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Chapter 8

General Discussion
Abstract

This thesis reports on studies that have investigated the role of several factors on the risk of arterial thrombosis. The thesis is organised in two sections. The first section (Chapters 2-5) deals with risk factors for the first arterial thrombotic event whereas the second section (Chapters 6 and 7) concerns risk factors for recurrence. The investigations touch several aspects of epidemiology, and make use of several observational study designs, encompassing case control, cohort and meta-analysis. Several statistical models were also applied ranging from logistic regression models, Cox proportional hazards time to event regression models as well as non-parametric statistics. Because of the heterogeneous nature, each investigation had specific problems in study design, data collection and analyses. A common line, however, is the haemostatic balance and its markers.

The studies are here summarised, and details of the methodology along with the possible implications in understanding the pathophysiology of arterial thrombosis are discussed. Additionally, a comparison between myocardial infarction and ischaemic stroke as well as hypothetic links between arterial and venous thrombosis are taken into consideration.
Summary and discussion about aetiology of arterial thrombosis

Anyone intuitively senses that explaining and predicting are different. Aetiological research aims to assess the presence or absence of a presumed causal relationship between a putative risk factor and a specific clinical condition. In this particular perspective, confounding and knowledge of the pathophysiology of the disease play a fundamental role.

Arterial thrombosis is a multicausal disease, in which a single complete causal mechanism (also called sufficient cause by Kenneth Rothman) requires the joint action of many component factors (component causes). A set of component causes occurring together may complete the causal mechanism, creating a sufficient cause and thus initiating the disease process.

Based on this model, the starting point to understand the aetiology of a disease is finding risk factors (component causes) for that particular disease. However, to find an association as a component cause of a disease, it is fundamental that we establish whether the association between this factor and the outcome is causal. In 1964, Hill proposed some criteria, which in turn were anticipated by the inductive canons of John Stuart Mill, that subsequently have widely been adopted to evaluate whether an association might be interpreted as causal. Those are (1) strength, (2) consistency, (3) specificity, (4) temporality, (5) biological gradient, (6) plausibility, (7) coherence, (8) experimental evidence, and (9) analogy. Rothman contends that Hill’s criteria fail to clearly distinguish causal from non-causal relations. The problem of causality can rarely, if ever, be resolved only on the basis of these criteria considered in an isolated manner, and establishing the nature of a given relationship (causal vs non causal) is a multidimensional process, demanding detailed knowledge on pathophysiological pathways. In summary, the problem of causality in biomedical research usually demands application of various kind of studies, that is experimental and observational studies as well. Nevertheless, a well performed epidemiological study can provide the starting point to assess the potential causal role of putative risk factors in human diseases.
Risk factors for new arterial thrombotic events

The first part of the thesis deals with risk factors for a first arterial thrombotic event and faces the problems related to aetiological research.

