reagent (Diagno-stica Stago)	63.5 ±9	30 d	clopidogrel 75 mg	20	0.722 ±0.1108^	0.7255 ±0.1203 ^	no effect	add-on therapy
Cross-sectional study												
Kamath, 2002 (European Heart Journal)	UK	atrial fibrillation	exclusion: NSAID, corticosteroids, hormone replacement	ELISA Techno-clone	72 ±8	unknown	ASA (75-325 mg)	60	-	1.665 (0.855-0.5137)	no effect	
					70 ±10		no anti-thrombotic therapy	93		1 (0.64-1.7)^^		
Bailey, 2015	UK	abdominal aortic aneurysm	statins, anti-hypertensive drugs	ELISA Sekisui Diagnostics	70.1 ±7	≥ 30 d	ASA 75 mg	46	-	0.357 (0.209-0.629)	no effect	
					71.6 ±7.3		not on ASA	50		0.351 (0.177-0.681) ^^		
Greilich, 1994	US	coronary artery disease	anti-hypertensive drugs	not reported	not reported	unknown	ASA (dose?)	10	-	1.076 ± 0.196	no effect	
							no ASA	4		1.638 ± 0.581^		
Reininger, 1996	Germany	peripheral arterial disease	anti-hypertensive drugs	ELISA Diagnostica Stago	59.5	unknown	ASA (dose?)	40	-	1.006 0.183	no effect	
							no ASA	52		0.942 0.152”		

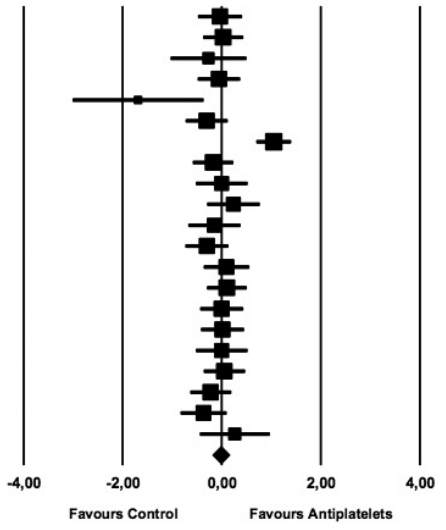
Table 1: Continued

	Location	Population	Co-medication	Test	Age (years)*	Exposure	Dose (daily)	Participants (n)	D-dimer before exposure (µg/ml)	D-dimer after exposure (µg/ml)	Conclusion	Details
Tohgi, 1993	Japan	after stroke	anti-hypertensive drugs	ELISA Dimer Test EIA Agen Ltd	63 ±11	>6 m	ticlopidine 200 mg	78	-	0.116± 0.076	no effect	
							aspirin 40 mg	42		0.108± 0.054		
							no anti-platelet	33		0.136± 0.11^		

** mean (±SD, when reported)*
*** mean (95% CI)*
^ mean ±SD
^^ median (IQR)
^^^ median (range)
“ mean ±SEM
“” geometric mean fold change
Abbreviations: d = days, w = weeks, m = months, ELISA = Enzyme-Linked Immuno Sorbent Assay, LPIA = latex-enhanced photometric immunoassays; OAC = oral anticoagulant, ASA = acetylsalicylic acid.

Figure 2: Forest plot for the effect of antiplatelet therapy on plasma D-dimer level

Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Aliberti et al. (1997)	-0,034	0,224	0,050	-0,473	0,404	-0,154	0,878
Bailey et al. (2015)	0,029	0,204	0,042	-0,371	0,430	0,143	0,886
Derhashnig et al. (2010)	-0,266	0,389	0,151	-1,029	0,496	-0,685	0,493
Eritsland et al. (1992)	-0,055	0,216	0,047	-0,479	0,369	-0,254	0,799
Greilich et al. (1994)	-1,687	0,672	0,452	-3,005	-0,370	-2,511	0,012
Kamath et al. (2002a)	-0,304	0,214	0,046	-0,723	0,115	-1,421	0,155
Kamath et al. (2002b)	1,050	0,176	0,031	0,705	1,395	5,961	0,000
Kim K.M. et al. (2009)	-0,175	0,207	0,043	-0,580	0,231	-0,845	0,398
Kim K.M., Kim H. et al. (2009a)	0,000	0,265	0,070	-0,519	0,519	0,000	1,000
Kim K.M., Kim H. et al. (2009b)	0,237	0,268	0,072	-0,289	0,762	0,882	0,378
Kim K.M., Kim H. et al. (2009c)	-0,148	0,266	0,071	-0,669	0,373	-0,556	0,578
Kim S.B. et al. (2002)	-0,300	0,220	0,048	-0,730	0,130	-1,367	0,172
Lip et al. (1996)	0,096	0,233	0,054	-0,360	0,552	0,414	0,679
O'Brien et al. (2016)	0,105	0,202	0,041	-0,292	0,501	0,517	0,605
Panchenko et al. (1997a)	0,000	0,220	0,048	-0,431	0,431	0,000	1,000
Panchenko et al. (1997b)	0,015	0,220	0,048	-0,415	0,446	0,069	0,945
Park et al. (2017)	0,000	0,265	0,070	-0,519	0,519	0,000	1,000
Reininger et al. (1997)	0,054	0,210	0,044	-0,359	0,466	0,254	0,799
Tohgi et al. (1993a)	-0,223	0,208	0,043	-0,631	0,185	-1,069	0,285
Tohgi et al. (1993b)	-0,366	0,235	0,055	-0,826	0,093	-1,562	0,118
Trellopoulos et al. (2014)	0,265	0,358	0,128	-0,436	0,965	0,740	0,459
	-0,015	0,085	0,007	-0,182	0,151	-0,183	0,855



Effect sizes are expressed as standardized mean difference (SMD) with its corresponding 95% confidence intervals (CI) using Cohen's d as the summary statistic. A random-effect model has been used for performance of the meta-analysis.

Figure 3: Leave-one-out sensitivity analysis of the effect of antiplatelet therapy on D-dimer level

Study name	Statistics with study removed						
	Point	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Aliberti et al. (1997)	-0,016	0,089	0,008	-0,191	0,159	-0,178	0,859
Bailey et al. (2015)	-0,020	0,090	0,008	-0,196	0,156	-0,219	0,826
Derhashnig et al. (2010)	-0,008	0,087	0,008	-0,179	0,162	-0,095	0,924
Eritsland et al. (1992)	-0,015	0,089	0,008	-0,190	0,161	-0,165	0,869
Greilich et al. (1994)	0,008	0,081	0,007	-0,150	0,166	0,100	0,921
Kamath et al. (2002a)	0,000	0,088	0,008	-0,171	0,172	0,004	0,996
Kamath et al. (2002b)	-0,075	0,053	0,003	-0,179	0,030	-1,400	0,162
Kim K.M. et al. (2009)	-0,008	0,089	0,008	-0,182	0,167	-0,085	0,933
Kim K.M., Kim H. et al. (2009a)	-0,017	0,089	0,008	-0,191	0,156	-0,196	0,844
Kim K.M., Kim H. et al. (2009b)	-0,028	0,088	0,008	-0,201	0,144	-0,319	0,750
Kim K.M., Kim H. et al. (2009c)	-0,010	0,088	0,008	-0,183	0,163	-0,116	0,908
Kim S.B. et al. (2002)	-0,000	0,088	0,008	-0,172	0,171	-0,002	0,998
Lip et al. (1996)	-0,023	0,089	0,008	-0,197	0,152	-0,255	0,799
O'Brien et al. (2016)	-0,024	0,090	0,008	-0,200	0,152	-0,268	0,789
Panchenko et al. (1997a)	-0,018	0,089	0,008	-0,193	0,158	-0,199	0,842
Panchenko et al. (1997b)	-0,019	0,090	0,008	-0,194	0,157	-0,209	0,835
Park et al. (2017)	-0,017	0,089	0,008	-0,191	0,156	-0,196	0,844
Reininger et al. (1997)	-0,021	0,090	0,008	-0,197	0,155	-0,234	0,815
Tohgi et al. (1993a)	-0,005	0,089	0,008	-0,178	0,169	-0,052	0,959
Tohgi et al. (1993b)	0,003	0,087	0,008	-0,167	0,173	0,034	0,973
Trellopoulos et al. (2014)	-0,026	0,087	0,008	-0,197	0,145	-0,295	0,768
	-0,015	0,085	0,007	-0,182	0,151	-0,183	0,855

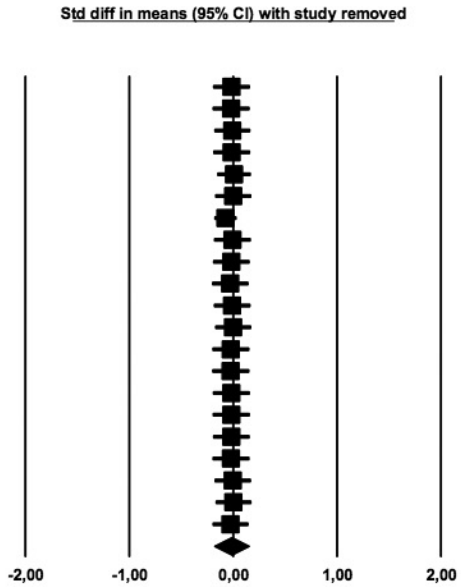


Figure 4a: Tabular presentation of QUADAS-2 results of the 17 studies included

Study	RISK OF BIAS				APPLICABILITY CONCERNS	
	PATIENT SELECTION	INDEX TEST	FLOW AND TIMING	CONFLICTS OF INTEREST	PATIENT SELECTION	INDEX TEST
Controlled trials						
Derhaschnig, 2010	☺	☺	☺	☹	☺	☺
Kim SB, 2002	☺	☹	☹	☹	☹	☺
Kim KM, Kim HW, 2009	☹	☺	☺	☹	☹	☺
O'Brien, 2016	☺	☺	☺	☹	☺	☺
Trellopoulos, 2014	☹	☺	NA	☺	☹	☺
Cohort studies						
Aliberti, 1997	☹	☺	☺	?	☺	☺
Eritsland, 1992	☹	☺	☹	☺	☹	☺
Kamath, 2002 (JACC)	☹	☺	?	☹	☹	☺
Kim KM, Kim H, 2009	☹	☺	☺	☺	☺	☺
Lip, 1996	☹	☺	?	?	☹	☺
Panchenko, 1997	☹	☺	☺	?	☺	☺
Park, 2017	☹	☺	☺	☹	☹	☺
Cross-sectional studies						
Kamath, 2002 (EHJ)	☹	☺	NA	☹	☹	☺
Bailey, 2015	☹	☺	NA	☺	☹	☺
Greilich, 1994	☹	?	NA	☺	☹	?
Reininger, 1996	☹	☺	NA	☺	☹	☺
Tohgi, 1993	☹	☺	NA	?	☹	☺

☺Low Risk *☹High Risk* *? Unclear Risk* *NA Not Applicable*

Figure 4b: Graphic display for QUADAS-2 results of the 17 studies included

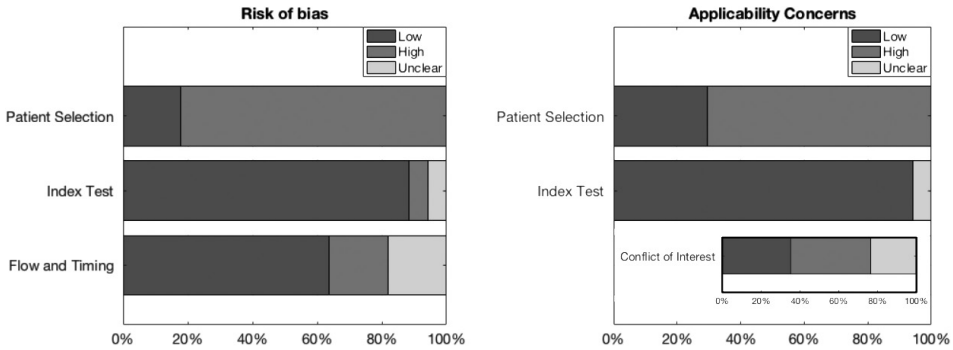
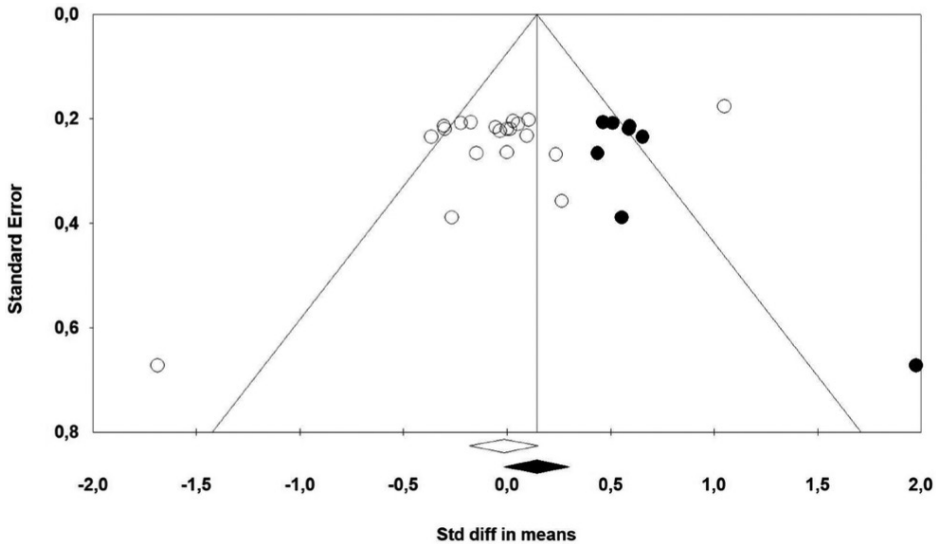


Figure 5: Funnel plot of publication bias of the included studies, according to the "trim and fill" method of Duval and Tweedie



Observed studies are shown as open circles, imputed studies are shown as filled circles.

Discussion

The goal of this analysis was to find out whether the D-dimer level is affected by antiplatelet treatment. If true, this change in D-dimer level could potentially affect the accuracy of clinical decision tools commonly used to diagnose VTE.

To answer this question, we performed a systematic review and meta-analysis of current literature, and finally selected 17 studies. We decided to include not only controlled trials but also cohort and cross-sectional studies. Except for the study by Kim et al. (2009), none of these studies showed a change in D-dimer levels in patients receiving antiplatelet drugs. In particular, the two randomized trials by Derhaschnig et al. and O'Brien et al., which are considered to be of superior quality (in terms of study design and population), confirmed this result.

Antiplatelet drugs inhibit platelet aggregation through different pathways. Low-dose aspirin inhibits cyclooxygenase 1, leading to decreased production of thromboxane$_2$, an important mediator of platelet aggregation. Clopidogrel and prasugrel inhibit platelet activation by preventing glycoprotein IIB/IIIa conformational change needed for fibrinogen binding.[35]

Beside their antithrombotic effect, antiplatelet drugs also have anti-inflammatory properties which might influence D-dimer levels as well.[5] Based on these observations, and the fact that use of aspirin is associated with a reduction in postoperative VTE risk[7], antiplatelet therapy could be expected to reduce D-dimer levels.

However, the study by Kim et al. was the only one that revealed a clear reduction of D-dimer levels, in patients on chronic dialysis receiving beraprost sodium, a prostacyclin analogue. Actually, there are at least three aspects we need to take into account when evaluating this finding. First, the risk of bias is high, especially when looking at the difference in the D-dimer level at baseline (0.87 ug/mL in the treatment group compared with 1.15 ug/mL in controls). Second, this favorable outcome could have been influenced by pharmaceutical industry sponsorship.[36] Third, this is one of the few studies that investigated beraprost sodium, whose mechanism of action differs from classical antiplatelet drugs such as aspirin or clopidogrel, possibly leading to a different impact on fibrinolysis (which, so far, has not been investigated).

All other included studies show no reduction of D-dimer levels in patients on treatment compared with controls (Figure 2). When looking at the effect sizes of the different studies on the forest plot, the study by Greilich et al. clearly stands out, since D-dimer level in treated group is much higher than in controls. This could be due to the small size of the treated group (only 4 patients).

For what concerns publication bias, the "trim and fill" adjustment method and Begg's rank correlation suggest that publication bias do not seem to affect the result.

When looking at potential limitations, we chose to exclude studies in which any cardiovascular event (ie, stroke) or intervention (ie, coronary angioplasty) occurred within 2 months from D-dimer measurement. The reason behind our decision is that these events can lead to an increase of D-dimer level, as an acute-phase reactant. In case of inclusion of studies with a recent cardiovascular event or intervention, a possible effect of antiplatelet drugs on the D-dimer level could have been misinterpreted or missed.

Furthermore, different immunoassays are used to measure D-dimer concentration. This variation may influence the size of D-dimer change, and thus the effect of antiplatelet drugs. However, we excluded studies in which nonstandardized D-dimer measurements were used. In our meta-analysis D-dimer levels were determined by validated assays, reason for which variability of laboratory tests is unlikely to influence outcome.

Beside these aspects, we also want to underline that our review and meta-analysis focuses specifically on antiplatelet drugs. Our work does not investigate the effect of other drugs that can also affect platelet aggregation, such as statins and dronedarone.[37, 38]

Recently, some of the investigators of this review performed a post-hoc analysis in the YEARS diagnostic study[39], comparing D-dimer levels among users and nonusers of antiplatelet drugs. Once more, use of antiplatelet drugs had no significant effect.[40]
In conclusion, antiplatelet drugs do not seem to influence D-dimer levels. Therefore, taken into account the limitations mentioned above, the accuracy of diagnostic algorithms for VTE should be high also in patients exposed to these drugs.

References

1. Chapin JC, Hajjar KA. Fibrinolysis and the control of blood coagulation. *Blood Rev* 2015;29(1):17-24.
2. Santos-Gallego CG, Bayon J, Badimon JJ. Thrombi of different pathologies: implications for diagnosis and treatment. *Curr Treat Options Cardiovasc Med*. 2010; 12(3):274-91.
3. Wells PS. Integrated strategies for the diagnosis of venous thromboembolism. *J Thromb Haemost* 2007;5 Suppl 1:41-50.
4. Adam SS, Key NS, Greenberg CS. D-dimer antigen: current concepts and future prospects. *Blood* 2009;113(13):2878-87.
5. Arazi HC, Badimon JJ. Anti-inflammatory effects of anti-platelet treatment in atherosclerosis. *Curr Pharm Des* 2012;18(28):4311-25.
6. Szczeklik A, Krzanowski M, Gora P, Radwand J. Antiplatelet drugs and generation of thrombin in clotting blood. *Blood* 1992;80(8):2006-11.
7. Hovens MM, Snoep JD, Tamsma JT, Huisman MV. Aspirin in the prevention and treatment of venous thromboembolism. *J Thromb Haemost* 2006;4(7):1470-5.
8. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med* 2009;151(4): 264-9.
9. Elias A, Bonfils S, Daoud-Elias M, Gauthier B, Sie P, Boccalon H, Boneu B. Influence of long term oral anticoagulants upon prothrombin fragment 1 + 2, thrombin-antithrombin III complex and D-Dimer levels in patients affected by proximal deep vein thrombosis. *Thromb Haemost* 1993;69(4):302-5.
10. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, Leeflang MM, Sterne JA, Bossuyt PM, QUADAS-2 group. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med* 2011;155(8): 529-36.
11. Duval S, Tweedie R. Trim and fill: A simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics* 2000; 56(2): 455-63.
12. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; 315(7109): 629-34.
13. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994;50(4): 1088-101.
14. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. *BMC Med Res Methodol* 2014;14:135.
15. Lakens D. Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. *Front Psychol* 2013;4: 863.
16. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. Hillsdale, NJ: Lawrence Erlbaum Associates; 1988.
17. Cooper H, Hedges LV. *The Handbook of Research Synthesis*. New York, NY: Russell Sage Foundation; 1994.
18. Derhaschnig U, Schweeger-Exeli I, Marsik C, Cardona F, Minuz P, Jilma B. Effects of aspirin and NO-aspirin (NCX 4016) on platelet function and coagulation in human endotoxemia. *Platelets* 2010;21(5):320-8.
19. Kim SB, Lee SK, Min WK, Chi HS, Park JS. Lack of effects of low-dose aspirin on high-sensitivity C-reactive protein, hemostatic factors, and troponin T in CAPD patients. *Perit Dial Int* 2002;22(6):721-3.

20. Kim KM, Kim HW, Lee JH, Chang JW, Park JS, Kim SB. Effects of beraprost sodium, an oral prostaglandin i2 analog, on hemostatic factors and inflammation in chronic peritoneal dialysis patients. *Perit Dial Int* 2009;29(2):178-81.
21. O'Brien MP, Hunt PW, Kitch DW, Klingman K, Stein JH, Funderburg NT, Berger JS, Tebas P, Ciagett B, Moisi D, Utay NS, Aweeka F, Aberg JA. A Randomized Placebo Controlled Trial of Aspirin Effects on Immune Activation in Chronically Human Immunodeficiency Virus Infected Adults on Virologically Suppressive Antiretroviral Therapy. *Open Forum Infect Dis* 2017;4(1):ofw278.
22. Trellopoulos G, Georgiadis GS, Nikolopoulos ES, Kapoulas KC, Georgakarakos EI, Lazarides MK. Antiplatelet treatment and prothrombotic diathesis following endovascular abdominal aortic aneurysm repair. *Angiology* 2014;65(9):783-7.
23. Aliberti G, Proietta M, Pulignano I. Platelet and hemocoagulative changes in elderly atherosclerotic patients after treatment with the antiaggregating drug picotamide. *Arch Gerontol Geriatr* 1997;25(2):193-200.
24. Eritsland J, Seljeflot I, Arnesen H, Smith P, Westvik AB. Effects of long-term treatment with warfarin on fibrinogen, FPA, TAT, and D-dimer in patients with coronary artery disease. *Thromb Res* 1992;66(1):55-60.
25. Kamath S, Blann AD, Chin BSP, Lip GYH. A prospective randomized trial of aspirin-clopidogrel combination therapy and dose-adjusted warfarin on indices of thrombogenesis and platelet activation in atrial fibrillation. J Am Coll *Cardiol* 2002;40(3):484-90.
26. Kim KM, Kim H, Chi HS, Park JS, Kim SB. Comparison of antiplatelet potency of sarpogrelate, aspirin, and beraprost in healthy volunteers according to in-vitro closure time. *Blood Coagul Fibrinolysis* 2010;21(3):262-5.
27. Lip GY, Lip PL, Zarifis J, Watson RD, Bareford D, Lowe GD, Beevers DG. Fibrin D-dimer and beta-thromboglobulin as markers of thrombogenesis and platelet activation in atrial fibrillation. Effects of introducing ultra-low-dose warfarin and aspirin. *Circulation* 1996;94(3):425-31.
28. Panchenko E, Eshkeeva A, Dobrovolsky A, Titaeva E, Podinovskaya Y, Hussain KM, Karpov Y. Effects of indobufen and pentoxifylline on walking capacity and hemostasis in patients with intermittent claudication: results of six months of treatment. *Angiology* 1997;48(3):247-54.
29. Park Y, Kim KH, Kang MG, Ahn JH, Jang JY, Park HW, Koh JS, Park JR, Hwang SJ, Lee HR, Kwak CH. Antiplatelet Therapy Combinations and Thrombogenicity in Patients with Non-Valvular Atrial Fibrillation. *Korean Circ J* 2017; 47(3): 366–376.
30. Kamath S, Blann AD, Chin BS, Lanza F, Aleil B, Cazenave JP, Lip GY. A study of platelet activation in atrial fibrillation and the effects of antithrombotic therapy. *Eur Heart J* 2002;23(22):1788-95.
31. Bailey MA, Aggarwal R, Bridge KI, Griffin KJ, Iqbal F, Phoenix F, Purdell-Lewis J, Thomas T, Johnson AB, Ariens RA, Scott DJ, Ajjan RA. Aspirin therapy is associated with less compact fibrin networks and enhanced fibrinolysis in patients with abdominal aortic aneurysm. *J Thromb Haemost* 2015;13(5):795-801.
32. Greilich PE, Carr ME, Zekert SL, Dent RM. Quantitative assessment of platelet function and clot structure in patients with severe coronary artery disease. *Am J Med Sci* 1994;307(1):15-20.
33. Reininger CB, Graf J, Reininger AJ, Spannagl M, Steckmeier B, Schweiberer L. Increased platelet and coagulatory activity indicate ongoing thrombogenesis in peripheral arterial disease. *Thromb Res* 1996;82(6):523-32.
34. Tohgi H, Takahashi H, Chiba K, Tamura K. Coagulation-fibrinolysis system in poststroke patients receiving antiplatelet medication. *Stroke* 1993;24(6):801-4.

35. Onselaer MB, Hardy AT, Wilson C, Sanchez X, Babar AK, Miller JLC, Watson SK, Bonna A, Philippou H, Herr AB, Mezzano D, Ariens RAS, Watson SP. Fibrin and D-dimer bind to monomeric GPVI. *Blood Adv.* 2017 Aug 15;1(19):1495-1504.
36. Lexchin J, Bero LA, Djulbegovic B, Clark O. Pharmaceutical industry sponsorship and research outcome and quality: systematic review. *BMJ* 2003; 326(7400): 1167.
37. F. Violi, C. Calvieri, D. Ferro, P. Pignatelli. Statins as antithrombotic drugs. *Circulation* 2013 Jan;127(2):251-7.
38. Zafar MU, Santos-Gallego CG, Smith DA, Halperin JL, Badimon JJ. Dronedarone exerts anticoagulant and antiplatelet effects independently of its antiarrhythmic actions. *Atherosclerosis* 2017 Nov;266:81-86.
39. van der Hulle T, Cheung WY, Kooij S, Beenen LFM, van Bemmel T, van Es J, Faber LM, Hazelaar GM, Heringhaus C, Hofstee H, Hovens MMC, Kaasjager KAH, van Klink RCJ, Kruip MJHA, Loeffen RF, Mairuhu ATA, Middeldorp S, Nijkeuter M, van der Pol LM, Schol-Gelok L et al. Simplified diagnostic management of suspected pulmonary embolism (the YEARS study): a prospective, multicentre, cohort study. *Lancet* 2017;390:289-297.
40. Schol-Gelok S, van der Hulle T, Biedermann JS, van Gelder T, Klok FA, van der Pol LM, Versmissen J, Huisman MV, Kruip MJHA. Clinical effects of antiplatelet drugs and statins on D dimer levels. *Eur J Clin Invest* 2018;48(7): e12944.

