Aus der Klinik für Neurologie mit Experimenteller Neurologie
der Medizinischen Fakultät der Charité – Universitätsmedizin Berlin

DISSERTATION

High sensitivity C-reactive protein in the ischemic stroke cohort

Hochsensitives C-reaktives Protein in Schlaganfallkohorten

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List of Abbreviations

AF atrial fibrillation.

ARHGEF26 rho guanine nucleotide exchange factor 26.

AS ankylosing spondylitis.

ASIST arterial stiffness in lacunar stroke and TIA study.

BI barthel index.

BMI body mass index.

BP blood pressure.

CADASIL cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy.

CARe cerebrovascular Aosta Registry.

CE cardioembolic.

CHANCE Clopidogrel in high-risk patients with acute non-disabling cerebrovascular events.

CHD coronary heart disease.

CI confidence interval.

CNSS Canadian neurological stroke scale.

CRP C-reactive protein.

CTA computer tomography angiography.

CVE cardiovascular events.

CXCL12 C-X-C motif chemokine ligand 12.

DBP diastolic blood pressure.

DM diabetes mellitus.

DWI diffusion weighted imaging.

EDNRA endothelin receptor type A.

EPO erythropoietin.

FBG fasting blood glucose.

GWAS genome-wide association studies.

Hcy homocystein.

HDL high density lipoprotein.

HMBG1 high mobility group box 1 protein.

HR hazard ratio.

hs-CRP high-sensitivity C-reactive protein.

HTN hypertension.

IBD inflammatory bowel diseases.

ICAM-1 intercellular adhesion molecule-1.

ICH intracranial hemorrhage.

IL interleukin.

IL6-R interleukin-6 receptor.

IQR interquartile range.

IS ischemic stroke.

LAA large artery atherosclerosis.

LACI lacunar infarcts.

LDL low density lipoprotein.

Lp(a) lipoprotein(a).

Lp-PLA2 lipoprotein-associated phospholipase A2.

MCP-1 monocyte chemoattractant protein-1.

MELAS mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes.

MI myocardial infarction.

MMP matrix metalloproteinase.

MMSE Mini-Mental State Exam.

MRA magnetic resonance angiography.

mRS modified Rankin scale.

NIHSS National Institutes of Health Stroke Scale.

NOMAS northern Manhattan study.

NORTHSTAR North West of England transient ischaemic attack and minor stroke.

OxVasc Oxford vascular study.

PACI partial anterior circulation infarcts.

PAI-1 plasminogen activator inhibitor-1.

PECAM-1 platelet and endothelial cell adhesion molecule 1.

POCI posterior circulation infarcts.

PRDX1 peroxiredoxin-1.

PROGRESS perindopril protection against recurrent stroke study.

PROSCIS-B prospective cohort with incident stroke—Berlin study.

PsA psoriatic arthritis.

QUIPS quality in prognostic studies.

RA rheumatoid arthritis.

RAGE receptor for advanced glycation end products.

RVE robust variance estimation.

SAMMPRIS stenting vs. aggressive medical management for preventing recurrent stroke in intracranial stenosis.

SBP systolic blood pressure.

SH2B3 SH2B adaptor protein 3.

SLE systemic lupus erythematosus.

SVD small vessel disease.

SVEP1 Sushi, von Willebrand factor type A, EGF and pentraxin domain containing 1.

TACI total anterior circulation infarcts.

TC total cholesterol.

TCD transcranial doppler.

TIA transient ischemic attack.

TOAST trial of ORG 10172 in acute stroke treatment.

tPA tissue plasminogen activator.

UND undetermined.

VCAM-1 vascular cell adhesion molecule-1.

WBC white blood cell count.

WHO World Health Organization.

Abstract

Introduction: Patients with ischemic stroke have a high risk of recurrent stroke and other vascular events. Currently, an accurate prediction of vascular risk in these patients is made difficult by the lack of markers with high prognostic value. High-sensitivity C-reactive protein (hs-CRP) has shown promise as a prognostic marker in both primary and secondary prevention of cardiovascular disease. Furthermore, as colchicine is emerging as a promising agent in secondary prevention in coronary disease, the association between inflammation and cerebrovascular disease needs to be examined more closely before similar interventions are applied for secondary stroke prevention. We systematically reviewed the existing literature to determine whether hs-CRP was associated with long-term vascular risk in ischemic stroke and transient ischemic attacks(TIA). Additionally, we conducted our own cohort study in Berlin to add to the available data.

Methods: The EMBASE and Medline databases were searched on 21 February 2021 for case-control or cohort studies composed of ischemic stroke or TIA patients that measured hs-CRP levels at baseline, and recorded vascular events or death during ≥ 12 months of follow-up. For the cohort study, first-ever ischemic stroke patients were consecutively enrolled at the three Charité university hospitals and followed up for 3 years. Hs-CRP was measured at admission. Recorded vascular endpoints included myocardial infarcts, recurrent ischemic strokes, and all-cause death.

Results: 27 studies (15752 patients) were included in the meta-analysis. In the cohort study, 533 ischemic stroke patients were included. When the highest quartile was compared against the lowest, hs-CRP was associated with vascular events during follow-up (adjusted HR: 2.04, CI:1.01-4.14).

Conclusion: There is evidence of an association between higher hs-CRP levels and increased vascular risk in patients with ischemic stroke or TIA, similar to the association seen in cardiovascular disease. This study adds to the evidence supporting the role of inflammation in the progression of cerebrovascular disease.

Zusammenfassung

Einleitung Patient(inn)en mit ischämischem Schlaganfall haben ein hohes Risiko für weitere Schlaganfälle und andere vaskuläre Ereignisse. Derzeit wird eine genaue Einschätzung des Rezidivrisikos bei diesen Patient(inn)en durch das Fehlen von Markern mit hohem prognostischen Wert erschwert. High-sensitivity C-reaktives Protein (hs-CRP) hat sich als prognostischer Marker sowohl bei der primären als auch bei der sekundären Prävention von Herz-Kreislauf-Erkrankungen als vielversprechend erwiesen. In dieser Studie wurde die vorhandene Literatur systematisch überprüft, um festzustellen, ob hs-CRP mit einem langfristigen vaskulären Risiko bei ischämischem Schlaganfall- und TIA-Patient(inn)en assoziiert ist. Zusätzlich haben wir in Berlin eine eigene Kohortenstudie durchgeführt, um die bereits verfügbaren Daten zu diesem Thema zu ergänzen.