Chapter 2 reports a study on the association between low levels of ADAMTS13 and the risk of a first myocardial infarction. The design is that of a case-control study based on individual patient data meta-analysis. The large sample size achieved allowed to investigate extreme levels of ADAMTS13, showing that only very low levels of ADAMTS13 are associated with an increased risk of myocardial infarction. This meta-analytical approach based on individual participant data has many potential advantages over meta-analysis of aggregate data, both statistically and clinically, that have been extensively described in Chapter 2.\textsuperscript{6,7} In order to establish if the observed association is causal, a further discussion is necessary. Firstly, because all included studies had a case-control design, we faced some limitations. In a case-control design blood samples can only be collected after the event, making it difficult to establish the temporality between the presumed cause and the effect.\textsuperscript{8} Secondly, publication bias, residual confounding, recall bias and survivor bias all play a role in the over- or underestimation of the causal effects of interest, even though particular attention has been given to the selection of the studies included in the meta-analysis and to the inclusion of possible sources of confounding in the statistical models. Therefore, even with a well-designed epidemiological study, how can it be said that ADAMTS13 is part of the causal mechanism of myocardial infarction? The previous knowledge of the disease pathophysiology could help. The hypothesis that ADAMTS13 is implicated in the pathophysiology of myocardial infarction is related to its regulatory function on von Willebrand Factor (VWF), which previously has been strongly associated with arterial thrombosis.\textsuperscript{9,10} Therefore, there is a strong biological plausibility that ADAMTS13 is involved in the pathophysiology of myocardial infarction. Furthermore, there is experimental evidence that supports a causal relationship between ADAMTS13 and myocardial infarction. ADAMTS13 knockout mice developed larger myocardial infarctions after coronary occlusion and showed decreased left ventricular function when compared with wild-type mice. Also, treatment with recombinant ADAMTS13 (rADAMTS13) reduced infarct size in wild-type mice.\textsuperscript{11-13} Finally, similar results were found in the Rotterdam prospective cohort, so our findings meet the criteria of consistency.\textsuperscript{14} All these considerations support a causal
relationship in the association between ADAMTS13 and myocardial infarction. Thanks to the IPD design, we also could perform additional analyses, i.e., of mediation and interaction between ADAMTS13 and VWF, in order to better elucidate the causal role of ADAMTS13 in myocardial infarction. We found that the effect of ADAMTS13 on the risk of myocardial infarction is not mediated by VWF, as might be expected. Therefore this study suggests that ADAMTS13 and VWF are both risk factors with different pathways, as also was shown in other studies.\textsuperscript{15,16} These observations can support the following sufficient cause model: even though ADAMTS13 activity below the lowest bound of the normal range (i.e. ~50\%) is high enough for cleavage of plasma VWF (as it has been shown in ADAMTS13 heterozygous individuals), locally, at sites of endothelial injury (component cause), the reduced ADAMTS13 activity may lead to a reduced cleavage of secreted large VWF multimers, and thereby contribute to local thrombus formation.

Chapter 3 reports a study on the association between pregnancy loss and both forms of arterial thrombosis in the frame of the original RATIO case-control study. This chapter is based on a case-control design. In this study, the concept of causation is more controversial. Indeed, pregnancy loss has a link with several comorbidities, including risk factors for arterial thrombosis (such as diabetes, hypertension, hyperlipidaemia, obesity and tobacco use).\textsuperscript{17} Moreover, pregnancy loss is also associated with hypercoagulability, and especially with the presence of antiphospholipid antibodies, a well-known prothrombotic condition.\textsuperscript{17-19} The aim of this chapter was to provide an improved understanding of the link between pregnancy loss and arterial thrombosis. Pregnancy loss itself, however, cannot be viewed as a cause of arterial thrombosis, but rather a proxy of a series of component causes. Being a composite measure, it violates the consistency assumption and causal inference on the exact aetiologic mechanism cannot be made. Indeed, even after adjustment for several variables (i.e., cardiovascular risk factors, cardiovascular family history and the presence of antiphospholipid antibodies) it is likely that residual confounding plays a role in the direction and strength of the association. We found that recurrent miscarriages and stillbirth were associated with ischaemic stroke whereas the risk of myocardial infarction was only marginally affected. When myocardial infarction and ischaemic stroke are two similar diseases with the same aetiology, it is likely that these markers would have similar associations. Therefore, even though no formal causal assertion can be made, these data at
least suggest that myocardial infarction and ischaemic stroke have some differential aetiologic mechanisms. This concept is further investigated in the following chapters.