Methods S1. Full search strategy on the effect of statins on D-dimer levels

Embase.com (Embase incl. Medline): 204 articles

('D dimer'/de OR 'fibrin fragment d'/de OR ('D-dimer' OR 'D-dimers' OR 'D1 dimer' OR 'fibrin fragment DD' OR 'fibrin fragment D' OR (crosslinked NEAR/3 fibrin* NEAR/3 degradation)):ab,ti) AND ('hydroxymethylglutaryl coenzyme A reductase inhibitor'/exp OR (((Hydroxymethylglutaryl OR HMG) NEAR/3 (CoA OR 'Coenzyme A') NEAR/3 (inhibitor*)) OR atorvastatin* OR bervastatin* OR cerivastatin* OR crilvastatin* OR dalvastatin* OR fluvastatin* OR fluindostatin* OR glenvastatin* OR lovastatin* OR mevinolin* OR mevastatin* OR compactin* OR pitavastatin* OR pravastatin* OR rosuvastatin* OR simvastatin* OR tenivastatin* OR Zocor* OR Lipitor* OR selektin* OR lescol* OR statin*):ab,ti) NOT ('Conference Abstract'/it OR 'case report'/de)

Medline Epub (Ovid): 72 articles

(("fibrin fragment D" OR "D-dimer" OR "D-dimers" OR D1-dimer*).mp. OR ("D-dimer" OR "D-dimers" OR "D1 dimer" OR "fibrin fragment DD" OR "fibrin fragment D" OR (crosslinked ADJ3 fibrin* ADJ3 degradation)).ab,ti.) AND ("Hydroxymethylglutaryl-CoA Reductase Inhibitors".mp. OR (((Hydroxymethylglutaryl OR HMG) ADJ3 (CoA OR "Coenzyme A") ADJ3 (inhibitor*)) OR atorvastatin* OR bervastatin* OR cerivastatin* OR crilvastatin* OR dalvastatin* OR fluvastatin* OR fluindostatin* OR glenvastatin* OR lovastatin* OR mevinolin* OR mevastatin* OR compactin* OR pitavastatin* OR pravastatin* OR rosuvastatin* OR simvastatin* OR tenivastatin* OR Zocor* OR Lipitor* OR selektin* OR lescol* OR statin*).ab,ti.)

Cochrane Central: 28 articles

(('D-dimer' OR 'D-dimers' OR 'D1 dimer' OR 'fibrin fragment DD' OR 'fibrin fragment d' OR (crosslinked NEAR/3 fibrin* NEAR/3 degradation)):ab,ti) **AND** ((((Hydroxymethylglutaryl OR HMG) NEAR/3 (CoA OR 'Coenzyme A') NEAR/3 (inhibitor*)) OR atorvastatin* OR bervastatin* OR cerivastatin* OR crilvastatin* OR dalvastatin* OR fluvastatin* OR fluindostatin* OR glenvastatin* OR lovastatin* OR mevinolin* OR mevastatin* OR compactin* OR pitavastatin* OR pravastatin* OR rosuvastatin* OR simvastatin* OR tenivastatin* OR Zocor* OR Lipitor* OR selektin* OR lescol* OR statin*):ab,ti)

Web of Science: 70 articles

TS=(((("D-dimer" OR (fibrin NEAR/1 fragment NEAR/1 (DD OR D)) OR (crosslinked NEAR/2 fibrin* NEAR/2 degradation))) AND ((((Hydroxymethylglutaryl OR HMG)

NEAR/4 (inhibitor*)) OR atorvastatin* OR bervastatin* OR cerivastatin* OR crilvastatin* OR dalvastatin* OR fluvastatin* OR fluindostatin* OR glenvastatin* OR lovastatin* OR mevinolin* OR mevastatin* OR compactin* OR pitavastatin* OR pravastatin* OR rosuvastatin* OR simvastatin* OR tenivastatin* OR Zocor* OR Lipitor* OR selektin* OR lescol* OR statin*))) AND DT=(Article OR Review)

Google Scholar: 100 articles (top relevant references)
"D-dimer"|"D-dimers"|"D1 dimer" statin|statins|"hydroxymethylglutaryl|HMG CoA reductase"

Methods S2. Full search strategy on the effect of antiplatelet drugs on D-dimer levels

Embase.com (Embase incl. Medline): 672 articles

('D dimer'/de OR ('D-dimer' OR 'D-dimers' OR 'D1 dimer' OR 'fibrin fragment DD' OR (crosslinked NEAR/3 fibrin* NEAR/3 degradation)):ab,ti) AND 'antithrombocytic agent'/exp OR ((platelet* OR thrombocyt*) NEAR/3 (inhibitor* OR antiaggregant* OR antagonist*)) OR (anti NEXT/1 (platelet* OR thrombocyt*)) OR antiplatelet* OR antithrombocyt* OR 'acetylsalylic acid' OR ((acetyl*) NEXT/1 (salicyl*)) OR aspirin* OR caprin* OR aspro* OR Easprin* OR ZORprin* OR aspegic* OR anagrelide* OR ataprost* OR atopaxar* OR beraprost* OR cangrelor* OR kengreal* OR kengrexal* OR cilostazol* OR clopidogrel* OR grepid* OR iscover* OR Plavix* OR dazoxiben* OR dehydrocilostazol* OR dipyridam* OR dipiridam* OR dipyrol* OR Persantin* OR elinogrel* OR abciximab OR eptifibatide* OR integrilin* OR integrelin* OR lefradafiban* OR lotrafiban* OR orbofiban* OR roxifiban* OR sibrafiban* OR tirofiban* OR Aggrastat* OR agrastat* OR xemilofiban* OR ifetroban* OR iloprost* OR ciloprost* OR Ventavist* OR indobufen* OR itazigrel* OR linotroban* OR nafazotrom* OR naxaprostene* OR octimibate* OR oxagrelate* OR pamicogrel* OR pentoxifylline* OR oxpentifylline* OR Trental* OR picotamide* OR plafibride* OR prasugrel* OR Effient* OR Efient* OR epoprostenol* OR cycloprostin* OR Flolan* OR prostavasin* OR Alpostadil* OR regrelor* OR samixogrel* OR sarpogrelate* OR satigrel* OR sulfinpyrazon* OR sulphinpyrazon* OR Anturan* OR taprostene* OR temanogrel* OR terbogrel* OR terutroban* OR ticagrelor* OR brilique* OR possia* OR ticlopidine* OR Ticlid* OR triflusal* OR disgren* OR thrombodipin* OR (thrombin* NEAR/3 (antagonist* OR block*)) OR vorapaxar* OR zontivity*):ab,ti) NOT ('Conference Abstract'/it OR 'case report'/de)

Medline Epub (Ovid): 233 articles

(("fibrin fragment D" OR "D-dimer" OR "D-dimers" OR D1-dimer*).mp. OR ("D-dimer" OR "D-dimers" OR "D1 dimer" OR "fibrin fragment DD" OR "fibrin fragment D" OR (crosslinked ADJ3 fibrin* ADJ3 degradation)).ab,ti.) AND ((platelet* OR thrombocyt*) ADJ3 (inhibitor* OR antiaggregant* OR antagonist*)) OR (anti ADJ (platelet* OR thrombocyt*)) OR antiplatelet* OR antithrombocyt* OR "acetylsalylic acid" OR ((acetyl*) ADJ (salicyl*)) OR aspirin* OR caprin* OR aspro* OR Easprin* OR ZORprin* OR aspegic* OR anagrelide* OR ataprost* OR atopaxar* OR beraprost* OR cangrelor* OR kengreal* OR kengrexal* OR cilostazol* OR clopidogrel* OR grepid* OR iscover* OR Plavix* OR dazoxiben* OR dehydrocilostazol* OR dipyridam* OR dipiridam* OR dipyrol* OR Persantin* OR elinogrel* OR abciximab OR eptifibatide* OR integrilin*

OR integrelin* OR lefradafiban* OR lotrafiban* OR orbofiban* OR roxifiban* OR sibrafiban* OR tirofiban* OR Aggrastat* OR agrastat* OR xemilofiban* OR ifetroban* OR iloprost* OR ciloprost* OR Ventavist* OR indobufen* OR itazigrel* OR linotroban* OR nafazotrom* OR naxaprostene* OR octimibate* OR oxagrelate* OR pamicogrel* OR pentoxifylline* OR oxpentifylline* OR Trental* OR picotamide* OR plafibride* OR prasugrel* OR Effient* OR Efient* OR epoprostenol* OR cycloprostin* OR Flolan* OR prostavasin* OR Alpostadil* OR regrelor* OR samixogrel* OR sarpogrelate* OR satigrel* OR sulfinpyrazon* OR sulphinpyrazon* OR Anturan* OR taprostene* OR temanogrel* OR terbogrel* OR terutroban* OR ticagrelor* OR brilique* OR possia* OR ticlopidine* OR Ticlid* OR triflusal* OR disgren* OR thrombodipin* OR (thrombin* ADJ3 (antagonist* OR block*)) OR vorapaxar* OR zontivity*).ab,ti.)

Cochrane Central: 71 articles

(('D-dimer' OR 'D-dimers' OR 'D1 dimer' OR 'fibrin fragment DD' OR 'fibrin fragment d' OR (crosslinked NEAR/3 fibrin* NEAR/3 degradation)):ab,ti) AND ((platelet* OR thrombocyt*) NEAR/3 (inhibitor* OR antiaggregant* OR antagonist*)) OR (anti NEXT/1 (platelet* OR thrombocyt*)) OR antiplatelet* OR antithrombocyt* OR 'acetylsalylic acid' OR ((acetyl*) NEXT/1 (salicyl*)) OR aspirin* OR caprin* OR aspro* OR Easprin* OR ZORprin* OR aspegic* OR anagrelide* OR ataprost* OR atopaxar* OR beraprost* OR cangrelor* OR kengreal* OR kengrexal* OR cilostazol* OR clopidogrel* OR grepid* OR iscover* OR Plavix* OR dazoxiben* OR dehydrocilostazol* OR dipyridam* OR dipiridam* OR dipyrol* OR Persantin* OR elinogrel* OR abciximab OR eptifibatide* OR integrilin* OR integrelin* OR lefradafiban* OR lotrafiban* OR orbofiban* OR roxifiban* OR sibrafiban* OR tirofiban* OR Aggrastat* OR agrastat* OR xemilofiban* OR ifetroban* OR iloprost* OR ciloprost* OR Ventavist* OR indobufen* OR itazigrel* OR linotroban* OR nafazotrom* OR naxaprostene* OR octimibate* OR oxagrelate* OR pamicogrel* OR pentoxifylline* OR oxpentifylline* OR Trental* OR picotamide* OR plafibride* OR prasugrel* OR Effient* OR Efient* OR epoprostenol* OR cycloprostin* OR Flolan* OR prostavasin* OR Alpostadil* OR regrelor* OR samixogrel* OR sarpogrelate* OR satigrel* OR sulfinpyrazon* OR sulphinpyrazon* OR Anturan* OR taprostene* OR temanogrel* OR terbogrel* OR terutroban* OR ticagrelor* OR brilique* OR possia* OR ticlopidine* OR Ticlid* OR triflusal* OR disgren* OR thrombodipin* OR (thrombin* NEAR/3 (antagonist* OR block*)) OR vorapaxar* OR zontivity*):ab,ti)

Web of Science: 220 articles

TS=((("D-dimer" OR (fibrin NEAR/1 fragment NEAR/1 (DD OR D)) OR (crosslinked NEAR/2 fibrin* NEAR/2 degradation))) AND ((platelet* OR thrombocyt*) NEAR/2 (inhibitor* OR antiaggregant* OR antagonist*)) OR (anti NEAR/1 (platelet* OR

thrombocyt*)) OR antiplatelet* OR antithrombocyt* OR "acetylsalylic acid" OR ((acetyl*) NEAR/1 (salicyl*)) OR aspirin* OR caprin* OR aspro* OR Easprin* OR ZORprin* OR aspegic* OR anagrelide* OR ataprost* OR atopaxar* OR beraprost* OR cangrelor* OR kengreal* OR kengrexal* OR cilostazol* OR clopidogrel* OR grepid* OR iscover* OR Plavix* OR dazoxiben* OR dehydrocilostazol* OR dipyridam* OR dipiridam* OR dipyrol* OR Persantin* OR elinogrel* OR abciximab OR eptifibatide* OR integrilin* OR integrelin* OR lefradafiban* OR lotrafiban* OR orbofiban* OR roxifiban* OR sibrafiban* OR tirofiban* OR Aggrastat* OR agrastat* OR xemilofiban* OR ifetroban* OR iloprost* OR ciloprost* OR Ventavist* OR indobufen* OR itazigrel* OR linotroban* OR nafazotrom* OR naxaprostene* OR octimibate* OR oxagrelate* OR pamicogrel* OR pentoxifylline* OR oxpentifylline* OR Trental* OR picotamide* OR plafibride* OR prasugrel* OR Effient* OR Efient* OR epoprostenol* OR cycloprostin* OR Flolan* OR prostavasin* OR Alpostadil* OR regrelor* OR samixogrel* OR sarpogrelate* OR satigrel* OR sulfinpyrazon* OR sulphinpyrazon* OR Anturan* OR taprostene* OR temanogrel* OR terbogrel* OR terutroban* OR ticagrelor* OR brilique* OR possia* OR ticlopidine* OR Ticlid* OR triflusal* OR disgren* OR thrombodipin* OR (thrombin* NEAR/2 (antagonist* OR block*)) OR vorapaxar* OR zontivity*))) AND DT=(Article OR Review)

Google Scholar: 100 articles (top relevant references)

"D-dimer"|"D-dimers"|"D1 dimer"|antiplatelet|antiplatelets|"platelet aggregation inhibitors"|"platelet antagonist"

Methods S3. QUADAS-2 – adapted for systematic review and meta-analysis on the effect of statins and antiplatelet drugs on D-dimer levels

Author, year study:
Phase 1: Draw a flow diagram for the primary study
Phase 2: Risk of bias and applicability judgments

DOMAIN 1: PATIENT SELECTION

A. Risk of Bias
Describe methods of patient selection:
Was a case-control design used? Yes/No/Unclear
Did the study avoid inappropriate exclusions? Yes/No/Unclear
Was the study randomized? Yes/No/Unclear
Are there important differences in baseline characteristics? Yes/No/Unclear
Did the study adjust for differences in baseline characteristics? Yes/No/Unclear

Could the selection of patients have introduced bias? RISK:LOW/HIGH/UNCLEAR

B. Concerns regarding applicability
Describe included patients (prior testing, presentation, intended use of index test and setting):

Is there concern that the included patients do not match the review question? CONCERN: LOW/HIGH/UNCLEAR

DOMAIN 2: INDEX TEST(S)
If more than one index test was used, please complete for each test.

A. Risk of Bias
Describe the index test and how it was conducted and interpreted:

Could the conduct or interpretation of the index test have introduced bias? RISK: LOW /HIGH/UNCLEAR

B. Concerns regarding applicability

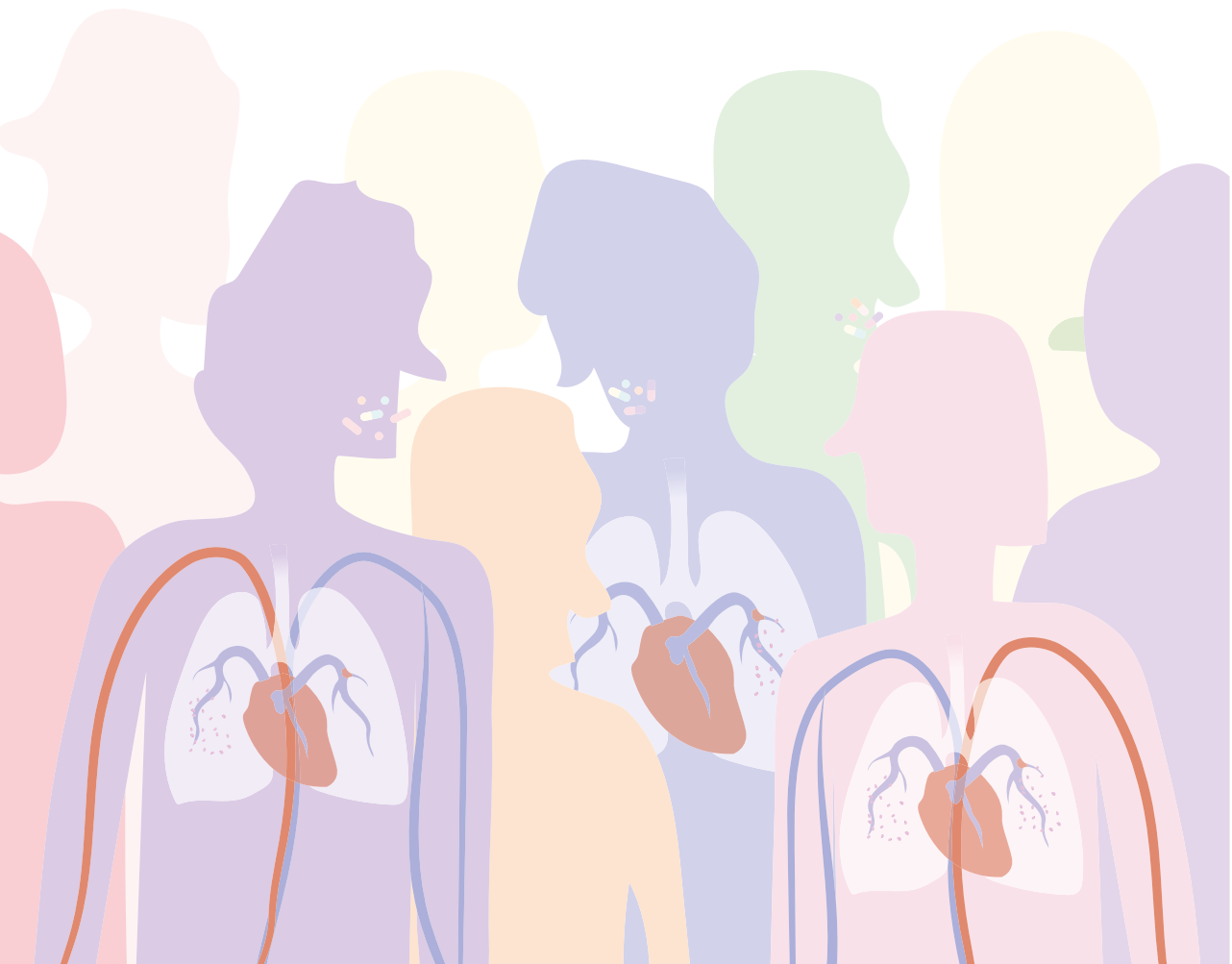
Is there concern that the index test, its conduct, or interpretation differ from the review question? CONCERN: LOW /HIGH/UNCLEAR

DOMAIN 4: FLOW AND TIMING

A. Risk of Bias

Describe any patients who did not receive the index test(s) (refer to flow diagram):
Describe the time interval and any interventions between index test(s):
Was there an appropriate interval between index test(s)? Yes/No/Unclear
Were all patients included in the analysis? Yes/No/Unclear

Could the patient flow have introduced bias? RISK: LOW /HIGH/UNCLEAR
Any conflicts of interest:



4

No effect of PCSK9 inhibitors on D-dimer and fibrinogen levels in patients with familial hypercholesterolemia

Suzanne Schol-Gelok
Annette M. H. Galema-Boers
Teun van Gelder
Marieke J. H. A. Kruip
Jeanine E. Roeters van Lennep
Jorie Versmissen

Biomed Pharmacother. 2018 Dec;108:1412-1414

Abstract

Statins are generally believed to have cardiovascular protective effects independent of low-density lipoproteincholesterol (LDL-C) lowering, such as antithrombotic effects characterized by a decrease in D-dimer levels. For the recently introduced Proprotein convertase subtilisin/kexin 9 (PCSK9) inhibitors antithrombotic effects are yet unknown. We determined the effect of starting PCSK9 inhibitors on D-dimer and fibrinogen levels as most robust markers for thrombogenicity in statin-intolerant patients with familial hypercholesterolemia. We determined D-dimer and fibrinogen levels before and after start of evolocumab (n=19) or alirocumab (n=11). Baseline median D-dimer levels were 0.34 mg/L (IQR 0.24-0.59 mg/L) and baseline median fibrinogen levels 3.2 g/L (IQR 2.88-3.63 g/L). At follow-up D-dimer levels (median 0.31 mg/L (IQR 0.25-0.59 mg/L; p=0.37), and fibrinogen levels (median 3.4 g/L (IQR 2.98-3.62 g/L); p=0.38) did not change significantly. We therefore conclude PCSK9 inhibitors do not seem to have a profound antithrombotic effect, although a more subtle effect can not been excluded.

Introduction

HMG-CoA reductase inhibitors, more commonly known as statins, are very effective in lowering LDL-cholesterol (LDL-C) and reducing risk of cardiovascular disease.[1] Statins are generally considered to have pleiotropic effects: cardiovascular protective effects independent of LDL-C lowering, with inhibition of geranylgeranylation of the Rho/Rho kinase pathway as one of the key mechanisms.[2,3] Most evidence exists for anti-inflammatory and anticoagulant effects. The latter effects lead to a lower risk of venous thrombosis as confirmed in a recent meta-analysis amongst 118,464 participants of randomized controlled trials: the risk of a primary venous thrombosis was 15% lower in the statintreated group.[4] Most studies showed in statin users lower levels of D-dimer and minor or transient effects on fibrinogen levels as most robust clinical markers for decreased thrombogenicity.[5,6]

Nowadays, Proprotein Convertase Subtilisin/Kexin 9 (PCSK9) inhibitors are widely introduced for high risk patients not reaching LDL-C targets despite maximum tolerated statins and ezetimibe, a Niemann-Pick C1-Like 1 inhibitor.[7] For PCSK9 inhibitors, antithrombotic effects have not been described. Considering the increasing frequency of prescribing PCSK9 inhibitors especially in patients with statin-associated side effects, it is important to assess the pleiotropic and in particular possible antithrombotic effects. In this scope, we determined D-dimer and fibrinogen levels in patients with familial hypercholesterolemia (FH) before and after starting PCSK9 inhibitors.

Methods

Study design

In the ErasmusMC University Medical Center, clinical data of all patients with familial hypercholesterolemia (FH) starting with PCSK9 inhibitors are documented in a standardized way including blood sampling before and after start.[8] The diagnosis FH is based on a causing pathogenic-mutation or the Dutch Lipid Clinic Network score of ≥ 6 representative of probable or definite FH.[9,10] Since blood sampling was part of standard care, this study was not subject to the Medical Research Human Subjects Act according to the Medical Ethical Research Committee. However, for use of clinical data for research purposes, informed consent of all patients was obtained. D-dimer and fibrinogen levels were determined before and between 14 days to one year after start of treatment with a PSCK9 inhibitor, using automated high-sensitive quantitative D-dimer (Innovance® D-dimer, Siemens, Marburg, Germany) and fibrinogen (Dade® Thrombin Reagent, Siemens, Marburg, Germany) assays.

Patient selection

Since statins are known to lower D-dimer levels potentially masking an additional effect by PCSK9 inhibitors, we decided to include patients not using statins. Statin intolerance is one of the major indications for reimbursement of PCSK9 inhibitors making this selection feasible.[11] We excluded patients of whom baseline or follow-up data or blood samples were missing. Additionally, patients with a cardiovascular event between baseline and follow-up and patients with injection-related hematomas or swelling to avoid confounding effects on D-dimer and fibrinogen levels.

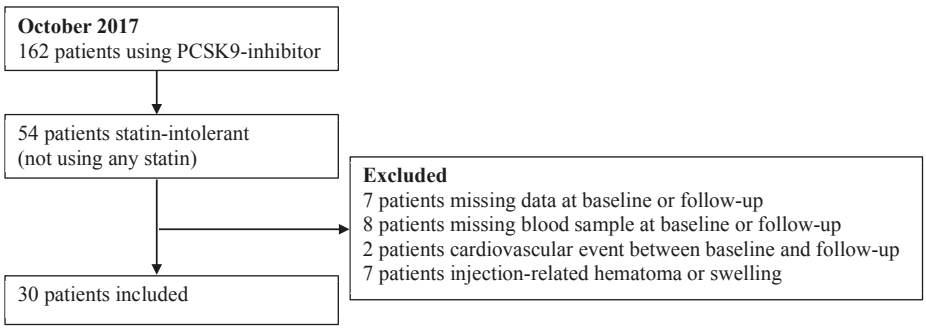
Statistical analysis

We aimed for at least 26 patients to have 80% power with a two tailed Wilcoxon paired test to find a clinically relevant difference in the mean concentration of D-dimer levels (the primary outcome) between baseline and follow-up of 10%, which is in line with earlier studies in statin patients.[5,12-14] We evaluated change in D-dimer and fibrinogen levels by a two tailed Wilcoxon paired test. All statistical analyses were carried out using 'IBM SPSS Statistics for Windows, version 21 (IBM Corp., Armonk, N.Y., USA)'.

Results

Until October 2017, 162 patients ≥18 years using a PCSK9 inhibitor were included in the database, of which 54 patients did not use statins because of statin associated side effects.[8] After exclusion, in most cases because of missing data or blood samples, 30 patients could be included (Figure 1; Table 1).

Figure 1: Flow chart of included and excluded patients.



Baseline characteristics are given in Table 1. Around half of the patients (43%) was male and the average age was 60 years. More than half of the patients were using antiplatelet or anticoagulant therapy due to prevalent cardiovascular disease. None of the patients had a severe renal insufficiency. All patients were on ezetimibe.

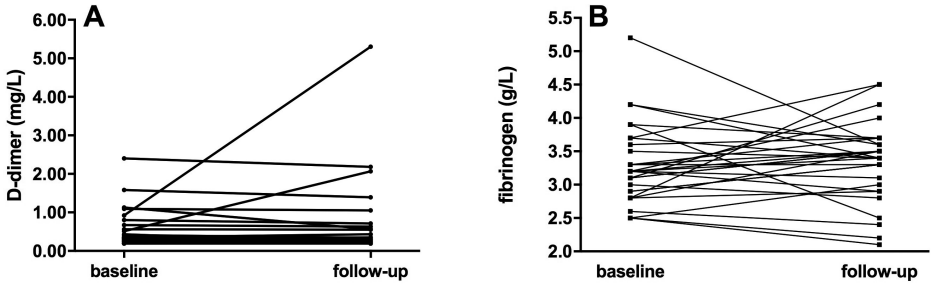
Table 1: Baseline characteristics of patients (n=30)

Description of characteristics	
Male (%)	13 (43.3%)
Age (years)	60 (11)
Body mass index (kg/m^2)	27 (3.2)
Pathogenic FH mutation	10 (33%)
Diabetes mellitus type 2	5 (16.7%)
Current smoker	3 (10 %)
History of cardiovascular events	19 (63.3%)
Estimated Glomerular Filtration Rate (CKD-Epi; ml/min)	75.5 (49.8-101.3
Current antiplatelet therapy	18 (60.0%)
Current anticoagulant therapy	1(3.3%)
Follow-up time (days)	28 (range 14-343)
LDL-cholesterol (mmol/L)	5.1 (4.1-6.2)
Total cholesterol (mmol/L)	7.1 (6.5-8.4)
Triglyceride (mmol/L)	2.2 (1.3-3.4)
HDL-cholesterol (mmol/L)	1.2 (0.9-1.7)
ApoB (g/L)	1.7 (1.3-1.8)
D-dimer (mg/L)	0.34 (0.24-0.59)
Fibrinogen (g/L)	3.2 (2.88-3.63)

Data are shown as mean values (SD); numbers (percentages) or median (25%-75% interquartile ranges) unless stated otherwise

Nineteen patients were treated with evolocumab 140 mg and 11 patients with alirocumab 150 mg subcutaneously every two weeks. Median follow-up time was 28 days (range 14-343 days). The mean D-dimer level at baseline was 0.52 mg/L (SD 0.49 mg/L), the median 0.34 mg/L (IQR 0.24-0.59 mg/L) and the mean fibrinogen level 3.28 g/L (SD 0.59 g/L) with a median of 3.2 g/L (IQR 2.88-3.63 g/L). The individual levels of D-dimer and fibrinogen for all patients are depicted in Figure 2.

Figure 2: Individual course of D-dimer and fibrinogen levels.



