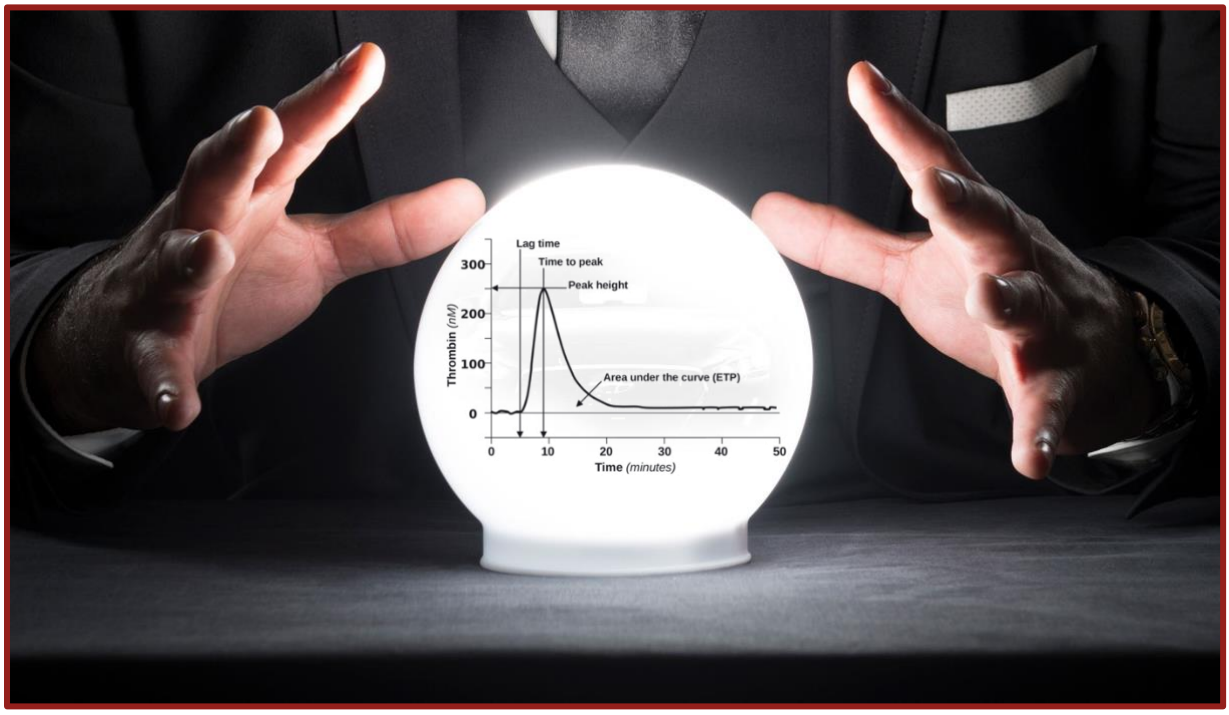
Predictive value of thrombin generation assessment for recurrent venous thromboembolism – a systematic review

Max Tijssen



Erasmus MC
University Medical Center Rotterdam

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Student:	Max Tijssen
Student number:	3834344
Email address:	m.b.tijssen@students.uu.nl
Institute:	Graduate School of Life Sciences – Utrecht University
Master:	Biomedical Sciences – Biology of disease
Project:	Writing Assignment

Institute:	Erasmus University Medical Center
Department:	Haematology
Supervisor:	Dr. Mandy Lauw
UU supervisor:	Dr. Rolf Urbanus

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Abstract

Background After a first venous thromboembolism (VTE), the risk of recurrence is significant (25% within 10 years). The ASH 2020 guideline recommends treating patients with idiopathic VTE with anticoagulation for indefinite time. Risk of recurrence can be assessed based on clinical prediction tools and D-dimer levels but none are regularly used. In this systematic review, we assessed the value of thrombin generation assessment (TGA) to predict recurrent thrombosis in patients with a first VTE.

Methods A systematic research was conducted in PubMed. We then summarised all data on thrombin generation and recurrent VTE. Quality and risk of bias of the included articles was assessed with the Newcastle-Ottawa scale.

Results Eleven studies were included; seven were of good quality according to the Newcastle-Ottawa Scale assessment, the other four were of moderate quality. 6 articles reported absolute values; all 6 reported peak thrombin, 4 reported on endogenous thrombin potential (ETP), only 2 reported on lag time and time to peak. Hazard ratios (HRs) were determined by 8 articles; 4 articles reported HRs for peak thrombin, 6 articled reported HRs for ETP and 2 articles reported HRs for lag time. Peak thrombin was found to be higher in patients with recurrent VTE than in patients without recurrence in four out of five articles reporting absolute values. Higher peak thrombin was also associated with a higher hazard ratio (HR) for recurrent VTE in all four articles reporting on HR and peak thrombin. Other thrombin generation parameters had weaker associations with recurrent VTE. The methodology between studies was inconsistent: the concentrations of tissue factor, phospholipids and substrate were not standardised, furthermore, the moment of blood sampling was not always consistent.

Conclusion Only a limited number of studies reporting on thrombin generation assessment and recurrent VTE were available. Between studies there was high variation in methods and reagents. Therefore, more research following a standardised protocol is needed to evaluate the predictive value of TGA.

Keywords: Thrombin generation – Venous thromboembolism – Recurrence – Prediction tool

Lay summary (Dutch)

Na een eerste veneuze trombose is de kans aanzienlijk dat een patiënt opnieuw trombose ontwikkeld. Daarom raden de recentste richtlijnen aan dat patiënten met niet-uitgelokte trombose langdurig behandeld worden met bloedverdunners (antistolling medicijnen). De behandelend artsen kunnen gebruik maken van vragenlijsten die het risico op trombose moeten inschatten. Dit kan gecombineerd worden met een laboratoriumtest voor D-dimeren, een bijproduct in de bloedstolling. Deze blijken in de praktijk weinig gebruikt te worden.

In deze review hebben we de literatuur samengevat over de voorspellende waarde van de trombine generatie test. Bij trombine generatie testen wordt er gemeten hoe actief het stollingssysteem is. Hiermee zouden patiënten beter geïdentificeerd kunnen worden die baat hebben bij langdurige antistolling evenals patiënten met een laag risico die mogelijk geen langdurige behandeling nodig hebben.