The coagulation balance is the main topic of the central part of the thesis. Chapter 4 and Chapter 5 deal both with the same question: does hypercoagulability play a similar role on the risk of the two main forms of arterial thrombosis, myocardial infarction and ischaemic stroke? The term hypercoagulability stands for an increased prothrombotic tendency, caused either by an elevated procoagulant activity or by a decreased anticoagulant activity. In these two chapters the aetiological question is crucial, but rather than referring to a single marker, it refers to the whole spectre of hypercoagulability. In Chapter 4 the question is investigated in the frame of the RATIO case-control study. The role of almost 30 markers of hypercoagulability on the risk of myocardial infarction is directly compared with their role on the risk of ischaemic stroke by using the relative odds ratio. We found that prothrombotic factors increased the risk of ischaemic stroke more than what they did for the risk of myocardial infarction, suggesting a different role of hypercoagulability in the aetiology of these two diseases, at least in young women. In Chapter 5 the same question is addressed in a meta-analysis on all studies available in the literature that investigated markers of hypercoagulability as a risk factor for myocardial infarction and ischaemic stroke. By applying a strict methodology, in order to obtain unbiased comparisons, we were able to collect data from 351 markers of hypercoagulability, derived from 31 study populations. The data analysis was similar to that in Chapter 4, with the calculation of the relative risk ratio, but now the picture of the coagulation system was much more complete. Results from this meta-analysis support the hypothesis that hypercoagulability plays a greater role on the risk of ischaemic stroke than on the risk of myocardial infarction, which was particularly true in the young populations. When we talk about causation in the comparison between two diseases, there are some major sources of bias that deserve to be discussed. Firstly, the presence of different study designs and statistical analyses should be taken into account. Differences in study design, data acquisition, data analyses and the underlying research questions in the separate studies can hamper the comparability between the two diseases. One strategy to overcome this problem is to include only studies that investigated both endpoints, and do within study comparisons. In this way data acquisition, control group recruitment, composition and data analysis are identical, minimising problems with comparability. We adopted this method in both chapters, restricting the analyses to the RATIO study in Chapter
4 and to studies in which both diseases had been investigated in Chapter 5. This had as the result that study design, control group composition, questionnaires and sample measurement, analyses and research questions were similar for the two relative risks. The second problem that had to be taken into consideration is that a different relative risk might represent differences in background absolute risks of diseases. Similar absolute risk differences between the two diseases for the marker of interests can lead to higher relative risk in the disease with the lower occurrence. However, myocardial infarction and ischaemic stroke have approximately the same incidence in the general population. Nevertheless, this is not the case in specific subgroups, for example, in patients with atrial fibrillation. This problem does not occur in Chapter 4, since patients with atrial fibrillation were not included, but the prevalence of patients with atrial fibrillation in the population investigated in Chapter 5 might have influenced the results. Nevertheless, we show that the hypothesis that the two diseases have different aetiological mechanisms is consistent among different populations and under various circumstances.

**Risk factors for recurrent arterial thrombotic events**

The second part of this thesis dealt with the risk of subsequent events and to some extent with prognosis. The aim was to give unbiased estimates of the probability of vascular recurrence and mortality after an arterial thrombotic event and to explore risk factors associated with the recurrent event. In order to address these two scopes, designs and approaches used have changed compared with the previous chapters. Chapter 6 and Chapter 7 were both based on a cohort study design, which has several advantages compared with case-controls studies, but also some drawbacks. The main advantage is that the cohort design allows to calculate absolute risks of multiple outcomes, at variance with case-control studies in which only relative risks (odds ratios) can be calculated. However, especially for long-term follow-up, the results from cohort studies can be severely biased by the participants who were lost to follow-up. A substantial number of subjects lost to follow-up can raise serious doubts about the validity of a study. The LiLAC cohort and the RATIO follow-up cohort did not suffer from this since in both studies we were able to follow almost all patients till the end of the study. Another issue regarding long-term follow-up is the presence of competing events. A patient may experience an event other than the one of interest, which alters the probability of
experiencing the event of interest. In the presence of many competing risk events, the Kaplan-Meier estimation procedure may not be directly applicable because a patient experiencing a competing risk event has to be censored in an informative manner (the censoring in the Kaplan-Meier is uninformative by definition).\textsuperscript{23-25} To overcome this problem in chapter 6 we applied a nonparametric estimation of cumulative incidence of the event of interest by taking the informative nature of censoring due to competing risks into account.\textsuperscript{24}