At follow-up D-dimer levels did not change significantly (median 0.31 mg/L (IQR 0.25-0.59 mg/L; p=0.37). Two patients had a steep rise and one a steep decline probably due to intercurrent disease at one of the time points although no clear clinical symptoms were present. Fibrinogen levels neither changed significantly after treatment with PCSK9 inhibitors (median 3.4 g/L (IQR 2.98-3.62 g/L); p=0.38). Effects were similar in users and non-users of antiplatelet therapy.

Discussion

We conclude that treatment with PCSK9 inhibitors did not change D-dimer or fibrinogen levels in statin-intolerant patients with FH. These findings suggest that PCSK9 inhibitors do not have antithrombotic effects.

Our findings are in contrast to the sparse pre-clinical studies, suggesting that PCSK9 might have pro-thrombogenic properties by increasing thrombin-antithrombin complexes and reducing anticoagulant zymogen protein C.[5] In mice, PCSK9 deficiency appeared protective against venous thrombosis.[15] In addition, in patients with higher fibrinogen levels and coronary artery disease, PCSK9 levels were significantly higher.[16] In this way, theoretically, PCSK9-inhibition could have an antithrombotic effect. However, our conclusions are based on actual treatment with PCSK9 inhibitors showing no effect on fibrinogen and D-dimer levels, the most robust clinical markers of thrombogenicity.

Although the sample size of our study is small compared to the large clinical trials assessing efficacy of PCSK9-inhibitors, it is one of the largest cohorts treated with PCSK9 inhibitors outside the clinical trials and the study was sufficiently powered to detect a clinically relevant difference in D-dimer levels.[8] Although we cannot exclude a

minor effect on D-dimer levels, overall and individual levels as shown in Figure 2 do not suggest such an effect. We chose to include only statin intolerant patients to eliminate the effect of statins well known for D-dimer lowering effects.[6] Although other factors influencing hemostatic parameters such as smoking and usage of antiplatelet therapy were not exclusion criteria, the effects on D-dimer levels are less well established than in the case of statins and the potentially confounding factor was the same at baseline and at follow-up.[17-19]

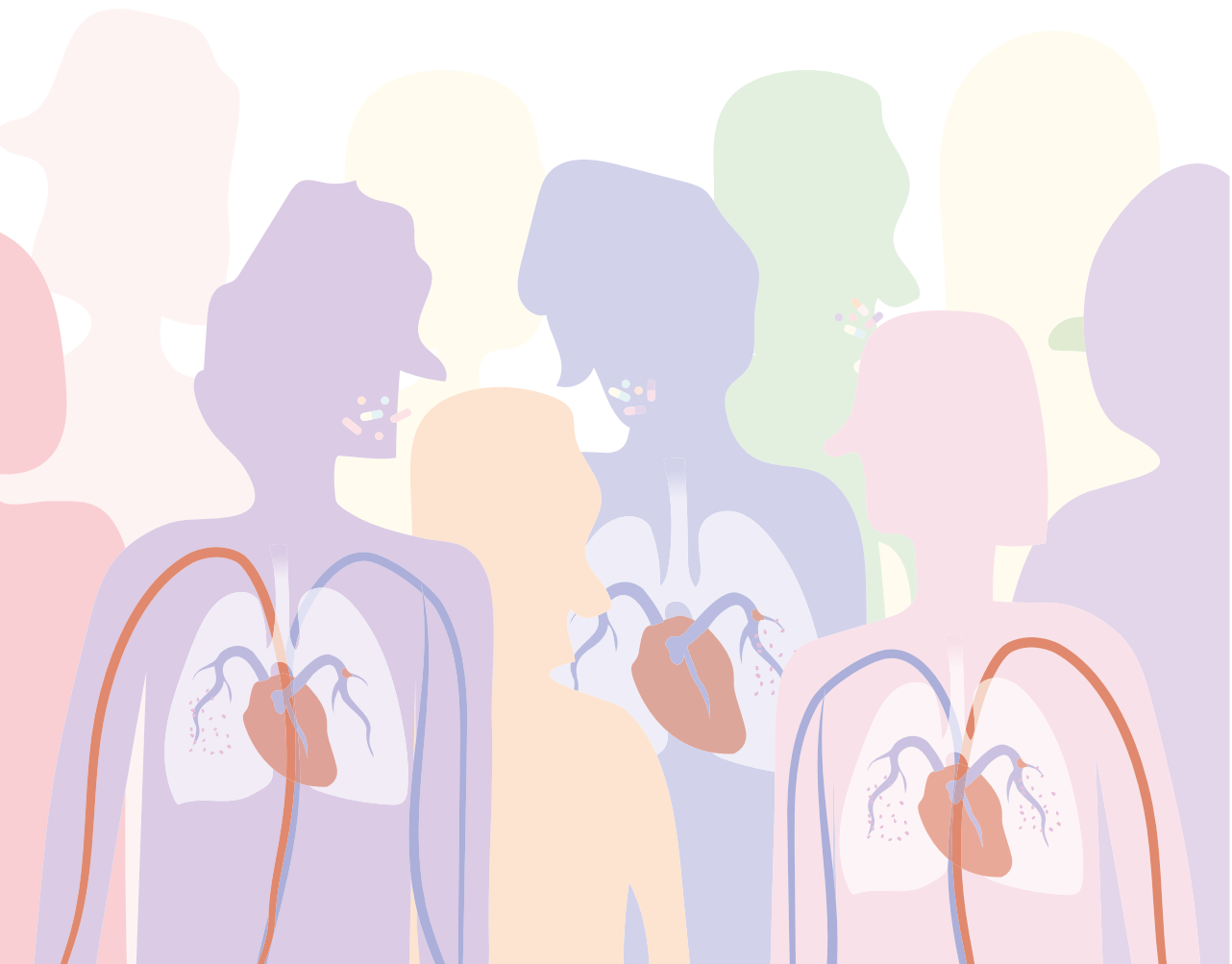
A limitation of our study is that follow-up measurement ranges between two and 49 weeks in our population, potentially leading to not detecting a putative transient effect on D-dimer and fibrinogen levels. However, in a sub analysis of 26 patients with a more narrow follow-up range between 17 and 60 days, we could still not identify a significant change. Also, due to the long term treatment, persistent changes are of most interest.

At the moment, guidelines recommend that at least three different statins should be tested before a PCSK9 inhibitor could be considered in patients at high risk of cardiovascular disease not reaching LDL-C target levels.[7,20] PCSK9 inhibitors are indisputably proven to reduce mean LDL-C levels, but based on our results they are unlikely to have antithrombotic effects in contrast to statins.[21] This stresses the recommendation in the guidelines to first extensively evaluate statin tolerance in the individual patient. Nonetheless, we acknowledge that the antithrombotic and other potential pleiotropic effects need to be studied in more detail to further evaluate the position of PCSK9 inhibitors in the treatment cascade of patients at high risk of cardiovascular disease.

References

1. Cholesterol Treatment Trialists C, Baigent C, Blackwell L, et al. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. *Lancet.* 2010;376(9753):1670-1681.
2. Oesterle A, Laufs U, Liao JK. Pleiotropic Effects of Statins on the Cardiovascular System. *Circ Res.* 2017;120(1):229-243.
3. Violi F, Calvieri C, Ferro D, Pignatelli P. Statins as antithrombotic drugs. *Circulation.* 2013;127(2):251-257.
4. Kunutsor SK, Seidu S, Khunti K. Statins and primary prevention of venous thromboembolism: a systematic review and meta-analysis. *Lancet Haematol.* 2017;4(2):e83-e93.
5. Bianconi V, Sahebkar A, Banach M, Pirro M. Statins, haemostatic factors and thrombotic risk. *Curr Opin Cardiol.* 2017;32(4):460-466.
6. Sahebkar A, Serban C, Mikhailidis DP, et al. Association between statin use and plasma D-dimer levels. A systematic review and meta-analysis of randomised controlled trials. *Thromb Haemost.* 2015;114(3):546-557.
7. Landmesser U, Chapman MJ, Stock JK, et al. 2017 Update of ESC/EAS Task Force on practical clinical guidance for proprotein convertase subtilisin/kexin type 9 inhibition in patients with atherosclerotic cardiovascular disease or in familial hypercholesterolaemia. *Eur Heart J.* 2017.
8. Galema-Boers AMH, Lenzen MJ, Sijbrands EJ, Roeters van Lennep JE. Proprotein convertase subtilisin/kexin 9 inhibition in patients with familial hypercholesterolemia: Initial clinical experience. *J Clin Lipidol.* 2017;11(3):674-681.
9. Watts GF, Gidding S, Wierzbicki AS, et al. Integrated guidance on the care of familial hypercholesterolaemia from the International FH Foundation. *Int J Cardiol.* 2014;171(3):309-325.
10. Hovingh GK, Davidson MH, Kastelein JJ, O'Connor AM. Diagnosis and treatment of familial hypercholesterolaemia. *Eur Heart J.* 2013;34(13):962-971.
11. Stroes ES, Thompson PD, Corsini A, et al. Statin-associated muscle symptoms: impact on statin therapy-European Atherosclerosis Society Consensus Panel Statement on Assessment, Aetiology and Management. *Eur Heart J.* 2015;36(17):1012-1022.
12. Wada H, Mori Y, Kaneko T, et al. Hypercoagulable state in patients with hypercholesterolemia: Effects of pravastatin. *CLIN THER.* 1992;14(6):829-834.
13. Trifiletti A, Lasco A, Scamardi R, et al. Long-term hemostatic effects of cholesterol-lowering therapy with atorvastatin. *Pathophysiol Haemost Thromb.* 2003;33(2):84-87.
14. Bolaman Z, Kadikoylu G, Özgel N, Yenisey C. Effects of atorvastatin on coagulation parameters and homocysteine in patients with primary hypercholesterolemia. *J Natl Med Assoc.* 2006;98(8):1273-1277.
15. Wang H, Wang Q, Wang J, et al. Proprotein convertase subtilisin/kexin type 9 (PCSK9) Deficiency is Protective Against Venous Thrombosis in Mice. *Sci Rep.* 2017;7(1):14360.
16. Zhang Y, Zhu CG, Xu RX, et al. Relation of circulating PCSK9 concentration to fibrinogen in patients with stable coronary artery disease. *J Clin Lipidol.* 2014;8(5):494-500.
17. Al Rifai M, DeFilippis AP, McEvoy JW, et al. The relationship between smoking intensity and subclinical cardiovascular injury: The Multi-Ethnic Study of Atherosclerosis (MESA). *Atherosclerosis.* 2017;258:119-130.
18. Cassar K, Bachoo P, Ford I, Greaves M, Brittenden J. Clopidogrel has no effect on D-dimer and thrombin-antithrombin III levels in patients with peripheral arterial disease undergoing peripheral percutaneous transluminal angioplasty. *J Vasc Surg.* 2005;42(2):252-258.

19. Tuut M, Hense HW. Smoking, other risk factors and fibrinogen levels. evidence of effect modification. *Ann Epidemiol*. 2001;11(4):232-238.
20. Fischer S, Julius U. Management of patients with statin intolerance. *Atheroscler Suppl*. 2017;30:33-37.
21. Stoekenbroek RM, Hartgers ML, Rutte R, de Wijer DD, Stroes ESG, Hovingh GK. PCSK9 inhibitors in clinical practice: Delivering on the promise? *Atherosclerosis*. 2017.



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Venous thrombosis during olanzapine treatment: a complex association

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Abstract

Olanzapine, a second generation antipsychotic, has previously been associated with an increased risk of venous thromboembolism (VTE).

In this mini-review we describe a case of a thirty-year-old schizophrenic patient who was diagnosed with a deep venous thrombosis (DVT) six months after starting olanzapine therapy, as well as seventeen other VTE cases in patients using olanzapine reported to the Netherlands Pharmacovigilance Centre Lareb. In 14 of these reports, patients had reported additional risk factors for VTE.

We found disproportionate Reporting Odds Ratios (RORs) in the global database VigiBase for olanzapine and the reactions deep vein thrombosis (ROR of 1.38 with a 95% CI (Confidence Interval) of 1.22-1.57) and pulmonary embolism (ROR of 1.99 with a 95% CI of 1.81-2.19).

The mechanism behind the association of olanzapine with VTE could be explained by two risk factors, substantial weight gain and lethargy, both common side effects of olanzapine. So far, a direct effect of olanzapine on platelet aggregation or coagulation has not been found.

Schizophrenic patients are more likely to have diagnostic delay in the diagnosis of VTE, as symptoms such as lethargy and impaired pain perception result in diminished pain perception and pain expression, while they are at increased risk of developing VTE. Currently no validated risk score is available for detection of psychiatric patients who might benefit from pharmacologic VTE prophylaxis. In patients developing a VTE while being treated with olanzapine, discontinuation of olanzapine could be considered based on the individual risk profile, control of psychotic symptoms and antipsychotic treatment options.

Introduction

Olanzapine is a second generation antipsychotic (SGA) commonly prescribed for treatment of positive symptoms of schizophrenic patients. Common side effects of SGAs include diabetes, metabolic syndrome, sexual dysfunction, hyperprolactinemia and weight gain, the latter more frequently seen in olanzapine.[1] A less known adverse event is that the use of olanzapine and other SGAs increase the risk of venous thromboembolism (VTE).[1,2]

VTE is a multifactorial disease with broadly two presenting entities: deep venous thrombosis (DVT) or pulmonary embolism (PE). Risk factors of VTE include immobility, sedation, previous VTE, active disease (e.g. infection or cancer), smoking, trauma, advanced age, male gender, hyperprolactinemia, antiphospholipid antibodies, obesity and certain genetic traits such as the factor V Leiden mutation.[2]

Both the Dutch website for drug information 'Farmacotherapeutisch Kompas' and the Dutch Summary of Product Characteristics (SmPC) of olanzapine mention VTE as an uncommon (0.1-1%) adverse drug reaction.[3,4] The SmPC of olanzapine describes no causal relationship has been established and that patients with schizophrenia often have acquired risk factors for VTE. They recommend identifying all possible risk factors for VTE and taking preventative measures. The annual incidence of VTE is 1 per 1000 in adult populations.[5] The risk of developing VTE is increased by 2.20 (odds ratio (OR), 1.22-3.95 (95% confidence interval (CI))) in patients using SGAs.[6]

We describe a case of a young man with a history of paranoid schizophrenia who presented with a DVT in our hospital (the Maasstad Hospital in Rotterdam, the Netherlands) while using olanzapine. By appraising the reported cases in the databases of the Netherlands Pharmacovigilance Centre Lareb and VigiBase (the worldwide pharmacovigilance database maintained by the WHO collaborating centre for international drug monitoring UMC (Uppsala Monitoring Centre in Sweden)), we further explored the possible association between olanzapine and VTE. Lastly we performed a brief literature review on the potential mechanisms behind this association.

Case report

A thirty-year-old man was diagnosed with a DVT in our hospital in March 2016, six months after initiating olanzapine treatment. He was admitted to a long-term psychiatric ward for treatment of his therapy resistant paranoid schizophrenia and comorbid cocaine and amphetamines addiction.

In July 2015, he suffered from continuous hallucinations and delusions despite having been treated with several antipsychotics. The Dutch multidisciplinary guideline for Schizophrenia advises clozapine treatment when two different types of antipsychotics are not effective.[7] However, our patient preferred olanzapine treatment and in August 2015 he was started on 300 mg olanzapine dosed by intramuscular injection once every four weeks.

By February 2016, he had gained 22 kg in weight (Body Mass Index (BMI) increase from 22.6 to 28.9) and had adopted a sedentary lifestyle, spending most of the day in bed. His high-density-lipoprotein (HDL) was reduced (0.83 mmol/l, reference level > 1 mmol/l) and his waist circumference had increased from 90 to 117 cm (reference < 102 cm). His blood pressure, glucose and triglyceride levels were normal. As only two out of five criteria obtained he did not qualify for metabolic syndrome.[8]

In March 2016, he presented at our hospital with a red swollen tender lower right leg. Ultrasound confirmed the diagnosis of DVT and the patient was started on anticoagulant treatment with a vitamin K antagonist with low molecular weight heparin injections until the desired prothrombin times were achieved.

Risk factors for DVT in this patient were male gender, active psychosis, use of olanzapine, lethargy and high BMI after his significant and ongoing weight gain. Persisting psychotic symptoms and the presence of DVT made us decide to discontinue olanzapine and start clozapine. Within the next few months our patient became more active and less psychotic, however his BMI increased further to 31 kg/m^2. Antithrombotic treatment was discontinued after three months, following the Dutch guidelines prevalent at that time.[9]

Discussion

Other reports from Lareb and VigiBase

From 2001 to the 26th of January 2018 Lareb received 18 reports of DVT or PE associated with the use of olanzapine, including the previously discussed case report, see Table 1 for further details.[10] In 14 of these reports, patients had additional risk factors for VTE.

Table 1: Reports received by the Netherlands Pharmacovigilance Centre Lareb of deep venous thrombosis or pulmonary embolism associated with the use of olanzapine[8]

Report details	**(number of reports)**
Gender	male (13), female (5)
Age	Range: 26-71 years old, median 45 years old, not reported in one case
Indications for olanzapine	Schizophrenia (6), psychosis (5), bipolar disorder/ mania (4), not reported (3)
Reported VTE (venous thromboembolism):	Deep vein thrombosis (6*), pulmonary embolism (12)
Latencies (time from starting olanzapine till event)	2 days – 13 years, median 5 months
Discontinuation of olanzapine	Continuation in same dose (5), continuation in reduced dose (3), discontinuation (5), unknown (5)
Follow-up	Recovery/recovering of VTE (12), no recovery at time of reporting (1), unknown (3), death of any cause with uncertain possible relationship with drug (2)
Pharmacologic treatment of VTE	Yes (15), unknown (3)
Concurrent risk factors	Possible risk factors reported (14): factor V Leiden (1), smoking (2), other antipsychotic drugs or oral contraception associated with thromboembolism as concomitant medication (4), immobilization (3), significant weight increase (5), reported Body Mass Index > 30 kg/m2 (2), family history with thromboembolism (1), significant comorbidity (2)

**In one of these reports the patient also experienced occlusion in an artery.*

VigiBase lists 241 reports on olanzapine (with a Reporting Odds Ratio (ROR) of 1.38 [95% CI 1.22-1.57]) of the reaction "deep veinous thrombosis" (DVT) and 441 (ROR 1.99 [95% CI 1.81-2.19]) reports of the reaction "pulmonary embolism"(PE), including the cases received by Lareb.[11] The ROR is a measure of disproportional reporting in the database, and in this case a significantly increased ROR indicates that DVT or PE is more often reported for olanzapine use than with other drugs in the database. The ROR has been developed as a method for signal detection; it is therefore a hypothesis generating tool.[12] The only other antipsychotic with a significantly increased ROR for these reactions was clozapine, with an ROR of 1.15 [95% CI 1.14-1.37, 473 reports] for DVT and an ROR of 2.15 [95% CI 2.02-2.29, 1024 reports) for PE. The RORs from VigiBase for various antipsychotics are described in detail in Table 2.

Table 2: Reporting Odds Ratios (ROR) from VigiBase

Antipsychotics	ROR for deep vein thrombosis		ROR for pulmonary embolism	
	ROR (95% CI)	N	ROR (95% CI)	N
Olanzapine	1.38 (1.22-1.57)	241	1.99 (1.81-2.19)	441
Clozapine	1.25 (1.14-1.37)	437	2.15 (2.02-2.29)	1024
Quetiapine	0.65 (0.55-0.77)	141	0.97 (0.86-1.10)	266
Risperidone	0.66 (0.57-0.77)	184	1.08 (0.98-1.20)	381
Sertinidole		0	4.19 (2.24-7.83)	10
Paliperidone	0.47 (0.35-0.62)	48	0.76 (0.63-0.93)	99
Aripiprazole	0.48 (0.38-0.60)	70	0.81 (0.69-0.95)	151
Lurasidone	0.35 (0.13-0.94)	4	0.83 (0.47-1.47)	12

Among the cases reported on in Lareb and VigiBase, the likelihood of a causal association between a drug and a reaction may vary as both are based on spontaneous reporting from various sources with different degrees of documentation.

Cases in the scientific literature

Various case reports in the scientific literature describe the association of olanzapine with VTE.[13-16] Most case reports describe male patients aged sixty years and older, although PE has also been described in a 28-year-old male patient shortly after starting olanzapine treatment.[5,15,16] Only a few population studies have reported on olanzapine specifically.[6] A 2014 meta-analysis reports an OR of 1.35 (95% CI 0.97–1.89, p = 0.08) for the risk of VTE in patients using olanzapine. The results of this meta-analysis should be interpreted with caution since the quality and inclusion criteria varied between studies leading to between-study heterogeneity. This resulted in a low statistical power and a wide and nonsignificant confidence interval for all included SGAs.[17] On the other hand a large case control study reported a significant OR for risk of VTE in patients using olanzapine of 1.49 (95% CI 1.07-2.08).[18]

Risk of VTE appears to be especially elevated in the first months of SGA treatment (OR 1.97; 95% CI 1.66-2.33). For users of antipsychotic drugs the risk was 56% higher compared to non-users of antipsychotic drugs (OR 1.56; 95% CI 1.39-1.75).[19]

However, current data can neither conclusively verify differences in occurrence rates of VTE between first- and second-generation antipsychotics nor identify which antipsychotic drugs have the lowest risk of VTE, though one might speculate that the risk of VTE is higher for clozapine than for other SGAs.[20]

Potential mechanism

The potential underlying mechanism explaining the higher risk of VTE during antipsychotic treatment is not yet fully clear. Various factors seem to play a role, especially metabolic syndrome, a common side effect of olanzapine treatment as 34% of schizophrenic patients taking olanzapine monotherapy fulfil its criteria.[21] Metabolic syndrome is a known risk factor of VTE.[22]

Immobilisation, a consequence of the lethargy caused by various antipsychotics, is linked to increased risk of VTE due to venous stasis and blood pooling in the lower extremities. Obesity is also an independent risk factor for VTE and although all SGAs are associated with some weight gain and increased appetite, olanzapine and clozapine have the most profound impact compared to non-SGA antipsychotics and placebo.[23-25] A database analysis comprising 3507 patients in 21 placebo- and active-controlled studies conducted in America, Australia, New Zealand and Europe showed that 48% of patients taking olanzapine experience > 7% weight gain within the first 12 weeks, and 57% of patients experience a significant weight gain within the first 6-12 months with a median weight gain of 0.7 kg per month, compared to placebo (incidence of weight gain in placebo was 13%).[24] This increase in body weight of at least 20% is more pronounced in inexperienced users of antipsychotics.[25] Only in olanzapine users a significant increase in weight was found when comparing the weight at > 38 weeks to the weight at six weeks after starting olanzapine.[25] Therefore, if a patient is already on olanzapine, switching to a different antipsychotic drug, such as haloperidol, might be indicated.[26] In one case, switching from olanzapine to asenapine resulted in 6.6% weight loss without further impairment of psychological functioning.[27]

There is less information available regarding the presence of lethargy in schizophrenic patients treated with antipsychotics. It is difficult to compare trials as they do not always give a clear description of somnolence, sedation, lethargy and hypersomnia. Nevertheless olanzapine, quetiapine, risperidone and especially clozapine are all associated with significantly more of these symptoms compared to placebo.[28] About 25-39% of patients taking olanzapine experience sleepiness, which is significantly more than placebo (26.2% compared to 15.3%), whereas somnolence is experienced by 26-46% of patients taking clozapine.[28,29] It has been postulated that in antipsychotic-induced somnolence blockade of histamine 1 receptors and α1 receptors play an important role.[28,30]

Other associated risk factors include raised levels of antiphospholipid antibodies and hyperprolactinemia. The exact roles of these risk factors in a clinical psychiatric setting still need to be determined.[31]

It is hypothesized that antipsychotics directly influence the risk of VTE, in particular second generation antipsychotics such as clozapine and olanzapine. Many antipsychotics antagonise the serotonin (5-HT2A) receptors. As these receptors are also present on platelets, the antipsychotic medication might influence platelet aggregation.[32] Paradoxically, most studies investigating this mechanism suggested a lower risk of VTE.

Almuqdadi et al. have shown that risperidone, and not olanzapine, leads to a clinically significant inhibition of platelet aggregation induced by serotonin. When adding these antipsychotics to a serotonin-epinephrine combination, both weak platelet agonists affecting respectively the 5-HT and the α2A-adenergic receptors on platelets, a dose-dependent inhibition of platelet aggregation was found. However, no statistically significant inhibitory effect on platelet aggregation was seen with olanzapine, possibly due to a lower 5-HT2A and α2A-adenergic receptor affinity compared to e.g. risperidone.[32] Another in vitro study indicates that clozapine and olanzapine show a strong inhibitory effect on ADP-stimulated platelet aggregation, which would also lower the risk of VTE.[33] Yet another in vitro study has shown increased platelet adhesion and aggregation for clozapine, but not for olanzapine.[31]

These results suggest that the serotonin receptor effect does not account for the increased risk of VTE while using SGA. In absence of a clear pathogenic mechanism to account for the associated higher risk of VTE in olanzapine users, we might conclude that this association is most probably caused by risk factors like substantial weight gain and lethargy, not by olanzapine itself.

Diagnosing VTE in psychiatric patients

VTE is known to have a diagnostic delay, the average delay in diagnosing PE is 8.6 days, where patient delay is on average 4.2 days and delay in primary care is 3.9 days on average.[34] 23.8% of patients are diagnosed at least a week after onset of symptoms. The absence of chest symptoms is associated with a diagnostic delay with an OR of 5.4 (95% CI 1.9-15).[35] Specifically, patients with a diagnostic delay were less likely to present with chest pain (24% vs 54%, $p = 0.003$) or pain during inspiration (9% vs 33%, $p = 0.011$) compared to patients without diagnostic delay.

Pain perception in schizophrenic patients is impaired in various ways, without an exact mechanism being clear.[36] Also, cognitive impairment and excess negative symptoms influence the expression of pain in this patient category.[37] For example, in a case report of a 75-year-old patient diagnosed with catatonic schizophrenia and with PE the patient could not express any pain symptoms, which stresses the fact that schizophrenic

patients are more likely to have diagnostic delay in the diagnosis of VTE.[38] As venous thromboembolism is an important cause of mortality and morbidity is it important to take this into account. An observational study including systematic venous ultrasound identified DVT in 10 out of 449 patients (2.2%) after 10 days admission to a psychiatric ward. Within 90 days 17 patients developed VTE including three symptomatic PEs.[39]

Options for prophylactic treatment

Identifying patients on antipsychotic therapy who have a high risk of developing a VTE might be difficult as no validated risk score is available that is able to detect which psychiatric patients might benefit from pharmacologic VTE prophylaxis. For olanzapine use alone, an OR of 1.35 for the risk of venous thrombosis does not seem to justify prophylactic treatment with low molecular weight heparins (LMWH). However, additional risk factors for VTE have been identified in psychiatric patients.

One risk score designed specifically for psychiatric patients includes the use of antipsychotics.[40] Other risk factors included are history of VTE, cancer (active/treated), age, acute infectious/respiratory disease, immobilization (including catatonia), hormone therapy, obesity (BMI > 30), dehydration and thrombophilia. Based on the presence of these risk factors patients could be categorized according to their low, medium or high risk of developing a DVT with the recommendation to start (LMWH in the medium and high risk populations. However, this risk score and the algorithm derived from it have not yet been validated in a larger setting and it is unclear whether the benefits of the prophylactic treatment with anticoagulants (e.g. LMWH) outweigh possible side effects, for example an increased risk of bleeding.