We hebben elf artikelen gevonden met onze zoekopdracht. Zeven daarvan waren van goede kwaliteit, de vier andere van gemiddelde kwaliteit op basis van de Newcastle-Ottawa score. 6 artikelen rapporteerden absolute getallen: vier van de zes artikelen schreven dat patiënten met een tweede trombose een hogere trombine piek hadden dan patiënten die niet opnieuw trombose ontwikkelden. Acht van de elf artikelen rapporteerden ‘hazard ratios’: het bleek dat het risico op een nieuwe trombose hoger werd als de trombine piek toenam. Andere parameters uit de trombine generatie test hadden een minder sterke associatie met een tweede trombose. Een probleem bij het vergelijken van alle studies was dat ze volgens verschillende protocollen werkten en met verschillende concentraties van de stoffen in het experiment.

Slechts een beperkt aantal studies kon geïncludeerd worden in onze review. Van deze artikelen verschilden de gerapporteerde methoden en uitkomsten aanzienlijk. Meer onderzoek volgens een gestandaardiseerd protocol is nodig om de voorspellende waarde van trombine generatie vast te kunnen stellen.

Introduction

Venous thromboembolism (VTE), the undesired development of clots in the venous blood circulation, can present as deep vein thrombosis (DVT) of the leg, pulmonary embolism (PE) or at other less typical locations. VTE can be provoked or unprovoked; unprovoked or idiopathic VTE indicates VTE development without a known provoking factor at the time of diagnosis, provoked VTE indicates VTE with a clear provoking factor such as major surgery, hospitalisation, and active cancer.[1]

Within ten years after the first VTE, the risk of a recurrent event is 25%.[2] The risk of recurrence is high with the first 180 days after the first VTE event and gradually decreases until a plateau is reached after 3-4 years.[2] Those who had a provoked event by a transient risk factor, have a lower chance of recurrence compared to those who had an unprovoked VTE (20% vs 28% within 10 years after the intimal event, respectively).[1,2] To prevent recurrent VTE, patients are treated with oral anticoagulants. Previous guidelines, like the ACCP 2008, advised to treat patients with unprovoked VTE with anticoagulants for 3 or 6 months.[3] The current ASH 2020 guideline on treating VTE advises indefinite treatment with anticoagulants for patients with an unprovoked VTE.[4] Prolonged anticoagulant treatment is accompanied by the risk of bleeding and its costs.[5] Moreover, a systematic review by Carrier showed that fatal bleeding events in patients treated with lifelong oral anticoagulants might outweigh the benefits of prevented fatal recurrent VTEs.[6]

To predict the VTE recurrence risk and to decide which patients would benefit from prolonged anticoagulant treatment, several considerations can be made. For instance, prediction tools can be used to assess the risk of recurrence of a thrombotic event and thereby to identify patients may or may not benefit from indefinite treatment. Validated prediction tools are the HERDOO2 score, the DASH score, and the Vienna prediction model. These models all consider sex and D-dimer levels; the DASH score combines this with age and hormone use, while the Vienna prediction model uses the location of the thrombus and the HERDOO2 includes body mass index, age and post-thrombotic signs to calculate the risk score.[7–9] A newly developed tool is the CONTINU-8 score, which considers unprovoked VTE, male sex, high FVIII level and presence of inflammation.[10] The advantage of this tool is that D-dimer is not a parameter and thus that anticoagulants do not need to be stopped during the assessment of the score. Like the VTE recurrence prediction tools, prediction tools have been developed to assess bleeding risk associated with anticoagulant use.[11,12] In a questionnaire published by De Winter et al., over 75% of internists and pulmonologists stated they rarely or not use a prediction tool to assess the risk of recurrent VTE events, displaying a gap between existing risk assessment tools and current practice of treating physicians.[13] Finally, patient preference and shared decision making should also be taken into account.[13]

Measuring the state of the haemostatic system with thrombin generation assessment (TGA) could potentially identify patients at high risk of recurrent VTE. In this assay, formed thrombin cleaves a peptide substrate which releases a fluorophore or chromophore; the substrate conversion is measured real-time.[14,15] Formation of thrombin is initiated by substitution of the plasma with $CaCl_2$ and phospholipids, and by adding TF as a trigger for coagulation. Several commercial assays are available to measure thrombin generation (TG): calibrated automated thrombinography (CAT or Thrombinoscope) by Stago, Technothrombin by Technoclone, and Innovance ETP by Siemens are commonly used. CAT and Technothrombin use a fluorescent substrate, whereas Innovance ETP uses a chromogenic substrate.[15] Parameters that can be measured through TGA are represented in figure 1. (1) lag time, the time between recalcification and initial thrombin formation; (2) peak thrombin, the highest concentration thrombin measured at one time point in the experiment; (3) time to peak, the time between recalcification of the plasma and the moment that peak thrombin is reached; (4) velocity

index, the slope of the thrombin generation curve between lag time and time to peak; (5) time to tail, the time between recalcification of the plasma and the moment that no thrombin is formed anymore; (6) endogenous thrombin potential (ETP), the area under the curve of the thrombin generation curve. The ETP represents all thrombin formed during the TGA.[16]

TGA has been used to assess thrombin generation profiles in research settings for patients with inherited bleeding disorders (i.e., haemophilia and Von Willebrand Disease), for monitoring of anticoagulant treatment and to assess the influence of risk factors of thrombosis on thrombin generation.[17] A previous review on the use of thrombin generation assays from 2008 included only two publications about thrombin generation and VTE recurrence. Their conclusion was that the data could not confirm nor deny a relationship between thrombin generation and recurrence yet. Ever since, more articles have been published on this topic.

The objective of the current review was to summarise the literature on the predictive value of thrombin generation assessment for VTE recurrence in adults with a first objectively confirmed VTE, including a meta-analysis if sufficient data was available.