Methoden EMBASE- und Medline-Datenbanken wurden am 21. Februar 2021 nach Fallkontroll- oder Kohortenstudien durchsucht, die sich aus Patient(inn)en mit ischämischem Schlaganfall zusammensetzten, die den hs-CRP-Spiegel zu Studienbeginn gemessen haben und vaskuläre Ereignisse oder Todesfälle während einer Nachbeobachtungszeit von ≥ 12 Monaten aufgezeichnet haben. Für die Kohortenstudie wurden Patient(inn)en mit ischämischem Schlaganfall an unseren drei Standorten eingeschlossen, und 3 Jahre lang nachverfolgt. Der hs-CRP-Spiegel wurde bei Aufnahme gemessen. Zu den aufgezeichneten vaskulären Endpunkten gehörten Myokardinfarkte, wiederkehrende ischämische Schlaganfälle und Tod.

Ergebnisse In die Meta-Analyse wurden 27 Studien mit 15752 Patient(inn)en eingeschlossen. In die Kohortenstudie wurden 533 Patient(inn)en mit ischämischem Schlaganfall eingeschlossen. Wenn das höchste hs-CRP-Quartil mit dem niedrigsten verglichen wurde, war hs-CRP mit vaskulären Ereignissen während des Follow-Ups assoziiert (adjustierte HR 4. vs. 1. Quartil: 2,06, CI: 1,01-4,14).

Schlussfolgerung Es gibt Hinweise für einen Zusammenhang zwischen höheren hs-CRP-Spiegeln und einem erhöhten vaskulären Rezidivrisiko bei Patient(inn)en mit ischämischem Schlaganfall oder TIA, ähnlich der Assoziation bei Koronarerkrankungen. Diese Studie ergänzt die Evidenz, die die Rolle von Entzündungen beim Fortschreiten von zerebrovaskulären Erkrankungen unterstützt.

0 Introduction

In patients with an ischemic stroke, once the acute phase has passed, the focus is shifted towards optimizing care to prevent further cerebrovascular events. Clinicians refer to guidelines based on large cohort studies and randomized controlled trials when deciding on the best medication regimen or whether to refer to vascular surgery or neuroradiology. Recent figures show that significant improvements have been made in the secondary prevention of stroke using this approach, with an estimated decrease per decade of about 1% in the annual recurrent stroke rate from 1960 to 2009 16, and the current rate estimated to be at about 4.98% per year.

Many of the risk factors for stroke have parallels in the secondary prevention of coronary heart disease. High blood pressure, high blood cholesterol levels, smoking, obesity, metabolic syndrome and diabetes mellitus each play important roles in the development of both coronary heart disease and cerebrovascular disease. Current approaches for secondary prevention of atherosclerotic stroke focus on platelet inhibition, reduction of cholesterol levels, control of hypertension, and in the case of symptomatic extracranial carotid stenosis, thrombendarteriectomy or stenting. In special populations, efforts are made to identify and address causes independent of general vascular risk factors, as several distinct mechanisms specific to cerebrovascular disease lead to a significant portion of ischemic strokes. These include dissections of cervical arteries, atrial fibrillation, vasculitis, the presence of right-left shunt, genetic conditions such as cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) and Fabry disease, among others. Targeted methods of secondary prevention, e.g., anticoagulation for atrial fibrillation, exist for some of these etiologies.

Though it is widely believed that inflammation plays a key role in the pathophysiology of

atherosclerosis, an active suppression or modulation of inflammatory state is currently not recommended in the secondary prevention of stroke. Recently, several randomized controlled trials investigated the effectiveness of anti-inflammatory therapies (canakinumab 37, colchicine 53, methotrexate 39) in preventing recurrent vascular events, including ischemic strokes, in patients with ischemic heart disease, and found mixed results. Interestingly, a low dose of colchicine (0.5 mg once daily) led to an additional decrease in the rate of stroke (HR 0.26, 95% CI 0.1-0.7) in a group of patients with recent myocardial infarction, and almost all of whom were already taking statins, aspirin, and another antiplatelet agent 53. Targeting inflammation, in addition to traditional secondary prevention strategies, could potentially lead to added vascular risk reduction in a population with advanced atherosclerotic disease, and may benefit ischemic stroke patients as well.

0.1 Atherosclerosis in inflammatory disease

The effect of inflammation on atherosclerotic disease is more pronounced in patients with chronic inflammatory disease, who tend to be younger and free of classic vascular risk factors. There is ample epidemiological evidence suggesting that chronic inflammatory diseases such as rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE) confer additional vascular risk, independent of traditional cardiovascular risk factors 41. A meta-analysis showed a comparable excess risk in cerebrovascular events among patients with RA, SLE, ankylosing spondylitis (AS), gout and psoriasis 58. This association is also seen in patients with inflammatory bowel diseases (IBD) 1,31. One explanation proposed for this phenomenon is that the chronic inflammatory state induced by those diseases leads to an accelerated atherosclerosis.

Evidence suggests that not only do the patients with rheumatic diseases have heightened cardiovascular risk, but they also have excess cardiovascular mortality, as the chronic inflammatory state promotes the build-up of unstable plaques with thin fibrous caps that are prone to rupture and tend to cause more severe vascular events 13. In a cross-sectional study where patients with rheumatoid arthritis with different levels of disease activity and controls underwent carotid ultrasounds, patients with more active RA had more unstable carotid plaques than controls or those in remission 48.