The Padua risk score has been validated in a larger clinical setting of medical inpatients and has been proven to distinguish between inpatients with high risk of VTE and patients with low risk. The risk factors involved are active malignancy, previous VTE, immobility, thrombophilia, trauma and/or surgery less than one month ago, age over 70 years, cardiovascular and or respiratory diseases, acute infection and or rheumatologic abnormalities, a BMI of more than 30 and hormonal therapy.[41]

Nevertheless, this model was not specifically tested in psychiatric patients and only in an acute hospital setting.[41] Therefore we also cannot use the Padua risk score in a psychiatric ward for deciding on whether to give pharmacological prophylactic treatment.

Conclusion

SGAs such as olanzapine are commonly prescribed for treatment of positive symptoms of schizophrenia, but are associated with a higher risk of VTE. This association is most likely explained by associated risk factors such as substantial weight gain and lethargy, both side effects of olanzapine. Further studies are required to determine the potential mechanisms of olanzapine on for example thrombogenicity and platelet aggregation.

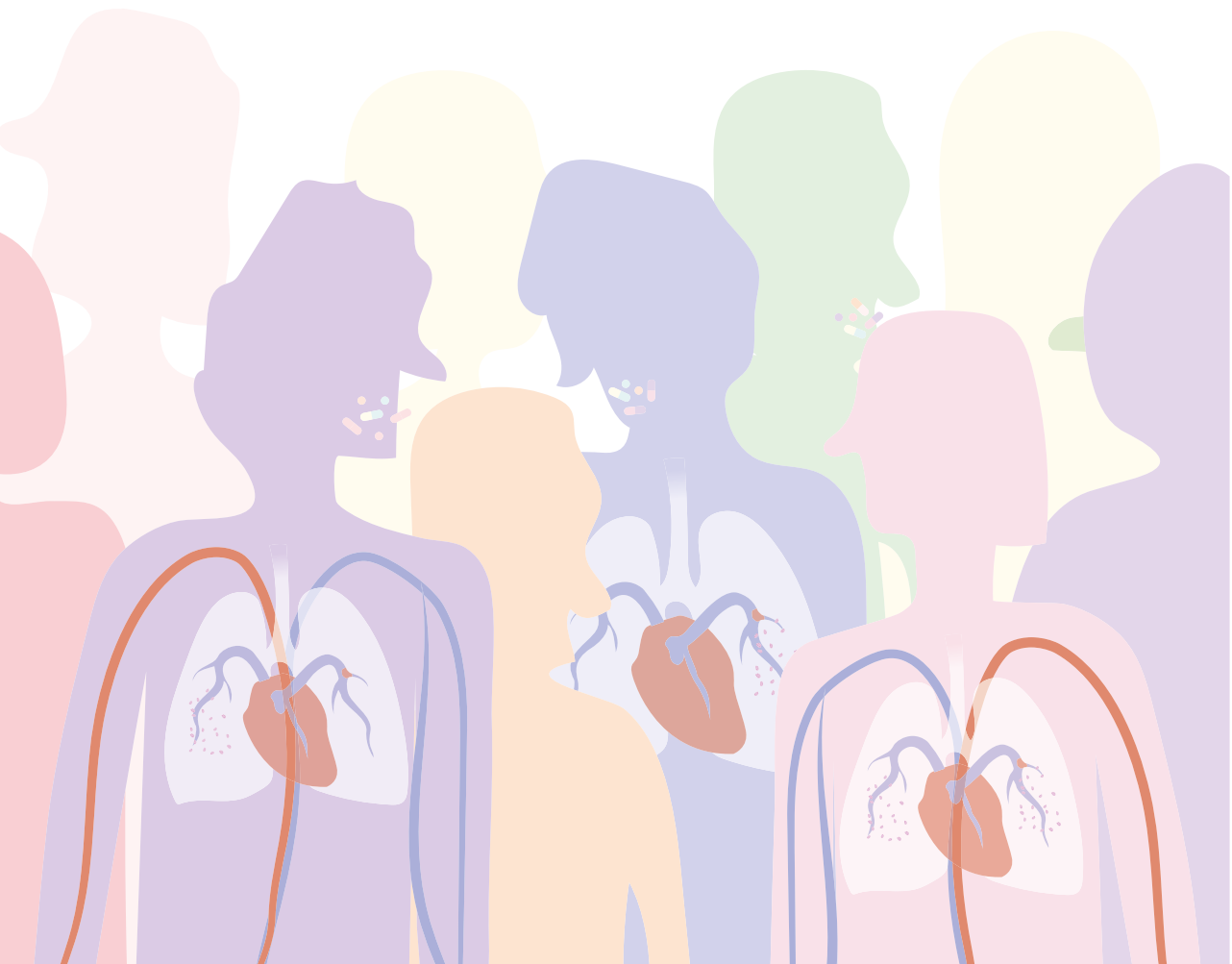
In general, it is important to realise that diagnosing VTE in schizophrenic patients can be more difficult, due to symptoms such as lethargy and impaired pain perception.
No validated risk score is available for detection of psychiatric patients who might benefit from pharmacological VTE prophylaxis. In patients who develop VTE while being treated with a SGA such as olanzapine, discontinuation of olanzapine might be considered based on individual risk profile, control of psychotic symptoms and antipsychotic treatment options.

References

1. Young SL, Taylor M, Lawrie SM. "First do no harm." A systematic review of the prevalence and management of antipsychotic adverse effects. *Journal of psychopharmacology (Oxford, England).* 2015;29(4):353-362.
2. Heit JA, Spencer FA, White RH. The epidemiology of venous thromboembolism. *J Thromb Thrombolysis.* 2016;41(1):3-14.
3. Zorginstituut Nederland. Farmacotherapeutisch Kompas. https://farmacotherapeutischkompas.nl.
4. Dutch SmPC olanzapine Zalasta 2.5/5/7.5/10/15/20 tablets. (version date 26 July 2012) http://www.ema.europa.eu/docs/nl_NL/document_library/EPAR-Product_Information/human/000792/WC500045945.pdf.
5. White RH. The epidemiology of venous thromboembolism. *Circulation.* 2003;107(23 Suppl 1):I4-8.
6. Zhang R, Dong L, Shao F, Tan X, Ying K. Antipsychotics and venous thromboembolism risk: a meta-analysis. *Pharmacopsychiatry.* 2011;44(5):183-188.
7. Multidisciplinaire richtlijn schizofrenie. 2012.
8. Kaur J. A comprehensive review on metabolic syndrome. *Cardiology research and practice.* 2014;2014:943162.
9. Richtlijn Diagnostiek, Preventie en Behandeling van Veneuze Trombo-embolie en Secundaire Preventie Arteriële Trombose. 2008.
10. The Netherlands pharmacovigilance centre Lareb database. Version date: 2018, access date 26-01-2018. www.lareb.nl.
11. WHO Global Individual Case Safety Reports database VigiBase (UMC Uppsala, Sweden), searched with VigiLyze. Version date 2018, access date 17-04-2018. http://www.vigiaccess.org/.
12. Kabel JS, van Puijenbroek EP. [Side effects of tramadol: 12 years of experience in the Netherlands] Bijwerkingen van tramadol: 12 jaar ervaring in Nederland. *Ned Tijdschr Geneeskd.* 2005;149(14):754-757.
13. Sheikhmoonesi F, Bahari Saravi SF. Deep venous thrombosis and atypical antipsychotics: three cases report. *DARU Journal of Pharmaceutical Sciences.* 2012;20(1):71.
14. Hagg S, Tatting P, Spigset O. Olanzapine and venous thromboembolism. *International clinical psychopharmacology.* 2003;18(5):299-300.
15. Maly R, Masopust J, Hosak L, Urban A. Four cases of venous thromboembolism associated with olanzapine. *Psychiatry and clinical neurosciences.* 2009;63(1):116-118.
16. Waage IM, Gedde-Dahl A. Pulmonary embolism possibly associated with olanzapine treatment. *BMJ (Clinical research ed.).* 2003;327(7428):1384.
17. Barbui C, Conti V, Cipriani A. Antipsychotic drug exposure and risk of venous thromboembolism: a systematic review and meta-analysis of observational studies. *Drug Saf.* 2014;37(2):79-90.
18. Parker C, Coupland C, Hippisley-Cox J. Antipsychotic drugs and risk of venous thromboembolism: nested case-control study. *BMJ (Clinical research ed.).* 2010;341:c4245.
19. Masopust J, Maly R, Valis M. Risk of venous thromboembolism during treatment with antipsychotic agents. *Psychiatry and clinical neurosciences.* 2012;66(7):541-552.
20. Jonsson AK, Schill J, Olsson H, Spigset O, Hagg S. Venous Thromboembolism During Treatment with Antipsychotics: A Review of Current Evidence. *CNS Drugs.* 2018;32(1):47-64.
21. Hirsch L, Yang J, Bresee L, Jette N, Patten S, Pringsheim T. Second-Generation Antipsychotics and Metabolic Side Effects: A Systematic Review of Population-Based Studies. *Drug safety.* 2017;40(9):771-781.

22. Ageno W, Di Minno MN, Ay C, et al. Association between the metabolic syndrome, its individual components, and unprovoked venous thromboembolism: results of a patient-level meta-analysis. *Arterioscler Thromb Vasc Biol.* 2014;34(11):2478-2485.
23. Zhao YJ, Lin L, Teng M, et al. Long-term antipsychotic treatment in schizophrenia: systematic review and network meta-analysis of randomised controlled trials. *BJPsych Open.* 2016;2(1):59-66.
24. Parsons B, Allison DB, Loebel A, et al. Weight effects associated with antipsychotics: a comprehensive database analysis. *Schizophrenia research.* 2009;110(1-3):103-110.
25. Bak M, Fransen A, Janssen J, van Os J, Drukker M. Almost all antipsychotics result in weight gain: a meta-analysis. *PloS one.* 2014;9(4):e94112.
26. Wang HH, Cai M, Wang HN, et al. An assessor-blinded, randomized comparison of efficacy and tolerability of switching from olanzapine to ziprasidone and the combination of both in schizophrenia spectrum disorders. *Journal of psychiatric research.* 2017;85:59-65.
27. Okazaki K, Yamamuro K, Kishimoto T. Reversal of olanzapine-induced weight gain in a patient with schizophrenia by switching to asenapine: a case report. *Neuropsychiatric disease and treatment.* 2017;13:2837-2840.
28. Fang F, Sun H, Wang Z, Ren M, Calabrese JR, Gao K. Antipsychotic Drug-Induced Somnolence: Incidence, Mechanisms, and Management. *CNS Drugs.* 2016;30(9):845-867.
29. Miller DD. Atypical antipsychotics: sleep, sedation, and efficacy. *Prim Care Companion J Clin Psychiatry.* 2004;6(Suppl 2):3-7.
30. Sekine Y, Rikihisa T, Ogata H, Echizen H, Arakawa Y. Correlations between in vitro affinity of antipsychotics to various central neurotransmitter receptors and clinical incidence of their adverse drug reactions. *European journal of clinical pharmacology.* 1999;55(8):583-587.
31. Hagg S, Jonsson AK, Spigset O. Risk of venous thromboembolism due to antipsychotic drug therapy. *Expert opinion on drug safety.* 2009;8(5):537-547.
32. Almuqdadi A, Bulatova N, Yousef AM. The effect of atypical antipsychotics on platelet aggregation. *Open Journal of Hematology 2016.*
33. Dietrich-Muszalska A, Rabe-Jablonska J, Nowak P, Kontek B. The first- and second-generation antipsychotic drugs affect ADP-induced platelet aggregation. *The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry.* 2010;11(2 Pt 2):268-275.
34. Walen S, Damoiseaux RA, Uil SM, van den Berg JW. Diagnostic delay of pulmonary embolism in primary and secondary care: a retrospective cohort study. *Br J Gen Pract.* 2016;66(647):e444-450.
35. Hendriksen JM, Koster-van Ree M, Morgenstern MJ, et al. Clinical characteristics associated with diagnostic delay of pulmonary embolism in primary care: a retrospective observational study. *BMJ Open.* 2017;7(3):e012789.
36. Antioch I, Ciobica A, Paulet M, Bild V, Lefter R, Timofte D. Pain manifestations in schizophrenia - clinical and experimental aspects in human patients and animal models. *Psychiatria Danubina.* 2015;27(2):142-152.
37. Urban-Kowalczyk M, Pigonska J, Smigielski J. Pain perception in schizophrenia: influence of neuropeptides, cognitive disorders, and negative symptoms. *Neuropsychiatric disease and treatment.* 2015;11:2023-2031.
38. Hu HC, Chiu NM. Delayed diagnosis in an elderly schizophrenic patient with catatonic state and pulmonary embolism. *Int J Gerontol.* 2013;7(3):183-185.
39. Patel R. Venous thromboembolism prophylaxis in mental healthcare: do the benefits outweigh the risks? *BJPsych Bull.* 2015;39(2):61-64.
40. Maly R, Masopust J, Hosak L, Konupcikova K. Assessment of risk of venous thromboembolism and its possible prevention in psychiatric patients. *Psychiatry and clinical neurosciences.* 2008;62(1):3-8.

41. Germini F, Agnelli G, Fedele M, et al. Padua prediction score or clinical judgment for decision making on antithrombotic prophylaxis: a quasi-randomized controlled trial. *Journal of thrombosis and thrombolysis.* 2016;42(3):336-339.



6

Rosuvastatin use increases plasma fibrinolytic potential: a randomised clinical trial

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Abstract

We conducted a study assess the effect of rosuvastatin use on fibrinolysis in patients with previous venous thromboembolism (VTE).

This was a post hoc analysis within the STAtins Reduce Thrombophilia (START) study (NCT01613794). Plasma fibrinolytic potential, fibrinogen, plasmin inhibitor, Plasminogen Activator Inhibitor-1 (PAI-1) and Thrombin Activatable Fibrinolysis Inhibitor (TAFI) were measured before and after four weeks of rosuvastatin or no treatment in participants with prior confirmed VTE after ending anticoagulant therapy. In the non-rosuvastatin group (n=121), plasma fibrinolytic potential and individual fibrinolysis parameters did not change at the end of the study versus the baseline, whereas in the rosuvastatin group (n=126), plasma fibrinolytic potential increased: mean clot lysis time decreased by 8.75 min (95% CI -13.8 to -3.72), and plasmin inhibitor levels and TAFI activity were lower at end of the study (-0.05 U/mL; 95% CI -0.07 to -0.02 and -4.77%; 95% CI -6.81 to -2.73 respectively). PAI-1 levels did not change and fibrinogen levels were 0.17 g/L (95% CI 0.04 to 0.29) higher.

In participants with prior VTE, rosuvastatin use led to an increased fibrinolytic potential compared with non-statin use. Our findings support the need for further studies on the possible role for statins in the secondary prevention of VTE.

Introduction

HMG-CoA (3-hydroxy-3-methyl-glutaryl-CoA) reductase inhibitors, more commonly known as statins, exert cardiovascular protective effects which are independent of LDL-cholesterol lowering, including antithrombotic effects.[1] A meta-analysis of 36 studies, including 23 randomised studies, showed a 15% risk reduction of statin use on first venous thromboembolism (VTE) events compared to placebo or no treatment.[2] Antithrombotic effects seem to be most robust for rosuvastatin.[3] However, the exact mechanism behind the antithrombotic effects of statins is not completely unraveled.[4,5] The fibrinolytic system, which plays an important role in dissolving thrombi, is likely to contribute to the antihrombotic effects. In two large observational studies hypofibrinolytic activity has been linked to an increased risk of VTE.[6,7] Also, statin use has been associated with lower levels of D-dimer and other fibrin degradation products (FDPs).[8] During fibrinolysis, the inactive proenzyme plasminogen is converted by tissue Plasminogen Activator (t-PA) or urokinase-type Plasminogen Activator (u-PA) to the active enzyme plasmin.[9,10] Several inhibitors of fibrinolysis are known, such as Plasminogen Activator Inhibitor-1 (PAI-1), which can inhibit these converting enzymes. The activated plasmin degrades fibrin into fibrin degradation products, and is inhibited by plasmin inhibitor. Additionally, thrombin converts fibrinogen into fibrin, but also activates Thrombin-Activatable Fibrinolysis Inhibitor (TAFI). A better understanding of the exact effect of statins on fibrinolysis will help in determining the position of statins in the treatment of prevention of (recurrent) VTE without increased risk of hemorrhage.

To test the effects of rosuvastatin use on fibrinolysis, we performed a post hoc analysis of the START (STAtins Reduce Thrombophilia-)study. Plasma fibrinolytic potential as well as fibrinogen, plasmin inhibitor (also called α2-antiplasmin), PAI-1 and TAFI were determined in blood samples before and after four weeks of rosuvastatin or no treatment in a population suspected to be hypercoagulable. Notably, D-dimer levels have been measured and analysed previously.[11]

Methods

Study design

This was a post hoc analysis within the STAtins Reduce Thrombophilia (START) study, a collaboration of three anticoagulation clinics in the Netherlands (Star-shl in Rotterdam, the Leiden Anticoagulation Clinic and Atalmedial, Hoofddorp), of which the design was previously described (clinical trials.gov NCT01613794).[11] This study was performed

in accordance with the Declaration of Helsinki, all participants gave written informed consent and the study was approved by the Medical Ethics Committee of the Leiden University Medical Center, Leiden, the Netherlands. The START trial was performed to evaluate the antithrombotic effects of statins.

Participants were included if they were 18 years or older with a prior (initial or recurrent) confirmed symptomatic proximal deep vein thrombosis or pulmonary embolism. Also, they should have stopped their vitamin K antagonist treatment for one month (to allow anticoagulant drugs to wear off) as decided by their treating physician. Before inclusion we did not check if the ultrasound or CTPA was negative for VTE. Reasons for exclusion were current use of statins or other lipid lowering drugs, or contraindications for rosuvastatin use. All those participants included were randomised to 28 days of treatment with rosuvastatin 20 mg or no study medication. Blood samples were collected during the randomisation visit and at the end of the study period.

Measurements

Blood sampling by venipuncture was performed using the Vacutainer system (Becton Dickinson), containing sodium citrate (final concentration 0.109 mol/L). Participants' blood was drawn between 8:00 am and 3:00 pm, and at the same hour of the day at the end of the study for logistical reasons, but also because of the circadian rhythm of especially clot lysis time and PAI-1.[12-14] Within 3 hours, blood samples were centrifuged at 2500 g for 15 min at 18˚C and stored afterwards at -80 ˚C in our biobank.

To study the plasma fibrinolytic potential, the plasma clot lysis time was measured by experienced technicians as described previously.[12] Platelet-poor plasma was diluted 1.7 times in buffer (25 mmol/L Hepes, 137 mmol/L sodium chloride, 3.5 mmol/L potassium chloride, 1% (w/v) BSA, pH 7.4) at room temperature and the diluted plasma (85 µl) was added to wells of a microtitre plate containing 15 µl of a reaction mixture. The reaction mixture contained the following components (with the final concentrations in the assay): tissue factor (TF, Innovin, 1000 times diluted; Dade Behring, Marburg, Germany), calcium chloride (17 mmol/L), t-PA (30 ng/ml, Actilyse, Boehringer Ingelheim, Ingelheim am Rhein, Germany) and phospholipid vesicles (10 µM, Rossix, Mölndal, Sweden). After mixing the diluted plasma with the reaction mixture on a plate shaker, each well was covered with 50 µl paraffin oil (Merck, Darmstadt, Germany) and the microtitre plate was placed into the preheated chamber of the microplate reader (VictorTM, PerkinElmer, Waltham, MA, USA). The optical density at 405 nm was measured every minute for 300 min at 37°C. The clot lysis time was measured in duplicate (time from the midpoint of minimum turbidity to maximum turbidity to the midpoint of maximum turbidity

to minimum turbidity). These midpoints were calculated using the Shiny app.[15,16] The intra- and inter-assay variation coefficients from our laboratory were previously shown 3.5% and 6.5% to 8% respectively.[12,17] PAI-1 activity was determined using a TriniLIZE PAI-1 activity bio-immunoassay (BIA) according to the instructions of the manufacturer (Tcoag, Wicklow, Ireland). Fibrinogen levels (Thrombin Reagent, Siemens) were measured using the von Clauss method, plasmin inhibitor levels using a chromogenic assay with reported units converted from % to U/mL (Stachrom, Stago; 100% = 1 U/mL) and TAFI activity using the Pefakit chromogenic assay in plasma (Pentapharm, Aesch, Switzerland) on a Sysmex CS5100 coagulation analyser (Siemens Healthcare Diagnostics B.V.). In this Pefakit assay, the amounts of TAFI in each plasma sample were activated by exogenous thrombin and thrombomodulin. These test results are therefore independent of the coagulation factors in the sample.

Study aim and endpoints

The primary aim of the present study was to assess the effect of rosuvastatin use on fibrinolysis and fibrinolysis parameters. The secondary aim was to evaluate the impact of each individual fibrinolysis parameter on overall plasma fibrinolytic potential after rosuvastatin use.

The primary endpoints of this study were plasma fibrinolytic potential and levels of fibrinogen, plasmin inhibitor, PAI-1 and TAFI before and after rosuvastatin and non-rosuvastatin use. The secondary endpoints of this study were the regression coefficient and the explained variance of the individual fibrinolysis parameters on a change in plasma fibrinolytic potential after rosuvastatin use.

Data analysis

The general characteristics of the participants are reported as means and ranges. For the primary endpoint, mean levels with 95% Confidence Interval (CI) of the plasma fibrinolytic potential and fibrinolysis parameters were calculated at the time of randomisation and at the end of the study period. All the parameters were normally distributed and we compared the mean values obtained at baseline and after four weeks of treatment. Because more men were randomised to non-rosuvastatin use and participants in this non-rosuvastatin group were older compared to the rosuvastatin users, we decided *a priori* to additionally perform an adjusted analysis for sex and age by methods of linear regression.

We furthermore prespecified a subgroup analysis in the group that did not report any signs or symptoms of an infection during the study, because inflammation leads

to a procoagulant state and consequently increased fibrinolysis.[18,19] Additionally, we performed a subgroup analysis comparing participants after an unprovoked or provoked first VTE , because an etiology including hypercoagulability and recurrence rate of VTE is expected to be different among these groups.[20] A two-sided P-value of 0.05 or lower was considered to indicate statistical significance.

For the secondary endpoint, results of the individual participants on change in plasma fibrinolytic potential, fibrinogen, plasmin inhibitor, PAI-1 and TAFI were standardized by calculating Z-scores in order to compare the relative strength of the various fibrinolysis parameters on plasma fibrinolytic potential. This Z-score is calculated for an observation in a participant as the number of standard deviations (SD) from the mean. To determine the relative impact on change of the various fibrinolysis parameters on the change in the plasma fibrinolytic potential, simple and also multiple linear regression analyses were performed in the rosuvastatin group. The standardised regression coefficient for a fibrinolysis parameter indicates the increase or decrease in SDs of the plasma fibrinolytic potential, when that particular fibrinolysis parameter increases per SD and all other variables in the model are unchanged. The R^2 was calculated, denoting (when multiplied by 100%) the explained variation of change in plasma fibrinolytic potential in rosuvastatin users by the individual, or the combined change in fibrinolysis parameters. All data was analysed using 'IBM SPSS Statistics for Windows, version 25'. Reporting of this study is in accordance with the CONSORT statement and the broader EQUATOR guidelines.[21]

Results

Study population

Between December 2012 and December 2016, a total of 255 participants were randomised: 131 assigned to the rosuvastatin group, and 124 to the non-rosuvastatin group. From 126 participants of the rosuvastatin group, and from 121 participants of the non-rosuvastatin group, blood samples were available for analysis of plasma fibrinolytic potential, fibrinogen, plasmin inhibitor, PAI-1 and TAFI. In one participant from the rosuvastatin group and in two participants from the non-rosuvastatin group PAI-1 levels could not be measured due to technological failure. Mean age was lower in rosuvastatin users (57 years, range 19-82) compared to non-rosuvastatin users (59 years, range 21-81) and the percentage of male participants was lower in rosuvastatin users (54%) compared to non-rosuvastatin users (69%) (Table 1). Other baseline characteristics were balanced. In the rosuvastatin group mean cholesterol levels were reduced by 1.96 mmol/L (95% CI

1.83-2.09) compared to 0.19 mmol/L (95%CI 0.10-0.27) in the non-rosuvastatin group, indicating good adherence to the rosuvastatin treatment.

Table 1: Baseline Characteristics

	Rosuvastatin-users (n=126)	**Non-rosuvastatin users (n=121)**
Age (years)	57 (19–82)	59 (21–81)
Male sex	68 (54)	84 (69)
Body mass index (kg/m2)	27.4 (19.2–43.5)	27.7 (17.2–43.2)
Current smoking	18 (14)	17 (14)
Hypertension	24 (19)	21 (17)
Diabetes	3 (2)	0 (0)
Baseline cholesterol (mmol/L)	5.61 (2.95–8.99)	5.59 (3.33–7.89)
Recurrent venous thrombosis	10 (8)	8 (7)
Unprovoked venous thromboembolism	57 (45)	64 (53)
Provoked venous thromboembolism	69 (55)	57 (47)
Provoked by:		
Surgery/trauma/immobilization	32 (25)	31 (26)
Travel >4 hours	22 (18)	14 (12)
Oestrogen use (% in women)	24 (41)	14 (38)
Pregnancy/puerperium (% in women)	0 (0)	2 (5)
Malignancy	2 (2)	8 (7)

Categorical variables are denoted as n (%) and continuous variables as mean (range).

The effect of rosuvastatin use on fibrinolysis

We found that in the rosuvastatin group, clot lysis time as indicator of the plasma fibrinolytic potential decreased by 8.75 min (95% CI -13.8 to -3.72), mean fibrinogen levels were 0.17 g/L higher (95% CI 0.04 to 0.29), plasmin inhibitor levels were 0.05 U/mL lower (95% CI -0.07 to -0.02) and TAFI activity was 4.77% lower (95% CI -6.81 to -2.73) at the end of study as compared with baseline, but PAI-1 levels did not differ (mean change -1.01 IU/mL; 95% CI -3.90 to 1.89). (Figure 1 and Table S1). In the non-rosuvastatin group, plasma fibrinolytic potential and individual fibrinolysis parameters did not change during follow-up (mean change clot lysis time -3.65 min; 95% CI -10.7 to 3.39). When we compared the change in plasma fibrinolytic potential during follow-up between the rosuvastatin and the non-rosuvastin group, the change in plasma fibrinolytic potential tended to be less for rosuvastatin users than for non-rosuvastatin users after adjustment for sex and age (-5.79 min; 95% CI -14.5 to 2.91) (Figure 1 and Table S1). For the fibrinolysis parameters, plasmin inhibitor levels and TAFI concentration were both lower at the end of the study in rosuvastatin users compared to non-rosuvastatin users

(-0.04 U/ml; 95% CI -0.08 to -0.003 and -3.78%; 95% CI -6.63 to -0.92 respectively). The mean change in fibrinogen and PAI-1levels did not differ between groups.

