

Unravelling von Willebrand Factor

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Valorisation

VALORISATION

The major goal of this thesis was to explore the role of active VWF in physiological haemostasis and pathological conditions associated with an increased risk of thrombosis (strenuous exercise, COPD, CKD) or a mild bleeding phenotype. This chapter first provides a short overview of the societal and economic burden of the overarching “problem” of cardiovascular disease. This is followed by a discussion of the potential opportunities for valorisation of the knowledge obtained through our studies, with valorisation referring to *“the process of creating value from knowledge, making knowledge suitable and/or available for economic and social use and for translation into competitive products, services, processes and new activities”*.¹

Cardiovascular disease: a deadly and costly burden

Cardiovascular diseases (CVD) represent the leading cause of death worldwide, with more than 4 million deaths each year.² Direct and indirect costs of CVD account for approximately 12% of the total European health care expenditures.³ Thrombosis is the most common underlying pathology of the three major cardiovascular disorders: ischemic heart disease (IHD), stroke, and venous thromboembolism (VTE), which includes both deep vein thrombosis (DVT) and pulmonary embolism (PE). Together, the two leading forms of arterial thrombosis, IHD (7 million deaths) and stroke (5.9 million deaths) collectively cause one in four deaths worldwide⁴, while epidemiological studies estimate the number of VTE-related deaths to be around 500.000 per year in the European Union alone.⁵ Estimates of the total economic impact of VTE, taking into account the lost economic output due to premature mortality, are as high as \$69 billion per year, and will continue to rise due to increasing longevity.⁶ The numbers presented here reflect the enormous disease- and economic burden of thrombosis, and hence project the potential benefits of prevention, in terms of reducing mortality/loss of productivity and healthcare costs. The subsequent paragraphs of this chapter will focus on how our findings may be relevant for society, therapy- and prevention strategies and diagnostic laboratory practice in the field of thrombosis and haemostasis.

Neutralization of the (active) VWF-platelet interaction

Spontaneous binding of platelets to active VWF occurs in a variety of diseases and can lead to thrombotic and/or bleeding complications that have a large impact on the quality of life, morbidity and mortality. In a number of common, multifactorial conditions associated with a prothrombotic phenotype, (e.g. COPD (**Chapter 6**) and CKD (**Chapter 7**)), increased active VWF levels are certainly not the only cause of the increased thrombotic risk. However, in some (generally rarer) conditions, active VWF is causally implicated in the haemostatic complications, such as in TTP (prevalence 1-13 per million⁷), VWD

type 2B (<<1 per 10.000⁸) and HELLP (0.17-0.85% of all live births⁹). Despite their low prevalence, the economic burden of these conditions is substantial. Exacerbations of acute TTP are treated with plasma exchange in conjunction with immunosuppression to remove UL-VWF and inhibitory autoantibodies, replenish ADAMTS13 and suppress new antibody production.¹⁰ Average healthcare costs for a hospitalization for incident acute TTP in the US were previously estimated to be \$56,347 (SD \$80,230), and are even higher in patients requiring prolonged plasma exchange.¹¹ For VWD type 2B, replacement therapy with purified VWF/FVIII concentrates is required to control recurrent bleeding and associated complications. The direct and indirect costs of such VWF/FVIII concentrate therapy is around €250,000 per year.¹² Also in more prevalent diseases, such as malaria (~219 million cases worldwide¹³) and dengue (96 million symptomatic, worldwide¹⁴), evidence demonstrates a link between active (UL-)VWF and haemostatic complications. These diseases mainly occur in developing countries with limited healthcare resources.

