Haemostasis monitoring

Chapter 8

General discussion and summary

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General discussion

In this thesis, I first have outlined the principles of haemostasis. Traditionally haemostasis is divided into a primary and secondary haemostasis part, both leading to fibrin formation; subsequent fibrin breakdown is regulated by fibrinolysis. The interplay of coagulation enzymes, blood platelets, and the vessel wall, is however more elaborate than what has been delineated in **chapter two**.[1,2] Instead of separate processes occurring in parallel, coagulation and fibrinolysis evolve at the same time. While the clot starts to grow due to the increased amount of activated platelets trapped in the expanding fibrin mesh, fibrinolytic enzymes start to shape and break down the growing clot right away. Moreover, a more 'modern' view on coagulation also adopts the influence of inflammatory processes on coagulation.[1,3]

However, it is sometimes more practical to consider distinct elements of the coagulation process, as most laboratory tests focus on a limited part of coagulation.[1] Platelet function testing is usually performed in isolated platelet rich plasma (PRP), thereby excluding the effect of the vessel wall and that of other blood constituents. Light transmission aggregometry (LTA) as the gold standard in platelet function testing in PRP might be replaced in the future by whole blood platelet function testing methods which adopt a more holistic view on haemostasis. Whole blood tests already have proven that they have added value compared to LTA, because of practical reasons including reduced time consumption and the ease of analysis and interpretation. Multiple electrode aggregometry (MEA or Multiplate) and the platelet function analyzer 100 (PFA-100) both use whole blood and thus, in theory, the effects of red blood cells are evaluated in these tests as well. One of the newest methods of haemostasis monitoring with focus on the platelet component are flow dependent microscopy analysis, such as pioneered in the laboratory of Prof. Heemskerk at the Maastricht University. A paper describing the protocol involved in these analyses has set the standard in these flow chamber experiments.[4] This technology incorporates whole blood,

flow, and can even mimic vessel wall elements. By varying different components in these chambers (low flow vs. high flow; different coating types; etc.), haemostasis under different conditions can by evaluated. A disadvantage of this technique remains the point-of-care aspect, because a well-equipped professional laboratory is needed. PFA-100, in contrast to MEA, also measures platelet function under flow conditions. This has made the PFA-100 an excellent screening tool for von Willebrand disease, as the multimeres unfold more or unfold less depending of the flow rate.

Viscoelastic whole blood tests, like rotational thromboelastometry (ROTEM) or thrombelastography (TEG), evaluate clot formation and breakdown as an overall process of haemostasis, similar to the cell-based concept of coagulation in vivo.[1] In contrast to pure plasma based tests, like the prothrombin time (PT) or activated partial thromboplastin time (aPTT), haemostasis is tested in a more holistic manner. During the analysis, clot formation and breakdown, facilitated by platelets together with the plasmatic and fibrinolytic enzymes, are visualized in real-time using graphs and numbers. This can be done bedside without involvement of specialized laboratory personnel using the latest addition to the rotational thromboelastometry portfolio of the ROTEM sigma or by using the TEG 6S from Haemonetics. On the other hand, the PT and aPTT, as the cornerstones of conventional plasma based coagulation tests, remain valuable as they specifically address medication effects of vitamin K antagonist and unfractionated heparin. Both PT and aPTT are still used as screening tests in suspected bleeding disorders in daily practice. For instance, a prolonged aPTT could have multiple causes (**table 2.1**) and by evaluating separate coagulation factors, an isolated deficiency in coagulation factor VIII leads to the diagnosis of haemophilia A. In contrast, standard viscoleastic whole blood point-of-care methods are insensitive for disorders such as haemophilia A. However, after tweaking of the protocols by varying TF amounts or by adding tissue type plasminogen activator (tPA), clot resistance in haemophilia A patients treated with recombinant activated FVII could be shown.[5,6] In acquired multifactorial disorders of haemostasis the use of viscoelastic tests is better applicable; in traumatic bleeding disorders ROTEM assesses both clot formation, clot stability, and

clot breakdown in one test cycle. Because multiple channels can be used at the same time, the effects of fibrinogen, heparin, and the contribution of an antifibrinolytic agent in these tests can be evaluated separately and in parallel. With the ROTEM sigma, all-in-one cartridges are employed to reduce the complexity of starting a measurement. The global haemostasis cartridge used in the TEG 6S has similar capabilities to the ROTEM sigma, but is different in the details.