The phenomenon of accelerated atherosclerosis is more obvious in younger patients with rheumatic diseases. In the Hopkins Lupus Cohort, the observed number of cardiovascular events was especially high in patients aged 18 to 39, with a relative risk, when compared to the expected number of CVE according to the Framingham risk formula, of 5.28 (95% CI 3.36-7.21) 25.

0.2 Atherosclerosis and inflammatory cells

A possible mechanism of how inflammation affects atherosclerosis can be inferred from the study of immune cells. *In vivo* experiments, and indirectly, observational studies, have shown that atherosclerosis, cholesterol levels, and the metabolic syndrome are intricately interconnected with the proliferation and differentiation of monocytes, macrophages and lymphocytes [29, 50, 11]. Patients with myeloproliferative neoplasms, that are often accompanied by leucocytosis, are at higher risk for vascular thrombosis, while this heightened risk was reduced in patients treated with hydroxyurea [2]. In both humans and mouse models, monocytosis of the classic monocyte subpopulation was associated with increased atherosclerotic lesion burden [29]. Interestingly, hypercholesterolemia itself led to monocytosis in mice, those monocytes in turn differentiating into macrophages to populate atherosclerotic plaques [49]. When peripheral blood of population-based cohorts were analyzed for mutations that were associated with clonal hematopoiesis, presence of those mutations was predictive of increased risk of cardiovascular mortality (HR of 1.9, 95% CI 1.1-3.5) [20].

0.3 Hs-CRP as a marker for inflammation and cardiovascular risk

One marker that can be used to measure the level of inflammation in a patient with atherosclerosis is C-reactive protein (CRP). Plasma levels of C-reactive protein are routinely used in clinical practice to varying degrees to monitor for signs of infection, success of antibiotic treatment, and activity level of rheumatic diseases such as giant cell arteritis. As a downstream systemic acute-phase reactant produced from the liver in response to interleukin-6, it is an unspecific marker of inflammation, while having the benefit of widely available and affordable assays. High-sensitivity analyses allow further stratification of values below the generally assumed pathological threshold of 5 mg/L. In population-based cohort studies of healthy individuals, hs-CRP measured at baseline was predictive of future vascular events [34, 38]. An individual patient data meta-analysis of people without previous history of vascular disease found a loglinear association between CRP concentration and risk of vascular events during follow-up [22]. In the Framingham cohort, those that had baseline CRP levels in the highest quartile had twice the risk of ischemic stroke or TIA during 12 to 14 years of follow-up [42].

The value of measuring CRP in the context of atherosclerosis is by no means undisputed [33]. Opponents emphasize the lack of a causal relationship between the acute phase reactant and progression of atherosclerosis. Mendelian randomization studies have failed to show causal link between CRP and coronary artery disease or ischemic stroke [61]. CRP, being a downstream marker of inflammation, does not play as undeniable a role in the pathogenesis of atherosclerosis as, for example, LDL-cholesterol. In the context of secondary prevention, the added value

of hs-CRP in estimating future vascular risk is even less clear, despite the seeming abundance of observational data. The interpretation observed correlation is complicated by CRP's lack of specificity. In the acute phase of an ischemic stroke, CRP levels increase depending on stroke severity [5, 7]. Perhaps because of its lack of specificity, and widely accessible measurement, studies have been published linking it to an indiscriminate number of outcomes, such as functional outcome, cognitive decline, the development of atrial fibrillation and depression.

However, a biomarker does not have to be causative in order to be useful clinically. Any cytokine or marker of inflammation may help in gauging the level of inflammation attributable to highly-active atherosclerosis or unstable plaques. It may also reflect systemic inflammation of other causes that would accelerate progression of existing atherosclerotic plaques.

Observational studies, which have looked at levels of inflammatory markers and risk of recurrent stroke, abound. They include substudies of large randomized controlled trials such as the CHANCE trial [57], PROGRESS study [24], and J-Stars [17], and large observational cohort studies, such as MITICO [3], CARe [6], NOMAS [45], OXVASC [44], and NORTHSTAR [47]. They each report varying degrees of association between levels of hs-CRP and vascular risk during follow-up, with some not reaching statistical significance. One has to also consider that, while we strive for a reduction in recurrence rates, a by-product of better secondary prevention is the need for larger studies to discover novel insights that reach statistical significance. This is especially true for observational studies that include multiple covariates in a model as adjustment [32].

Meta-analytical studies have been performed in an attempt to combine what seems to be a multitude of observational data regarding CRP's association with vascular recurrence [27]. Despite the abundance of data, a quantitative synthesis of the effect sizes was possible for only a handful of studies, largely due to the heterogeneity in reporting. Others have looked at CRP in ischemic stroke patients and mortality [60], or functional outcome [56].

The purpose of this paper is to review published data on the levels of hs-CRP and vascular risk in patients with stroke, reporting also the results of our cohort study. The results of this study, in turn, may help in deciding whether to conduct interventional studies with anti-inflammatory drugs in the secondary prevention of ischemic stroke.

1 Methods

1.1 Cohort study

Consecutive patients admitted to one of our university hospitals in Berlin were recruited to our study within 7 days of admission between January 2010 and May 2013 (PROSpective Cohort with Incident Stroke—Berlin - PROSCIS-B study). Patients were included if they had an ischemic stroke, were aged 18 years or older, and were followed up for 3 years. Those excluded were patients with previous stroke, brain tumor, brain metastasis, or those who were participating in an intervention study. Each patient or their legal representative gave written informed consent for study participation. The PROSCIS-B study was approved by the Ethics Committee of the Charité—Universitätsmedizin Berlin (EA1/218/09). In the present study, we excluded patients with severe ischemic stroke, as defined by National Institutes of Health Stroke Scale (NIHSS) scores of >16, as well as patients with an hs-CRP of more than 50 mg/l, and a WBC of more than 14/nl. The exclusion of extremes of CRP or patients with leucocytosis has been previously done in CRP studies, as in Mengozzi et al. 2020 [28], as they are an indication of other irrelevant causes of high CRP levels such as acute infections. Information collected at baseline included age, sex, routine laboratory measures, measures of functional status and stroke severity, risk factor profile including current smoking, past medical history of arterial hypertension, coronary heart disease, peripheral arterial disease and diabetes mellitus.