Neutralization of the interaction between VWF and platelets appears to be an attractive strategy to prevent microthrombi formation (as in TTP) and excessive platelet clearance (with resulting thrombocytopenia and bleeding), thereby reducing the disease burden and societal and economic impact. This strategy was previously explored by others (**Chapter 10**, Table 1), of which the bivalent VHH caplacizumab is the most successful.^{15,16} As demonstrated in **Chapter 2**, our VHH S-VWFA1 could have beneficial properties over caplacizumab as it specifically inhibits only active VWF. However, it is debatable whether one should pursue the development of this VHH and the possible ensuing clinical studies in light of the financial burden of drug development programmes (estimated to be around 200-400 million US dollars¹⁷), given that caplacizumab is already available and approved, and TTP is a rare ("ultra-orphan") disease. In addition, the increased bleeding risk associated with caplacizumab appears mild and manageable and only required VWF/FVIII concentrate replacement therapy in a small percentage of patients in the phase III HERCULES trial.¹⁰ Of note, the costs for treating a typical acute TTP episode with caplacizumab (Cabliivi®) is \$270,000. One argument in favour of our VHH is that it would be theoretically more suitable for prophylactic long-term use than caplacizumab. Caplacizumab induces increased clearance of VWF and FVIII, which would ultimately lead to a more severe bleeding risk,¹⁵ while targeting only active VWF would allow for prevention (instead of rescuing) of acute TTP episodes. This would also reduce the associated costs of hospitalization and other treatment modalities.

Hypothetically, caplacizumab may also benefit patients with other conditions/complications related to excessive platelet-VWF interactions and consequent thrombocytopenia. In conditions with a bleeding phenotype, such as VWD type 2B, the increased

bleeding risk associated with caplacizumab treatment needs extra consideration. In these conditions, the S-VWFA1 VHH, which is expected to pose no/less risk of bleeding due to its specificity for active VWF, may be beneficial in terms of safety.

Altogether, in terms of valorisation, there are still many open questions around the possible “market size” of the VHH and its value proposition (since an alternative, caplacizumab, is available). Given the very early stage of its development, significant resources would be needed to reach a viable and valuable point for patent application. It is uncertain whether the potential benefits of the S-VWFA1 VHH outweigh the costs associated with drug development.

Raising awareness on the prothrombotic effects of physical stress

Compelling evidence demonstrates that physical activity reduces premature mortality and prevents a variety of chronic medical conditions, including CVD.^{18,19} However, there is also evidence of sudden cardiac death and thromboembolic events in athletes, and clearly, there are risks associated with vigorous and/or prolonged physical activity.²⁰ Although our findings confirm previous studies on the prothrombotic response to exercise²¹, in particular of the endothelium, these experimental findings in small groups of healthy individuals may not directly have a socio-economic impact. Nevertheless, future larger studies may be able to resolve some of the important gaps in knowledge (as discussed in **Chapter 10**) and contribute to the public awareness of the thrombotic risks associated with strenuous exercise in untrained individuals.

Research into the effects of exercise is (clinically) relevant for several situations and groups of individuals. Although our studies focused on the effects of cycling on coagulation, other forms of exercise, such as (long-distance) running, may cause similar effects. Running is one of the world’s most popular sports²² and marathon events continue to grow annually²² (called “marathon fever”), especially among middle-aged, non-elite runners.²³ An increased frequency of sudden cardiac death has been observed in middle-aged men during marathons since the year 2000.^{24,25} Atherosclerotic heart disease was the main cause of marathon-related sudden cardiac death in participants over the age of 40.²⁶ These studies clearly demonstrate an increase in marathon-related cardiac arrests in middle-aged men, while there is a declining rate of sudden cardiac deaths in the general population.²⁷ Siegel et al. previously studied inflammatory- and coagulation markers in pre- and post-race blood samples of middle-aged male participants of the Boston marathon.²⁸ Post-race samples showed neutrophilia and elevated interleukin-6 (IL-6) and C reactive protein (CRP), in combination with elevated fibrinogen, VWF, D-dimer and platelet activation, together indicating a pro-inflammatory and procoagulant state.²⁸ Protection of susceptible runners from atherosclerotic complications during

marathons may be feasible by low-dose aspirin before the race.²⁹ Although our findings indicate that endothelial activation appears more important for the procoagulant state during/following exercise than platelet activation (which is targeted by aspirin), aspirin also has anti-inflammatory effects, which may reduce endothelial activation as well. Thus, pre-race low-dose aspirin may provide antithrombotic benefit for “at-risk” runners during the race and for the 24 hours of high post-race cardiac risk.