Thrombin as the central controlling enzyme is involved in clot formation as well as in clot breakdown. Basically, too much thrombin could lead to a prothrombotic state, while too little thrombin is seen in bleeding disorders. Another innovation in the field of coagulation monitoring enables measuring the amount of thrombin generated using the calibrated automated thrombogram.[7] Essentially, the amount of thrombin formed is measured using a fluorogenic substrate based analysis. The thrombin generation test used to be a plasma based analysis, but it has evolved towards a point-of-care whole blood device, quite recently.[7]

Similarly to the shift in the use of conventional plasma based tests to whole blood based point-of-care tests, a shift in transfusion practice takes place, from the use of whole blood products, to fractionated blood components, and further to purified, isolated coagulation proteins. Especially in traumatic bleeding, the replacement of whole blood by fractioned transfusion products, like red blood cell concentrates, fresh frozen plasma or cryoprecipitate, and platelet concentrates is noticeable. More recently, purified coagulations protein like fibrinogen concentrate or recombinant activated FVII and the use of tranexamic acid have gained much interest as a more goal-directed therapeutic approach in acute bleeding disorders.[8]

Pitfalls of whole blood point-of-care methods of haemostasis testing are that, although its use is simple, validation of the method and quality insurance over time needs to be established. Deviations of results due to, for instance, inadequate filling of blood collection tubes, improper use and wear-and-tear of point-of-care devices need to be addressed when being used in daily clinical care. Physicians and other non-laboratory medical personnel can be educated to perform tests on the spot, but in the end they are not sufficiently qualified to

address these matters. Besides, their focus should be on taking care of patients, not on laboratory equipment. For all laboratory tests, pre-analytical variables need to be controlled as much as possible to ensure quality control.[9] Haemolysis will disturb optical plasmatic coagulation assays, low platelet counts and anaemic conditions will affect whole blood platelet function tests like PFA-100 and Multiplate, as we showed in **chapter three**. By correcting the results for these pre-analytical differences among healthy volunteers we were able to define dynamic reference intervals. The effect of red blood cells seemed to be important in the PFA-100 only and not in the Multiplate, based on the different methods used in both devices. The PFA-100 acts by adding flow to the measuring system and thus red blood cells improve platelet adhesion by pushing them towards the outer layer of the bloodstream.[10] I stated in my introduction chapter: 'for effective primary haemostasis, a sufficient number of functional platelets needs to be present in the body'. In analogy, a sufficient number of platelets needs to be present to measure their function. The fact that the results of the Multiplate device needs to be adjusted for the individual platelet count has been described earlier, however, we could show for the first time how to interpretate whole blood point-of-care platelet function results in patients in need for a biopsy of a previously transplanted kidney. This study presented in **chapter four,** revealed that in chronic kidney disease (CKD) patients prone for developing bleeding events, because of thrombocytopenia (present in 6%), anaemia (63%), frequent use of the antiplatelet medication acetylsalicylic acid (17%), and uraemia (73%), potential bleeders could not be identified. None of these risk factors, potentially contributing to in vivo platelet dysfunction, emerged as in vitro predictors of bleeding complications. In conclusion, (prior) use of acetylsalicylic acid seemed to be the only predictor of a bleeding event after biopsy of a transplanted kidney. We expected that either PFA-100 or MEA or both would help identify patients prone to bleeding disorders, but as can be observed from **figure 4.1 and 4.2**, patients with a positive bleeding endpoint were distributed all over the charts. It did not matter if results were or were not adjusted for thrombocytopenia or anaemia. Most strikingly, we observed an in vitro platelet dysfunction in almost half of the patients when using MEA (**table 4.3**). One could argue that

