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Chapter 1

General Introduction
Chapter 1

Abstract

This thesis reports on risk factors involved in the aetiology and prognosis of the two main forms of arterial thrombosis, namely myocardial infarction and ischaemic stroke. The majority of risk factors under study are related to coagulation, and particularly to its pro-coagulant activity. There is also an underlying question in most of the chapters, which is whether the risk factors being investigated act differentially in myocardial infarction and ischaemic stroke. Finally, there is a discussion about the implications and interpretation of the findings from an aetiological point of view, and for the development of future research. As an introduction to this thesis, this chapter provides background information on myocardial infarction and ischaemic stroke. Moreover, it describes the study populations used to investigate the hypotheses along with an overview of the subsequent chapters.
Introduction

Myocardial infarction

Acute myocardial infarction remains a leading cause of morbidity and mortality worldwide.\(^1\) Myocardial infarction occurs when myocardial ischaemia, a diminished blood supply to the heart, exceeds a critical threshold and overwhelms myocardial cellular repair mechanisms designed to maintain normal operating function and homeostasis. Ischaemia at this critical level for an extended period results in irreversible myocardial cell damage and death. The myocardial ischaemia can be the result of an increased myocardial metabolic request, a decreased delivery of oxygen and nutrients to the myocardial tissue, or both. An interruption in blood flow occurs for example when a thrombus is superimposed over an ulcerated or unstable atherosclerotic plaque, resulting in coronary occlusion. A coronary artery stenosis caused by atherosclerosis or a dynamic stenosis associated with the vasospasm of the coronary artery can also limit the supply of oxygen and nutrients and precipitate a myocardial infarction.

Since 1998, the age-standardised incidence of myocardial infarction in the Netherlands decreased from 6.2 to 3.8 per 1000 person-year in 2007 in men and from 3.2 to 2.1 per 1000 person-year in 2007 in women.\(^2\) In absolute terms, the decline in myocardial infarction incidence was smaller in the young (35-54 years: \(-3.8\%\)) than in middle-aged individuals (55-84 years: \(-5.3\%\)). Similarly, the annual percentage change in incidence was larger in men than women.\(^2\) Incidences strongly differ per age category, and age is their strongest determinant. Myocardial infarction recurrence rates vary widely among studies and age populations, ranging at one year from 5.6% to 57% in autopsy-based population studies.\(^3,4\) Out-of-hospital mortality decreased from 24.3% in 1998 to 20.6% in 2007 in men and from 33.0% to 28.9% in women. Hospitalised case-fatality declined from 2003 onwards. Similar pictures were found in other European countries and in the U.S.A.\(^5-7\) In the latter, the annual incidences are slightly higher, with approximately 660 000 Americans having a new coronary attack each year.\(^8\) Coronary heart disease alone caused approximately 1 out of 7 deaths in the U.S.A. in 2013. Even though incidences in developed countries are decreasing, a substantial
burden of morbidity persists: the overall estimated prevalence of coronary heart disease in the Netherlands is 3 % in women and 5 % in men.\textsuperscript{9}

Seven primary risk factors for myocardial infarction have been identified so far, mostly associated with the development of atherosclerotic coronary artery disease: tobacco use, physical inactivity, obesity, family history of arterial disease, hyperlipidaemia, diabetes mellitus and hypertension.\textsuperscript{1}

\textbf{Ischaemic stroke}

A stroke is defined as an acute loss of neurological function due to an abnormal perfusion of brain tissue. The majority of strokes are ischaemic (87%), and commonly result from an arterial obstruction by a thrombus or embolus, with the occurrence of cerebral infarction. The other strokes are haemorrhagic, either into the parenchyma or into the subarachnoid space. A transient ischaemic attack (TIA) is a brief episode of neurological dysfunction, with clinical symptoms typically lasting less than one hour, without evidence of acute infarction.\textsuperscript{10}