Figure 1: Effects of rosuvastatin on measures of fibrinolysis

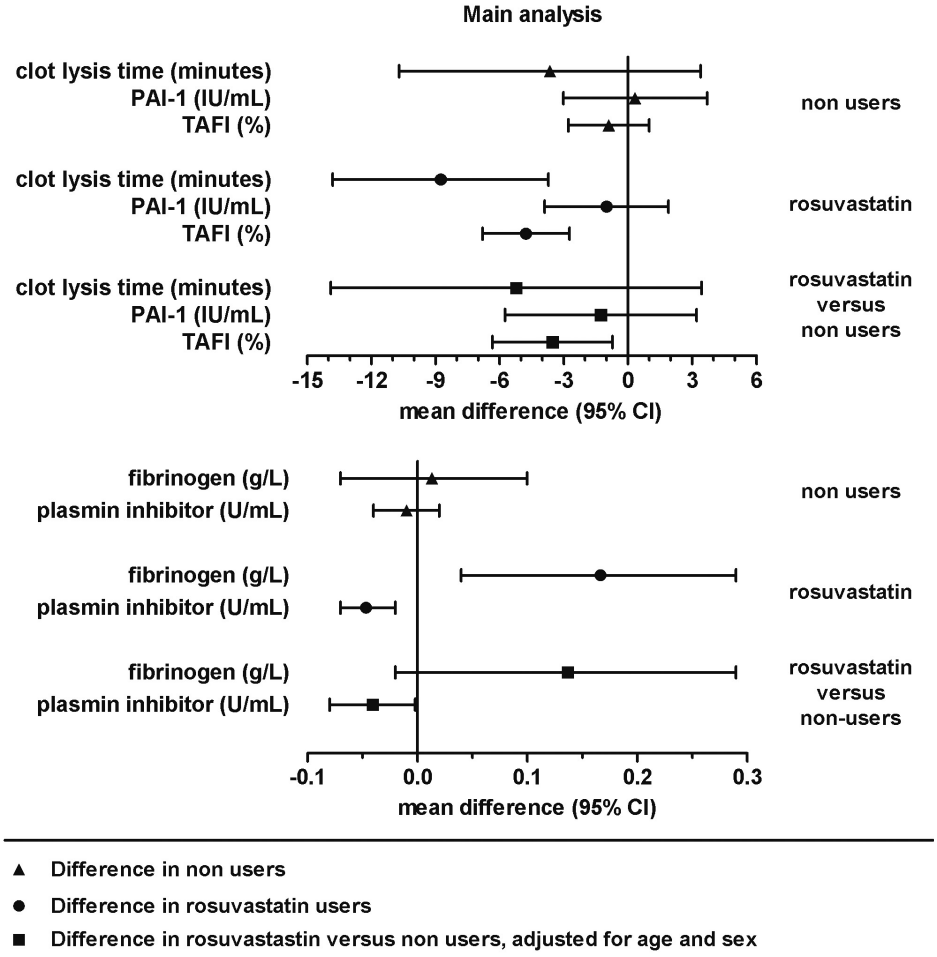


Table S1: Effects of rosuvastatin on measures of fibrinolysis

	Mean levels									
	Baseline	End of study	Mean* change (95% CI)		Mean difference† in change (95% CI)		Mean difference‡ in change (95% CI)		Mean^ change (95% CI)		Mean difference# in change (95% CI)	
Plasma fibrinolytic potential												
Clot lysis time (minutes)												
Non users	85.4	81.8	-3.65	(-10.7 to 3.39)	Reference		Reference		-3.62	(-10.8 to 3.52)	Reference	
Rosuvastatin users	84.6	75.8	-8.75	(-13.8 to -3.72)	-5.09	(-13.7 to 3.46)	-5.22	(-13.9 to 3.44)	-9.41	(-14.3 to -4.52)	- 5.79	(-14.5 to 2.91)
Fibrinolysis parameters												
fibrinogen (g/L)												
Non users	2.90	2.91	0.01	(-0.07 to 0.10)	Reference		Reference		0.02	(-0.07 to 0.10)	Reference	
Rosuvastatin users	2.90	3.07	0.17	(0.04 to 0.29)	0.15	(-0.001 to 0.30)	0.14	(-0.02 to 0.29)	0.10	(0.003 to 0.20)	0.08	(-0.06 to 0.21)
plasmin inhibitor (U/mL)												
Non users	1.15	1.14	-0.01	(-0.04 to 0.02)	Reference		Reference		-0.01	(-0.04 to 0.01)	Reference	
Rosuvastatin users	1.17	1.12	-0.05	(-0.07 to -0.02)	-0.04	(-0.07 to 0.002)	-0.04	(-0.08 to -0.002)	-0.05	(-0.07 to -0.02)	-0.04	(-0.08 to -0.003)
Plasminogen Activator Inhibitor-1(PAI-1) (IU/mL)												
Non users	29.9	30.2	0.33	(-3.03 to 3.69)	Reference		Reference		0.10	(-3.26 to 3.46)	Reference	
Rosuvastatin users	28.4	27.3	-1.01	(-3.90 to 1.89)	-1.33	(-5.73 to 3.06)	-1.27	(-5.75 to 3.21)	-1.20	(-4.15 to 1.75)	-1.26	(-5.77 to 3.25)
Thrombin Activatable Fibrinolysis Inhibitor (TAFI) (%)												
Non users	106.4	105.5	-0.90	(-2.79 to 0.99)	Reference		Reference		-0.90	(-2.83 to 1.03)	Reference	
Rosuvastatin users	105.3	100.6	-4.77	(-6.81 to -2.73)	-3.87	(-6.64 to -1.10)	-3.54	(-6.34 to -0.73)	-5.07	(-7.17 to -2.97)	-3.78	(-6.63 to -0.92)

** Paired analysis*
† Between comparison analysis
‡ Between comparison analysis, adjusted for age and sex
^ Paired analysis; eight participants who reported an infection at time of end of study excluded
 # Between comparison analysis, adjusted for age and sex; 8 participants who reported an infection at time of end of study excluded
Abbreviation: CI indicates confidence interval

In the subgroup analysis of patients who did not report any symptoms or signs of infection (n=239), results in both groups were similar to the main analysis. When we compared results of participants after an unprovoked or provoked VTE, mean change in plasma fibrinolytic potential appeared larger in the group of rosuvastatin users after a provoked VTE as compared to the unprovoked group (-11.3 min; 95% CI -19.0 to -3.59 compared to -5.67 min; 95% CI -11.9 to 0.57). For fibrinogen and TAFI, change in levels or concentration at the end of study in rosuvastatin users was more evident in the subgroup after provoked VTE (Table S2).

Table S2: Effects of rosuvastatin use on measures of fibrinolysis separated in subgroups of participants with unprovoked or provoked venous thromboembolism

		Mean levels					
		Baseline	End of study	Mean* change (95% CI)		Mean difference† in change (95% CI)	
Plasma fibrinolytic potential							
Clot lysis time (minutes)							
Non users	Unprovoked	80.8	77.8	-2.99	(-11.9 to 5.91)	Reference	
Rosuvastatin users	Unprovoked	81.6	75.9	-5.67	(-11.9 to 0.57)	-2.67	(-13.7 to 8.34)
Non users	Provoked	90.7	86.3	-4.40	(-15.8 to 7.01)	Reference	
Rosuvastatin users	Provoked	87.1	75.8	-11.3	(-19.0 to -3.59)	-6.90	(-20.1 to 6.35)
Fibrinolysis parameters							
fibrinogen (g/L)							
Non users	Unprovoked	2.85	2.92	0.07	(-0.05 to 0.19)	Reference	
Rosuvastatin users	Unprovoked	2.93	3.03	0.10	(-0.12 to 0.32)	0.03	(-0.22 to 0.28)
Non users	Provoked	2.95	2.90	-0.05	(-0.18 to 0.08)	Reference	
Rosuvastatin users	Provoked	2.88	3.10	0.22	(0.08 to 0.36)	0.26	(0.07 to 0.45)
plasmin inhibitor (U/mL)							
Non users	Unprovoked	1.13	1.12	-0.02	(-0.05 to 0.01)	Reference	
Rosuvastatin users	Unprovoked	1.14	1.09	-0.05	(-0.09 to -0.01)	-0.03	(-0.08 to 0.02)
Non users	Provoked	1.17	1.17	0.00	(-0.04 to 0.04)	Reference	
Rosuvastatin users	Provoked	1.19	1.15	-0.04	(-0.08 to -0.005)	-0.04	(-0.10 to 0.01)

Table S2: Continued

		Mean levels					
		Baseline	**End of study**	**Mean* change (95% CI)**		**Mean difference† in change (95% CI)**	
Plasminogen Activator Inhibitor-1(PAI-1) (IU/mL)							
Non users	Unprovoked	30.1	27.6	-2.44	(-6.50 to 1.63)	Reference	
Rosuvastatin users	Unprovoked	29.7	27.7	-2.09	(-6.91 to 2.74)	0.35	(-5.85 to 6.55)
Non users	Provoked	26.7	33.2	3.49	(-2.04 to 9.01)	Reference	
Rosuvastatin users	Provoked	27.2	27.1	-0.11	(-3.48 to 3.46)	-3.60	(-9.89 to 2.69)
Thrombin Activatable Fibrinolysis Inhibitor (TAFI) (%)							
Non users	Unprovoked	107.3	105.7	-1.61	(-4.23 to 1.01)	Reference	
Rosuvastatin users	Unprovoked	104.6	101.0	-3.60	(-6.65 to -0.54)	-1.99	(-5.95 to 1.98)
Non users	Provoked	105.3	105.2	-0.11	(-2.91 to 2.70)	Reference	
Rosuvastatin users	Provoked	106.0	100.2	-5.74	(-8.52 to -2.96)	-5.63	(-9.58 to -1.69)

** Paired analysis † Between comparison analysis CI: Confidence interval*

The impact of individual fibrinolysis parameters on change in plasma fibrinolytic potential in rosuvastatin users

Among the fibrinolysis parameters, the only change in plasmin inhibitor was associated with change in plasma fibrinolytic potential in rosuvastatin users (Table 2). The regression coefficient of this association was 0.18 (95% CI 0.002 to 0.352). The explained variance in this model was 3% (R^2 0.03). Including all the fibrinolytic parameters in a multiple regression model increased the explained variance of change in plasma fibrinolytic potential to 5% (R^2 0.05).

Table 2: Mean impact in change in plasma fibrinolytic potential with one SD increase in fibrinolytic parameter between baseline and end of study in rosuvastatin users

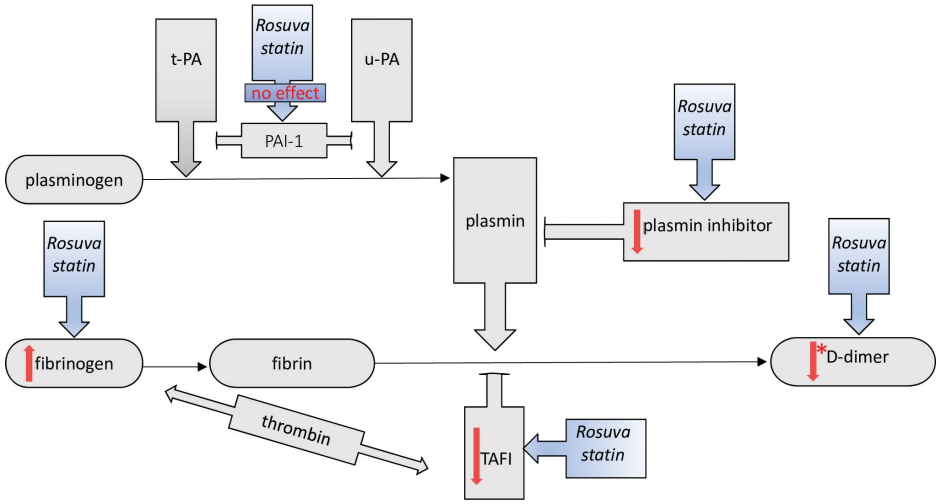
	Simple model†		**Multiple model‡**	
	β (95%CI)	**R^2**	**β (95%CI)**	**R^2**
Change in fibrinogen (g/L)	-0.02 (-0.20 to 0.16)	0.00	-0.04 (-0.22 to 0.14)	0.05
Change in plasmin inhibitor (U/mL)	0.18 (0.002 to 0.35)	0.03	0.18 (0.003 to 0.36)	
Change in Plasminogen Activator Inhibitor-1(PAI-1) (IU/mL)	0.001 (-0.18 to 0.18)	0.00	0.04 (-0.14 to 0.21)	
Change in Thrombin Activatable Fibrinolysis Inhibitor (TAFI) (%)	-0.13 (-0.31 to 0.04)	0.02	-1.26 (-0.30 to 0.05)	

Abbreviations: SD standard deviation; CI indicates confidence interval
†In each of the four different single models, plasma fibrinolytic potential was the dependent variable, and only one of the fibrinolytic parameters was the independent variable.
‡Plasma fibrinolytic potential was the dependent variable and all four fibrinolytic parameters simultaneously were independent variables.

Discussion

Our randomised study in those participants with prior VTE who stopped anticoagulant treatment showed that four weeks of 20 mg/day rosuvastatin use led to an increased plasma fibrinolytic potential, whereas non-use did not lead to increased fibrinolysis. The difference in change in fibrinolytic potential in users versus non-users did, however, not reach statistical significance. Among the fibrinolysis parameters, plasmin inhibitor and TAFI decreased during use of rosuvastatin and the changes in rosuvastatin users compared to non-users also differed significantly. Fibrinogen levels were increased after four weeks of rosuvastatin use, but PAI-1 levels did not change (Figure S1). The variance in change in plasma fibrinolytic potential between the baseline and the end of the study was explained for 3% by the change in plasmin inhibitor and not by other individual fibrinolysis parameters. Notably, there is a relationship between clot formation and lysis, [22] suggesting that increased fibrinolytic potential by rosuvastatin could be secondary to decreased coagulation. However, we also found decreased levels of TAFI and plasmin inhibitor, which support a direct pro-fibrinolytic effect of rosuvastatin. The changes in fibrinolytic potential and the individual fibrinolysis parameters in rosuvastatin users could be clinically relevant, though this should be investigated in prospective larger studies.

Figure S1: Increased plasma fibrinolytic potential by rosuvastatin 20 mg/day for four weeks



Schematic overview of effects of rosuvastatin 20 mg/day on fibrinolysis
Grey arrows: positive influence; Grey blocked end: negative influence.
Red arrows/ symbols: effects of rosuvastatin 20 mg/day on fibrinolysis parameters:
Red up arrow: increase; red down arrow: decrease; red down arrow: lower levels in rosuvastatin group compared to non-statin group; 'no effect': no effect*

This is the first study in participants with prior VTE, showing that 28 days use of rosuvastatin increases plasma fibrinolytic potential and lowers plasma levels of TAFI and plasmin inhibitor. Direct effects on plasma fibrinolytic potential were also measured earlier after a very short (three days) use of atorvastatin in a non-randomised study.[23] Another before-after study evaluated plasma fibrinolytic potential after simvastatin therapy, and presented a mean shortening in clot lysis time of 12.9 min after a three-month treatment with simvastatin 40 mg.[24] Reduced plasma fibrinolytic potential (i.e. longer clot lysis times) has been shown to be associated with higher risk of VTE.[3,6,7] Our findings corroborate fibrinolytic activity being increased by rosuvastatin and that it might therefore decrease the risk of recurrent VTE.

Results on the individual fibrinolytic parameters are primarily in line with the results on plasma fibrinolytic potential. Specifically, lower levels of plasmin inhibitor and TAFI will lead to reduced inhibition of fibrinolysis, consequently resulting in shorter clot lysis times and therefore increased global fibrinolytic potential. Decreased TAFI levels were also previously observed in 35 patients with hyperlipidaemia after eight weeks of treatment with simvastatin and in another study in which 44 patients with hypercholesterolaemia

were treated with atorvastatin.[25,26] Studies on plasmin inhibitor are scarce with only one comparable study on 24 patients with primary dyslipidaemia treated with simvastatin and 18 patients treated with pravastatin for 12 weeks.[27] In the simvastatin group, plasmin inhibitor did not change after treatment, but in the pravastatin group the levels decreased. Remarkably, in our study PAI-1 levels did not change after rosuvastatin use even though we expected this fibrinolytic parameter to have a high impact on the plasma fibrinolytic potential.[28] Fibrinogen levels were also unexpectedly higher after rosuvastatin use in our study. Since fibrinogen is associated with pro-inflammatory and pro-coagulants effects, one would expect that statins would decrease fibrinogen levels. In a meta-analysis of 14 other studies including patients with high cholesterol or stable coronary disease, also no effect of statin treatment on PAI-1 levels and fibrinogen was found either.[29] For rosuvastatin specifically, a reduction in fibrinogen levels after six months of treatment was shown in 24 patients with rheumatoid arthritis and in 100 patients with metabolic syndrome, but in another study fibrinogen levels did not change after three months in 17 patients with type 2 diabetes.[30-32] D-dimer levels, a FDP generated in the blood clot during fibrinolysis *in vivo*, were already measured in the START study. Measured D-dimer levels were higher at the end of the study in non-rosuvastatin users, but remained unchanged in the rosuvastatin group.[11] After withdrawal of anticoagulant treatment, D-dimer levels normally increase, which is called the rebound phenomenon.[33,34] D-dimer levels are the result of the amount of available fibrin and the fibrinolytic potential. Rosuvastatin is expected to lower fibrin levels by reducing clotting factors and by its anti-inflammatory effects, and to increase the fibrinolytic potential. [9,11,35] Because of the lack of increase in D-dimer levels in rosuvastatin users during follow-up, we expect that the effect of lowering fibrin levels is stronger than the effect of increasing fibrinolytic potential.

Interestingly, in the subgroup analysis results on increased plasma fibrinolytic potential, decreased plasmin inhibitor and TAFI seemed to be more robust after a provoked VTE than unprovoked VTE. Karasu et al. on the other hand, reported that provoked VTE showed a 1.5-fold increased risk of VTE in the presence of hypofibrinolysis whereas for unprovoked VTE, hypofibrinolysis was associated with a higher 2.1-fold increased risk.[36] This contradiction might be explained by a different or stronger additional impact of rosuvastatin use on hypofibrinolysis in patients after provoked VTE rather than unprovoked VTE, or a reduced impact in our study for performing this subanalysis.

To evaluate the impact of each individual fibrinolysis parameter on overall plasma fibrinolytic potential after rosuvastatin use, we performed an additional regression analysis. Results suggest minor impact of change of individual fibrinolysis parameters with only a small significant impact of plasmin inhibitor. This was unexpected, because

in another study TAFI and PAI-1 levels explained the majority of the variance in clot lysis time.[28] However in our analysis we only evaluated the change in parameters according to the change in plasma fibrinolytic potential.

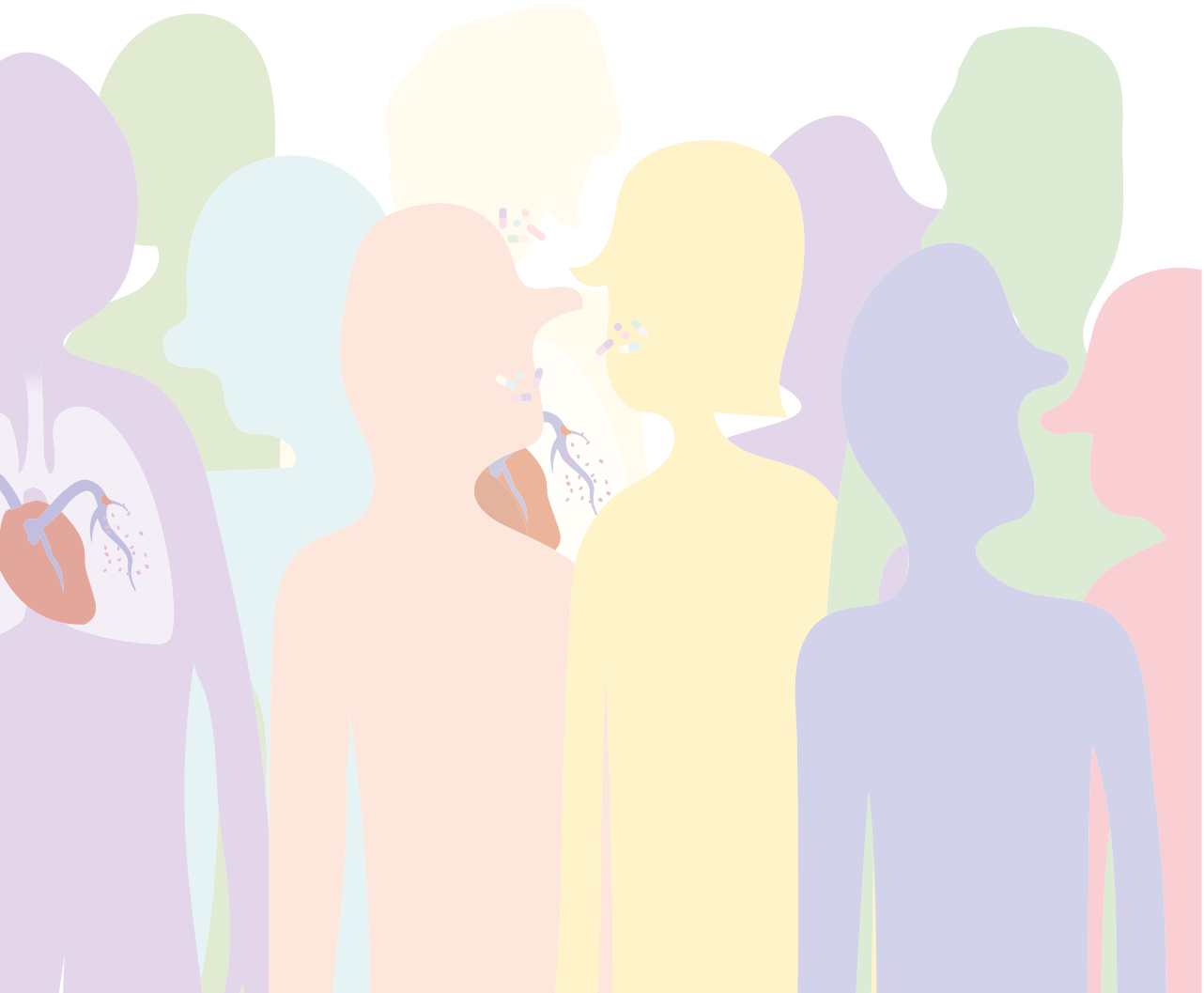
Some aspects of this study warrant comment. The START study was open label and we noticed a difference in the distribution of sex and age between the groups. However, since we evaluated fibrinolysis parameters and we decided a priori to correct our analysis for these possible confounders, it is unlikely that both factors influenced our results. Another aspect is that we only tested the effect of rosuvastatin, a hydrophilic type of statin. Hydrophilic statins, such as pravastatin and rosuvastatin have different properties to lipophilic statins, such as simvastatin, atorvastatin and fluvastatin. Cellular uptake of hydrophilic types of statins for example, is only present in hepatocytes but not in extrahepatic cells, whereas lipophilic statins penetrate cell membranes and enter cells in any organ.[37] The exact effect on fibrinolysis might therefore be different for hydrophilic statins than for rosuvastatin. Nevertheless, our findings on increased fibrinolysis after rosuvastatin treatment underline the suggestion that statins could be prescribed to patients with prior VTE who are considered to be at high risk of anticoagulation-related bleeding.

In conclusion, we found that in participants with prior VTE who stopped anticoagulant treatment, four weeks of rosuvastatin use led to an increased plasma fibrinolytic potential, decreased plasmin inhibitor and TAFI and higher fibrinogen levels compared to non-statin users, whereas PAI-1 levels did not change. Variance in change in plasma fibrinolytic potential could only be explained for a small part by change in plasmin inhibitor and not by the other individual fibrinolysis parameters. This increase in fibrinolytic potential and formerly reported anticoagulant effects after rosuvastatin treatment support the need for further studies on the possible role for statins in the secondary prevention of VTE.