von Willebrand factor (vWF) might play a pivotal regulatory role in CKD. VWF levels are increased when age advances, just as the incidence of CKD increases when growing older. On the other hand, uraemia seen in CKD can result in vWF dysfunction.[11] This notion and the observation that Ivy bleeding times (IBT) were prolonged in von Willebrand disease (vWD, especially in type III vWD) made that desmopressin used to be given to patients with prolonged IBT awaiting a kidney biopsy. A direct causal effect of uraemia in CKD on bleeding events is nowadays put in doubt and anaemia seems to be the main culprit.[11] Although specific haemostatic defects are found in uraemia, growing evidence suggests that in several chronic conditions affecting haemostasis (e.g. in CKD or in cirrhotic liver disease) a reset of the haemostatic balance (homeostasis) is occurring.[11,12] Because of the abundance of regulatory coagulation proteins, homeostasis of coagulation can be controlled more precisely and effectively, unlike by the means of a simple on/off-switch. This renewed balance seen in cirrhosis and uraemia is unstable and could tip easily to either side, resulting in thrombosis or bleeding.[11,12] As such, the term 'uraemic thrombocytopathy' seems to be more of an in vitro laboratory finding, than a clinical sign of bleeding due to platelet dysfunction. This conclusion is supported by the fact that we were unable to find a link between elevated or high blood urea concentrations and increased bleeding endpoints (non-significant odds ratios) in our study.

Bleeding is mostly multifactorial, but slight (or large) disorders in a specific part of the haemostatic system can result in major bleeding complications even after minor trauma. The pathogenesis of traumatic bleeding is an interesting phenomenon.[13,14] Endothelial damage initiates the formation of the tenase complex by expressing tissue factor (TF) to the blood on TF bearing cells after which thrombin is produced in abundance while further being amplified through the Josso loop. Thrombin with its central controlling role in haemostasis is halted when in complex with endothelial thrombomodulin. Next, protein C will be activated by the thrombin-thrombomodulin complex and fibrin formation by the diminishing thrombin will be further attenuated. Activated protein C also negates PAI-1; PAI-1 is then unable to block the by endothelial damage released tPA. The end result is anticoagulation and hyperfibrinolysis in which hyperfibrinolysis

is predominant.[15] One can argue that homeostasis will find a renewed balance in haemostasis, but in traumatic bleeding this sophisticated system seems to fail.[14] The overt bleeding, tissue hypoxemia due to shock, shedding of the glycocalyx, subsequent acidosis, inflammatory response, and need for fluid resuscitation all contribute to a lethal triad of dilutional consumptive coagulopathy by trauma: acute coagulopathy of traumatic shock (ACoTS).[14] Viscoelastic methods are considered the best tools for fast and adequate detection of hyperfibrinolysis in trauma,[16,17] however mild to moderate fibrinolysis can easily be missed even by viscoelastic tests.[18] Our research of the validated tPA induced fibrinolysis assay for ROTEM was not performed in ACoTS, because of lack of steady inclusion rates of trauma patients at that time. On the other hand, the antifibrinolytic effects of the tranexamic acid could be demonstrated effectively in the group of post cardiac surgery patients using our method (**chapter five**). The euglobulin clot lysis time (ECLT) as the plasma based gold standard assay for fibrinolysis detection, was insensitive for tranexamic acid therapy. On the other hand, good agreement between our method and the ECLT on hypofibrinolysis detection in sepsis was apparent. The high antifibrinolytic effect of PAI-1 together with the prothrombotic potential due to high fibrinogen levels were deemed to be responsible for the results in both tests. From a scientific point of view likely, the tPA induced fibrinolysis protocol for ROTEM should be tested in ACoTS, but after the CRASH -2 trial and subsequent analyses,[19] most, if not all, trauma patients will be treated with tranexamic acid before haemostasis monitoring can be initiated. Blood sampling before tranexamic acid treatment could prove the hypothesis that our method is able to distinguish fast and reliable between trauma patients if hyperfibrinolysis is not, moderately, or overtly present. Thereby, it would be possible to guide tranexamic acid therapy and administer it to those who would benefit from it and refrain clinicians from using a regime of 'tranexamic acid for all'.[14] To me this seems to be an interesting investigational study. Of note, a similar non-validated method has been performed in a study using the TEG and thus this concept seems to be promising for the validated ROTEM method as well.[20] Another interesting opportunity of research, could be to address the systemic effects of intravenous thrombolytic therapy for