Quantification of active VWF in thrombo-inflammatory conditions

The socio-economic burden of COPD and CKD

In **Chapters 6** and **7**, we studied active VWF (and other haemostatic parameters) in two thrombo-inflammatory conditions, namely COPD and CKD. Both are chronic, progressive conditions with a high prevalence: the Global Burden of Disease Study reported a prevalence of 251 million cases of COPD and 752.7 million people with impaired kidney function globally in 2016.^{30, 31} Not surprisingly, both are also associated with a tremendous economic burden. For COPD patients, the periodic deteriorations called exacerbations pose the highest financial burden due to supplementary treatment and increased hospital admissions.³² With regard to CKD, high-income countries typically spend more than 2–3% of their annual healthcare budget on the treatment of end-stage kidney disease.³³

Active VWF in COPD and CKD

Importantly, both conditions are independently associated with an increased risk of thrombotic cardiovascular events, which contribute significantly to the overall disease morbidity and mortality.^{34, 35} As endothelial activation in response to the inflammatory state in these conditions is known to contribute to the thrombotic risk, we assessed whether circulating active VWF levels were increased in patients with COPD (**Chapter 6**) and CKD (**Chapter 7**). This was indeed confirmed³⁶, and is likely a consequence of the increased release of VWF by the inflamed endothelium in combination with decreased ADAMTS13 activity, due to inhibition by inflammatory mediators and saturation with the excessive VWF antigen.³⁷ However, it is important to emphasize that in these multifactorial conditions active VWF is only one of the various haemostatic factors that are altered in response to the chronic inflammatory state, as discussed in **Chapter 10**.

Diagnostic value of active VWF

One important question with regard to valorisation is: is there added (diagnostic) value of measuring active VWF levels, and if so, in which conditions? In valorisation terms, this question pertains to the value proposition: which problem does active VWF quantification solve? Based on the discussion in **Chapter 10**, the conclusion would be that large, clinical outcome studies need to be performed first to answer the question on

the clinical usefulness of active VWF measurements. Current evidence only supports a possible role of active VWF measurements in the monitoring of TTP patients.^{15, 16, 38, 39} In most conditions, including mild bleeding disorders (**Chapter 8**) active VWF correlates well with established laboratory tests (VWF antigen, ristocetin cofactor activity)⁴⁰, but the additional diagnostic value is not clear at the moment.

Commercially available active VWF kit for research?

Despite the current absence of large clinical outcome studies, measuring active VWF levels has proven very valuable in unraveling the pathophysiological mechanisms of haemostatic complications in a variety of conditions.⁴¹⁻⁴⁵ Currently, the value of active VWF quantification thus seems to lie in the research field. Thus, possible "customers" would be research groups studying conditions associated with increased thrombotic risk or bleeding tendency due to thrombocytopenia. It is therefore important to make our assay (**Chapter 3**) widely available to other researchers, for instance in the form of a "kit" with pre-coated ELISA plates and all required buffers and antibodies. Previous experiments (unpublished data) have shown that coating of the VHH in a buffer containing trehalose to (vacuum sealed) ELISA plates provided good stability for at least 1 month when stored at 4°C. Further validation studies of these active VWF kits are required, but may ultimately lead to a commercially available kit.

Active VWF in clinical laboratories?

If large clinical outcome studies would prove additional diagnostic value for active VWF quantification, implementation in the clinical chemistry laboratory requires significant modifications to the current manual ELISA assay. In this form, the assay is labour-intensive and time-consuming. Thus, a high-throughput assay that can be run on existing automated laboratory analysers should be developed to make the test "hospital laboratory-friendly". Regarding market potential, possible partners for co-development would be large companies specialized in the development of laboratory assays and analysers in the field of haemostasis. Besides the automation of the assay, an important aspect to consider is the comparability of results between laboratories.⁴⁶ The active VWF assay uses normal pooled plasma as a reference to which sample results are normalized, which may result in between-laboratory bias, as discussed in **Chapters 3 and 10**. Thus, it is important that the potential manufacturer of the assay develops a (lyophilized) reference plasma for normalization of assay results.⁴⁷ This allows for comparison of results between laboratories and hence patient populations.

CONCLUSION

Most of the research presented in this thesis should be regarded as explorative work, with the aim to generate hypotheses for future, more large-scale studies. Although there is a long road ahead to fully explore the therapeutic and diagnostic opportunities of the S-VWFA1 and active VWF assay, our findings are the first steps in that direction, and may hence guide research that will have a socio-economic impact in the future.

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