Ischaemic stroke has several aetiologies and classifications, of which the most adopted is the TOAST.\textsuperscript{11,12} The TOAST classification has five categories: large-artery atherosclerosis, embolism, small-vessel disease, stroke of other determined aetiology, and stroke of undetermined aetiology. This classification reflects the mechanisms underlying the reduction in blood flow causing ischaemia. (i) Large-artery atherosclerosis accounts for a stenosis or occlusion of the major intra- and extra-cranial arteries due to the deposition of an atherosclerotic plaque, its rupture or a prolonged low-flow state due to relative hypotension. (ii) Embolism can be the consequence of thrombi resulting from turbulent or stagnant flow states in the heart. These thrombi can dislodge and occlude blood vessels in the intracranial circulation. The most common cause of cardioembolic stroke is atrial fibrillation, but emboli can also originate from cardiac valves or large arterial vessels. (iii) Changes in the arterial vasculature of small perforating arteries that result in the narrowing of the vessel lumen and the eventual occlusion may lead to lacunar infarcts. Chronic hypertension, as well as hyperlipidaemia, smoking, and diabetes are all causes of this kind of vessel damage. (iv) Vasculopathies, genetic disorders, and metabolic disorders are rare causes of other determined aetiologies. (v) Finally, in a significant number of cases (30-40%), no clear
explanation can be found for an ischaemic stroke event, despite an extensive diagnostic evaluation. These strokes are classified as strokes of undetermined aetiology, or cryptogenic strokes. The average age-adjusted incidence of ischaemic stroke in the Netherlands is 1.9 per 1000 person-years and as for myocardial infarction, the incidence rises with age.\textsuperscript{13} Overall, the incidence of ischaemic stroke is slightly decreasing. From 2003 to 2013, the stroke death rates declined among the elderly from 5.3 to 2.4 per 1000 person-years and among the young from 0.05 to 0.02 per 1000 person-years.\textsuperscript{1} Stroke recurrence is reported to be as high as 13\% at one year.\textsuperscript{14} Only about 10\% of all strokes occur in people aged 18 to 50 years-old. However, in this age category the burden of the disease is the highest considering the long life expectancy.

Major risk factors for ischaemic stroke are the same as for myocardial infarction, as well as atrial fibrillation and carotid stenosis.

**The RATIO case-control study**

The RATIO (Risk of Arterial Thrombosis In relation to Oral contraceptives) is a multicentre, population-based, case-control study performed in the Netherlands between 1995 and 1998, and it serves as the basis for the questions posed in Chapters 3 and 4.\textsuperscript{15,16} The coordinating centre is the department of Clinical Epidemiology at the Leiden University Medical Center. The RATIO study included young women (between 18 and 49 years old) with either myocardial infarction or ischaemic stroke. Exclusion criteria were TIA (defined as an event lasting <24 hours), haemorrhagic stroke, venous sinus thrombosis, carotid artery dissection, history of cardiovascular or cerebrovascular diseases, severe illness, aphasia, or cognitive impairment interfering with the questionnaire, and not speaking Dutch. The population-based group of control women was identified by random-digit dialing.\textsuperscript{17} In this method, telephone numbers are randomly produced and dialled at any time during the day at least 7 times or until a successful connection is made. Eligible as controls were women who were aged 18 to 49 years and who did not meet the exclusion criteria that were used for selecting patients. The control group has been frequency matched with cases for age, area of residence, and calendar year. Finally, in recruitment of control subjects, women in the older age groups were over-sampled to minimise the age difference between the patients and control women.\textsuperscript{16}
Therefore, adjustment for matching variables in the statistical models is always indicated. A structured and standardised questionnaire was used to collect information about classic cardiovascular risk factors, family history, oral contraceptive use as well as obstetrical history. The questions referred to the period before the index date (the date of myocardial infarction or ischaemic stroke for patients and mid-year of the same year for controls).