A combined endpoint of recurrent stroke, MI, and all-cause death within 3 years was defined as the primary endpoint. Information regarding recurrent strokes and MIs were either self-reported during follow-up interviews or obtained through surveying the hospital's medical records. Events were validated independently by two senior vascular neurologists not involved

in the PROSCIS study. Records of death were obtained from the Berlin local registration office.

For univariate analysis, the Student's t-test was used to compare normally distributed continuous predictors, and Pearson's chi-square tests were used for categorical variables. The Cox proportional hazard model was used to estimate hazard ratios (HR). Data preparation and analysis was done in IBM SPSS Statistics for Windows, version 24 (IBM Corp, Armonk, NY). Missing data was handled using multiple imputation.

1.2 Systematic review and meta-analysis

The EMBASE (1980 to Feb. 16, 2021) and Medline (1946 to Feb. 16, 2021) databases were searched on 16 February 2021 with both subject headings and text searches for stroke and C-reactive protein. The MeSH terms used were: cerebrovascular disorders, brain ischemia, cerebrovascular accident, brain infarction, intracranial embolism and thrombosis, and C-reactive protein. The EMTREE subject headings used included: cerebrovascular accident, stroke, brain infarction, brain ischemia, cerebrovascular disease, and C-reactive protein. Additional search terms were used to identify articles with abstracts, titles, or keywords containing these words: ischemic stroke, cerebrovascular accident, transient ischemic attack, TIA, cerebral infarction, and C-reactive protein, or CRP (see also 1.2.1 and 1.2.2). The references of relevant reviews and articles were hand-searched for further candidate studies.

1.2.1 Embase search strategy

1. cerebrovascular disease/ or exp brain infarction/ or exp brain ischemia/ or exp cerebrovascular accident/ or exp carotid artery disease/ or cerebral artery disease/ or exp occlusive cerebrovascular disease/ or stroke/

2. ((brain or cerebr$ or intracran$ or intracerebral or cerebell$ or hemispher$ or infratentorial or supratentorial or MCA) adj5 (isch?emi$ or infarct$ or thrombo$ or emboli$ or occlus$ or hypoxi$)).tw.

3. (stroke$ or apoplex$ or cerebral vasc$ or cerebrovasc$ or cva or attack$ or brain vasc$ or isch?emi$ attack$ or tia?).tw.

4. 1 or 2 or 3

5. exp C Reactive Protein/

6. (crp or hs?crp or c-reactive protein or c reactive protein).tw.

7. 5 or 6

8. 4 and 7
9. remove duplicates from 8

1.2.2 MEDLINE search strategy

1. cerebrovascular disorders/ or exp brain ischemia/ or carotid artery diseases/ or cerebrovascular accident/ or exp brain infarction/ or exp hypoxia-ischemia, brain/ or exp intracranial arterial diseases/ or exp „intracranial embolism and thrombosis"/ or stroke/
2. ((brain or cerebr$ or cerebell$ or intracran$ or intracerebral) adj5 (isch?emi$ or infarct$ or thrombo$ or emboli$ or occlus$ or hypoxi$)).tw.
3. (stroke$ or apoplex$ or cerebral vasc$ or cerebrovasc$ or cva or isch?emi$ attack$).tw.
4. ((transi$ adj3 isch?em$ adj3 attack$) or TIA$1).tw.
5. or/1-4
6. exp C-Reactive Protein/
7. (c reactive protein or crp or hs?crp or c-reactive protein).mp.
8. 6 or 7
9. exp mortality/ or exp incidence/ or exp follow-up studies/ or exp cohort studies/
10. (mortality or prognos* or predict* or course).tw.
11. 9 or 10
12. 5 and 8

Published journal articles of studies were included in this review if they were based on a prospective cohort study that included patients older than 18 years with ischemic strokes and/or transient ischemic attacks, the follow-up duration was at least three months, and endpoints included vascular death, or vascular events (including acute myocardial infarction, recurrent stroke or TIA) during the follow-up period. If two or more publications were based on the same cohort, the article with more relevant data (e.g. results on vascular events) was included. Studies that primarily investigated another marker of interest, but also reported an effect size for CRP in the same model, were not included.

To report a summary of the included studies, information on the characteristics of each cohort, such as age, percentage of male patients, distribution of stroke etiology, specific inclusion and exclusion criteria, and vascular recurrence rate per year of follow-up, was collected using a standardized form. In addition, relevant aspects of study protocols, including definition of outcome events, duration of follow-up, time-point of venous blood sampling, and variables used in multivariate models were recorded. Finally, adjusted ORs, RRs or HRs of CRP for vascular events were extracted to calculate a summary effect measure. The quality of included studies was evaluated using the "Quality in Prognostic Studies" tool [14] (see also Table 1.1).

Table 1.1: Quality in Prognostic Studies (QUIPS) Tool

Domains	Prompting Items for Consideration
Study Participation	a. Adequate participation in the study by eligible persons b. Description of the source population or population of interest c. Description of the baseline study sample d. Adequate description of the sampling frame and recruitment e. Adequate description of the period and place of recruitment f. Adequate description of inclusion and exclusion criteria
Study Attrition	a. Adequate response rate for study participants b. Description of attempts to collect information on participants who dropped out c. Reasons for loss to follow-up are provided d. Adequate description of participants lost to follow-up e. There are no important differences between participants who completed the study and those who did not
Prognostic Factor Measurement	a. A clear definition or description of the PF is provided b. Method of PF measurement is adequately valid and reliable c. Continuous variables are reported or appropriate cut points are used d. The method and setting of the measurement of PF is the same for all study participants e. Adequate proportion of the study sample has complete data for the PF f. Appropriate methods of imputation are used for missing PF data