massive pulmonary or venous thromboembolism or of intra-arterial treatment for acute ischemic stroke. A PubMed search revealed 31 hits when searching for "(thrombolysis viscoelastic) OR (thrombolysis TEG) OR (thrombolysis ROTEM)", of which only two looked at systemic effects of intravenous thrombolysis therapy in thrombotic disorders.[21,22] Systemic effects of thrombolytic therapy on lysis parameters could not be demonstrated using standard TEG[21] or ROTEM[22] analysis in these studies.

As time is of the essence in bleeding scenarios and early diagnosis and treatment saves lives,[17,18] we have also looked at early prediction models of thrombocytopenia and hypofibrinogenemia in cardiac surgery patients for which ROTEM was introduced as standard care in the Maastricht UMC+ (**chapter six**). We envisioned that ROTEM would replace conventional plasma based coagulation assays for multifactorial bleeding diagnosis. Prerequisites were that ROTEM would do this faster and give more information than conventional laboratory tests, and all of this with preferably only one tube of (citrate anticoagulated) blood. Treatment protocols such as those used in **chapter seven** normally recommend using amplitude at ten minutes (A10) on EXTEM and FIBTEM to measure overall clot strength and the contribution of fibrinogen. Platelets and fibrinogen, being the most crucial end products in a formed clot, could be deduced from the ROTEM graphs and numbers. FIBTEM as a measurement of clot strength invoked by fibrinogen had a strong positive correlation with fibrinogen levels analysed by the Clauss method. Using the amplitude at five minutes (FIBTEM A5; Pearson's *r* of 0.87 in respect to Clauss' fibrinogen levels) meant five minutes of time gain over using A10. By subtraction of the amplitude of the FIBTEM from the EXTEM, we could calculate the platelet contribution to the clot strength. The newly introduced PLTEM for estimating platelet counts correlated strongly with conventionally measured platelet counts (PLTEM A5; $r = 0.85$). The PLTEM amplitude at five minutes correlated even better with platelet counts than amplitudes at ten minutes or when maximum clot strength was reached (MCF). In a letter to the editor,[23] we re-assessed this PLTEM parameter after a publication which stated that clot elasticity (CE) should be used for evaluating platelet contribution to the formed clot. In hindsight, our more

straightforward approach seemed to be more simple and superior in estimating platelet counts.[23] It is now possible to use one tube of citrate anticoagulated whole blood to get reliable insights pertaining fibrinogen level, platelet count, overall clot strength, and available fibrinolysis in cardiac surgery patients within 15 minutes of arrival of the sample at the laboratory. Conventional laboratory tests for PT, aPTT, platelet counts and Clauss' determination of fibrinogen require more blood and about 25 minutes longer to give full results, while not giving any information on fibrinolysis parameters.

