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Proefschriften

Contribution of clinical chemistry in the diagnostic procedure of deep venous thrombosis

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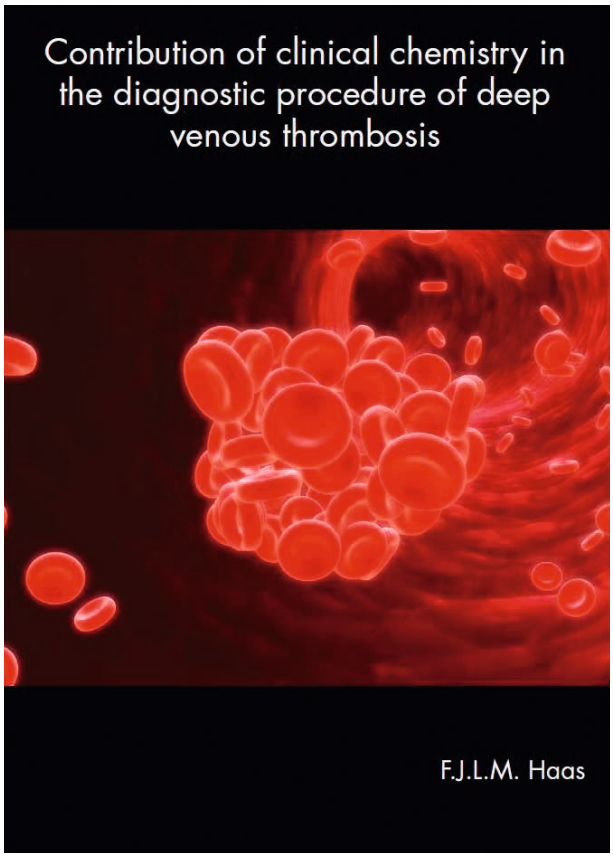
Venous thrombosis

Formation of a clot (thrombosis) is the result of an imbalance between procoagulant, anticoagulant and fibrinolytic factors in arteries or veins. In case of venous thrombosis, this imbalance and clot formation is often due to acute and transient risk factors. Symptoms may occur suddenly or with a delay of some days or even weeks. Venous thromboembolism (VTE) is a common denominator, including superficial or deep venous thrombosis (DVT) of the leg and pulmonary embolism (PE). VTE is one of the most common cardiovascular disorders in industrialized countries, affecting about 5% of all people during their lifetime (1;2).

The mean Dutch figures of fatal outcome over the years 2000 - 2010 were for men respectively woman in case of DVT 7 and 14, for PE 190 and 300 (http://statline.cbs.nl). However, especially PE is a frequently missed diagnosis: 22% of the PE cases are not diagnosed before causing death. The early fatality of DVT is lower (1- and 7- day survival rates of respectively 97.0% and 96.2%), as compared to PE (1- and 7- day survival rates of 63.6% and 59.1%) (3).

There is a strong age-dependency of VTE, as shown in three large studies in France, USA and Norway (4-6), from 1 in 100.000 per year in childhood to nearly 1 in 100 in the elderly. From the patients with VTE, nearly 70% is older than 60 years and nearly 25% is over 80 years old (7). Most patients with VTE are male. Only in the reproductive period of woman (20 - 45 years old), there is a higher number of female patients. Whether gender is an independent risk factor for the first and recurrent VTE, is however not fully clarified (8).



Diagnosis and exclusion of DVT

Thrombosis is a result of coagulation with a key role of the enzymatic conversion of fibrinogen in fibrin monomers (FM) by thrombin. The thrombus will be removed by the enzymatic conversion of the cross linked fibrin by plasmin (fibrinolysis) in fibrin degradation products (FbDP). In some situations there is also an enzymatic degradation of fibrinogen (fibrinogenolysis) in fibrinogen degradation products (FgDP). A closer look to the process of coagulation scheme is helpful to gather information about the formation or presence of a thrombus (Figure 1).

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One of the most important fibrin degradation products is the D-dimer. D-dimers can be found during both coagulation and fibrinolysis.
With the development of specific monoclonal antibodies against epitopes of the D-dimer molecule, measurement of D-dimer levels became feasible (9). The first D-dimer tests were qualitative tests (10), followed by quantitative ELISA's (11). Because the ELISA is very laborious test, this type of D-dimer assays was only used in studies. With the launch of the Vidas® ELFA (a so-called fast ELISA) (12) and the latex D-dimer assays, both measured on dedicated instruments or analyzers, the application of D-dimer tests in direct patient care became possible (13).