\textbf{Chapter 6} investigated the role of concomitant headache on vascular recurrences and mortality in minor stroke and transient ischaemic attack (TIA) of non-cardioembolic origin. The study was conducted in the setting of the LiLAC cohort study. We found that patients who experienced concomitant headache at the time of a minor stroke or TIA had a lower risk of long-term vascular death. Moreover, they had a reduced vascular recurrence compared with patients without concomitant headache, even when several potential predictors were taken into account. In order to give unbiased estimations of cumulative incidence of vascular recurrences, we accounted for competing risk events (death) by a two-step process.\textsuperscript{24,26} In the first step, we calculated the Kaplan-Meier estimate of the overall survival for any event. Note that both the events of interest as well as the competing risk events are considered ‘events’. In the second step, the conditional probabilities of experiencing the event of interest were calculated. The cumulative incidences were estimated with the obtained non-parametric cumulative incidence function.\textsuperscript{23} Therefore, the internal validity of the study was not influenced by this potential bias. However, the prognostic value of headache on the risk of recurrence and death is not strong enough to have clinical relevance, and this study does not suggest it to be a marker with clinical prognostic value. Nevertheless, because the association with the outcome persisted after taking into account several other potential confounders, the findings of this study might be used to provide insight into causality of stroke, suggesting that patients presenting with headache may have an aetiologically different type of stroke than patients with other stroke presentations. This hypothesis has been recently supported by other evidence.\textsuperscript{27,28} Therefore, despite the absence of a formal causal question, this study also touches on the aetiological aspect of ischaemic stroke.

\textbf{Chapter 7} describes a study on mortality rates after ischaemic stroke and myocardial infarction. Risk factors associated with vascular recurrences were also investigated, including the role of hypercoagulability. The analyses are based on the follow-up of the RATIO study.
We compared patients with myocardial infarction, ischaemic stroke, and subjects without arterial thrombosis for long-term mortality and vascular recurrences. This study showed that women who suffered from a major arterial thrombotic event had a high risk of death and vascular recurrences for decades after the first event. Additionally, we found that vascular recurrences are true to type (i.e., the recurrence rate for cerebrovascular events is higher in patients with ischaemic stroke whereas the rate of cardiac events is higher in patients presenting with myocardial infarction). The rates of death and recurrences were likely to be underestimated. To be able to be included in the RATIO case-control study, patients must have survived for a specific period of time (median 1.5 years) after the acute event (myocardial infarction or ischaemic stroke). This inevitably led to an underestimation of the true incidence rates, and it affected the external validity of our results. However, it is unlikely that it influenced the comparison between myocardial infarction and ischaemic stroke, because it is reasonable to assume that it acts equally on the two groups of patients. Therefore, the direct comparison between myocardial infarction and ischaemic stroke on the role of hypercoagulability on vascular recurrences is valid.

After the description of hypercoagulability in Chapters 4 and 5, we built a coagulation score in Chapter 7 in order to reflect a personal prothrombotic propensity. Despite the low statistical power of our analysis related to the relatively low number of outcomes, we found that high levels of the score (reflecting a prothrombotic state) increased the risk of vascular recurrences in ischaemic stroke patients but not in myocardial infarction patients. This finding may be interpreted together with our findings in the previous chapters: hypercoagulability plays a greater role in ischaemic stroke than in myocardial infarction, on both the risk of the first and recurrent events.
Comparison between ischaemic stroke and myocardial infarction and links with venous thrombosis