Implementation of a ROTEM-based treatment protocol cannot only give faster results, but at the same time it can save costs. In my **seventh chapter** we sought to investigate the impact of introducing ROTEM-based treatment strategies in cardiac surgery patients. Using two cohorts, one in which conventional laboratory tests were used and another in which ROTEM-based decisions were made to monitor and guide haemostasis in cardiac surgery patients, it was possible to show reductions in the use of allogeneic blood products, reduction of hospital length of stay, and cut down in overall costs. Mortality rates were not reduced upon the implementation of a ROTEM-guided transfusion algorithm, likewise, benefit on mortality could not be demonstrated in a recent systematic review and meta-analysis of seven randomized controlled trials.[24] Relative risk was in favour of ROTEM guidance at 0.55, but this was not significant with a 95% confidence interval of 0.28-1.10.[24] Subgroup analysis of coronary artery bypass graft (CABG) surgery and of high risk cardiac surgery based on EuroScore, showed also overall costs reduction by using ROTEM-guided transfusion protocols. Both CABG surgery and high EuroScore were chosen in order to reflect homogenous subgroups of cardiac surgery patients. In the isolated CABG surgery subgroup the amount of blood loss and the number of rethoracotomies were reduced at the expense of using more fibrinogen. In the high EuroScore subgroup, blood loss and hospital length of stay were reduced as the two most remarkable findings.

Point-of-care viscoelastic monitoring of haemostasis and platelet function testing seems to be the way forward, as new modalities and research opportunities are introduced regularly, further fine-tuning accuracy, precision, and efficacy of

these tests. Point-of-care whole blood thrombin generation seems to be the next step in assessing the intricacies of secondary haemostasis (and more), while flow chamber testing will boost research at the primary haemostasis side (and more) to a point-of-care level which ROTEM made us become familiar with. Monitoring of fibrinolysis and the anticoagulation pathways of haemostasis has still a long way to go in the point-of-care era, but our rTPA induced ROTEM fibrinolysis assay may have set this first step forward providing a robust point-of-care concept for the future.

Summary in English

From **chapter two** we have learned that haemostasis monitoring can be done in plasma based or whole blood setups. Every single contributing element to haemostasis can be assessed in order to pinpoint a diagnosis. Point-of-care testing differs from conventional laboratory testing in that it has the premisses of enabling bedside application in an easy and fast manner. Platelet function testing is based on the ability to use platelet agonists to induce platelet aggregation. Different assays exist each with their own advantages and disadvantages. The overall ruling seems to be that in order to adequately test platelet function, sufficient amounts of platelets needs to be present in the measuring sample. Correcting for platelet counts, like we did in **chapter three**, seems to overcome this problem. That bleeding complications are mostly multifactorial and therefore highly unpredictable, is apparent from our study in **chapter four**. A thorough investigation using point-of-care platelet function testing in transplanted kidney biopsies could show that, although half of the patients seem to have an in vitro platelet function defect, almost none of the patients experienced bleeding complications. In **chapter five**, our validated method of whole blood viscoelastic testing of the fibrinolytical pathway was proven to be an innovative addition to the conventional plasma based fibrinolysis assays. We could show that the diagnosis of a hypofibrinolytical state could be made quickly in septic patients, while this was not possible by using regular viscoelastic tests. The use of a fibrinolysis blocker (tranexamic acid) in cardiac surgery patients was evidently

picked up by our method in contrast to the conventional plasma based euglobulin clot lysis time assay. The holistic cell based concept in haemostasis testing has proven that whole blood viscoelastic point-of-care assays, like rotational thromboelastometry (ROTEM) can adequately and efficiently diagnose haemostatic disturbances in bleeding scenarios. That time can be saved by using ROTEM is even more evident from our studies in **chapter six**. Twenty-five minutes could be saved in diagnostic speed over conventional laboratory tests by using the results after just five minutes with ROTEM. Using implemented ROTEM-guided transfusion protocols we were able to reduce hospital length of stay after cardiac surgery and have potentially reduced total costs, as was shown in **chapter seven**. In conclusion, with point-of-care devices in haemostasis monitoring it is feasible to accurately and quickly pinpoint disorders in platelet function, coagulation, and fibrinolysis. The clinical impact of these findings is however uncertain, but eventually, it might be possible to save patients' lives and reduce healthcare costs.