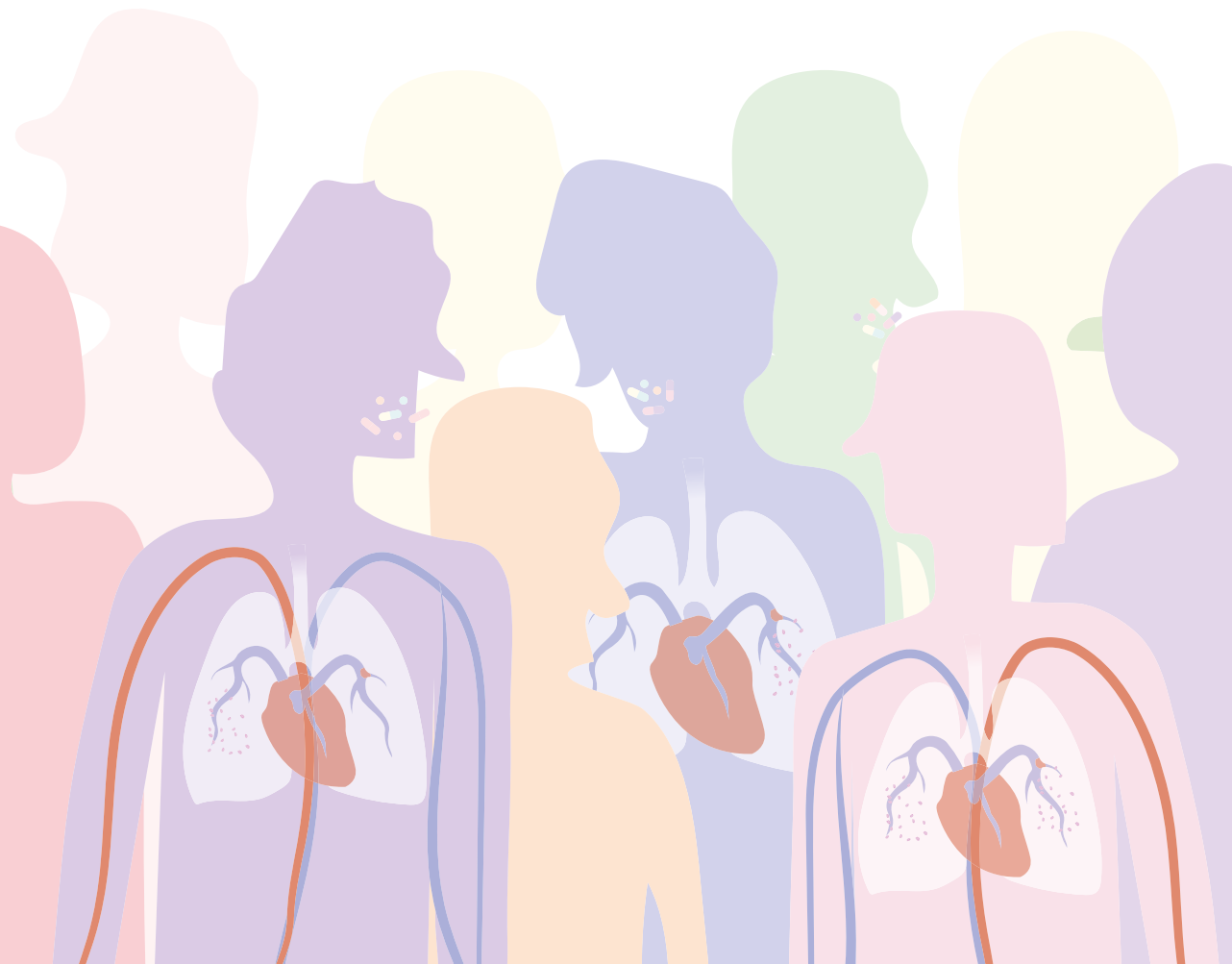
References

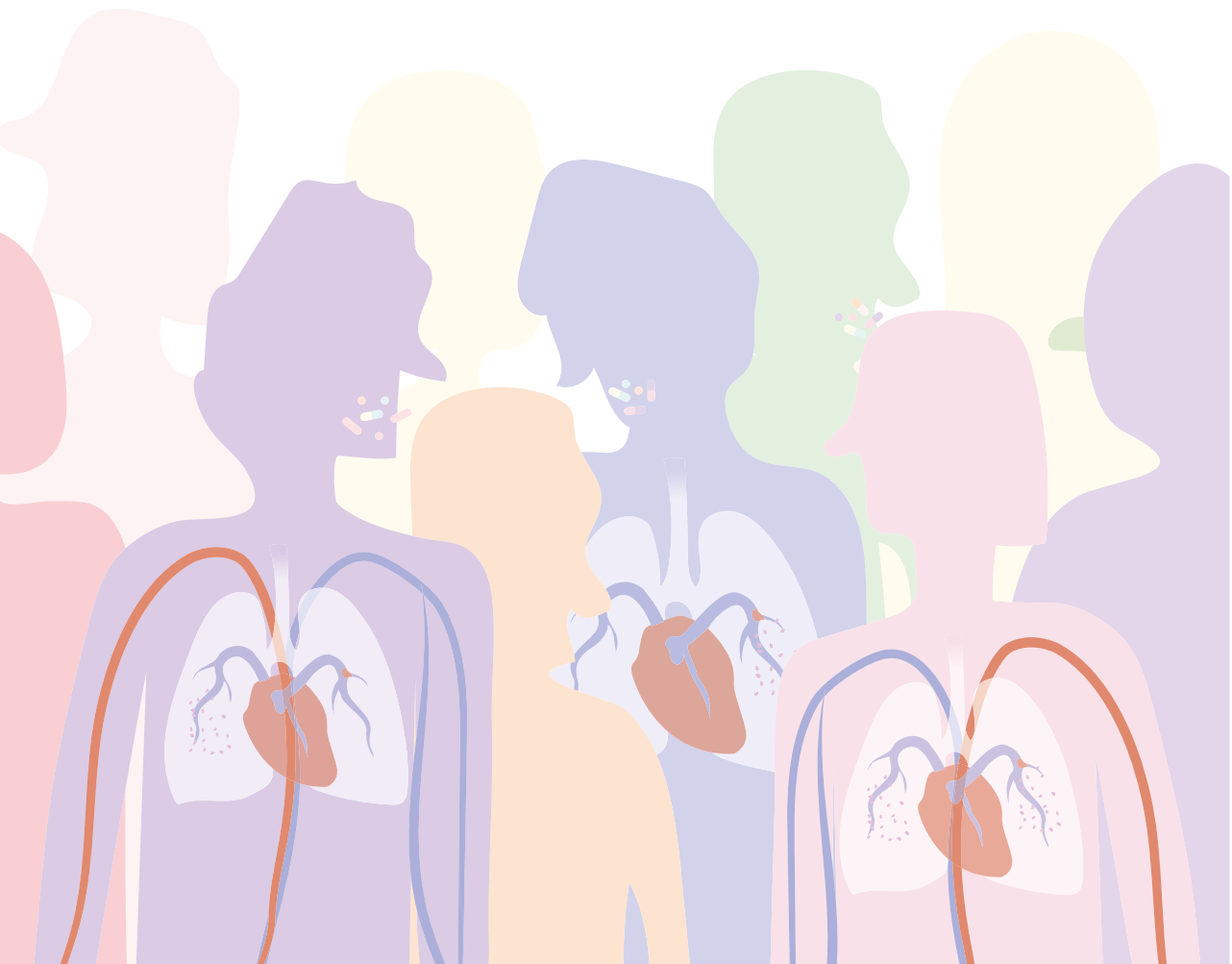
1. Oesterle A, Laufs U, Liao JK. Pleiotropic Effects of Statins on the Cardiovascular System. *Circ Res.* 2017;120(1):229-243.
2. Kunutsor SK, Seidu S, Khunti K. Statins and primary prevention of venous thromboembolism: a systematic review and meta-analysis. *Lancet Haematol.* 2017;4(2):e83-e93.
3. Glynn RJ, Danielson E, Fonseca FA, et al. A randomized trial of rosuvastatin in the prevention of venous thromboembolism. *N Engl J Med.* 2009;360(18):1851-1861.
4. Pignatelli P, Carnevale R, Pastori D, et al. Immediate antioxidant and antiplatelet effect of atorvastatin via inhibition of Nox2. *Circulation.* 2012;126(1):92-103.
5. Tannous M, Cheung R, Vignini A, Mutus B. Atorvastatin increases ecNOS levels in human platelets of hyperlipidemic subjects. *Thromb Haemost.* 1999;82(5):1390-1394.
6. Meltzer ME, Lisman T, Doggen CJ, de Groot PG, Rosendaal FR. Synergistic effects of hypofibrinolysis and genetic and acquired risk factors on the risk of a first venous thrombosis. *PLoS Med.* 2008;5(5):e97.
7. Lisman T, de Groot PG, Meijers JC, Rosendaal FR. Reduced plasma fibrinolytic potential is a risk factor for venous thrombosis. *Blood.* 2005;105(3):1102-1105.
8. Schol-Gelok S, Morelli F, Arends LR, et al. A revised systematic review and meta-analysis on the effect of statins on D-dimer levels. *Eur J Clin Invest.* 2019;49(8):e13130.
9. Rijken DC, Lijnen HR. New insights into the molecular mechanisms of the fibrinolytic system. *J Thromb Haemost.* 2009;7(1):4-13.
10. Zorio E, Gilabert-Estelles J, Espana F, Ramon LA, Cosin R, Estelles A. Fibrinolysis: the key to new pathogenetic mechanisms. *Curr Med Chem.* 2008;15(9):923-929.
11. Biedermann JS, Kruip M, van der Meer FJ, et al. Rosuvastatin use improves measures of coagulation in patients with venous thrombosis. *Eur Heart J.* 2018;39(19):1740-1747.
12. Talens S, Malfliet JJ, Rudez G, et al. Biological variation in tPA-induced plasma clot lysis time. *Thromb Haemost.* 2012;108(4):640-646.
13. Johansen LG, Gram J, Kluft C, Jespersen J. Chronobiology of coronary risk markers in Greenland Eskimos: a comparative study with Caucasians residing in the same Arctic area. *Chronobiol Int.* 1991;8(5):352-360.
14. Kluft C, Jie AF, Rijken DC, Verheijen JH. Daytime fluctuations in blood of tissue-type plasminogen activator (t-PA) and its fast-acting inhibitor (PAI-1). *Thromb Haemost.* 1988;59(2):329-332.
15. Longstaff C, subcommittee on f. Development of Shiny app tools to simplify and standardize the analysis of hemostasis assay data: communication from the SSC of the ISTH. *J Thromb Haemost.* 2017;15(5):1044-1046.
16. Website. https://drclongstaff.shinyapps.io/clotlysisCL_2019/. 2019-05-16.
17. Guimaraes AH, de Bruijne EL, Lisman T, et al. Hypofibrinolysis is a risk factor for arterial thrombosis at young age. *Br J Haematol.* 2009;145(1):115-120.
18. Reitsma PH, Branger J, Van Den Blink B, Weijer S, Van Der Poll T, Meijers JC. Procoagulant protein levels are differentially increased during human endotoxemia. *J Thromb Haemost.* 2003;1(5):1019-1023.
19. Vazquez-Garza E, Jerjes-Sanchez C, Navarrete A, Joya-Harrison J, Rodriguez D. Venous thromboembolism: thrombosis, inflammation, and immunothrombosis for clinicians. *J Thromb Thrombolysis.* 2017;44(3):377-385.

20. Kearon C, Ageno W, Cannegieter SC, et al. Categorization of patients as having provoked or unprovoked venous thromboembolism: guidance from the SSC of ISTH. *J Thromb Haemost.* 2016;14(7):1480-1483.
21. Schulz KF, Altman DG, Moher D, Group C. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *BMJ.* 2010;340:c332.
22. Longstaff C. Measuring fibrinolysis: from research to routine diagnostic assays. *J Thromb Haemost.* 2018;16(4):652-662.
23. Zolcinski M, Ciesla-Dul M, Undas A. Effects of atorvastatin on plasma fibrin clot properties in apparently healthy individuals and patients with previous venous thromboembolism. *Thromb Haemost.* 2012;107(6):1180-1182.
24. Undas A, Topor-Madry R, Tracz W. Simvastatin increases clot permeability and susceptibility to lysis in patients with LDL cholesterol below 3.4 mmol/l. *Pol Arch Med Wewn.* 2009;119(6):354-359.
25. Guven GS, Atalar E, Yavuz B, et al. Simvastatin treatment improves endothelial function and increases fibrinolysis in patients with hypercholestrolemia. *J Natl Med Assoc.* 2006;98(4):627-630.
26. Bruni F, Pasqui AL, Pastorelli M, et al. Effect of atorvastatin on different fibrinolyis mechanisms in hypercholesterolemic subjects. *Int J Cardiol.* 2004;95(2-3):269-274.
27. Doncheva NI, Nikolov KV, Vassileva DP. Lipid-modifying and pleiotropic effects of gemfibrozil, simvastatin and pravastatin in patients with dyslipidemia. *Folia Med (Plovdiv).* 2006;48(3-4):56-61.
28. Meltzer ME, Lisman T, de Groot PG, et al. Venous thrombosis risk associated with plasma hypofibrinolysis is explained by elevated plasma levels of TAFI and PAI-1. *Blood.* 2010;116(1):113-121.
29. Balk EM, Lau J, Goudas LC, et al. Effects of statins on nonlipid serum markers associated with cardiovascular disease: a systematic review. *Ann Intern Med.* 2003;139(8):670-682.
30. Tam LS, Li EK, Shang Q, et al. Effects of rosuvastatin on subclinical atherosclerosis and arterial stiffness in rheumatoid arthritis: a randomized controlled pilot trial. *Scand J Rheumatol.* 2011;40(6):411-421.
31. Polenova NV, Vaulin NA, Masenko VP, Iavelov IS, Gratsianskii NA. [Rosuvastatin and fenofibrate in patients with diabetes and low high density lipoprotein cholesterol: comparison of changes of lipid levels and some markers of inflammation]. *Kardiologiia.* 2009;49(2):9-14.
32. Bostan C, Yildiz A, Ozkan AA, Uzunhasan I, Kaya A, Yigit Z. Beneficial effects of rosuvastatin treatment in patients with metabolic syndrome. *Angiology.* 2015;66(2):122-127.
33. Martinez C, Katholing A, Folkerts K, Cohen AT. Risk of recurrent venous thromboembolism after discontinuation of vitamin K antagonist treatment: a nested case-control study. *J Thromb Haemost.* 2016;14(7):1374-1383.
34. Cundiff DK. Clinical evidence for rebound hypercoagulability after discontinuing oral anticoagulants for venous thromboembolism. *Medscape J Med.* 2008;10(11):258.
35. Kata D, Foldesi I, Feher LZ, Hackler L, Jr., Puskas LG, Gulya K. Rosuvastatin enhances anti-inflammatory and inhibits pro-inflammatory functions in cultured microglial cells. *Neuroscience.* 2016;314:47-63.
36. Karasu A, Baglin TP, Luddington R, Baglin CA, van Hylckama Vlieg A. Prolonged clot lysis time increases the risk of a first but not recurrent venous thrombosis. *Br J Haematol.* 2016;172(6):947-953.
37. van Vliet AK, van Thiel GC, Huisman RH, Moshage H, Yap SH, Cohen LH. Different effects of 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors on sterol synthesis in various human cell types. *Biochim Biophys Acta.* 1995;1254(1):105-111.



General discussion and Summary





7

General discussion and Summary

General discussion and Summary

Physicians are facing diagnostic challenges on a daily basis. To increase the accuracy of the diagnostic process they often request diagnostic tests to detect or to rule out the presence of a disease. A wide range of medical tests, ranging from laboratory tests, physiological tests and radiologic procedures are available to support the physician in detecting or excluding diseases. For diagnostic tests in the domain clinical chemistry typically test results obtained in a reference population are compared with the test results obtained in patients with the disease to establish performance data such as positive and negative predictive value. When the diagnostic test is able to make a substantial difference between the pre- and post-test likelihood of the condition of interest this will support the use of the test in the diagnostic process. In general, diagnostic tests with high sensitivity are commonly used to rule out diseases and diagnostic tests with high specificity to correctly identify those patients with the disease.[1]

Among all diseases at the emergency department (ED), venous thrombo-embolism (VTE), including deep vein thrombosis (DVT) and pulmonary embolism (PE), might be one of the most challenging diagnoses. One of the challenges in the diagnostic process of VTE is to select imaging procedures and/or laboratory tests that provide acceptable post-test probability while at the same time avoiding exposing all patients to extensive diagnostic procedures. Patients with suspected VTE may present with a variety of non-specific symptoms, like dyspnoea, thoracic pain or swelling of an extremity. Many symptoms associated with VTE are mild and could also point to other diagnoses, for example pneumonia or musculoskeletal pain.[2] This may eventually lead to a diagnostic delay. For diagnosis of PE in the Netherlands, diagnostic delay has been estimated to be 8.6 days, with 4.2 days on average because of patient delay and 3.9 days of primary care doctor delay.[3] The absence of chest pain is specifically associated with a fivefold higher risk in diagnostic delay.[4] Notably, when VTE is missed as diagnosis this may lead to severe morbidity or even death. Therefore, the physician requires reliable diagnostic instruments to safely rule out VTE. The adherence to the validated diagnostic strategies is unfortunately variable.[5,6] This can be explained by the sometimes hectic work environment at the EDs and the complexity of some algorithms used for ruling out VTE. Furthermore, physicians receive only limited comparative feedback on adherence to guidelines, and they tend to rely on their own judgment and personal experience to decide on which diagnostic procedures to choose from. Based on clinical experience or by using a clinical decision rule with a standardized scoring scheme, the pre-test probability of a VTE can be estimated. The most commonly used clinical decision rule in DVT and PE was introduced by Wells et al.[7,8] The Wells score

for PE consists of seven different items and is sequential (Table 1). In clinical practice the physician has to score those different items and calculate the score. In patients with low probability of VTE, a normal D-dimer test result safely excludes PE or DVT without performance of imaging tests. VTE guidelines therefore recommend combining clinical decision rules and measurement of D-dimer levels to identify patients in whom DVT or PE may be ruled out without performing imaging tests.[9,10] In this way patients with low probability of VTE and a negative D-dimer do not need an imaging test, such as compression venous ultrasonography in the diagnosis of DVT or computed tomography pulmonary angiography (CTPA) for PE.[11,12] CTPA leads to radiation exposure, high healthcare costs, time consumption and possible allergic reactions and contrast-induced nephropathy.[11,13] Also, most CTPA results are negative for PE, indicating that selection of patients needing a CTPA is yet not optimal and several patients are exposed to avoidable radiation. In this scope, we have performed a study to improve the diagnostic strategy and therapeutic management in VTE for specific patient groups and to investigate the influence of co-medication on haemostatic biomarkers.

Table 1: Wells score[7]

Item	Points
Clinical signs and symptoms of DVT	3
An alternative diagnosis is less likely than PE	3
Heart rate greater than 100/min	1.5
Immobilization or surgery in the previous 4 weeks	1.5
Previous DVT or PE	1.5
Haemoptyisis	1
Active malignancy	1

DVT: deep venous thrombosis; PE: pulmonary embolism
In total ≤ 4 points: PE unlikely (low clinical pretest probability): measurement of D-dimer level; if normal (< 500ng/mL) PE could be safely excluded
In total > 4 points: PE likely (high clinical pretest probability): performance of CTPA (computed tomography pulmonary angiography), measurement of D-dimer level not indicated.

Chapter 2 of this thesis focuses on the optimal diagnostic strategy of PE in different (sub) populations. First we present a new diagnostic algorithm for PE: The YEARS algorithm.[14] This YEARS algorithm was designed to be more easily applied in clinical practice than already existing clinical decision rules such as the Wells score, and to further decrease the number of required CTPA imaging. Three items of the original Wells clinical decision rule were found to be the most predictive for PE. These items were clinical signs of DVT, hemoptysis, and PE being the most likely diagnosis.[15] If one of these items is present in combination with a classic D-dimer test threshold below 500 ng/mL or a higher

threshold of below 1000 ng/mL in absence of one of the YEARS items it was expected to safely exclude PE without performance of CTPA. In the YEARS study we evaluated the safety and efficiency of the YEARS diagnostic algorithm in a prospective multicenter cohort consisting of 2944 patients with clinically suspected PE. This prospective study showed that the YEARS algorithm safely excluded acute PE and led to a statistically significant absolute 14% decrease in the need for CTPA imaging compared to the Wells score in combination with a D-dimer threshold of 500 ng/mL. In this study we found a 0.43% 3-month VTE incidence in patients not subjected to CTPA. This was in line with other sequential algorithms.[14,16,17] The YEARS algorithm has now been implemented in many hospital protocols. However, some researchers suggest that prior to large scale implementation the YEARS algorithm first needs validation in another cohort.[18] They argue that D-dimer levels were measured before clinical assessment, that hemoptysis was uncommon, and that determination of PE as the most likely diagnosis may have been influenced by the a priori knowledge of the other clinical prediction rules like the Wells score. In my experience these arguments do not hold. YEARS criteria were determined before D-dimer levels were made available. Also, the physician should always be aware of all risk factors for VTE. Presence of a risk factor for VTE in the individual patient will influence the choice to select PE as the most likely diagnosis. This may also be partly the reason that this item is most predictive for PE in the Wells algorithm.[15]

An additional factor influencing the complexity of the diagnostic workup of VTE is that D-dimer levels and other haemostatic biomarkers can be influenced by patient characteristics such as age, presence of an active malignancy, infection or pregnancy and treatment with certain drugs.[19-22] The influence of these factors on D-dimer levels potentially could affect the diagnostic performance of the clinical decision rules. To make it even more complex, many drugs and risk factors like immobility, active infection, cancer, pregnancy, trauma, advanced age, antiphospholipid antibodies, obesity and genetic traits such as the factor V Leiden mutation could also influence the risk of VTE.[23] Another algorithm, the age-adjusted D-dimer cut-off (ADJUST) strategy, also showed that it was able to reduce the number of CTPAs in patients suspected of PE.[24] In this ADJUST strategy, the Wells score is used in combination with a D-dimer cut-off (10 * age) ng/mL for patients aged 50 years or older. For example, the D-dimer cut-off of a 70-year old patient is set to 700 ng/mL and for a 90-year old patient to 900 ng/mL. Integration of this ADJUST strategy in the YEARS study cohort however did not improve efficiency and led to more missed diagnoses, although one should keep in mind that this was a post hoc analysis.[25] A different analysis stressing the advantages of the YEARS algorithm showed that implementation of the YEARS algorithm was associated with a shorter length of stay at the emergency department and also reduced costs compared

with the ADJUST strategy.[26] Also, use of the YEARS algorithm is associated with a lower frequency of diagnosing isolated subsegmental PE, probably by the reduction of CTPAs.[27] After improvement of CTPA imaging, isolated subsegmental PE has been more often diagnosed, but the risk of fatal PE remained unaffected.[28] As mentioned above, lower frequency of VTE diagnoses in the YEARS study was not associated with a higher risk of recurrent VTE during follow-up.

Because of the important role of D-dimer levels in diagnostic algorithms for VTE, we studied the literature for the influence of co-medication on D-dimer levels. Among all co-medication, antiplatelet drugs and statins, both frequently prescribed drugs, might influence D-dimer levels. To investigate if D-dimer cut-offs may need to be adjusted in statin and/or antiplatelet therapy users, in Chapter 2.2 we additionally performed a post-hoc analysis in the YEARS study database.[29] We found that statin use was associated with 15% decrease in D-dimer levels, and also found that there was no association between antiplatelet therapy and D-dimer levels. We then reclassified patients using statins within the YEARS algorithm using 15 % lower D-dimer thresholds. This model resulted in lower specificity (0.42 compared to 0.33) with no difference in false negative tests. Consequently, adjusting D-dimer cut-offs for statin use did not result in a safer diagnostic strategy. Based on our study, even though post-hoc, there is no need for adjusting D-dimer cut-off values for statin users within the YEARS algorithm. The D-dimer cut-off values seem to be chosen safely also for statin users. Avoidance of unnecessary complexity is the preferred option, and we support the KISS principle (Keep It Simple & Straightforward).

The post-hoc analysis on statins and antiplatelet drugs leads to the second aim of this thesis: to investigate the influence of co-medication on measurable indicators of haemostasis, such as D-dimer, fibrinogen, plasminogen activator inhibitor-1 (PAI-1), tissue-type plasminogen activator (t-PA) and antiplasmin, or VTE risk. In general, measurable indicators, more commonly referred to as biomarkers, can be used for screening, diagnosing or monitoring the activity of a disease, to guide targeted therapy or to assess therapeutic response.[30,31] In Chapter 3, we provide an overview of the literature evaluating the effect of statins and antiplatelet drugs on D-dimer levels. A previously performed meta-analysis on association between statin use and D-dimer levels was hampered by several serious statistical/methodological flaws.[32] In this meta-analysis, that included nine randomized controlled trials, a significant reduction of D-dimer levels of 0.988 μg/ml (95%CI: -1.590 to -0.385, p=0.001) in statin users was reported. This suggests a tremendous clinical impact of statin use on D-dimer levels. However, this estimate is inappropriate since the used Cohen's d effect size should be dimension less and can

be interpreted as a standardized difference.[33] Triggered by this inaccuracy, we further elucidated the used methods and results and found several important shortcomings. Our main concerns, next to misuse of Cohen's d, are incorrect extraction of data from original studies and unreported assumptions. To reevaluate the association between statins and D-dimer levels, in Chapter 3.1 we systematically reviewed all published articles on the influence of statins on D-dimer levels and conducted a novel meta-analysis.[34] In an opinion article published in the journal Circulation, it is stated that many physicians incorrectly believe that there is something magical about a meta-analysis.[35] Our meta-analysis on the association between statins and D-dimer levels stresses that the quality of a meta-analysis is highly dependent on interpretation of the original data and the methodology used by the researchers. We must realize that a meta-analysis is a mathematical method for combining data, which is not weighted by the quality but by the quantity of the observations. Also, if criteria for inclusion of articles in the meta-analysis differ, this may lead to other conclusions. Therefore, results of a meta-analysis should be interpreted with caution and critical discussion about methods used in the separate studies is necessary. In our meta-analysis, in total we included 18,052 study participants from 7 randomized controlled trials, 11 cohort and 4 cross-sectional studies conducted in humans with data on D-dimer levels and the effect of statin use. We found significantly lower D-dimer levels in those patients receiving statin treatment than in controls (Cohen's d: -0.165, 95% CI -0.234; -0.096), a small but robust effect and not driven by any single study. In Chapter 3.2, we performed a separate systematic review and meta-analysis on the influence of antiplatelet drugs on the D-dimer level.[36] We included five controlled trials, seven cohort studies and five cross-sectional studies conducted in humans, with a drug exposure time of at least 7 days. Meta-analysis using the same Cohen's d effect size involved 1117 participants and resulted in no difference of D-dimer levels between users or non-users of antiplatelet drugs (Cohen's d: -0.015, 95% CI -0.182; 0.151). We concluded that antiplatelet drugs do not influence D-dimer levels. The small effect of statin use, but not of antiplatelet drugs on D-dimer levels is in line with the results of our analysis within the YEARS study, as reported in Chapter 2.2. . The results of our meta-analyses in combination with our analysis in the YEARS study confirm that there is no need for adjusting D-dimer cut-off values for statin and/or antiplatelet users within the YEARS algorithm.

As shown in both Chapter 2.2 and 3.1, statin use has been shown to be associated with lower D-dimer levels. Statins in general are believed to have pleiotropic anticoagulant effects.[37] It is unclear whether other cholesterol lowering drugs also do influence hemostasis and haemostatic biomarkers. Potential anticoagulant effects could influence future guidelines in their choice for specific lipid lowering drugs in the treatment of

hypercholesterolemia patients. Therefore, in Chapter 4, we evaluated the effects of more recently introduced cholesterol lowering drugs: Proprotein convertase subtilisin/kexin 9 (PCSK9) inhibitors on D-dimer and fibrinogen levels in patients with familial hypercholesterolemia (FH).[38] PCSK9 inhibitors are monoclonal IgG2-antibodies and indicated for patients at high risk for cardiovascular disease, who do not reach LDL-cholesterol targets despite maximum tolerated statins and ezetimibe (a Niemann-Pick C1 Like 1 inhibitor). This patient group not reaching the LDL-cholesterol targets has so far primarily consisted of patients with FH. We determined D-dimer and fibrinogen levels in 30 statin intolerant FH patients before and after starting PCSK9 inhibitors treatment. After a median follow-up time of 28 days, both D-dimer levels and fibrinogen levels did not change significantly. These findings suggest that PCSK9 inhibitors do not have antithrombotic effects. It also stresses to completely evaluate statin tolerance in the individual patient, as recommended in the guidelines.[39] Nonetheless, we did not investigate all haemostatic biomarkers and we acknowledge that other potential pleiotropic effects need to be studied in more detail. In a recent review several possible and hypothetical interactions between PCSK9 and the haemostatic system are discussed, including a decrease in clotting factor VIII by PCSK9 inhibition.[40] All of these effects however need confirmation by actually measuring haemostatic biomarkers in humans using PCSK9 inhibitors. Nowadays statins and ezetimibe are by far less expensive than PCSK9 inhibitors. When perhaps in the future, due to a lower cost more patients are treated with of PCSK9 inhibitors, it will be even more important to have figured out potential pleiotropic effects of this class of drugs. When among the cholesterol lowering drugs, only statins are proven to have pleiotropic effects, this reinforces the current guidelines to prescribe statins as first choice lipid lowering drug in patients at high risk of cardiovascular disease.

To further evaluate the antithrombotic properties of statins, we tested the effects of prolonged rosuvastatin use on fibrinolysis. From literature we know that when fibrinolytic activity is diminished, the risk of VTE is higher.[41-43] In Chapter 6, we tested plasma fibrinolytic potential as well as fibrinogen, PAI-1, plasmin inhibitor and Thrombin Activatable Fibrinolysis Inhibitor (TAFI) before and after four weeks of rosuvastatin or no treatment in participants with a prior confirmed VTE .[44] We found that rosuvastatin use led to an improved fibrinolysis profile compared to non-use. After treatment with rosuvastatin plasma fibrinolytic potential improved significantly: mean clot lysis time decreased by 8.75 minutes (95% CI -13.8 to -3.72), fibrinogen levels were higher, plasmin inhibitor levels and TAFI activity were lower, but PAI-1 levels did not change. This improvement in fibrinolysis and formerly reported anticoagulant effects after statin treatment warrant further studies on the possible role for statins in the secondary prevention of VTE.