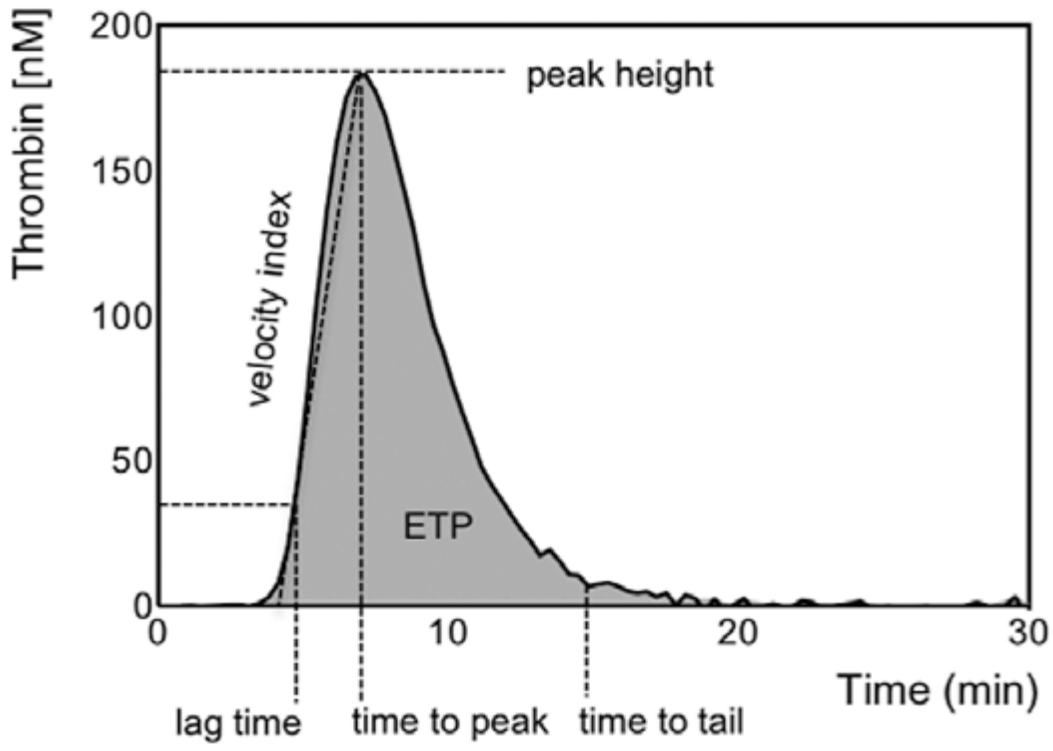

Figure 1: Thrombin generation curve with its parameters. Lag time, peak thrombin, time to peak, velocity index, time to tail and endogenous thrombin potential (ETP). Figure from Loeffen et al.[16]

Materials & methods

Data sources and searches

We conducted a systematic literature search for articles reporting on thrombin generation assessment and recurrent venous thromboembolism using PubMed. Additional articles were identified through screening references of identified literature. The last search was completed on May 3rd, 2021. There was no restriction on publication date, while only publication written in English or Dutch were included in the review process. Keywords used to identify relevant articles were synonyms of 'thrombin generation assessment', 'venous thromboembolism' and 'recurrence'. The complete search strategy is available in supplement I.

Study selection

We screened titles and abstracts and full-text articles were downloaded of records that reported on VTE and thrombin generation assessment. Full-text articles were reviewed for inclusion based on predetermined in- and exclusion criteria. We included articles that met the following requirements: (1) patients were diagnosed with an objectively confirmed first venous thromboembolism; (2) thrombin generation was measured with TGA; (3) the article reported recurrent venous thromboembolism as an outcome; (4) thrombin generation data was available for both the group with and without recurrent venous thromboembolism. Reasons to exclude articles were (1) articles reporting on children; (2) articles without objectively confirmed VTE; (3) articles not reporting on VTE recurrence.

Data extraction & quality assessment

We extracted data from the included articles using a data extraction form. The extracted data included: author, journal, year of publication, number of patients, patient characteristics, thrombin generation methods, results (absolute values and hazard ratios of VTE recurrence, associations with thrombin generation assessment), and statistical results.

The Newcastle-Ottawa scales for cohort studies and case-controls studies was used to assess the quality and the risk of bias of the articles. Cohort studies were awarded stars for criteria mentioned under the topics of selection, comparability, and outcome, with a maximum of 9 stars. The obtained stars were converted into a percentage. Studies with a percentage between 0% and 35% were considered of poor quality, studies scoring between 35% and 70% were considered of moderate quality and articles awarded scores above 70% were considered of good quality.

Results

Search results

Our search strategy identified 250 records in PubMed and we identified 2 additional records via screened references of identified articles. 219 entries were excluded based on title and abstract. 33 records were downloaded for full-text assessment. 22 full-text articles were excluded because of the following reasons: no thrombin generation measured (N = 11), article did not report on VTE recurrence (N = 6), no assessment of association between thrombin generation and VTE recurrence mentioned (N = 2). Three articles reported on the same study population (N = 3). Finally, 11 articles were included in this review (Figure 2). Some articles reported absolute values of thrombin generation parameters (N = 6), whereas others reported odds ratios, risk ratios or hazard ratios of thrombin generation parameters and VTE recurrence (N = 8). We approached authors of articles that did not report absolute values of TG parameters in order to obtain more data. Unfortunately, this did not result in additional data.

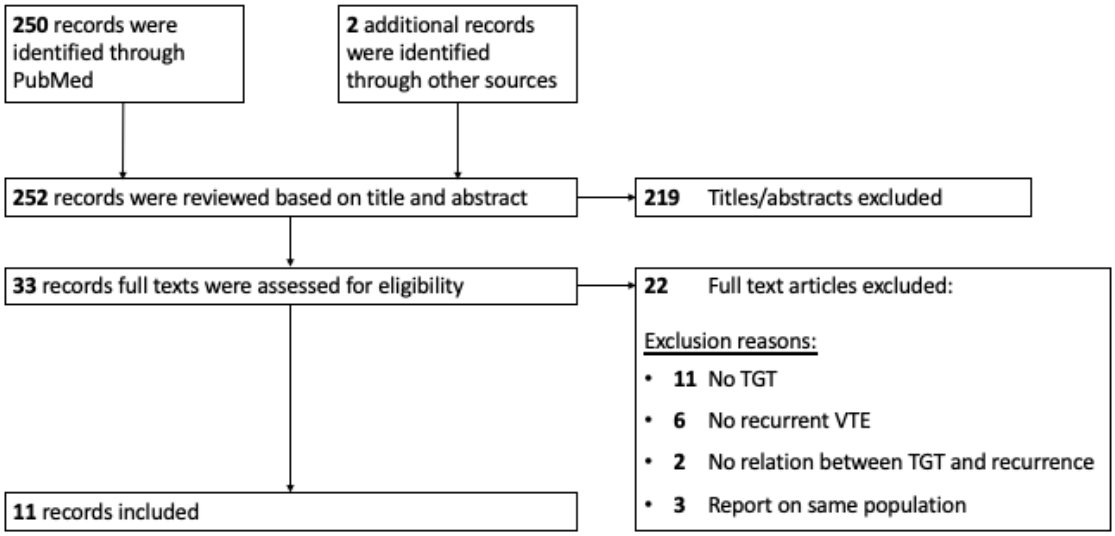

Figure 2: Flow diagram of literature review.