Summary in Dutch

In **hoofdstuk twee** hebben we gezien dat het controleren van de bloedstolling kan met zowel op bloedplasma als op volbloed gebaseerde technieken. Elk los element dat bijdraagt aan de stolling kan in kaart worden gebracht om tot een diagnose te komen met deze technieken. Point-of-care testen verschillen van de conventionele laboratorium testen in het feit dat ze aan het bed van de patient gebruikt kunnen worden op een gemakkelijke en snelle wijze. De basis om bloedplaatjes functie te meten is dat stolsels geïnduceerd worden met bloedplaatjesactivatoren. Er zijn verscheidene testen, elk met hun eigen voor- en nadelen. In het algemeen geldt dat er een voldoende aantal bloedplaatjes nodig is om hun functie nauwkeurig te kunnen meten. Door te corrigeren voor het bloedplaatjesaantal conform het onderzoek in **hoofdstuk drie**, lukte het ons dit probleem te omzeilen. Dat bloedingscomplicaties meestal meerdere oorzaken hebben en daarom ook moeilijk voorspelbaar zijn, is gebleken uit het onderzoek in **hoofdstuk vier**. Ondanks grondig onderzoek met point-of-care bloedplaatjesfunctietesten voorafgaand aan nierbiopten van patiënten na een niertransplan-

tatie, was het niet mogelijk, ondanks dat bijna de helft van hen een in vitro bloedplaatjes functiedefect leek te hebben, een voorspelling te doen over bloedingscomplicaties. In **hoofdstuk vijf** hebben we laten zien dat onze gevalideerde volbloed viscoelasticiteits methode om fibrinolyse metingen te verrichten een innovatieve aanvulling is op de reguliere plasma gebaseerde fibrinolyse methodes. Een verlaagde fibrinolyse capaciteit in patiënten met bloedvergiftiging (sepsis) kon makkelijk en snel worden opgespoord, terwijl dit niet mogelijk was met de gebruikelijke viscoelastische methodes. Het gebruik van een fibrinolyse remmer (tranexaminezuur) in een patiëntenpopulatie na hartchirurgie kon duidelijk teruggevonden worden met onze methode in tegenstelling tot de conventionele in plasma uitgevoerde euglobuline clotlysis tijd. De holistische benadering van het meten van bloedstolling heeft reeds bewezen dat volbloed point-of-care methodes zoals ROTEM (rotational tromboelastometry) nauwkeurig en efficiënt stollingsafwijkingen in bloedingssituaties kunnen aantonen. Dat er tijdswinst valt te behalen door ROTEM in te zetten werd duidelijk uit de studies die in **hoofdstuk zes** zijn beschreven. Het is mogelijk 25 minuten eerder een diagnose te stellen met ROTEM dan dat dit met de gebruikelijke metingen kon. Hiervoor gebruikten we de ROTEM resultaten na vijf minuten. Verder was het mogelijk om vernieuwde op ROTEM gebaseerde transfusieprotocollen in de klinische praktijk in te voeren. In **hoofdstuk zeven** konden we aantonen dat de ligduur in het ziekenhuis van de patiënten na hartchirurgie verminderd konden worden alsmede de hiermee gemoeide kosten. Concluderend, met point-of-care methodes om de bloedstolling te vervolgen is het mogelijk om nauwkeurig en snel afwijkingen in de bloedplaatjes functie, de stollingscascade en het fibrinolytische systeem aan te duiden. De exacte klinische relevantie van al deze afwijkingen in stollingswaarden is nog onduidelijk, maar mogelijk kan met deze methodes toekomstige patiëntenlevens en ziekenhuiskosten bespaard worden.

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