In total, 248 participants suffered from myocardial infarction, 203 from ischaemic stroke and 925 women served as healthy controls. All qualifying events occurred between 1990 and 1997 and cases were included from 1995 to 1998. In a subset of these women blood samples were collected for blood measurement of clotting factors and DNA analyses (205 participants with myocardial infarction, 125 with ischaemic stroke and 638 controls). Buccal swabs were collected if blood could not be sampled (13 participants with myocardial infarction, 15 with ischaemic stroke and 129 controls). To compensate for the loss of statistical power in the ischaemic stroke group, a further sample of 50 women who presented with an ischaemic stroke at the University Medical Center Utrecht were additionally recruited between 1996 and 2001. In total, blood samples were available from 205 cases with myocardial infarction, 175 cases with ischaemic stroke and 638 control subjects. Blood was collected after a median of 69 months (range 38 - 117) for myocardial infarction and 95 months for ischaemic stroke cases (range 23-146), thereby ensuring blood was sampled after the acute phase to minimise the risk of reverse causation. All participants gave informed consent and the study was approved by the ethics committees of the participating hospitals.

Because the RATIO study focused on patients with a young age of onset, it has several advantages for the purpose of this thesis. Firstly, it is highly suitable to study non age-related risk factors, such as coagulation markers. Since age-related risk factors are prevalent in ageing patient groups, they may mask the effects of non-age-related risk factors. Secondly, because myocardial infarction and ischaemic stroke are so prevalent in ageing populations, research into the causes and consequences of these diseases often target these age categories. The RATIO study helps to fill this gap in knowledge. Thirdly, studies into cardiovascular diseases are also disproportionally targeted at certain types of disease. The proportion of clinical research aimed at identifying new causes of myocardial infarction is larger than the studies that target ischaemic stroke, even when the difference in the incidence of these diseases is taken into account. The RATIO study offers the unique opportunity to
study both forms of arterial thrombosis, myocardial infarction and ischaemic stroke, and to compare them within the same population. This makes the comparison easier and more reliable than when it is done between different studies with different designs and populations. Finally, falls in cardiovascular incidence in recent years have been reported to be greater in men and in the elderly than in women and the young. Therefore, the focus of the RATIO on women and on young patients made it particularly interesting in order to try to address those disparities.

**The RATIO follow-up study**

The RATIO follow-up study is a cohort study, described in detail in Chapter 7. It aims to provide answers to the need of further knowledge of secondary prevention strategies in young patients with myocardial infarction and ischaemic stroke.\textsuperscript{22,23} Using data linkage between the original RATIO case-control study and existing national databases with follow-up data, provided by the Central Bureau of Statistics (CBS) in the Netherlands, the RATIO follow-up study was built. Not only does this study make it possible to determine the mortality and recurrence rate of myocardial infarction and ischaemic stroke, but also to investigate the coagulation risk factors related to prognosis and to compare these aspects between myocardial infarction and ischaemic stroke. The results from the RATIO follow-up project provide a direct insight of the consequences of cardiovascular diseases in young women.

Of the original study group, 226 women with myocardial infarction, 160 with ischaemic stroke, and 782 controls were linked to the national databases and included in the follow-up. With a previously reported recurrence rate and mortality in young patients with stroke of 0.5-3.6 per 100 person-year and 0.6-1.4 per 100 person-year respectively, and a follow up of 15 years, we expected a priori to find 15 to 84 recurrences of stroke and 17 to 38 deaths in the ischaemic stroke group.\textsuperscript{18,19,24} In patients with young onset myocardial infarction, reported rates of recurrence and mortality are 1.8-2.2 and 1.2-2.0 per 100 person-year, leading to 59-70 expected recurrences and 40-64 expected deaths in the myocardial infarction group.\textsuperscript{25}
The LiLAC study

The LiLAC (Life Long After Cerebral Ischaemia) is a cohort study that serves as the basis for the investigation presented in Chapter 6, in order to answer the question about the role of concomitant headache in predicting vascular recurrences and death after a TIA or a minor stroke.