Outcome Measurement	a. A clear definition of the outcome is provided b. Method of outcome measurement used is adequately valid and reliable c. The method and setting of outcome measurement is the same for all study participants
Study Confounding	a. All important confounders are measured b. Clear definitions of the important confounders measured are provided c. Measurement of all important confounders is adequately valid and reliable d. The method and setting of confounding measurement are the same for all study participants e. Appropriate methods are used if imputation is used for missing confounder data f. Important potential confounders are accounted for in the study design g. Important potential confounders are accounted for in the analysis
Statistical Analysis and Reporting	a. Sufficient presentation of data to assess the adequacy of the analytic strategy b. Strategy for model building is appropriate and is based on a conceptual framework or model c. The selected statistical model is adequate for the design of the study d. There is no selective reporting of results

Quantitative analysis and synthesis was performed in RStudio using R (Version 4.1.2 "Bird Hippie"). The summary effect size was calculated using inverse-variance weighted meta-analysis. The random-effects model was used because the true effect size of each study was likely to differ due to differences in the study population, and the varying hs-CRP thresholds used. The DerSimonian-Laird approach was used to estimate between-studies variance. Heterogeneity was estimated using the Cochran's Q and I2 statistic.

More comprehensive analysis was attempted using methods described in the literature, that take into account multiple effect sizes taken from the same study and their interdependence. One method was applied that was described by Pustejovsky et al., among others [15, 51, 36, 54, 55, 52], which uses robust variance estimation to estimate the variance of β, b and is implemented in the package `robumeta`. The model, as described in Tanner-Smith et al. 2016 [52], can be written as follows:

$$y_{ij} = \beta_0 + u_i + e_{ij} \tag{1.1}$$

Where y_{ij} is the ith effect size of the jth study, β_0 is the average population effect, $Var(u_j) = \tau^2$ is the between-study variance, and e_{ij} is the residual for the ith effect size of the jth study. The correlated effects were chosen as the dependence structure, where the inverse variance weights were estimated as follows:

$$w_{ij} = \frac{1}{k_j(v_{.j} + \tau^2)} \tag{1.2}$$

Where $v_{.j}$ is the mean of the within-study sampling variances for the k_jth effect size in study j, τ^2 is the estimate of the between-study variance, and k_j is the number of effect sizes in study j [52].

Another way to take into account dependent effect sizes is multi-level meta-analysis. An example is a three-level meta-analysis with this model:

$$y_{ij} = \beta_R + u_{(2)ij} + u_{(3)ij} + e_{ij} \tag{1.3}$$

Where y_{ij} is the ith effect size of the jth study, β_R is the average population effect, $Var(e_{ij})$ is the sampling variance, and $Var(u_{(2)ij}) = \tau^2_{(2)}$ and $Var(u_{(3)j}) = \tau^2_{(3)}$ are the second and third level variances [4]. Cheung et al. 2014 [4] describe an implementation of this model using structural equations modeling in the `metaSEM` package for R. This package was also used to calculate a summary effect.

The code used is within the R environment shown below.

1.2.3 R code

```r
# Load libraries
library(ggplot2)
library(metaviz)
library(meta)
library(metafor)
library(tidyverse)
library(dmetar)
library(PerformanceAnalytics)
library(readxl)
library(robumeta)
library(clubSandwich)
library(kableExtra)
library(grid)
library(wildmeta)
library(metaSEM)

# Load data
noncontinuous <- read_excel("Data/SR/Noncontinuous.xlsx")
allincludedv1 <- read_excel("Data/SR/allincludedv1.xlsx")
cutoff_cve_es <- read_excel("EScutoffcve.xlsx")
recstroke_notcont<- read_excel("contstroke.xlsx")
noncontrs <- read_excel("noncontrs.xlsx")

# Independent ES
m.gen.rs <- metagen(TE = LnES,
                    seTE = LnSE,
                    studlab = studlab,
                    data = recstroke_notcont,
                    sm = "HR",
                    comb.fixed = FALSE,
                    comb.random = TRUE,
                    method.tau = "REML",
                    hakn = TRUE)

m.gen.cve <- metagen(TE = LnES,
                    seTE = LnSE,
                    studlab = studlab,
                    data = cutoff_cve_es,
                    sm = "HR",
                    comb.fixed = FALSE,
                    comb.random = TRUE,
                    method.tau = "REML",
```

```
                  hakn = TRUE)

forest.meta(m.gen.rs,
            sortvar = TE,
            leftlabs = "studlab")

forest.meta(m.gen.cve,
            sortvar = TE,
            leftlabs="studlab"
)

# RVE
robu_intercept <- robu(formula=LnES ~ 1, modelweights="CORR", data=allincludedv1,
                       studynum=Main_ID, var.eff.size = var, rho=.8, small=TRUE)
sensitivity(robu_intercept)
print(robu_intercept)

robu_intercept_noncontrs <- robu(formula=LnES ~ 1,  modelweights="CORR", data=noncontrs,
                                 studynum=ID, var.eff.size = var, rho=.8, small=TRUE)
print(robu_intercept_noncontrs)

# multilevel using metafor
full.model <- rma.mv(yi = LnES,
                     V = var,
                     slab = Study_ID,
                     data = noncontrs,
                     random = ~ 1 | Study_ID/ES_ID,
                     test = "t",
                     method = "REML")

forest(full.model)
full.model2 <- rma.mv(yi = LnES,
                     V = var,
                     slab = Main_ID,
                     data = allincludedv1,
                     random = ~ 1 | Main_ID/EffectSize_ID,
                     test = "t",
                     method = "REML")

forest(full.model2)

# metaSEM
fit1 <-meta3(y=LnES, v=var,cluster= Main_ID, data = allincluded)
summary(fit1)
```

```r
fit2 <-meta3(y=LnES, v=var,cluster= ID, data = noncontrs)
summary(fit2)

# Create prisma flowchart
prisma(found = 7781,
       found_other = 4279,
       no_dupes = 7860,
       screened = 7860,
       screen_exclusions = 13,
       full_text = 57,
       full_text_exclusions = 30,
       qualitative = 27,
       quantitative = 2,
       extra_dupes_box = TRUE)
```