General characteristics of the included studies

We included nine prospective studies and two case-control studies with a prospective follow-up period. A general overview with the patient population, TGA methods and conclusion of the included studies can be found in Table 1. A meta-analysis of the included data was deemed impossible as there was too much heterogeneity in patient populations and TGA methods.

Cohort studies

Hron et al. (2006) aimed to identify patients at low risk of recurrence by measuring TGA.[18] The study included 914 patients, all cases were unprovoked VTE. Blood was collected three weeks after withdrawal of VKA. During follow-up, 100 patients developed recurrent VTE (11%).

Tripodi et al. (2008) measured TGA to investigate its association with recurrent VTE.[19] 254 patients were included in the study, 52% of the cases were unprovoked VTE. Blood was taken one month after withdrawal of anticoagulants. During follow-up 34 patients developed recurrent VTE (13%).

Eichinger et al. (2008) investigated the possibilities to predict recurrent VTE by measurement of TGA and D-dimer levels.[20] 861 patients with an objectively confirmed VTE were included in the study, all initial cases were unprovoked VTE. Patients entered the study after withdrawal of anticoagulants, blood was obtained at a median time of 13 months after entering the study. During follow-up 15% of the patients developed recurrent VTE.

Besser et al. (2008) investigated thrombin generation in patients with unprovoked VTE.[21] In the prospective cohort study 188 patients were included. Blood samples were obtained 2-3 months after discontinuation of anticoagulants. 15% of the patients developed recurrent VTE during follow-up.

Sonnevi et al. (2011) studied thrombin generation in women with VTE in a prospective cohort study.[22] The study included 148 women, 28% of the cases were unprovoked. Blood samples were obtained 6 months after the initial event and at least 3 weeks after discontinuation of anticoagulants. During follow-up 9% of the patients developed recurrent VTE

Chaireti et al. (2012) looked into thrombin generation and its relation towards VTE.[23] 115 patients were included in the study, 66% of the cases were unprovoked. Blood was drawn at the time of the VTE and at 1 to 2 months after discontinuation of oral anticoagulants. During follow-up 35% of the patients developed recurrent VTE. This study is the only one to report on thrombin generation values at the time of VTE.

Siudut et al. (2016) investigated fibrin clot permeability and its relationship with post-thrombotic syndrome.[24] In their experiments, TGA was also measured and recurrent VTE was mentioned. The study included 243 patients with objectively confirmed VTE, 49% of the cases was unprovoked. Blood samples were drawn after three months after the diagnosis. Patients who were treated with VKAs were switched to LMWH, blood was drawn 12 hours after the last LMWH injection. During the follow-up of 1 year, 26 patients developed recurrent VTE (13%).

Zabczyk et al. (2016) explored the association between fibrin clot phenotype and recurrent PE in a prospective cohort study, as part of this study TGA was also measured.[25] 156 patients with PE alone or PE and DVT were included, 57% of the cases were unprovoked. Patients were included if they completed 3-month anticoagulant therapy. Blood samples were taken 4 weeks after discontinuation of treatment. 39 patients developed recurrent PE or DVT during follow-up (25%).

Cieslik et al. (2018) measured thrombin generation parameters as part of a study towards altered plasma clot properties and the risk of DVT recurrence in a prospective cohort study.[26] 320 patients with a first objectively confirmed VTE were included, 50% of the cases experienced unprovoked VTE. Patients were treated with vitamin K antagonists (VKAs). Before blood sampling, VKA treatment was replaced with low-molecular weight heparin (LMWH), LMWH treatment was stopped for a minimum of 16 hours before blood sampling. After blood sampling, patients returned to VKA treatment. During follow up 77 patients developed recurrent DVT (25%), 231 remained recurrence free; 12 patients were lost during follow-up.

Case-control studies

Van Hylckama Vlieg et al. (2007) looked into the predictive value of thrombin generation for a first VTE and recurrent VTE.[27] Plasma samples were included for 360 patients and 404 controls. Controls were friends or partners of the patients and were matched based on sex and age. 52% of the cases were unprovoked VTE. Plasma samples were obtained at least three months after discontinuation of anticoagulants. During follow-up 59 patients developed recurrent VTE (16%).

Van Hylckama Vlieg et al. (2015) investigated the association between the risk of a first VTE and recurrent VTE and thrombin generation and D-dimer levels.[28] The study included 626 patients with a first VTE and 361 controls, with controls being partners of patients. 50% of the cases were unprovoked VTE. Blood samples were taken when patients and controls were not on anticoagulants. During follow-up 13.7% of the controls developed recurrent VTE.

Association between thrombin generation and recurrent thrombosis

Of the 11 identified articles, 6 reported absolute values for TG parameters, 8 reported hazard ratios for different TG parameters and 1 reported relative risk for peak thrombin. Absolute values of peak thrombin were reported by 6 studies (Hron et al.[18], Tripodi et al.[19], Chaireti et al.[23], Siudut et al.[24], Zabczyk et al.[25] and Cieslik et al.[26]). ETP was reported by 4 studies (Tripodi et al.[19], Chaireti et al.[23], Zabzcyk et al.[25] and Cieslik et al.[26]). Lag time was reported by two studies (Tripodi et al.[19] and Chaireti et al.[23]). Time to peak was reported by 3 studies (Chaireti et al.[23], Zabczyk et al.[25] and Cieslik et al.[26]). An overview of the reported absolute values can be found in Table 2. Hazard ratios for peak thrombin were reported by 4 studies (Tripodi et al.[19], Sonnevi et al., Zabczyk et al.[25] and Cislik et al.[26]). HRs were reported for ETP by 6 studies (Tripodi et al.[19], Eichinger et al., Besser et al.[21], Sonnevi et al.[22], Van Hylckama Vlieg et al. (2007)[27] and Van Hylckama Vlieg et al. (2015)[28]). HRs were reported for lag time by 2 studies (Tripodi et al.[19] and Sonnevi et al.[22]). The reported hazard ratios have been combined in Table 3.