In this section, we discuss the differences between myocardial infarction and ischaemic stroke in more detail, and compare these diseases with other forms of thrombosis. Myocardial infarction and ischaemic stroke were contrasted in almost all chapters of this thesis. Strokes with evident sources of cardiac emboli were excluded, when possible, from this comparison. In Chapter 2, in which a direct comparison was not possible, we looked at other studies in the literature that reported on the association between ADAMTS13 and ischaemic stroke. This indirect comparison suggests that ADAMTS13 is a stronger risk factor for ischaemic stroke than for myocardial infarction. In Chapter 3 the comparison is made with the same study population (the RATIO case-control) and we found that pregnancy loss increases the risk of ischaemic stroke whereas that of myocardial infarction was affected only marginally. As already discussed above, pregnancy loss here is a proxy of several causes, one of which is very likely a prothrombotic state. Chapters 4 and 5 focus both on the direct comparison between myocardial infarction and ischaemic stroke, and they conclude that there is an imbalance in the role of hypercoagulability in the two main forms of arterial thrombosis. Finally, in Chapter 7, the comparison between the two diseases focuses on recurrences, and we found that hypercoagulability has a prognostic value in predicting recurrences after stroke but not after myocardial infarction. The conclusions of all those investigations support the hypothesis that hypercoagulability plays a greater role in the pathogenesis of ischaemic stroke than on that of myocardial infarction.

Several mechanisms can explain this observation. Arterial thrombosis most frequently occurs after the rupture or erosion of an unstable atherosclerotic plaque that exposes thrombogenic elements to blood, such as collagen, von Willebrand factor, fibrinogen, fibronectin and laminin. This results in platelet adhesion, activation and aggregation under the conditions of the rapid blood flow of arteries. Activated platelets can also provide negatively-charged surfaces that harbour coagulation factors and markedly potentiate cell-based thrombin generation and blood coagulation. Atherosclerosis and arterial thrombosis have traditionally been considered two distinct processes. However, it is becoming increasingly clear that the cellular and biochemical interactions underlying thrombosis are also directly relevant to atherosclerosis. Indeed, the coagulation reaction linked to fibrin generation may
contribute to the rapid progression of atherosclerotic plaque, creating an insidious cycle, which eventually leads to the catastrophic ischaemic event. Therefore, the first explanation for the different role of hypercoagulability on myocardial infarction and ischaemic stroke can be found in differences between the vessel walls and flow patterns of the coronary arteries, the carotids, intracranial arteries and the cerebral microvasculature. These differences can influence the way that asymptomatic thrombi are formed and their promotion of the rapid progression of atherosclerotic lesions. Another consideration is the effect of flow deceleration on the blood clotting process. Once a critical stenosis has developed as a result of the atherothrombotic process and vasoconstriction, blood flow recirculation and stagnation downstream from the site of plaque injury becomes more prominent. Blood coagulation is favoured under such conditions, leading to the propagation of a fibrin-rich and red-cell-rich thrombus (the fibrin tail). Also, in this case the different diameters and muscular components of the vessel walls might be implicated in the differences between the two diseases.

Ischaemic stroke has several aetiologies, in which it differs from myocardial infarction where the cause is almost invariably the atherosclerotic plaque. According to the most used classification, the TOAST, stroke can be classified into 5 categories, each with its own causes and consequences: cardioembolism, large-artery atherosclerosis, small-vessel occlusion, stroke of other determined aetiology and stroke of undetermined aetiology. Risk-factor profiles differ across ischaemic stroke subtypes, including the most debated category of ‘stroke of undetermined cause’ (cryptogenic). Cryptogenic stroke represents one third of all transient ischemic attacks (TIA) or ischaemic strokes, and half of the ischaemic strokes in the young. It has been proposed that cryptogenic events might often be caused by occult arterial sources of thromboembolism, paroxysmal atrial fibrillation (AF), patent foramen ovale (PFO), or cardiac structural abnormalities. However, even with a detailed investigation, only a minority of patients with cryptogenic stroke have been found to have potentially unstable plaques in arterial vessels, some of which are probably coincidental. Similarly, although long-term monitoring of heart rhythm identifies paroxysmal AF in up to a third of patients with cryptogenic events, the relevance of these mostly short episodes of AF for cryptogenic stroke is uncertain. Unfortunately, we were not able to investigate differences between subtypes of ischaemic stroke in the projects described in this thesis, since the necessary data were not available neither in the RATIO population, nor in the available
literature included in the systematic review. However, the concept that cryptogenic events have the fewest atherosclerotic markers and no excess of cardioembolic markers made it the most interesting candidate for the observed difference in hypercoagulability between myocardial infarction and ischaemic stroke. If there is such a link, it might have important implications in the management, prophylaxis and treatment of those patients and therefore, it deserves to be elucidated in further studies.