2 Results

2.1 Cohort study

2.1.1 Patient characteristics

Patients with mild to moderate ischemic stroke (NIHSS < 16) were recruited to our cohort study at three locations of our university hospital, from January 2010 until June 2013. Of the 621 patients with mild-to-moderate ischemic stroke that were included in the main study, 585 had available hs-CRP measurements. 5 patients did not consent to having their biomarkers analyzed. After excluding patients with CRP values above 50 mg/L or those with leukocyte counts above 14/nl, who were considered likely to have acute infections, 533 patients remained and were ultimately included in the analysis. Baseline characteristics of the entire cohort and those included in this study are shown in Table 2.1. In Table 2.2, baseline characteristics according to hs-CRP levels dichotomized using a cutoff of 3 mg/l are shown. Overall, patients with higher hs-CRP levels tended to be older, and more overweight and hypertensive. More of the patients who had a higher hs-CRP also had atrial fibrillation, and a higher NIHSS at baseline.

2.1.2 Recurrent vascular events during follow-up

During 3 years of follow-up, 42 recurrent strokes and 5 myocardial infarctions were recorded. 50 patients died during the follow-up period. Table 2.3 shows baseline characteristics according to whether recurrent events occurred during follow-up. Age, and history of atrial fibrillation, coronary heart disease, and diabetes mellitus were significantly associated with the occurrence of a vascular event or death during follow-up.

Table 2.1: Baseline characteristics of PROSCIS-B cohort and patients included in this study

	All patients (n=621)	Included (n=533)
Age (mean, SD)	66.96 (12.99)	66.6 (13.0)
Sex, male (n, %)	379 (61%)	330(61.9%)
Smoker, current (n, %)	171 (27.5%)	154 (28.9%)
Atrial fibrillation (n, %)	132 (21.3%)	102 (19.1%)
Diabetes mellitus (n, %)	137 (22.1%)	116 (21.8%)
Hypertension (n, %)	406 (65.4%)	343 (64.4%)
Coronary artery disease (n, %)	99 (15.9%)	89 (16.7%)
Peripheral artery disese(n, %)	42 (6.8%)	33 (6.2%)
NIHSS (median, IQR)	2 (1-4)	2 (1-4)
BMI (mean SD)	27.54 (5.01)	27.44 (4.71)
TOAST criteria (n, %)		
Large-artery atherosclerosis	167 (27%)	141 (26.5%)
Cardioembolism	145 (22%)	121 (22.7%)
Small vessel disease	96 (15%)	90 (16.9%)
Other determined	22 (4%)	15 (2.8%)
Undetermined	191 (31%)	166 (31.1%)
hs-CRP in mg/l (median, IQR)	4.77 (1.77-12.5)	4.2 (1.67-10.15)
Sampling at day (median, IQR)	4 (3-5)	4 (3-5)

2.1.3 Hs-CRP and recurrent vascular events or death

When patients in the top CRP quartile were compared to those of the lowest quartile, they appeared to be at higher risk of suffering a recurrent vascular event or death during follow-up, after adjusting for other vascular risk factors (Table 2.4). Figure 2.2 shows the crude Kaplan-Meier curves for the combined vascular endpoint, with each color representing a subgroup belonging to a quartile of hs-CRP. In Table 2.5, effect sizes calculated using different contrasts of hs-CRP in the Cox proportional hazards model with other vascular risk factors are shown. Hs-CRP shows an especially high association with death from all causes, while when one looks at the occurrence of only recurrent stroke or vascular events, there was no association with hs-CRP.

2.2 Systematic review

2.2.1 Search results

7860 titles and abstracts were screened after removing duplicate references. 57 full texts were accessed, and 30 were excluded (13 because the outcome only included functional outcome or mortality, 5 due to duplicate reporting, 7 because the follow-up was less than 3 months, 4 due

Table 2.2: Baseline characteristics of patients with hs-CRP $<$ 3 mg/l and $\geq$ 3 mg/l

	hs-CRP	
	$<$ 3 mg/l (N=220)	$\geq$ 3 mg/l (N=313)
Age (mean, SD)	64.3 (13.3)	68.2 (12.6)
Sex, male (n, %)	156 (70.9%)	174 (55.6%)
Smoking status (n, %)	65 (30.1%)	89 (57.8%)
Diabetes (n, %)	42 (19.1%)	74 (23.6%)
BMI (kg/m2, mean, SD)	26.5 (3.76)	28.1 (5.21)
Atrial fibrillation (n, %)	29 (13.2%)	73 (23.3%)
Hypertension (n, %)	120 (54.5%)	223 (71.2%)
CHD (n, %)	31 (14.1%)	58 (18.5%)
PAD (n, %)	10 (4.5%)	23 (7.30%)
NIHSS (mean, SD)	2.61 (2.45)	3.66 (3.28)
log(hs-CRP) (mean, SD)	0.60 (0.50)	0.80 (0.50)

Notes: Baseline characteristics according to hs-CRP level, either less than, or equal to or greater than 3 mg/l.

to the study design being a cross sectional study, see also Figure 2.3). Included in the qualitative analysis were 27 studies, with 15752 patients.

2.2.2 Data extraction

From each included study, information was extracted using a form that included study type, size of cohort, inclusion and exclusion criteria, outcome or endpoints examined, follow-up duration, other markers measured in addition to hs-CRP, method of CRP measurement, blood sample collection time, baseline characteristics of the cohort, and overall recurrence rates. In addition, the reported effect sizes were extracted along with the methods used to calculate them (comparison method, endpoint, effect size type, adjustment).

46 reported effect sizes were collected, composed of 8 odds ratios, 33 hazard ratios, and 3 relative risks. 12 effect sizes were calculated with continuous log-transformed hs-CRP values, 18 using a cutoff-value that dichotomized the study cohort, and 16 with tertiles, quartiles or quintiles.