Peak thrombin

The association between absolute values of peak thrombin and recurrent VTE was determined by five studies. Hron et al. showed that patients with VTE recurrence had a significantly higher peak thrombin than patients without recurrence (mean: 420 vs. 349 nM, $p < 0.001$).[18] Tripodi et al. also reported on peak thrombin: in their study, patients with recurrence had a higher peak thrombin than patients without VTE recurrence (mean: 232 vs. 187 nM, $p = 0.005$).[19] Siudut et al. did not find an association between patients with and without VTE recurrence: TGA showed no difference in peak thrombin (median: 221 vs. 233 nM, $p = 0.78$).[24] Zabczyk et al. found that peak thrombin was higher in patients with recurrent PE or DVT than in patients without recurrence (mean: 284 vs. 245 nM, $p < 0.002$).[25] Cieslik et al. found that peak thrombin was higher in patients with recurrent VTE compared to patients without recurrence (median: 286 vs. 239 nM, $p < 0.0001$).[26]

Four studies reported hazard ratios for peak thrombin levels. Tripodi et al. divided patients' thrombin generation data into tertiles. Patients in the highest tertile of peak thrombin (> 237 nM) had a higher risk of recurrent VTE (HR 3.09) compared to patients in the lowest tertile (≤ 150 nM).[19] Sonnevi et al. calculated that an increase of 10nM peak thrombin correlated with a HR of 1.16 for recurrent VTE.[22] Zabczyk et al found that the risk of recurrent PE of patients with peak thrombin above 269 nM was higher than those under 269 nM (HR = 3.66).[25] Cieslik et al. determined that for each increase of 100 nM peak thrombin, the HR for recurrent VTE was 2.67.[26] Hron et al. were the only to report a relative risk. They found that the relative risk of recurrent thrombosis was 1.04 for each increase in peak thrombin by 10 nM.[18]

ETP

The association between absolute ETP values and recurrent VTE was investigated by three studies. Tripodi et al. did not find a difference in ETP between patients with and without recurrence (mean: 1502 vs.1361 nM·min, $p = 0.122$).[19] In the study of Zabczyk et al. ETP was higher in patients with recurrent VTE (mean: 1615 vs.1566 nM·min, $p < 0.004$).[25] Similarly, ETP was higher in patients with recurrent VTE compared to patients without recurrence in the study of Cieslik et al. (median: 1622 vs.1510 nM·min, $p < 0.0001$).[26]

Five articles reported hazard ratios for ETP and recurrent VTE. Tripodi et al. found that patients in the highest tertile of ETP (> 1565 nM·min) had a 2.54 higher risk of recurrence than patients in the lowest tertile (≤ 1172 nM·min).[19] Eichinger et al. calculated the HR for each increase of 1% in ETP: 1.011. When patients were divided into patients with low ETP (< 100%) and high ETP (> 100%), the patients in the high ETP group had a HR of 1.7 for recurrent VTE.[20] Besser et al. found an association between an increase of 100nM·min in ETP and increased risk of recurrent VTE (HR 1.25). Sonnevi et al. found that

ETP correlated with recurrent VTE: an increase of 10 nM·min results in a HR of 1.03.[22] Van Hylckama Vlieg et al. (2007) determined a 90th percentile in controls (2109 nM·min). Patients with an ETP above 2109 nM·min were found to have a HR of 1.1 to develop recurrent VTE compared with patients with a lower ETP.[27] Van Hylckama Vlieg et al. (2015) determined the ETP in the plasma of controls and took the 90th percentile (2052 nM·min) as a cut-off value for their risk assessment. Patients who had an ETP above this level had a HR of 1.3 for recurrent VTE compared to patients who have an ETP lower than 2052 nM·min.[28]

Lag time & Time to peak

Tripodi et al. was the only study to report on lag time values and recurrent VTE. They did not identify a difference in lag time between patients with and without recurrence (mean: 11.9 vs. 12.8 min, p = 0.319). Tripodi et al. also calculated hazard ratios for lag time and VTE recurrence; patients in the lowest tertile (< 10.7 min) had a higher risk of recurrence (HR 2.29) compared with patients in the highest tertile (> 13.8 min).[19]

Two studies reported on time to peak. Zabczyk et al. reported that time to peak was shorter in patients with recurrence (mean: 265 vs. 317 s, p = 0.001).[25] Cieslik et al. found that time to peak did not differ between groups (median for both: 4.67 min, p = 0.26).[26]

TGA at multiple time points

Chaireti et al. was the only article to measure thrombin generation at the time of the VTE and after discontinuation of anticoagulants. The reported values represent the TGA assay at the time of the initial event and are therefore reported separately in this review. There was a discrepancy between the p value reported in the text and in the figure; we here report the p value given in the text.

Chaireti et al. reported higher peak thrombin in patients without recurrence (p = 0.058). No difference in ETP was found between groups (p = 0.111). The authors also reported that TG parameters differ after discontinuation of anticoagulants. After anticoagulant withdrawal, peak thrombin was found to be higher in patients with recurrence, however this result was not significant (p = 0.059). Patients with recurrent VTE had longer lag time than patients without recurrence (median: 5.2 vs. 4.2 min, p < 0.001). Finally, patients with VTE recurrence had a longer time to peak (median: 8.7 vs. 7.2 min, p = 0.034).[23]

Thrombin generation methods

Calibrated automated thrombinography

Nine of the eleven included articles used the CAT method to measure thrombin generation. Most studies used 5 pM TF to trigger thrombin generation (Besser et al.[21], Chaireti et al.[23], Siudut et al.[24], Zabczyk et al.[25] and Cieslik et al.[26]). 1 pM TF was used by Tripodi et al.[19] and Van Hylckama Vlieg et al. (2015)[28], while Sonnevi et al.[22] used both 1 and 10 pM TF. The phospholipid concentration was also often described. Siudut et al.[24], Zabczyk et al.[25] and Cieslik et al.[26] used 4mM PL in their experiments. 4 µM PL was used by Besser et al.[21] and Chaireti et al.[23]. While Sonnevi et al.[22] used 30 µM PL and Tripodi et al.[19] used 1 µM PL. Van Hylckama Vlieg et al. (2015)[28] did not report on the concentration of PL used. The substrate concentration was not mentioned by all authors, but when mentioned the substrate concentration varied widely. Siudut et al.[24], Zabczyk et al.[25] and Cieslik et al.[26] used 2.5 mM substrate. Sonnevi et al.[22] used 0.32 mM substrate, while Tripodi et al.[19] and Besser et al.[21] used a final concentration of 0.417 mM substrate. Van Hylckama Vlieg et al. (2007)[27] used an adapted protocol of the original CAT. They performed the experiment with 15 pM TF, 4 µM PL, and 2.5 nM substrate. The plasma in the experiment was diluted 1:4 in HEPES buffer.