Information about the coagulation scheme can be obtained by:
thrombin (thrombin generation assay)
fibrin monomer (FM)
fibrin degradation products (FbDP)
fibrinogen degradation products (FgDP)

The D-dimer has proven to be a very useful tool in the diagnostic procedure of VTE.
Different D-dimer assays have been evaluated in the diagnosis of VTE. The general conclusion is that the combination of sensitivity and specificity is not high enough to exclude VTE, based on only the results of a D-dimer test (14-25). With sensitivity beneath 100% for almost all D-dimer tests, there is a small but unacceptable risk for sending home patients with a VTE. The relatively low specificity of most D-dimer tests, results in the need for additional investigations to exclude VTE. Therefore, the combination of D-dimer tests and a clinical decision rule (CDR) score is used. Wells has introduced a CDR, initially with three levels (low, moderate and high) (26), later simplified to two levels (unlikely and likely) (27). In patients with high D-dimer levels or a high CDR-score, additional imaging procedures are necessary. The CDR has only been validated for outpatients. For the primary care, an adapted decision rule has been developed and evaluated (28;29).
D-dimer levels are not only high in patients with VTE. Many non-pathological (e.g. high age or surgery) and pathological (e.g. inflammation or malignancy) conditions are associated with elevated D-dimer levels (30). It is, therefore, necessary to improve the contribution of the clinical chemistry in the diagnostic workup of VTE.

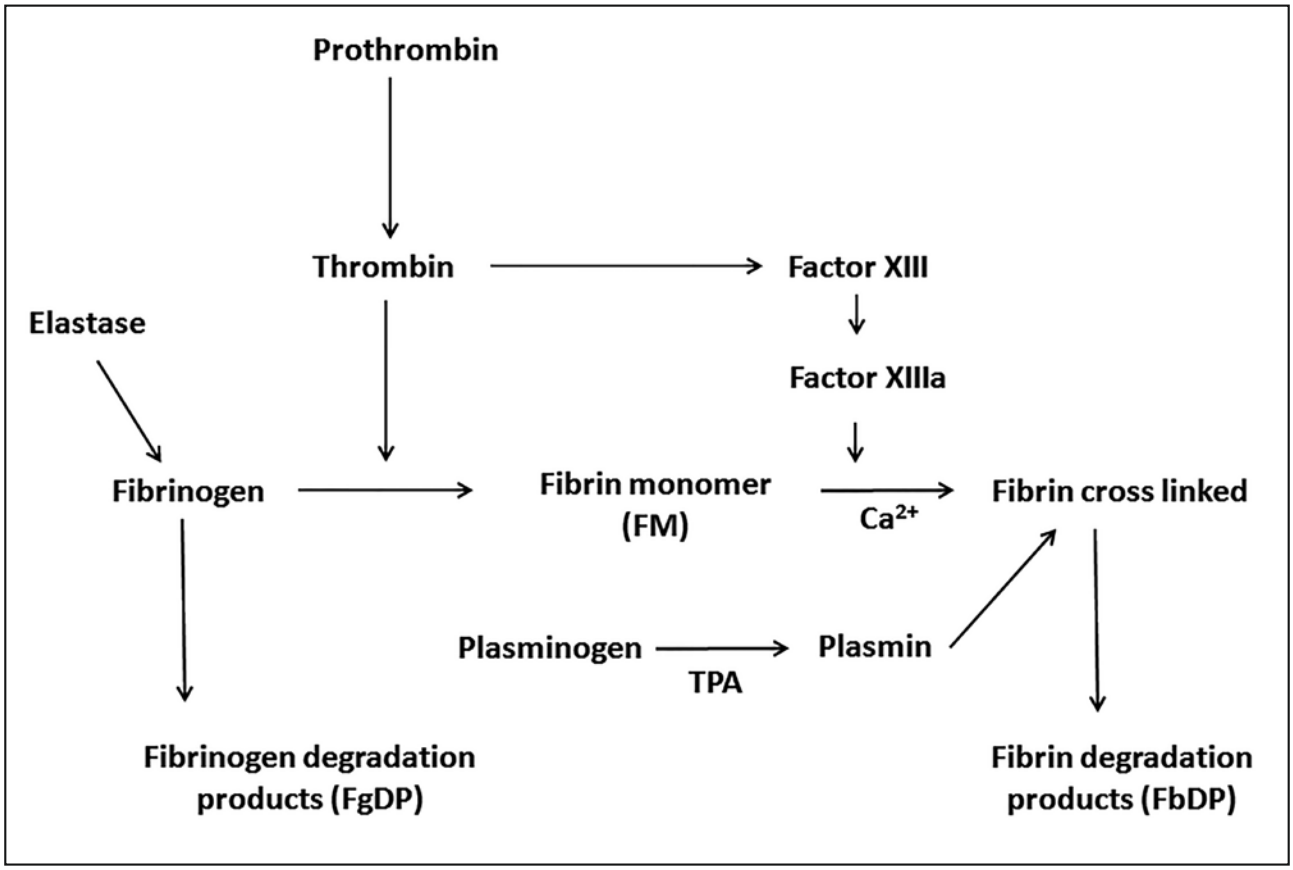
Outline of the thesis
The aim of this thesis is the improvement of clinical chemical techniques, used in the diagnostic workup of patients suspected for DVT, resulting in a safe exclusion of DVT as well as a reduction of false positive results. To realize this aim, the specificity of the used technique(s) must increase without reduction of a sensitivity of 100%.
This reduction may be achieved by:

- more specific assays; we have evaluated current and new laboratory assays, measuring the forming of intermediate or end products of coagulation (Figure 1),
- the combination of these new assay results with the D-dimer assay, as second decision criterion or correction of the D-dimer value,
- changing the cut-off value of the D-dimer assay in special subgroups of patients.

Because the highest number of false positive results is obtained in the elderly, we have focused on this subgroup of patients.

Like the D-dimer assay, additional assays should be available in a routine laboratory setting, 24 hours a day with an acceptable turnaround time. A further prerequisite is that the selected and evaluated assays should be commercially available or under development. For all evaluation studies we used the patient database of a management study, investigating the use of a quantitative D-dimer assay in combination with the Wells score in the exclusion of DVT in symptomatic outpatients. (31). In **Chapter 2,** a review of D-dimer in relation to DVT and PE is presented, based on the text used in the Dutch CBO Guideline *'Diagnostiek, preventie en behandeling van veneuze trombo-embolie en secundaire preventie van arteriële trombose'* (32).
The use of heparin plasma instead of the standard citrated plasma for the measurement of D-dimer is described in **Chapter 3.** The possibility of using heparin plasma is of great logistic importance, because the turnaround time of the D-dimer assay can be reduced and the usefulness as diagnostic tool may be enhanced.
In **Chapter 4,** the performance of four D-dimer assays is discussed and compared to the results obtained with Tina-quant® in the mentioned previous management study (31).
In **Chapter 5,** the results of the D-dimer assay in combination with the Wells score in the elderly are presented. Because the D-dimer level increases with age, the specificity decreases with a reduction in its clinical applicability in the elderly.
The combination of the D-dimer assay and CDR is not very helpful in the exclusion of VTE in the elderly. The contribution of other intermediate and end products of the coagulation scheme (Figure 1) is, therefore, investigated. The results of two quantitative FM assays in combination with the Tina-quant D-dimer assay and CDR in the diagnostic workup are given in **Chapter 6.**
Currently, a fixed cut-off value of D-dimer (regardless the age of the patient) is used. A higher specificity (less false positive results) may be obtained by applying age dependent cut-off values. In **Chapter 7,** a new D-dimer assay (Innovance™ D-dimer) is introduced with special emphasis on the results of age-adapted cut-off values.
Coagulation results also in thrombin formation. Therefore, the thrombin generation assay (TGA) as diagnostic tool in patients suspected for having VTE is of interest. TGA according to Hemker (33-35) is not feasible in a routine patient care setting due to the high turnaround time and the need of specialized technicians. New TGAs, such as the Endogenous Thrombin Potential (Innovance®-ETP), are not

Figure 1. Simplified coagulation scheme



TPA = tissue plasminogen activator

hampered by these factors and can be used for routine application (36;37). In **Chapter 8,** the Innovance®-ETP as new TGA is evaluated, as stand-alone assay and in combination with the standard procedures of the diagnostic workup of VTE. The performance of TGA and D-dimer/CDR is especially tested in the elderly.

It is known that some thrombin generation assays are sensitive for thrombophilic factors. In **Chapter 9,** the relation between ETP parameters and the most common thrombophilic risk factors is investigated in DVT negative and DVT positive patients.

End products of the coagulation scheme (Figure 1), such as fibrinogen elastase degradation product and another fibrin degradation product than D-dimer, may also have a role in the diagnostic procedure of VTE. The results of measurements of these products, alone and in combination with some D-dimer assays, are presented in **Chapter 10.**

In **Chapter 11** a compilation of the results of this thesis and the future perspectives are presented.

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