2.2.3 Quality assessment

The quality in prognostic studies (QUIPS) tool was used to assess the methodological quality of included studies (see also Hayden 2013 [14]), which is summarized in Table 2.8. Overall, participants in the included studies were appropriate for the research question. Few studies reported on the proportion of participants lost to follow-up. In addition to the criteria mentioned for confounding, studies were assessed on whether they excluded other causes of CRP

Table 2.3: Baseline characteristics, grouped by recurrence.

	Events during follow-up	
	No (N=443)	Yes (N=90)
Age (mean, SD)	65.4 (13.1)	72.3 (10.7)
Sex, male (n, %)	274 (73.1%)	56 (74.7%)
Smoking status (n, %)	136 (30.7%)	18 (16.7%)
Diabetes (n, %)	87 (23.2%)	29 (38.7%)
BMI (kg/m2, mean, SD)	27.5 (4.64)	27.3 (5.12)
Atrial fibrillation (n, %)	72 (19.2%)	30 (37.3%)
Hypertension (n, %)	279 (74.4%)	64 (70.7%)
CAD (n, %)	63 (16.8%)	26 (34.7%)
PAD (n, %)	24 (6.4%)	9 (12.0%)
NIHSS (mean, SD)	3.13 (2.97)	3.71 (3.15)
log(hs-CRP) (mean, SD)	0.60 (0.50)	0.80 (0.50)

Notes: Baseline variables according to occurrence of vascular events during follow-up.

elevation, such as tumor, acute infection, or other known rheumatic diseases. Two studies 35, 23 did not report the results of multivariate, adjusted analyses.

2.2.4 Qualitative summary

Table 2.6 and Table 2.4 summarize extracted data from the included studies. Cohort sizes ranged from 71 to 3044 patients, average ages ranged from 58 to 83, 48 to 90% of the included cohorts had hypertension, and the most frequent follow-up duration was 1 year. The Dade-Behring nephelometer was most often used to measure hs-CRP. One study 59 included both hemorrhagic and ischemic strokes.

Table 2.4: HRs for CVE, per CRP quartile

Variables	Model 1 HR (95% CI)	P	Model 2 HR (95% CI)	P
Age	1.04 (1.02 – 1.06)	0.000	1.04 (1.02 - 1.06)	0.000
Sex	1.16 (0.74 – 1.81)	0.512	1.22 (0.78 - 1.9)	0.385
NIHSS	1.34 (0.85 – 2.13)	0.211	1.46 (0.93 - 2.3)	0.100
Diabetes mellitus	1.47 (0.92 – 2.34)	0.105	1.91 (1.19 - 3.04)	0.007
CAD	1.67 (1.03 – 2.71)	0.036		
Hypertension	0.87 (0.54 – 1.41)	0.579		
PAD	1.90 (0.94 – 3.85)	0.076		
Atrial fibrillation	1.50 (0.94 – 2.39)	0.086		
CRP quartiles				
1st				
2nd	1.95 (0.99 – 3.84)	0.054	1.99 (1.01 - 3.92)	0.046
3rd	1.35 (0.67 – 2.73)	0.407	1.48 (0.73 - 2.98)	0.275
4th	2.0 (1.03 – 3.87)	0.040	2.19 (1.14 - 4.2)	0.019

Notes: HRs for each quartile of hs-CRP, calculated using the multivariate Cox proportional hazards model, including covariates for which HRs are shown.

2.2.5 Quantitative summary

Overall, there was significant heterogeneity in the methods used to calculate effect sizes. First, several criteria were applied to make the effect sizes more comparable, and one effect size was included per study. When effect sizes using dichotomizing cut-off values for the outcome of combined vascular events were combined, the overall HR was 2.35 (95% CI: 1.54-3.6), with significant heterogeneity (P=0.002, Figure 2.4).

When effect sizes looked at the outcome of stroke recurrence and hs-CRP noncontinuously (either cutoff, or highest vs. lowest N-tile), the overall HR was 1.53 (95% CI: 1.13-2.07), also with significant heterogeneity (P=0.009, Figure 2.5).

Multilevel meta-analysis was performed both on a selection of effect sizes, as well as on all extracted effect sizes. Including noncontinuous contrasts (cutoff or highest vs. lowest N-tile) and the outcome of CVE in a multi-level model resulted in a pooled estimate of 1.91(95% CI: 1.41-2.59).

Taking into account all the extracted effect sizes using the `metaSEM` package resulted in a pooled estimate of 1.77 (95% CI: 1.41-2.21) and level 3 I^2 of 83.9%. Figure 2.6 depicts a graphical representation of the multilevel meta-analysis, created using code provided by Fernandez-Castilla et al. [12]. With the RVE estimator, a pooled effect size of 1.3 (95% CI: 1.09-1.55) with an I^2 of 85.4% was calculated.

Table 2.5: Hazard ratios for multiple outcomes using varying hs-CRP contrasts

	CVE	Vascular recurrence	Recurrent stroke	Death
Cutoff (3 mg/l)	1.77(1.10-2.80)	0.96 (0.51-1.80)	1.10 (0.51-2.35)	2.41 (1.36-4.29)
Tertile	1.91(1.08-3.34)	0.73 (0.37-1.43)	0.95 (0.40-2.23)	3.85 (1.62-9.13)
Quartile	2.04(1.01-4.14)	0.86 (0.39-1.89)	0.92 (0.35-2.46)	8.38 (1.95-36.01)
Quintile	1.98(0.86-4.54)	0.74 (0.29-1.86)	0.82 (0.26-2.62)	12.43 (1.63-94.87)
log(hs-CRP)	1.57(0.92-2.53)	0.67 (0.39-1.23)	0.76 (0.38-1.55)	2.93 (1.56-5.5)
per SD of hs-CRP	1.25(0.99-1.59)	0.84 (0.63-1.11)	0.88 (0.62-1.24)	1.71 (1.25-2.33)

Notes: HRs calculated using the multivariate Cox proportional hazards model for the outcome of combined vascular events (CVE) including death and vascular events, vascular recurrence (recurrent stroke, TIA, myocardial infarction, revascularizations), recurrent stroke only, and death from all causes. Included in the model are age, sex, NIHSS, diabetes, CAD, and hypertension. Each HR was calculated using different methods of comparing hs-CRP (cutoff, N-tile, continuous - per log or per SD).