Other thrombin generation assessment methods

Hron et al. and Eichinger et al. did not measure thrombin generation with CAT. Hron et al.[18] used the TechnoThrombin assay. Their assay included 71.6 pM TF and 3.2 µM PL, the substrate concentration was not stated. Eichinger et al.[20] used a TGT method by Dade Behring; unfortunately, TF, PL and substrate concentration were kept confidential by the manufacturer. Multiple studies explored TGA in the presence of thrombomodulin to investigate the functioning of the protein C system. Tripodi et al.[19], performed TGA in presence and absence of 4 nM thrombomodulin. Van Hylckama Vlieg et al. (2007)[27] ran the experiments in the presence of 7nM thrombomodulin. Sonnevi et al.[22] did not add thrombomodulin to their experiments but instead, they performed the experiment in the absence and presence of 5 nM activated protein C.

Quality of the included studies

Five of the nine included cohort studies were of good quality, the other four articles were of moderate quality (table 4). All studies clearly describe how the cohorts were chosen and how VTE was objectively confirmed. Four studies did not mention specifically how it was assessed that signs of post-thrombotic syndrome were not seen as recurrent VTE. Seven out of nine articles did not mention if studies correct for age or sex and were therefore not awarded a star, despite the baseline characteristics being similar. All articles stated that recurrence was confirmed with ultrasound. Follow-up was shorter than 24 months in one article, all other studies had a follow-up of 24 months or longer.

The studies by Van Hylckama Vlieg (2007[27] and 2015[28]) were case-control studies with an included prospective follow-up. Since the follow-up period is most important in our review, we have assessed the follow-up description of the case-control studies. Both studies are of good quality; the 2007 study lacked information about cases lost in follow up and on how it was assessed that signs of post-thrombotic syndrome were not seen as recurrent VTE.

Discussion

The ASH 2020 guideline advises indefinite anticoagulant treatment for patients with an unprovoked VTE with a low bleeding risk. We summarised all available literature on thrombin generation and recurrent VTE in order to assess the predictive value of thrombin generation for recurrent VTE. Most of the studies that were identified in the initial search did not report on thrombin generation assessment, others did not report on recurrent VTE and were therefore excluded. To include as many studies as possible we included studies performing any TGA method. Finally, eleven articles were eligible for inclusion in our review process.

Predictive value of thrombin generation assessment

The majority of studies reporting on peak thrombin and recurrent VTE showed increased peak thrombin levels in patients with recurrence. Similarly, HRs indicated that the risk of recurrence increased with higher peak thrombin levels. Although ETP, lag time and time to peak were also mentioned in some articles, the evidence on their predictive value for VTE recurrence was less strong. The TGA parameter peak thrombin might have the potential to become a tool for predication of recurrent venous thromboembolism; it is the most reported parameter and is often associated with higher risk of recurrent VTE.

Provoked vs unprovoked

Only 3 out of the 11 articles solely included patients with unprovoked VTE. In the other 8 articles the unprovoked cases were between 28% and 66% of all included cases. Since the risk of recurrent VTE is lower in patients with a provoked VTE compared to patients with unprovoked VTE, the number of patients with provoked VTE could have influenced whether there was a significant difference between

groups or not. Since we only identified 11 articles, of which 3 with only unprovoked cases, we did not exclude studies with provoked cases.

TGA methods and outcomes

A major issue when comparing thrombin generation data between the identified studies was the use of different protocols and reagents. Even though nine of eleven included articles used the CAT assay, there was still variety in reported methods.

The used concentration of tissue factor in the included studies varied highly between 1 and 15 pM for CAT measurements, Hron et al. used the TechnoThrombin assay which used 71.6 pM TF. Tissue factor concentration highly influences the TGA and the derived TG parameters.[15,29] Between 1 and 15 pM, lag time, time to peak and ETP are highly dependent on the TF concentration, at higher concentrations stabilisation occurs; peak thrombin does not reach stabilisation at 30 pM yet.[30] The large variation in TF concentrations made a comparison between the absolute values of TG parameters of the included studies impossible and therefore hindered a meta-analysis.

The phospholipid concentrations in our included studies had large variation and were between 1 µM and 30 mM. The study of Tripodi et al. was the only study to use a concentration below 3 µM (1 µM). Siudut et al., Cieslik et al. and Zabczyk et al. used 4 mM PL. Like the tissue factor concentration, the influence of different concentrations of PL can influence thrombin generation parameters. Low phospholipid concentrations do influence TG parameters, however, from 3 µM PL, lag time, time to peak and ETP are no longer influenced by PL concentration; peak thrombin does not stabilise before 8 µM PL is reached.[14,30] At concentrations above 10 µM PL, contact activation could influence TG.[14]

Another important variation was found in the reported substrate concentration. The substrate concentrations reported were between 2.5 nM and 2.5 mM. Like the TF and PL concentration, the substrate concentration also influences the TG parameters: at higher substrate concentrations, peak thrombin and ETP are also higher.[14]

Besides differences in TGA protocols, the moment of blood sampling was not always consistent between articles. TGA measures the state of the haemostatic system, at the time of the VTE this is expected to be different than months after the initial event. Ten of eleven studies measured TG in samples obtained several months after the initial event, after discontinuation of anticoagulants. However, the reported TG values by Chaireti et al. were from blood samples that were obtained at the time of the initial event, which makes the study hard to compare with the other studies.

Another issue when comparing the included studies was the variation in parameters of reported outcomes. 6 out of 11 articles reported absolute values for TGA. Seven out of eleven reported hazard ratios and one article reported relative risk. When hazard ratios were reported, sometimes it was given as 'HR per' (i.e., 10 nM), other times the HR was given for two groups (i.e., ≤ 269 vs. > 269 nM). When absolute values were reported it also varied which parameter was given: peak thrombin was always reported, ETP in 4 out of 6 articles, lag time and time to peak were only reported in 2 articles.

To improve comparability between studies on TGA and VTE recurrence, we suggest using a standardised protocol for future studies. TF concentration should be equal between studies since all TG parameters are influenced by the TF concentration. To avoid influence by contact activation, PL concentration should be below 10 µM. A possibility would be to use the CAT-Thrombinoscope kit with PPP reagent, this kit includes 5 pM TF, 4 µM PL and a standard concentration substrate. Furthermore, timing of blood sampling, used TGA parameters and methodology of reporting the association should be more harmonized in order to allow for a proper comparison between studies and a meta-analysis on the predictive value of TGA for VTE recurrence.

Conclusion

Studies on TGA and VTE recurrence are rare, publishes studies have large variations between used protocols and reagents which makes comparison between them difficult. Of all parameters, peak thrombin currently seems to have the biggest potential, however the number of publications on peak thrombin is still limited. A standardised protocol is necessary so that large cohort studies can be performed and so that predictive value of TG can be determined. Once determined, TGA could be integrated into already available prediction tools like the HERDOO2 and DASH score to improve their usefulness for treating physicians.