That certain subpopulations need more attention in the diagnostic management of VTE becomes even more clear in Chapter 5. Based on anecdotal clinical suspicions, we explored the association between olanzapine and the incidence of VTE.[45] Diagnosing VTE in schizophrenic patients can be more difficult, due to symptoms such as lethargy and impaired pain perception.[46,47] This could lead to more diagnostic delays and underdiagnosis. The paradox of these diagnostic challenges is that olanzapine, a drug frequently subscribed in schizophrenic patients, is associated with an increased risk of VTE. The Reporting Odds Ratio (ROR) in the VigiBase (the worldwide pharmacovigilance database maintained by the WHO collaborating centre for international drug monitoring) of olanzapine and DVT is 1.38 (1.22-1.57(95% CI)) and of olanzapine and PE 1.99 (1.81-2.19). This ROR has been developed to generate a tool for signal detection between a side effect and a certain drug.[48] It is however based on spontaneous reporting from various sources with different degrees of documentation and should therefore be interpreted with caution. In line with the data from VigiBase, a meta-analysis reports an OR of 1.35 (95% CI 0.97-1.89, p = 0.08) for the risk of VTE in patients using olanzapine.[49] This association is most likely explained by associated risk factors such as substantial weight gain and lethargy, both side effects of olanzapine. Further studies are required to determine the potential mechanisms of olanzapine on for example thrombogenicity and platelet aggregation. According to the ROR of the Vigibase, clozapine also seems to be associated with an increased risk of VTE, while other second generation antipsychotics like quetiapine and risperidone are not. Discontinuation of olanzapine after diagnosis of VTE might be considered based on individual risk profile, control of psychotic symptoms and antipsychotic treatment options. The observed higher risk of VTE in the pharmacovigilance reports of olanzapine and clozapine stresses the importance of the pharmacovigilance databases in the notice of certain important side effects.

General conclusions and future perspectives

In conclusion, the first important finding of this thesis is that the simplified diagnostic YEARS algorithm for suspected acute pulmonary embolism can be safely applied in patients presenting in the hospital, also in statin and/or antiplatelet medication users. Secondly, we showed that statins but not antiplatelet drugs and PCSK9 inhibitors influence D-dimer levels and that in participants with prior VTE, rosuvastatin use led to an improved fibrinolysis profile compared to non-statin use. Additionally, diagnosis of VTE in schizophrenic patients remains difficult and co-medication like olanzapine seems to be associated with an increased risk of VTE. All these results give more insight

both in the use of diagnostics algorithms for ruling out VTE in case of co-medication and in the pathophysiology of anticoagulant effects of certain co-medication.

In this thesis we partly focused on the effect of co-medication on the safety of diagnostic algorithms for ruling out VTE. One more specific challenge is to test the safety of diagnostic algorithms in pregnant women. This population has an increased risk of VTE and also mean levels of haemostatic biomarkers differ from general population. It is well known that D-dimer levels physiologically rise during pregnancy.[50] In pregnant women diagnostic algorithms for ruling out VTE are therefore difficult to apply. One could speculate that pregnant women suspected to have PE with high probability on a clinical decision rule, but with a low D-dimer level, should not have a CTPA. Application of the revised Geneva score, one of the diagnostic algorithms for ruling out PE comparable with the Wells score, was already tested by Rhigini et al in this population.[51] This study showed that this score could be safely used, but in only 11.7% of the women PE could be ruled out without additional imaging tests. In this scope, the YEARS algorithm has been adapted for pregnancy.[52] Pregnant women suspected to have a pulmonary embolism with clinical signs of a DVT first underwent compression ultrasonography of the symptomatic leg to confirm or rule out a DVT. When DVT was not confirmed, the YEARS algorithm was applied. The proportion of women not needing a CTPA was high (39%) and the algorithm could be applied safely. Especially in the first trimester efficiency was highest with 65% avoidance of CTPAs, whereas in the third trimester 32% of CTPAs could be avoided. A little beyond the scope of this thesis, but important to realize is that in the pediatric population in general, diagnosis of PE remains a challenge. VTE is a relative rarity in children and it is unknown if D-dimer levels could be used in combination with clinical risk stratification algorithms. Also children usually present with vague non-typical symptoms. [53] In the guideline of the American Society of Hematology in 2018 covering the treatment of pediatric VTE , many recommendations are made based on extrapolation of adult data.[54] Additional research in this specific population is required, especially in understanding the natural history of asymptomatic thrombosis, but also on the determination of the diagnosis of VTE.[55]

One of the most vital future perspectives is that physicians need to become more aware of the importance to keep reporting to pharmacovigilance databases any unexpected case of VTE that might be related to a certain drug. This will increase our knowledge of the risk of thrombosis and possibly will prevent new events. More education on the role of physicians in pharmacovigilance during pharmacotherapy lectures at medical and pharmacy school and support for reporting suspected adverse events by the (hospital) pharmacy will help to achieve this.

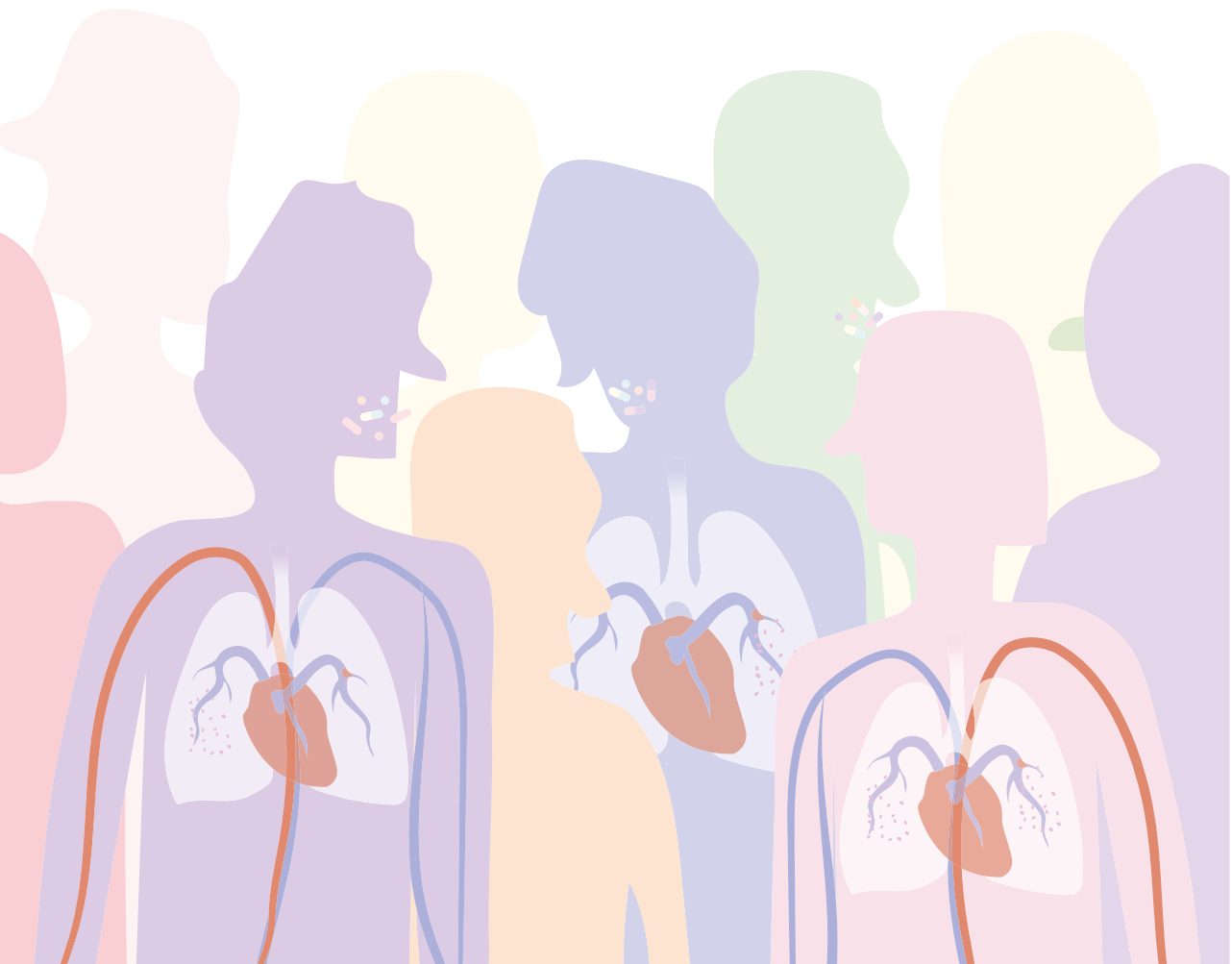
Finally, we provide with the evidence from the rosuvastatin study a reasonable and necessary basis for an interventional study to establish the effectiveness of statins in reducing the risk of VTE. In the extended PROLONG II prospective study D-dimer levels were tested one year after stopping anticoagulation therapy for unprovoked VTE.[56] Patients in whom D-dimer levels became and remained abnormal after the third month had a higher risk of recurrence of VTE than the patients in whom D-dimer levels remained normal. It would in the future therefore be conceivable to perform a randomized controlled trial in patients with an increased risk of hemorrhage to test if statins reduce the risk of recurrent thrombosis in this specific population. It would also be interesting to test D-dimer levels while treatment with a statin or placebo is started in this specific selected population with increased risk of hemorrhage. If the proposed randomized controlled study would show that statin use in patients with an increased risk of hemorrhage is associated with a reduction in recurrent thrombosis and lower levels of D-dimer, this might lead to adjustment of the guidelines in the long-term treatment of VTE in this specific population. One could then speculate to recommend use of statins after initial treatment with anticoagulant therapy. A lower dose of anticoagulation therapy is also an option in this situation and should of course be weighted against benefits and risks, including side effects such as myopathy, of statin treatment.

References

1. Baeyens JP, Serrien B, Goossens M, Clijsen R. Questioning the "SPIN and SNOUT" rule in clinical testing. *Arch Physiother.* 2019;9:4.
2. Meyer G, Roy PM, Gilberg S, Perrier A. Pulmonary embolism. *BMJ.* 2010;340:c1421.
3. Walen S, Damoiseaux RA, Uil SM, van den Berg JW. Diagnostic delay of pulmonary embolism in primary and secondary care: a retrospective cohort study. *Br J Gen Pract.* 2016;66(647):e444-450.
4. Hendriksen JM, Koster-van Ree M, Morgenstern MJ, et al. Clinical characteristics associated with diagnostic delay of pulmonary embolism in primary care: a retrospective observational study. *BMJ Open.* 2017;7(3):e012789.
5. Newnham M, Stone H, Summerfield R, Mustfa N. Performance of algorithms and pre-test probability scores is often overlooked in the diagnosis of pulmonary embolism. *BMJ.* 2013;346:f1557.
6. Teismann NA, Cheung PT, Frazee B. Is the ordering of imaging for suspected venous thromboembolism consistent with D-dimer result? *Ann Emerg Med.* 2009;54(3):442-446.
7. Wells PS, Anderson DR, Rodger M, et al. Derivation of a simple clinical model to categorize patients probability of pulmonary embolism: increasing the models utility with the SimpliRED D-dimer. *Thromb Haemost.* 2000;83(3):416-420.
8. Wells PS, Owen C, Doucette S, Fergusson D, Tran H. Does this patient have deep vein thrombosis? *JAMA.* 2006;295(2):199-207.
9. Konstantinides SV, Torbicki A, Agnelli G, et al. 2014 ESC guidelines on the diagnosis and management of acute pulmonary embolism. *Eur Heart J.* 2014;35(43):3033-3069, 3069a-3069k.
10. Raja AS, Greenberg JO, Qaseem A, et al. Evaluation of Patients With Suspected Acute Pulmonary Embolism: Best Practice Advice From the Clinical Guidelines Committee of the American College of Physicians. *Ann Intern Med.* 2015;163(9):701-711.
11. Sarma A, Heilbrun ME, Conner KE, Stevens SM, Woller SC, Elliott CG. Radiation and chest CT scan examinations: what do we know? *Chest.* 2012;142(3):750-760.
12. Konstantinides SV. 2014 ESC Guidelines on the diagnosis and management of acute pulmonary embolism. *Eur Heart J.* 2014;35(45):3145-3146.
13. Kooiman J, Klok FA, Mos IC, et al. Incidence and predictors of contrast-induced nephropathy following CT-angiography for clinically suspected acute pulmonary embolism. *J Thromb Haemost.* 2010;8(2):409-411.
14. van der Hulle T, Cheung WY, Kooij S, et al. Simplified diagnostic management of suspected pulmonary embolism (the YEARS study): a prospective, multicentre, cohort study. *Lancet.* 2017;390(10091):289-297.
15. van Es J, Beenen LF, Douma RA, et al. A simple decision rule including D-dimer to reduce the need for computed tomography scanning in patients with suspected pulmonary embolism. *J Thromb Haemost.* 2015;13(8):1428-1435.
16. Pasha SM, Klok FA, Snoep JD, et al. Safety of excluding acute pulmonary embolism based on an unlikely clinical probability by the Wells rule and normal D-dimer concentration: a meta-analysis. *Thromb Res.* 2010;125(4):e123-127.
17. van Es N, Kraaijpoel N, Klok FA, et al. The original and simplified Wells rules and age-adjusted D-dimer testing to rule out pulmonary embolism: an individual patient data meta-analysis. *J Thromb Haemost.* 2017;15(4):678-684.
18. Tritschler T, Kraaijpoel N, Le Gal G, Wells PS. Venous Thromboembolism: Advances in Diagnosis and Treatment. *JAMA.* 2018;320(15):1583-1594.

19. Douma RA, van Sluis GL, Kamphuisen PW, et al. Clinical decision rule and D-dimer have lower clinical utility to exclude pulmonary embolism in cancer patients. Explanations and potential ameliorations. *Thromb Haemost.* 2010;104(4):831-836.
20. Harb TS, Zareba W, Moss AJ, et al. Association between inflammatory markers, hemostatic, and lipid factors in postinfarction patients. *Am J Cardiol.* 2003;91(9):1120-1123.
21. Chabloz P, Reber G, Boehlen F, Hohlfeld P, de Moerloose P. TAFI antigen and D-dimer levels during normal pregnancy and at delivery. *Br J Haematol.* 2001;115(1):150-152.
22. Crop MJ, Siemes C, Berendes P, van der Straaten F, Willemsen S, Levin MD. Influence of C-reactive protein levels and age on the value of D-dimer in diagnosing pulmonary embolism. *Eur J Haematol.* 2014;92(2):147-155.
23. Anderson FA, Jr., Spencer FA. Risk factors for venous thromboembolism. *Circulation.* 2003;107(23 Suppl 1):I9-16.
24. Righini M, Van Es J, Den Exter PL, et al. Age-adjusted D-dimer cutoff levels to rule out pulmonary embolism: the ADJUST-PE study. *JAMA.* 2014;311(11):1117-1124.
25. van der Pol LM, van der Hulle T, Cheung YW, et al. No added value of the age-adjusted D-dimer cut-off to the YEARS algorithm in patients with suspected pulmonary embolism. *J Thromb Haemost.* 2017;15(12):2317-2324.
26. van der Pol LM, Dronkers CEA, van der Hulle T, et al. The YEARS algorithm for suspected pulmonary embolism: shorter visit time and reduced costs at the emergency department. *J Thromb Haemost.* 2018;16(4):725-733.
27. van der Pol LM, Bistervels IM, van Mens TE, et al. Lower prevalence of subsegmental pulmonary embolism after application of the YEARS diagnostic algorithm. *Br J Haematol.* 2018.
28. Carrier M, Righini M, Wells PS, et al. Subsegmental pulmonary embolism diagnosed by computed tomography: incidence and clinical implications. A systematic review and meta-analysis of the management outcome studies. *J Thromb Haemost.* 2010;8(8):1716-1722.
29. Schol-Gelok S, van der Hulle T, Biedermann JS, et al. Clinical effects of antiplatelet drugs and statins on D-dimer levels. *Eur J Clin Invest.* 2018;48(7):e12944.
30. Rifai N, Gillette MA, Carr SA. Protein biomarker discovery and validation: the long and uncertain path to clinical utility. *Nat Biotechnol.* 2006;24(8):971-983.
31. Biomarkers Definitions Working G. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther.* 2001;69(3):89-95.
32. Sahebkar A, Serban C, Mikhailidis DP, et al. Association between statin use and plasma d-dimer levels: A systematic review and meta-analysis of randomised controlled trials. *Thromb Haemost.* 2015;114(3):546-557.
33. Cohen J. *Statistical Power Analysis for the Behavioral Sciences.* Lawrence Erlbaum Associates; 1988.
34. Schol-Gelok S, Morelli F, Arends LR, et al. A revised systematic review and meta-analysis on the effect of statins on D-dimer levels. *Eur J Clin Invest.* 2019;49(8):e13130.
35. Packer M. Are Meta-Analyses a Form of Medical Fake News? Thoughts About How They Should Contribute to Medical Science and Practice. *Circulation.* 2017;136(22):2097-2099.
36. Morelli F, Schol-Gelok S, Arends LR, et al. Effect of Antiplatelet Drugs on D-Dimer Levels: A Systematic Review and Meta-analysis. *J Cardiovasc Pharmacol.* 2019;73(6):343-351.
37. Violi F, Calvieri C, Ferro D, Pignatelli P. Statins as antithrombotic drugs. *Circulation.* 2013;127(2):251-257.
38. Schol-Gelok S, Galema-Boers J, van Gelder T, Kruip M, Roeters van Lennep JE, Versmissen J. No effect of PCSK9 inhibitors on D-dimer and fibrinogen levels in patients with familial hypercholesterolemia. *Biomed Pharmacother.* 2018;108:1412-1414.

39. Landmesser U, Chapman MJ, Stock JK, et al. 2017 Update of ESC/EAS Task Force on practical clinical guidance for proprotein convertase subtilisin/kexin type 9 inhibition in patients with atherosclerotic cardiovascular disease or in familial hypercholesterolaemia. *Eur Heart J.* 2017.
40. Paciullo F, Momi S, Gresele P. PCSK9 in Haemostasis and Thrombosis: Possible Pleiotropic Effects of PCSK9 Inhibitors in Cardiovascular Prevention. *Thromb Haemost.* 2019.
41. Lisman T, de Groot PG, Meijers JC, Rosendaal FR. Reduced plasma fibrinolytic potential is a risk factor for venous thrombosis. *Blood.* 2005;105(3):1102-1105.
42. Meltzer ME, Lisman T, Doggen CJ, de Groot PG, Rosendaal FR. Synergistic effects of hypofibrinolysis and genetic and acquired risk factors on the risk of a first venous thrombosis. *PLoS Med.* 2008;5(5):e97.
43. Orsi FA, Cannegieter SC, Lijfering WM. Statin Therapy to Revert Hypercoagulability and Prevent Venous Thromboembolism: A Narrative Review. *Semin Thromb Hemost.* 2019.
44. Schol-Gelok S, de Maat MPM, Biedermann JS, et al. Rosuvastatin use increases plasma fibrinolytic potential: a randomised clinical trial. *Br J Haematol.* 2020.
45. Dijkstra ME, van der Weiden CFS, Schol-Gelok S, et al. Venous thrombosis during olanzapine treatment: a complex association. *Neth J Med.* 2018;76(6):263-268.
46. Antioch I, Ciobica A, Paulet M, Bild V, Lefter R, Timofte D. Pain manifestations in schizophrenia - clinical and experimental aspects in human patients and animal models. *Psychiatr Danub.* 2015;27(2):142-152.
47. Urban-Kowalczyk M, Pigonska J, Smigielski J. Pain perception in schizophrenia: influence of neuropeptides, cognitive disorders, and negative symptoms. *Neuropsychiatr Dis Treat.* 2015;11:2023-2031.
48. Kabel JS, van Puijenbroek EP. [Side effects of tramadol: 12 years of experience in the Netherlands]. *Ned Tijdschr Geneeskd.* 2005;149(14):754-757.
49. Barbui C, Conti V, Cipriani A. Antipsychotic drug exposure and risk of venous thromboembolism: a systematic review and meta-analysis of observational studies. *Drug Saf.* 2014;37(2):79-90.
50. Gutierrez Garcia I, Perez Canadas P, Martinez Uriarte J, Garcia Izquierdo O, Angeles Jodar Perez M, Garcia de Guadiana Romualdo L. D-dimer during pregnancy: establishing trimester-specific reference intervals. *Scand J Clin Lab Invest.* 2018:1-4.
51. Righini M, Robert-Ebadi H, Elias A, et al. Diagnosis of Pulmonary Embolism During Pregnancy: A Multicenter Prospective Management Outcome Study. *Ann Intern Med.* 2018.
52. van der Pol LM, Tromeur C, Bistervels IM, et al. Pregnancy-Adapted YEARS Algorithm for Diagnosis of Suspected Pulmonary Embolism. *N Engl J Med.* 2019;380(12):1139-1149.
53. Thacker PG, Lee EY. Pulmonary embolism in children. *AJR Am J Roentgenol.* 2015;204(6):1278-1288.
54. Monagle P, Cuello CA, Augustine C, et al. American Society of Hematology 2018 Guidelines for management of venous thromboembolism: treatment of pediatric venous thromboembolism. *Blood Adv.* 2018;2(22):3292-3316.
55. Biss TT, Rajpurkar M, Williams S, et al. Recommendations for future research in relation to pediatric pulmonary embolism: communication from the SSC of the ISTH. *J Thromb Haemost.* 2018;16(2):405-408.
56. Cosmi B, Legnani C, Tosetto A, et al. Usefulness of repeated D-dimer testing after stopping anticoagulation for a first episode of unprovoked venous thromboembolism: the PROLONG II prospective study. *Blood.* 2010;115(3):481-488.



8

Samenvatting (Dutch summary)

Samenvatting

Dagelijks komen artsen uitdagingen tegen bij het stellen van diagnoses. Om de nauwkeurigheid van een diagnostisch proces te verbeteren maken zij vaak gebruik van testen om een ziekte te kunnen aantonen of uitsluiten. Er is een groot aantal medische testen, zoals laboratorium testen, fysische testen en radiologische procedures, beschikbaar om de arts te ondersteunen in het detecteren of uitsluiten van een ziekte. Resultaten van diagnostische testen worden over het algemeen vergeleken met resultaten van deze test in een referentiepopulatie om de waarde van een test, zoals de positieve en negatieve voorspellende waarde, te kunnen vaststellen. Als de test inderdaad een substantieel verschil kan maken tussen de voor- en achterafkans van een bepaalde ziekte, dan kan de test worden ingezet in een diagnostisch proces. Diagnostische testen met een hoge sensitiviteit worden in het algemeen gebruikt om een ziekte uit te sluiten en diagnostische testen met een hoge specificiteit om een ziekte bij patiënten vast te stellen.[1]

Te midden van alle aandoeningen op de spoedeisende hulp (SEH), is de diagnose veneuze trombo-embolie (VTE) misschien wel één van de meest uitdagende. Onder VTE vallen de diagnoses diep veneuze trombose (DVT), stolsels in de diepe vaten van de armen of benen en longembolieën, stolsels in de longslagaderen. Eén van de uitdagingen in het diagnostische proces van een VTE is om beeldvormende diagnostiek en/of laboratorium testen te selecteren om veilig de diagnose VTE te kunnen stellen of uitsluiten. Daarbij moet ook vermeden worden alle patiënten uitgebreide diagnostiek te laten ondergaan. Patiënten presenteren patiënten die verdacht worden van een VTE zich met uitlopende lichamelijke klachten, zoals kortademigheid, pijn ter plaatse van de ribben of zwelling van een extremiteit. Veel lichamelijke klachten die geassocieerd worden met een VTE zijn mild en kunnen ook bij andere diagnoses passen, zoals bijvoorbeeld een longontsteking of spiergerelateerde pijnklachten.[2] Dit kan uiteindelijk tot een diagnostische vertraging leiden. Voor de diagnose longembolie in Nederland is de diagnostische vertraging op 8,6 dagen berekend, met gemiddeld 4,2 dagen vertraging toegerekend aan de patiënt zelf en 3,9 dagen aan (huis)artsen.[3] Met name is het ontbreken van pijn ter plaatse van de ribben geassocieerd met een vijfvoudig verhoogd risico op diagnostische vertraging.[4] Het is daarnaast belangrijk om te beseffen dat als de diagnose VTE gemist wordt, kan dit leiden tot grote gezondheidsschade of zelfs de dood van een patiënt. Daarom heeft de arts betrouwbare diagnostische strategieën nodig om een VTE veilig te kunnen uitsluiten. Deze diagnostische strategieën worden helaas niet altijd goed toegepast.[5,6] Dit kan worden verklaard door de soms hectische werkomstandigheden op de SEH en de complexiteit van sommige algoritmes die worden gebruikt om een VTE uit te sluiten. Gebaseerd op klinische ervaring of door het gebruik van een klinische

beslisregel met gestandaardiseerde scoringssystemen kan de voorafkans op een VTE worden ingeschat. De meest gebruikte klinische beslisregel voor de diagnostiek naar DVT en longembolie is geïntroduceerd door Wells et al.[7,8] De Wells score voor longembolie bevat zeven verschillende items (Tabel 1) en is stapsgewijs. De arts moet in de klinische praktijk de verschillende items scoren en de punten bij elkaar optellen. In patiënten met een lage voorafkans op het hebben van een VTE kan een niet verhoogde D-dimeer waarde - een afbraakproduct van stolsels dat meestal verhoogd is als een patiënt een VTE heeft - een longembolie of DVT veilig uitsluiten zonder beeldvormend onderzoek. Daarom wordt in VTE richtlijnen geadviseerd om een klinische beslisregel te combineren met de bepaling van de hoogte van een D-dimeer om die patiënten te kunnen identificeren waarbij een longembolie of DVT kan worden uitgesloten zonder beeldvormend onderzoek.[9,10] Patiënten met een lage kans op een VTE en een negatieve D-dimeer hebben dan bijvoorbeeld geen echografie voor verdenking DVT of een scan van de longslagaders (CTPA) nodig.[11,12] Een CTPA immers zorgt voor blootstelling aan straling, hogere zorgkosten, tijdsinvestering en mogelijke allergische reacties en contrast geïnduceerde nefropathie.[11,13] Daarnaast worden er op de meeste CTPA beelden geen longembolieën gezien, wat erop wijst dat de selectie van patiënten die daadwerkelijk een CTPA moet ondergaan nog niet optimaal is en dat sommige patiënten worden blootgesteld aan straling die wellicht vermijdbaar is. In dit kader hebben we verschillende onderzoeken verricht om de diagnostische strategie en therapie voor VTE in specifieke patiëntengroepen te verbeteren, en om te onderzoeken wat de effecten zijn van bepaalde geneesmiddelen op stollingsparameters en het risico op het krijgen van een VTE.

Tabel 1: Wells score[7]

Item	Punten
klinische tekenen van trombosebeen	3
longembolie meest waarschijnlijke diagnose	3
hartfrequentie > 100/min	1.5
immobilisatie of grote operatie <4 weken	1.5
trombosebeen of longembolie in de voorgeschiedenis	1.5
Bloed ophoesten	1
actieve kanker	1

Totaal ≤ 4 punten longembolie onwaarschijnlijk (lage klinische voorafkans): indicatie voor bepaling D-dimeer, indien deze niet verhoogd is (< 500ng/mL) kan een longembolie veilig worden uitgesloten
Totaal > 4 punten longembolie waarschijnlijk (hoge klinische voorafkans): indicatie voor beeldvorming middels CTPA (CT-scan van de longslagaders), geen D-dimeer bepaling.