Moreover, there is another interesting interpretation of these findings. The classical paradigm of the pathophysiology of thrombus formation began with the pathologist Virchow, who in the mid-1800s postulated three major causes of thrombosis: changes in the vessel wall, changes in the blood flow, and changes in the blood composition. This broad classification is still valid. However, we are used to consider that changes in blood flow and blood composition are mainly valid for venous thrombosis, whereas changes in vessel wall (atherosclerosis) are the cause of arterial thrombosis. Partly because of the obvious anatomical differences, as well as their distinct clinical presentations, arterial thrombosis and venous thrombosis were traditionally considered separate diseases, with different pathophysiological mechanisms. In this thesis, risk factors such as pregnancy loss and markers of hypercoagulability were investigated. However, the main risk factors for arterial thrombosis include hypertension, hyperlipidaemia, smoking, and diabetes mellitus, and those are still the most important. In the last decade, there has been an increasing awareness about the association between venous and arterial thrombosis. However, the nature of this association is unclear. Several attempts have been made to find links between arterial risk factors and venous thrombosis, with diverging results. According to the findings of a recent meta-analysis, except for cigarette smoking, traditional arterial cardiovascular risk factors are not associated with an increased risk of venous thrombosis. Atherosclerosis itself might have the potential to promote the development of thrombotic disorders in the venous system and the two clinical conditions might be simultaneously triggered by the activation of coagulation, in both the arterial and the venous system. Because the effect of hypercoagulability in arterial thrombosis is usually much less pronounced than in venous thrombosis, the results of this thesis lead to the consideration that ischaemic stroke shares similarities with venous thrombosis, at least for the major role of hypercoagulability. Patients with venous thrombosis have been found to have an excess rate of arterial thrombosis compared with the general population (and to lesser extent patients with arterial thrombosis.
seem to have more venous thrombotic complications).\textsuperscript{61-66} Studies that have investigated arterial events after venous thrombosis reported that rates of ischaemic stroke are higher than those of myocardial infarction.\textsuperscript{64} This finding was also present in a meta-analysis with long term follow-up, especially for unprovoked venous thrombotic events.\textsuperscript{63} These are intriguing findings that together with our observations may stimulate new studies to explore the hypothesis whether the excess in ischaemic stroke events after venous thrombosis might be explained by hypercoagulability.

If the relationship between venous thrombosis and ischaemic stroke is stronger than that with myocardial infarction, there may also be clinical implications. In the era of the direct oral anticoagulants (DOACs), such as dabigatran, which inhibits thrombin, and rivaroxaban, apixaban, and edoxaban, which inhibit factor Xa, the primary and secondary prophylaxis of ischaemic stroke might need to be more similar to that of venous thrombosis than of myocardial infarction.\textsuperscript{67-70} It has been suggested that DOACs are safer than vitamin K antagonists but as efficacious for prevention of venous thrombosis, as well as for stroke in atrial fibrillation.\textsuperscript{71} Our findings suggest that they might be also helpful in other stroke categories, for instance for the secondary prevention of stroke of undetermined origin. A few trials testing this hypothesis in patients with stroke of undetermined cause are already ongoing.\textsuperscript{72} It remains the challenge to identify those individuals at high risk for arterial thrombosis and implement safe and effective antithrombotic strategies that can prevent thrombotic vascular occlusion both for the first event and for recurrences. This thesis overturns the one-size-fits-all approach in arterial thrombosis and suggests to tailor antithrombotic therapies.


7. Stewart LA, Tierney JF. To IPD or not to IPD? Advantages and disadvantages of systematic reviews using individual patient data. Eval Health Prof. 2002;25(1):76-97.


42. Hanson E, Jood K, Karlsson S, Nilsson S, Blomstrand C, Jern C. Plasma


55. Pomp ER, Rosendaal FR, Doggen CJ. Smoking increases the risk of