Abbreviations

CAT	Calibrated automated thrombinography
DVT	Deep venous thrombosis
ETP	Endogenous thrombin potential
HR	Hazard ratio
LMWH	Low molecular weight heparin
PE	Pulmonary embolism
TF	Tissue factor
TG	Thrombin generation
TGA	Thrombin generation assessment
VKA	Vitamin K antagonists
VTE	Venous thromboembolism

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Supplementary data

Supplement I - Search strategy

P: Adults with a first unprovoked objectified venous thromboembolism
I: Abnormal thrombin generation time (endogenous thrombin potential or clot lysis time)
C: Normal thrombin generation time (endogenous thrombin potential or clot lysis time)
O: Objectified recurrent venous thromboembolism

Objectives
To summarise the literature on whether an altered thrombin generation time is predictive for the risk of a recurrent event in adults with a first objectively confirmed unprovoked venous thromboembolism.

Search
Venous thromboembolism, defined as deep vein thrombosis of the leg, pulmonary embolism, upper limb vein thrombosis, cerebral vein thrombosis or splanchnic vein thrombosis – objectively confirmed by imaging.

MESH Terms
Venous thrombosis
OR Pulmonary Embolism
OR Venous thromboembolism
OR sinus thrombosis, intracranial
Recurrence

Free search (title, abstract, all fields)
“deep vein thrombosis” OR "deep venous thrombosis” OR “venous thrombosis” OR “venous thromboembolism” OR “pulmonary embolism” OR “thrombosis” OR “vein thrombosis”
AND
“Thrombin generation test” OR “Thrombin generation assay” OR “Endogenous Thrombin potential” OR “Clot lysis Time” OR “calibrated automatic thrombography” OR “thrombin generation” OR “thrombin generation potential”
AND
Recurrent OR Recurrence OR Second OR repeated OR repeat

Search: (250 hits, 03-05-2020)
((("Venous Thrombosis"[Mesh] OR "Pulmonary Embolism"[Mesh] OR "Sinus Thrombosis, Intracranial"[Mesh] OR "Venous Thromboembolism"[Mesh]) OR ("deep vein thrombosis"[All Fields] OR "deep venous thrombosis"[All Fields] OR "venous thrombosis"[All Fields] OR "venous thromboembolism"[All Fields] OR "pulmonary embolism"[All Fields] OR "thrombosis"[All Fields] OR "vein thrombosis"[All Fields])) AND ("Recurrence"[Mesh] OR ("recurrence"[All Fields] OR "recurrent"[All Fields] OR "second"[All Fields] OR "repeated"[All Fields] OR "repeat"[All Fields]))) AND ("thrombin generation potential"[All Fields] OR "thrombin generation test"[All Fields] OR "endogenous thrombin potential"[All Fields] OR "clot lysis time"[All Fields] OR "thrombin generation"[All Fields] OR "calibrated automated thrombography"[All Fields])

Table 1: Summary of included studies

Study	Design	**Population**, n (% male) **Age**, y - median [IQR] / mean (±SD). **Unprovoked cases**, %	TGA method	Reported TG parameters	Recurrent VTE rate,		Conclusion
					Recurrent VTE / Total	%	
Hron et al.[18] 2006	Prospective cohort	914 (44,6%) 47 y (±16) 100%	TechnoThrombin 71,6pM TF / 3,2 µM PL No [substrate] stated	Peak thrombin	100/914	10.9%	Thrombin generation assessment can identify patients at low risk of recurrence.
Tripodi et al.[19] 2008	Prospective cohort	254 (54.3%) 66 y [20-84] 100%	CAT 1pM TF / 1µM PL 0.417 mM substrate ±4nM TM	Peak thrombin ETP Lag time	34/254	13.4%	Peak thrombin, ETP and lag time associate with recurrent VTE.
Eichinger et al.[20] 2008	Prospective cohort	383 (44.5%) Age n/a 100%	DadeBehring ETP [TF], [PL] or [substrate] not provided by manufacturer	ETP	130/861	15.1%	ETP associates with recurrent VTE and can be of predictive value.
Besser et al.[21] 2008	Prospective cohort	188 (48.4%) 66 y [20-100] 52.7%	CAT 5pM TF / 4µM PL 0.417 mM substrate	ETP	29/188	15.4%	A higher rate of unprovoked recurrence was found in the patients with an ETP above the 50th percentile.
Sonnevi et al.[22] 2011	Prospective cohort	148 (0%) 45 y [18-63] 27.7%	CAT 1 & 10 pM TF / 30µM PL 0.32 mM substrate ± 5nM APC	Peak thrombin ETP Lag time	13/148	8.8%	High thrombin generation at low TF concentration correlates with development of recurrent VTE.
Chaireti et al.[23] 2012	Prospective cohort	115 (42.6%) 61.2 y (±17.4) 66.1%	CAT 5pM TF / 4µM PL No [substrate] stated	Peak thrombin ETP Lag time Time to peak	40/115	34.8%	Patients without recurrent VTE had elevated ETP at the time of the first event.
Siudut et al.[24] 2016	Prospective cohort	197 (54.3%) 45 y [33-54] 49.2%	CAT 5pM TF / 4mM PL 2.5 mM substrate	Peak thrombin	26/197	13.2%	No statement about thrombin generation.
Zabczyk et al.[25] 2016	Prospective cohort	156 (52.6%) 44 y (±13) 57.1%	CAT 5pM TF / 4mM PL 2.5 mM substrate	Peak thrombin ETP Time to peak	39/156	25.0%	Thrombin generation can be a promising indicator for risk of recurrence. Suggested prognostic value specifically for peak thrombin in recurrence of PE.
Cieslik et al.[26] 2018	Prospective cohort	320 (48.4%) 46 y [36-54] 49.7%	CAT 5pM TF / 4mM PL 2.5 mM substrate	Peak thrombin	77/308	25.0%	Higher peak thrombin predicts DVT recurrence.
Van Hylckama Vlieg et al.[27] 2007	Case-control with prospective follow-up	*360 (*42.6%*)* *47 y (16-70)** 51.9%	CAT 15pM TF / 4µM PL 2.5 nM substrate +7nM TM	ETP	59/360	16.4%	No association between high ETP and recurrent VTE
Van Hylckama Vlieg et al.[28] 2015	Case-control with prospective follow-up	598 (55.4%) 53.4 y [18-75] 49.8%	CAT 1pM TF No [PL] or [substrate] stated	ETP	86/626	13.7%	Lack of association between recurrent VTE and thrombin generation.