Hoofdstuk 2 van dit proefschrift legt de nadruk op een optimale diagnostische strategie voor longembolie in verschillende (sub)populaties. Allereerst presenteren we een nieuwe

diagnostische strategie voor longembolie, waarbij gebruik wordt gemaakt van het YEARS algoritme.[14] Dit YEARS algoritme is zo ontworpen dat het makkelijker kan worden toegepast in de klinische praktijk dan de reeds bestaande beslisregels, zoals de Wells score, en om het aantal benodigde CTPA scans terug te dringen. Drie items uit de originele Wells klinische beslisregel bleken het meest bepalend te zijn om de diagnose longembolie te voorspellen. Deze items waren: klinische tekenen van een DVT, bloed ophoesten, en de diagnose longembolie als meest waarschijnlijke diagnose.[15] Indien één van deze items aanwezig is in combinatie met een klassieke D-dimeertest beneden 500ng/mL óf een hogere afkaapwaarde van beneden 1000 ng/mL bij ontbreken van één van de YEARS items, werd verwacht dat longembolie veilig kon worden uitgesloten zonder een CTPA. In het YEARS onderzoek hebben we de veiligheid en de efficiëntie van het YEARS diagnostische algoritme bepaald door in verschillende ziekenhuizen 2944 patiënten te vervolgen die verdacht werden van het hebben van een longembolie. Dit onderzoek toonde aan dat het YEARS algoritme veilig kon worden gebruikt en ook tot 14% minder CTPAs leidde in vergelijking met de Wells score, die toegepast werd met een D-dimeer waarde beneden 500ng/mL. We constateerden dat slechts 0,43% van de patiënten die geen CTPA hadden ondergaan binnen 3 maanden een VTE ontwikkelde. Dit was geheel binnen de verwachting vergeleken met andere algoritmes.[14,16,17] Momenteel wordt het YEARS algoritme al in veel ziekenhuisprotocollen gebruikt. Sommige onderzoekers echter zouden voordat het YEARS algoritme verder wordt ingevoerd eerst nog bevestiging van de resultaten in een ander onderzoek willen zien.[18] Hun belangrijkste kritiekpunten zijn dat de D-dimeer waarden al gemeten waren voordat de arts de klinische inschatting maakte, dat bloed ophoesten te weinig voorkwam en dat het scoren van het item 'longembolie meest waarschijnlijke diagnose' mogelijk beïnvloed is doordat de artsen ook andere algoritmes, zoals de Wells score kennen. In mijn ogen zijn deze argumenten niet valide. In de praktijk werden de YEARS criteria bepaald voordat de D-dimeer waarden bekend waren. Ook moet de arts altijd alle risicofactoren voor het hebben van een VTE meewegen. Het hebben van een risicofactor voor VTE in de individuele patiënt zal de keuze tot het stellen van 'longembolie meest waarschijnlijke diagnose' beïnvloeden. Dit kan deels ook de reden zijn dat dit het meest voorspellende item is in het Wells algoritme.[15] Verder nog te benoemen is dat hoewel op oudere leeftijd de D-dimeer waarde hoger is, het meenemen van de leeftijd in een aangepast YEARS algoritme niet leidde tot verbetering van de diagnostische strategie.[19]

Omdat D-dimeer waarden een belangrijke rol hebben in de diagnostische algoritmes voor VTE, onderzochten wij in de literatuur wat er bekend is over de invloed van medicatie op de D-dimeer waarden. Hierin vonden we dat plaatjesaggregatieremmers, geneesmiddelen die het samenklonteren van bloedplaatjes remmen, en statines, geneesmiddelen die

het cholesterol verlagen, de D-dimeer waarde zouden kunnen beïnvloeden. Daarom onderzochten we in hoofdstuk 2.2 van dit proefschrift door middel van een post-hoc analyse in het YEARS onderzoek of de D-dimeer afkapwaarde moet worden aangepast voor patiënten die plaatjesaggregatieremmers en/of statines gebruiken.[20] We stelden vast dat gebruik van statines geassocieerd is met 15% lagere D-dimeer waarden, maar dat er géén associatie is tussen gebruik van plaatjesaggregatieremming en de waarde van de D-dimeer. Daarna maakten we een model waarin de statine gebruikers in het YEARS algoritme een 15% lagere afkapwaarde kregen. Dit aangepaste algoritme zorgde er echter niet voor dat de diagnose VTE minder vaak gemist werd in deze groep. Daarom lijkt er geen noodzaak om de D-dimeer afkapwaarden in het YEARS algoritme aan te passen voor statinegebruikers. De D-dimeer afkapwaarden lijken ook voor statinegebruikers veilig te zijn. We willen dan ook onnodige complexiteit vermijden en het houden bij hetzelfde algoritme, ongeacht gebruik van statines.

De post-hoc analyse leidt ons naar het tweede doel van dit proefschrift: onderzoek naar de effecten van bepaalde geneesmiddelen op stollingsparameters, zoals D-dimeer, fibrinogeen, plasminogeen activator inhibitor-1 (PAI-1), tissue-type plasminogeen activator (t-PA) en antiplasmine, en op het risico op het krijgen van een VTE. In het algemeen worden meetwaarden in het bloed, ook wel biomarkers genoemd, gebruikt voor screening, diagnose stellen, het vervolgen van activiteit van een bepaalde ziekte of het vervolgen van het effect van een bepaalde behandeling.[21,22] In hoofdstuk 3 verschaffen we een overzicht van de literatuur betreffende de invloed van plaatjesaggregatieremmers en statines op de D-dimeer waarde. Een eerder overzicht in de vorm van een meta-analyse, die de literatuur over de associatie tussen statinegebruik en D-dimeerwaarden samenvat en analyseert, bevatte serieuze statistische en methodologische tekortkomingen.[23] Om deze associatie opnieuw te bepalen hebben wij in hoofdstuk 3.1 systematisch alle gepubliceerde artikelen over de invloed van statines op de D-dimeer waarde beoordeeld en een nieuwe meta-analyse uitgevoerd.[24] In een opinieartikel dat gepubliceerd is in het tijdschrift Circulation staat dat veel artsen erg veel waarde hechten aan uitkomsten van een meta-analyse en dit als de waarheid beschouwen.[25] Onze meta-analyse over de associatie tussen statines en de D-dimeer waarde benadrukt dat de kwaliteit van een meta-analyse sterk afhankelijk is van de interpretatie van de originele data en de methodologie die gebruikt wordt door de onderzoekers. Daarom moeten de resultaten van een meta-analyse altijd voorzichtig worden geïnterpreteerd en is kritische discussie over de onderzoeksmethode noodzakelijk. In onze meta-analyse includeerden we in totaal 18.052 deelnemers uit verschillende soorten onderzoeken. We constateerden dat patiënten die statine behandeling ondergingen significant lagere D-dimeer waarden hadden in vergelijking met controle patiënten, een klein maar robuust effect.

In hoofdstuk 3.2 verrichtten wij een aparte meta-analyse betreffende het effect van plaatjesaggregatieremmers op de D-dimeer waarde.[26] Wij includeerden 17 verschillende onderzoeken waarbij deelnemers tenminste 7 dagen een plaatjesaggregatieremmers hadden gebruikt. In totaal werden 1117 deelnemers geïncludeerd. De meta-analyse toonde geen verschil in D-dimeer waarde tussen gebruik of geen gebruik van plaatjesaggregatieremmers. We concludeerden dat plaatjesaggregatieremmers de waarde van de D-dimeer niet lijkt te beïnvloeden. Het kleine effect van het gebruik van statines en het ontbreken van een effect van plaatjesaggregatieremmers komt overeen met de resultaten van onze analyse in het YEARS onderzoek, beschreven in hoofdstuk 2.2. De resultaten van onze meta-analyses in combinatie met onze analyse in het YEARS onderzoek bevestigen dat er geen noodzaak is tot het aanpassen van de D-dimeer afkapwaarde in het YEARS algoritme voor statine en/of plaatjesaggregatiegebruikers.

Zoals is aangetoond in hoofdstuk 2.2 en 3.1 is gebruik van statines geassocieerd met lagere D-dimeer waarden. In het algemeen staan statines er bekend om dat zij antistollingseffecten hebben.[27] Het is echter onduidelijk of andere cholesterolverlagende geneesmiddelen ook invloed hebben op de stolling en meetwaarden van de stolling. Mogelijke antistollingseffecten kunnen de keuze voor bepaalde cholesterol verlagende geneesmiddelen voor de behandeling van patiënten met een hoog cholesterol in de richtlijnen beïnvloeden. Daarom onderzochten we in hoofdstuk 4 de effecten van de nieuwere cholesterol verlagende geneesmiddelen: Proprotein convertase subtilisin/kexin 9 (PCSK9) remmers op D-dimeer en fibrinogeen waarden in patiënten met familiaire hypercholesterolemie (FH).[28] PCSK9 remmers zijn monoklonale IgG2-antilichamen die het cholesterolgehalte verlagen. Ze kunnen worden voorgeschreven aan patiënten met Familiaire Hypercholesterolemie (FH) die een hoog risico hebben op hart- en vaatziekten, maar die de cholesterol streefwaarden niet bereiken ondanks maximaal verdraagbare doseringen van statines en ezetemibe (een andere cholesterolverlager). Wij bepaalden D-dimeer en fibrinogeen waarden voor en na het starten van behandeling met PCSK9 remmers in 30 FH patiënten die geen statines verdroegen. Na een gemiddelde van 28 dagen waren zowel de D-dimeer als de fibrinogeen waarden niet significant veranderd. Deze resultaten wekken de suggestie dat PCSK9 remmers geen antitrombotische effecten hebben. Ook benadrukken de resultaten om statine verdraagzaamheid in patiënten volledig te testen, zoals ook wordt aanbevolen in de richtlijnen.[29] Niettemin hebben wij niet alle biomarkers van de stolling onderzocht en zouden andere mogelijk bijkomstige voordelen van PCSK9 remmers ook moeten worden onderzocht.

Om de mogelijke effecten van statines op de stolling nog verder te kunnen bepalen, onderzochten we de invloed van rosuvastatine gebruik op de fibrinolyse, het proces

waarbij de bloedstolsels weer worden opgeruimd. We weten uit de literatuur dat wanneer de fibrinolytische activiteit verminderd is, het risico op een VTE hoger is.[30-32] In hoofdstuk 6 bepaalden we in onderzoeksdeelnemers die eerder een VTE hadden, voor en na vier weken rosuvastine behandeling of geen behandeling met een statine de waarden van de fibrinolyse.[33] We constateerden dat het gebruik van rosuvastine gebruik tot een verbeterd fibrinolyse profiel leidde wanneer vergeleken werd met geen gebruik van rosuvastine. Na behandeling met rosuvastatine verbeterde het fibrinolytisch potentieel significant, fibrinogeen waarden waren hoger, plasmin inhibitor waarden en TAFI activiteit waren lager, maar PAI-1 waarden veranderden niet. Deze verbetering in fibrinolyse en de eerder vermelde antistollingseffecten na statinebehandeling bekrachtigen de noodzaak van verdere onderzoeken naar de mogelijke rol van statines in de secundaire preventie van VTE.

Dat bepaalde subpopulaties meer aandacht nodig hebben in de diagnostische strategie van VTE wordt duidelijk in hoofdstuk 5. Aangemoedigd door ervaringen in de kliniek en casuïstiek uit de medische literatuur onderzochten we de associatie tussen gebruik van het geneesmiddel olanzapine en de incidentie van VTE.[34] De diagnose VTE kan in schizofrene patiënten erg moeilijk zijn doordat deze patiënten vaker loomheid vertonen en een veranderde pijnperceptie hebben.[35,36] Dit kan leiden tot diagnostische vertragingen en onderschatting van het ziektebeeld. De paradox van deze diagnostische uitdaging is dat olanzapine, een geneesmiddel dat frequent wordt voorgeschreven in deze patiëntengroep, geassocieerd is met een verhoogd risico op VTE. In overeenstemming met data uit Vigibase, de wereldwijde bijwerkingendatabase, rapporteerde een meta-analyse een 35% verhoogd relatief risico op VTE in patiënten die olanzapine gebruiken.[37] Deze associatie lijkt het beste verklaard te worden door een aantal bijwerkingen van olanzapine, zoals gewichtstoename en loomheid. Andere onderzoeken zijn nodig om mogelijke mechanismen van olanzapine op bijvoorbeeld stolling en plaatjesaggregatie vast te stellen. Clozapine lijkt op basis van de data in Vigibase ook geassocieerd te zijn met een verhoogd risico op VTE, terwijl andere tweede generatie antipsychoctica zoals quetiapine en risperidon hiermee niet geassocieerd zijn. Stoppen van olanzapine na een VTE zou moeten worden overwogen op basis van individueel risico profiel, controle van psychotische kenmerken en behandelmogelijkheden met andere antipsychotica. Met behulp van meldingen in de bijwerkingencentra konden olanzapine en clozapine worden geassocieerd met een hogere kans op VTE. Dit bevestigt het grote belang van deze bijwerkingendatabases om belangrijke bijwerkingen op te kunnen sporen.

Algemene conclusies en toekomstperspectief

Concluderend, de eerste belangrijke bevinding van dit proefschrift is dat het gesimplificeerde diagnostische YEARS algoritme voor verdenking longembolie veilig kon worden gebruikt in patiënten die zich in het ziekenhuis presenteren, ook in statine en/of plaatjesaggregatie gebruikers. Ten tweede toonden we aan dat statines de D-dimeer waarde beïnvloeden, maar plaatjesaggregatieremmers en PCSK9 remmers niet, en dat in onderzoekdeelnemers, die eerder een VTE hadden, rosuvastine gebruik tot een verbeterd fibrinolyse profiel leidt dan zonder statine gebruik. Daarnaast blijft de diagnose van VTE in schizofrene patiënten moeilijk en zijn een aantal geneesmiddelen, zoals olanzapine, geassocieerd met een verhoogd risico op VTE. Al deze bevindingen geven meer inzicht in zowel het gebruik van diagnostische algoritmes om een VTE uit te sluiten in het geval van medicatiegebruik als in de pathofysiologische effecten op de stolling van bepaalde medicatie.

In dit proefschrift concentreren we ons ten dele op de effecten van medicatie op de veiligheid van diagnostische algoritmes naar VTE. Een andere specifieke uitdaging is om de veiligheid van een diagnostisch algoritme in zwangere vrouwen te onderzoeken. Deze populatie heeft een verhoogd risico op VTE en gemiddelde stollingswaarden zijn anders dan in de algemene bevolking. Het is bekend dat D-dimeer waarden tijdens de zwangerschap hoger worden.[38] Daarom zijn diagnostische algoritmes om VTE uit te sluiten moeilijk toe te passen bij zwangere vrouwen. Het is daarom te verwachten dat zwangere vrouwen met een hoge kans op een longembolie, maar een lage D-dimeer waarde waarschijnlijk geen CTPA hoeven te ondergaan. Na eerder testen van een ander algoritme, is ook de YEARS score aangepast voor zwangerschap.[39,40] Zwangere vrouwen die verdacht werden van het hebben van een longembolie en die ook klinische tekenen van een DVT hadden, ondergingen eerst een echografie van het been om een DVT te bevestigen of uit te sluiten. Indien er geen DVT kon worden aangetoond, werd het YEARS algoritme toegepast. Het aantal vrouwen dat op deze manier geen CTPA nodig had was hoog (39%) en het aangepaste algoritme kon ook veilig worden toegepast. Vooral in het eerste trimester was de efficiëntie het hoogst met 65% vermijding van CTPAs, waarbij in het derde trimester 32% van de CTPAs kon worden vermeden. Een beetje buiten het bereik van dit proefschrift, maar belangrijk om te realiseren, is dat diagnostiek naar longembolie bij kinderen een uitdaging blijft. VTE is relatief zeldzaam in kinderen en het is onduidelijk of D-dimeer waarden kunnen worden gebruikt in combinatie met klinische beslisregels. Ook presenteren kinderen zich vaak met vage, niet typische lichamelijke klachten. [41] In de richtlijn over de behandeling van VTE bij kinderen uit 2018 van de Amerikaanse Vereniging van Hematologie, worden veel aanbevelingen gemaakt

door extrapolatie van gegevens van volwassenen.[42] Extra onderzoek in deze specifieke populatie is nodig om het ontstaan van VTE te begrijpen, maar ook om de diagnostiek van VTE te versnellen en verbeteren.[43]

Eén van de meest essentiële toekomstperspectieven is dat artsen zich meer bewust moeten worden van het belang om te blijven melden aan bijwerkingencentra in het geval van een onverwachte diagnose van VTE, die gerelateerd zou kunnen zijn aan het gebruik van een bepaald geneesmiddel. Dit zou de kennis van tromboserisico's verbeteren en mogelijk toekomstige voorvallen voorkomen. Hoewel zorgverleners op basis van de Geneesmiddelenwet sinds 2007 verplicht zijn ernstige bijwerkingen te melden gebeurt dit nog slechts in beperkte mate. Meer educatie over dit onderwerp gedurende farmacotherapie onderwijs tijdens de studie geneeskunde en farmacie, alsmede ondersteuning bij het melden van mogelijke bijwerkingen door de (ziekenhuis) apotheek zou kunnen helpen om dit toekomstperspectief te verwezenlijken.

Tenslotte ondersteunen we met de resultaten verkregen in het rosuvastatine onderzoek een redelijke en noodzakelijke basis voor een interventieonderzoek naar de effectiviteit van statines op het verminderen van het risico op VTE. In het verlengde PROLONG II onderzoek werden D-dimeer waarden bepaald 1 jaar na het stoppen van antistollingsbehandeling voor niet uitgelokte VTE.[44] Patiënten waarbij de D-dimeer waarde na 3 maanden abnormaal werd en bleef, hadden een hoger risico op recidief (terugkeer) van een VTE dan patiënten waarbij de D-dimeer waarde normaal bleef. Daarom zou het in de toekomst denkbaar zijn om een gerandomiseerd gecontroleerd onderzoek uit te voeren naar het effect van statines in het verlagen van het risico op recidief VTE in patiënten met een verhoogd bloedingsrisico. Het zou daarbij ook interessant zijn om de D-dimeer waarden te meten gedurende behandeling met statines of placebo in deze geselecteerde groep met verhoogd risico op bloedingen. Als dit voorgestelde onderzoek zou aantonen dat statine gebruik in patiënten met een verhoogd risico op bloedingen is geassocieerd met een verlaagd risico op recidief VTE, dan zou dit kunnen leiden tot aanpassen van de richtlijnen in lange termijn behandeling van VTE in deze specifieke populatie. Men zou dan statines kunnen aanbevelen na initiële behandeling met antistollingstherapie. Een lagere dosis antistolling is ook een optie in deze situatie en moet natuurlijk worden afgewogen tegen de voor- en nadelen van statine behandeling, waarbij ook rekening moet worden gehouden met de bijwerkingen van statines, zoals spierverval.

Referenties

1. Baeyens JP, Serrien B, Goossens M, Clijsen R. Questioning the "SPIN and SNOUT" rule in clinical testing. *Arch Physiother.* 2019;9:4.
2. Meyer G, Roy PM, Gilberg S, Perrier A. Pulmonary embolism. *BMJ.* 2010;340:c1421.
3. Walen S, Damoiseaux RA, Uil SM, van den Berg JW. Diagnostic delay of pulmonary embolism in primary and secondary care: a retrospective cohort study. *Br J Gen Pract.* 2016;66(647):e444-450.
4. Hendriksen JM, Koster-van Ree M, Morgenstern MJ, et al. Clinical characteristics associated with diagnostic delay of pulmonary embolism in primary care: a retrospective observational study. *BMJ Open.* 2017;7(3):e012789.
5. Newnham M, Stone H, Summerfield R, Mustfa N. Performance of algorithms and pre-test probability scores is often overlooked in the diagnosis of pulmonary embolism. *BMJ.* 2013;346:f1557.
6. Teismann NA, Cheung PT, Frazee B. Is the ordering of imaging for suspected venous thromboembolism consistent with D-dimer result? *Ann Emerg Med.* 2009;54(3):442-446.
7. Wells PS, Anderson DR, Rodger M, et al. Derivation of a simple clinical model to categorize patients probability of pulmonary embolism: increasing the models utility with the SimpliRED D-dimer. *Thromb Haemost.* 2000;83(3):416-420.
8. Wells PS, Owen C, Doucette S, Fergusson D, Tran H. Does this patient have deep vein thrombosis? *JAMA.* 2006;295(2):199-207.
9. Konstantinides SV, Torbicki A, Agnelli G, et al. 2014 ESC guidelines on the diagnosis and management of acute pulmonary embolism. *Eur Heart J.* 2014;35(43):3033-3069, 3069a-3069k.
10. Raja AS, Greenberg JO, Qaseem A, et al. Evaluation of Patients With Suspected Acute Pulmonary Embolism: Best Practice Advice From the Clinical Guidelines Committee of the American College of Physicians. *Ann Intern Med.* 2015;163(9):701-711.
11. Sarma A, Heilbrun ME, Conner KE, Stevens SM, Woller SC, Elliott CG. Radiation and chest CT scan examinations: what do we know? *Chest.* 2012;142(3):750-760.
12. Konstantinides SV. 2014 ESC Guidelines on the diagnosis and management of acute pulmonary embolism. *Eur Heart J.* 2014;35(45):3145-3146.
13. Kooiman J, Klok FA, Mos IC, et al. Incidence and predictors of contrast-induced nephropathy following CT-angiography for clinically suspected acute pulmonary embolism. *J Thromb Haemost.* 2010;8(2):409-411.
14. van der Hulle T, Cheung WY, Kooij S, et al. Simplified diagnostic management of suspected pulmonary embolism (the YEARS study): a prospective, multicentre, cohort study. *Lancet.* 2017;390(10091):289-297.
15. van Es J, Beenen LF, Douma RA, et al. A simple decision rule including D-dimer to reduce the need for computed tomography scanning in patients with suspected pulmonary embolism. *J Thromb Haemost.* 2015;13(8):1428-1435.
16. Pasha SM, Klok FA, Snoep JD, et al. Safety of excluding acute pulmonary embolism based on an unlikely clinical probability by the Wells rule and normal D-dimer concentration: a meta-analysis. *Thromb Res.* 2010;125(4):e123-127.
17. van Es N, Kraaijpoel N, Klok FA, et al. The original and simplified Wells rules and age-adjusted D-dimer testing to rule out pulmonary embolism: an individual patient data meta-analysis. *J Thromb Haemost.* 2017;15(4):678-684.
18. Tritschler T, Kraaijpoel N, Le Gal G, Wells PS. Venous Thromboembolism: Advances in Diagnosis and Treatment. *JAMA.* 2018;320(15):1583-1594.

19. van der Pol LM, van der Hulle T, Cheung YW, et al. No added value of the age-adjusted D-dimer cut-off to the YEARS algorithm in patients with suspected pulmonary embolism. *J Thromb Haemost.* 2017;15(12):2317-2324.
20. Schol-Gelok S, van der Hulle T, Biedermann JS, et al. Clinical effects of antiplatelet drugs and statins on D-dimer levels. *Eur J Clin Invest.* 2018;48(7):e12944.
21. Rifai N, Gillette MA, Carr SA. Protein biomarker discovery and validation: the long and uncertain path to clinical utility. *Nat Biotechnol.* 2006;24(8):971-983.
22. Biomarkers Definitions Working G. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther.* 2001;69(3):89-95.
23. Sahebkar A, Serban C, Mikhailidis DP, et al. Association between statin use and plasma d-dimer levels: A systematic review and meta-analysis of randomised controlled trials. *Thromb Haemost.* 2015;114(3):546-557.
24. Schol-Gelok S, Morelli F, Arends LR, et al. A revised systematic review and meta-analysis on the effect of statins on D-dimer levels. *Eur J Clin Invest.* 2019;49(8):e13130.
25. Packer M. Are Meta-Analyses a Form of Medical Fake News? Thoughts About How They Should Contribute to Medical Science and Practice. *Circulation.* 2017;136(22):2097-2099.
26. Morelli F, Schol-Gelok S, Arends LR, et al. Effect of Antiplatelet Drugs on D-Dimer Levels: A Systematic Review and Meta-analysis. *J Cardiovasc Pharmacol.* 2019;73(6):343-351.
27. Violi F, Calvieri C, Ferro D, Pignatelli P. Statins as antithrombotic drugs. *Circulation.* 2013;127(2):251-257.
28. Schol-Gelok S, Galema-Boers J, van Gelder T, Kruip M, Roeters van Lennep JE, Versmissen J. No effect of PCSK9 inhibitors on D-dimer and fibrinogen levels in patients with familial hypercholesterolemia. *Biomed Pharmacother.* 2018;108:1412-1414.
29. Landmesser U, Chapman MJ, Stock JK, et al. 2017 Update of ESC/EAS Task Force on practical clinical guidance for proprotein convertase subtilisin/kexin type 9 inhibition in patients with atherosclerotic cardiovascular disease or in familial hypercholesterolaemia. *Eur Heart J.* 2017.
30. Lisman T, de Groot PG, Meijers JC, Rosendaal FR. Reduced plasma fibrinolytic potential is a risk factor for venous thrombosis. *Blood.* 2005;105(3):1102-1105.
31. Meltzer ME, Lisman T, Doggen CJ, de Groot PG, Rosendaal FR. Synergistic effects of hypofibrinolysis and genetic and acquired risk factors on the risk of a first venous thrombosis. *PLoS Med.* 2008;5(5):e97.
32. Orsi FA, Cannegieter SC, Lijfering WM. Statin Therapy to Revert Hypercoagulability and Prevent Venous Thromboembolism: A Narrative Review. *Semin Thromb Hemost.* 2019.
33. Schol-Gelok S, de Maat MPM, Biedermann JS, et al. Rosuvastatin use increases plasma fibrinolytic potential: a randomised clinical trial. *Br J Haematol.* 2020.
34. Dijkstra ME, van der Weiden CFS, Schol-Gelok S, et al. Venous thrombosis during olanzapine treatment: a complex association. *Neth J Med.* 2018;76(6):263-268.
35. Antioch I, Ciobica A, Paulet M, Bild V, Lefter R, Timofte D. Pain manifestations in schizophrenia - clinical and experimental aspects in human patients and animal models. *Psychiatr Danub.* 2015;27(2):142-152.
36. Urban-Kowalczyk M, Pigonska J, Smigielski J. Pain perception in schizophrenia: influence of neuropeptides, cognitive disorders, and negative symptoms. *Neuropsychiatr Dis Treat.* 2015;11:2023-2031.
37. Barbui C, Conti V, Cipriani A. Antipsychotic drug exposure and risk of venous thromboembolism: a systematic review and meta-analysis of observational studies. *Drug Saf.* 2014;37(2):79-90.

38. Gutierrez Garcia I, Perez Canadas P, Martinez Uriarte J, Garcia Izquierdo O, Angeles Jodar Perez M, Garcia de Guadiana Romualdo L. D-dimer during pregnancy: establishing trimester-specific reference intervals. *Scand J Clin Lab Invest.* 2018:1-4.
39. Righini M, Robert-Ebadi H, Elias A, et al. Diagnosis of Pulmonary Embolism During Pregnancy: A Multicenter Prospective Management Outcome Study. *Ann Intern Med.* 2018.
40. van der Pol LM, Tromeur C, Bistervels IM, et al. Pregnancy-Adapted YEARS Algorithm for Diagnosis of Suspected Pulmonary Embolism. *N Engl J Med.* 2019;380(12):1139-1149.
41. Thacker PG, Lee EY. Pulmonary embolism in children. *AJR Am J Roentgenol.* 2015;204(6):1278-1288.
42. Monagle P, Cuello CA, Augustine C, et al. American Society of Hematology 2018 Guidelines for management of venous thromboembolism: treatment of pediatric venous thromboembolism. *Blood Adv.* 2018;2(22):3292-3316.
43. Biss TT, Rajpurkar M, Williams S, et al. Recommendations for future research in relation to pediatric pulmonary embolism: communication from the SSC of the ISTH. *J Thromb Haemost.* 2018;16(2):405-408.
44. Cosmi B, Legnani C, Tosetto A, et al. Usefulness of repeated D-dimer testing after stopping anticoagulation for a first episode of unprovoked venous thromboembolism: the PROLONG II prospective study. *Blood.* 2010;115(3):481-488.