The LiLAC is based on the Dutch TIA Trial (DTT), a double blind, randomised study for the comparison of two doses of aspirin (30mg or 283 mg) in preventing vascular events in patients who had had a TIA or a minor stroke between 1986 and 1989. For logistical reasons, in the LiLAC study only patients from the 24 hospitals that had enrolled at least 50 patients in the DTT (2473 of the original 3150) were included. In order to be enrolled in the DTT patients must have had a TIA (symptoms lasting for less than 24 h) or an ischaemic stroke (symptoms persisting for more than 24 h), but non-disabling (modified Rankin grade < 3), within the past three months. Patients with potential sources of embolism in the heart or conditions other than atherosclerosis that might have caused the cerebral ischaemia were excluded. In the Dutch TIA trial details of the history and the presence of vascular risk factors of each patient were recorded at baseline by a standardised questionnaire. The list also contained a number of detailed multiple-choice questions about the nature and time course of the symptoms. One question pertained to the presence and nature of headache. Follow-up in the DTT has been carried out until 1990. In the LiLAC study, follow-up was extended to the period between March, 2001, and December, 2003. Follow-up data were obtained from the clinicians involved in the DTT, the general practitioners and directly from patients, relatives or acquaintances. Twenty-six patients were completely lost to follow-up after close-out of the DTT. Seven patients were lost because they moved abroad and 19 for unknown reasons.

The LiLAC has several strengths that are useful for the purpose of this thesis. Firstly, it included a large sample of patients with a cerebrovascular accident, ensuring that patients with headache were a numerically sufficient group to allow enough statistical power. Secondly, the LiLAC is one of the longest follow-up studies available in the literature for patients with ischaemic stroke, thereby ensuring that outcomes of interest (vascular recurrences and death) were well represented. With a prevalence of headache at baseline of
17% among participants and an alpha error of 0.05, LiLAC has a power of 80% in detecting a relative risk of 0.86 or more extreme.
Outline of this thesis

In Chapter 2 we present an individual patient data (IPD) meta-analysis and investigate the relationship between ADAMTS13, the von Willebrand Factor cleaving protease, and myocardial infarction. The IPD design was preferred over a meta-analysis of aggregate data, since we realised that only a few studies were available in the literature and we had the possibility to collect a large population sample, a goal hardly achievable with a single centre study.

A standard case-control study analysis, based on the RATIO study, is presented in Chapter 3. It investigates the relationship between pregnancy loss and both forms of arterial thrombosis. In this chapter we built a comprehensive statistical model in order to deal with all possible confounders and mediators that can affect the relationship of interest. Chapters 4 and 5 share similarities. We used an unconventional approach in both chapters in order to compare the role of hypercoagulability in the aetiology of myocardial infarction and ischaemic stroke. In Chapter 4 we did this comparison within the frame of the RATIO case-control study, and we calculated relative odds ratios (ROR) for all the prothrombotic markers studied in the RATIO study to assess the difference in effects between the two diseases. We used a similar approach in Chapter 5, where the same question is investigated within the studies published in the literature, with the calculation of relative risk ratios (RRR). The direct comparison was maintained by selecting only those studies that have investigated the same marker in both diseases within the same study population. Chapter 6 addresses the relationship between concomitant headache and vascular recurrences after TIA or minor strokes. It is based on the LiLAC cohort study. In Chapter 7 the long term follow-up of the RATIO study is presented. Participants were followed until 2012. Results focus on mortality and on the relationship between hypercoagulability and fatal and non-fatal recurrences after myocardial infarction and ischaemic stroke. Finally, in Chapter 8 we report a summary of the major findings and a general discussion on causality and prediction. Moreover, the comparison between myocardial infarction and ischaemic stroke and possible links with venous thrombosis are discussed in more detail.


9. Leening MJ, Siregar S, Vaartjes I, Bots ML, Versteegh MI, van Geuns RJ,