* Of bigger cohort of 474 patients.
Abbreviations: APC, activated protein C; CAT, calibrated automated thrombinography; ETP, endogenous thrombin potential; HR, hazard ratio; IQR, interquartile range; PL, phospholipids; SD, standard deviation; TF, tissue factor; TG, thrombin generation; TM, thrombomodulin; VTE, venous thromboembolism.

Table 2: Absolute values of thrombin generation parameters compared in patients with and without recurrent VTE

Study	TGA method	N total	Recurrent VTE (N)	No recurrent VTE (N)	Parameter	Recurrent VTE		No recurrent VTE		Interpretation
						Median/Mean	IQR/SD	Median/Mean	IQR/SD	
Hron et al.[18] 2006	TechnoThrombin	914	100	814	Peak thrombin	420	111	349	108	Peak thrombin higher in patients with recurrent VTE.
Tripodi et al.[19] 2008	CAT	254	34	220	Peak thrombin	232	82	187	89	Peak thrombin higher in patients with recurrent VTE. No difference in ETP or lag time.
					ETP	1502	446	1361	499	
					Lag time	11.9	5.7	12.8	4.7	
Chaireti et al.[23] 2012	CAT	115	40	75	Peak thrombin	261	125	302	91	Lag time and time to peak longer in patients with recurrent VTE. Peak thrombin higher in patients with recurrence (p = 0.058). No difference in ETP.
					ETP	1491	536	1671	514	
					Lag time	5.2	4.1	4.2	1.6	
					Time to peak	8.7	5.0	7.2	2.2	
Siudut et al.[24] 2016	CAT	197	26	171	Peak thrombin	221	194-320	233	198-304	No difference in peak thrombin between patients with and without recurrence.
Zabczyk et al.[25] 2016	CAT	156	39	117	Peak thrombin	285	66	245	69	ETP & peak thrombin higher in patients with recurrent VTE. Time to peak shorter in recurrent VTE.
					ETP	1615	70	1566	96	
					Time to peak	4.42	3.90-5.40	5.28	4.37-6.70	
Cieslik et al.[26] 2018	CAT	308	77	231	Peak thrombin	286	241-352	239	202-280	ETP & peak thrombin higher in patients with recurrent VTE
					ETP	1622	1465-1791	1510	1304-1638	
					Time to peak	4.67	4.22-5.33	4.67	4.21-5.52	

Abbreviations: CAT, calibrated automated thrombinography; ETP, endogenous thrombin potential; IQR, interquartile range; SD, standard deviation; TGA, thrombin generation assay; VTE, venous thromboembolism.

Table 3: Hazard ratios for recurrent VTE

Study	TGA method	N total	Recurrent VTE (N)	No recurrent VTE (N)	Parameter	HR per	Univariate			Multivariate		
							HR	95% CI	P value	HR	95% CI	P value
Tripodi et al.[19] 2008	CAT	254	34	220	Peak thrombin	≤ 150 vs. > 237 nM	3.09	(1.31-7.32)	-	2.65	(1.10-6.39)	-
					ETP	≤ 1174 vs. > 1565 nM·min	2.54	(1.05-6.12)	-	2.41	(0.99-5.86)	-
					Lag time	> 13,8 vs. ≤ 10.7	2.29	(0.93-5.62)	-	3.07	(1.23-7.66)	-
Eichinger et al.[20] 2008	Dade Behring ETP	861	130	731	ETP	Increase in ETP with 1%	1.011	(1.0-1.02)	0.06	1.014	(1.0-1.03)	0.06
Besser et al.[21] 2008	CAT	188	29	159	ETP	100 nM·min	-	-	-	1.25	(1.01-1.55)	-
Sonnevi et al.[22] 2011	CAT	148	13	135	Peak thrombin	10 nM	1.16	(1.05-1.28)	0.007	-	-	-
					ETP	10 nM·min	1.03	(1.01-1.05)	0.023	-	-	-
					Lag time	1 minute increase	0.8	(0.6-1.3)	0.42	-	-	-
Zabczyk et al.[25] 2016	CAT	156	39	117	Peak thrombin	≤ 269 vs. > 269 nM	3.66	(1.60-8.80)	0.002	-	-	-
Cieslik et al.[26] 2018	CAT	308	77	231	Peak thrombin	100 nM	2.67	(2.03-3.53)	< 0.001	1.46	(1.09-1.96)	0.01
Van Hylckama Vlieg et al.[27] 2007	CAT	360	59	301	ETP	High vs. low (above 90th percentile)	-	-	-	1.1	(0.5-2.2)	-
Van Hylckama Vlieg et al.[28] 2015	CAT	626	86	540	ETP	≤2052.2 vs. >2502.2 nM·min	1.3	(0.8-2.3)	-	-	-	-

Abbreviations: CAT, calibrated automated thrombinography; CI, confidence interval; ETP, endogenous thrombin potential; HR, hazard ratio; TGA, thrombin generation assay; VTE, venous thromboembolism.

Table 4: Newcastle-Ottawa scale rating

	Selection				Comparability		Outcome					
	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study	Study controls for sex	Study controls for age	Assessment of outcome	Follow-up long enough for outcomes to occur (>24 months)	Adequacy of follow up of cohorts	**Total stars awarded**		
Hron et al.[18] 2006	★	★	★	★	★	★	★	★	★	9/9	100%	Good
Tripodi et al.[19] 2008	★	★	★	★	★	★	★	★	★	9/9	100%	Good
Eichinger et al.[20] 2008	★	★	★	★	-	-	★	★	★	7/9	77.7%	Good
Besser et al.[21] 2008	★	★	★	★	-	-	★	★	★	7/9	77.7%	Good
Sonnevi et al.[22] 2011	★	★	★	-	-	-	★	★	★	6/9	66.6%	Moderate
Chaireti et al.[23] 2012	★	★	★	-	-	-	★	★	★	6/9	66.6%	Moderate
Siudut et al.[24] 2016	★	★	★	-	-	-	★	-	★	5/9	55.6%	Moderate
Zabczyk et al.[25] 2016	★	★	★	-	-	-	★	★	★	6/9	66.6%	Moderate
Cieslik et al.[26] 2018	★	★	★	★	-	-	★	★	★	7/9	77.7%	Good
Van Hylckama Vlieg et al.[27] 2007	★	★	★	-	★	★	★	★	-	7/9	77.7%	Good
Van Hylckama Vlieg et al.[28] 2015	★	★	★	★	★	★	★	★	★	9/9	100%	Good

* Case-control studies with prospective follow-up.