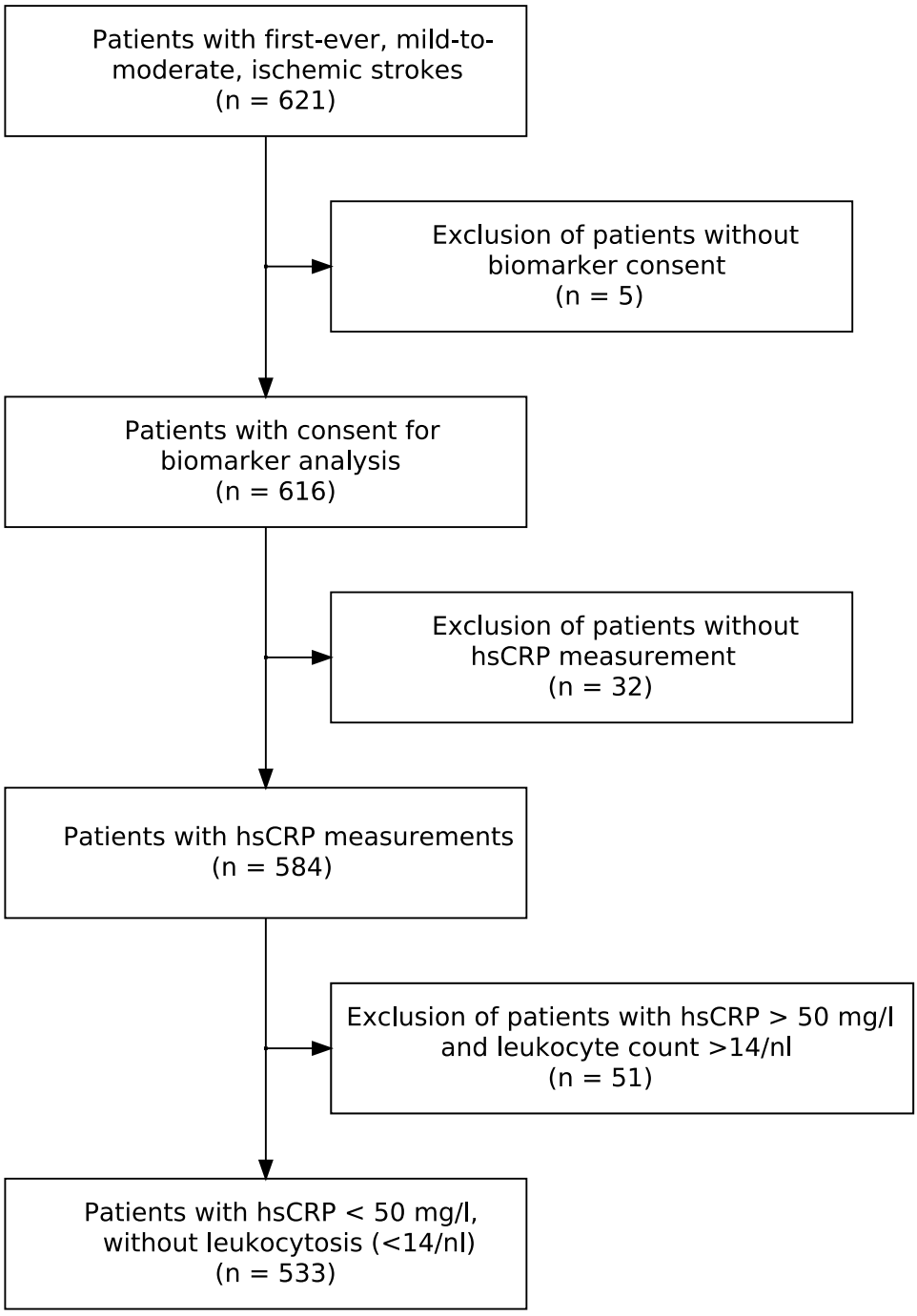


Figure 2.1: Flowchart of included patients

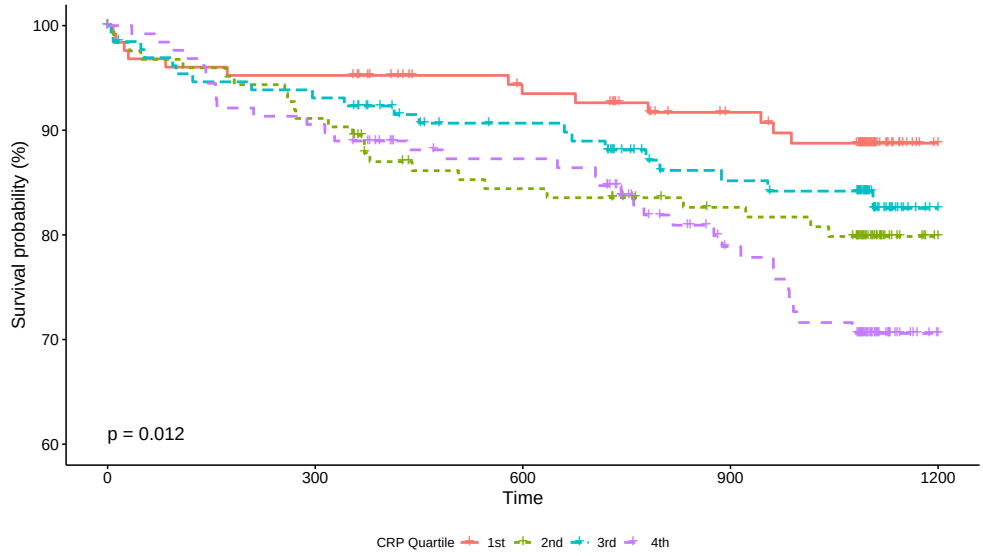


Figure 2.2: Kaplan-Meier curve by CRP Quartile, for the combined vascular endpoint

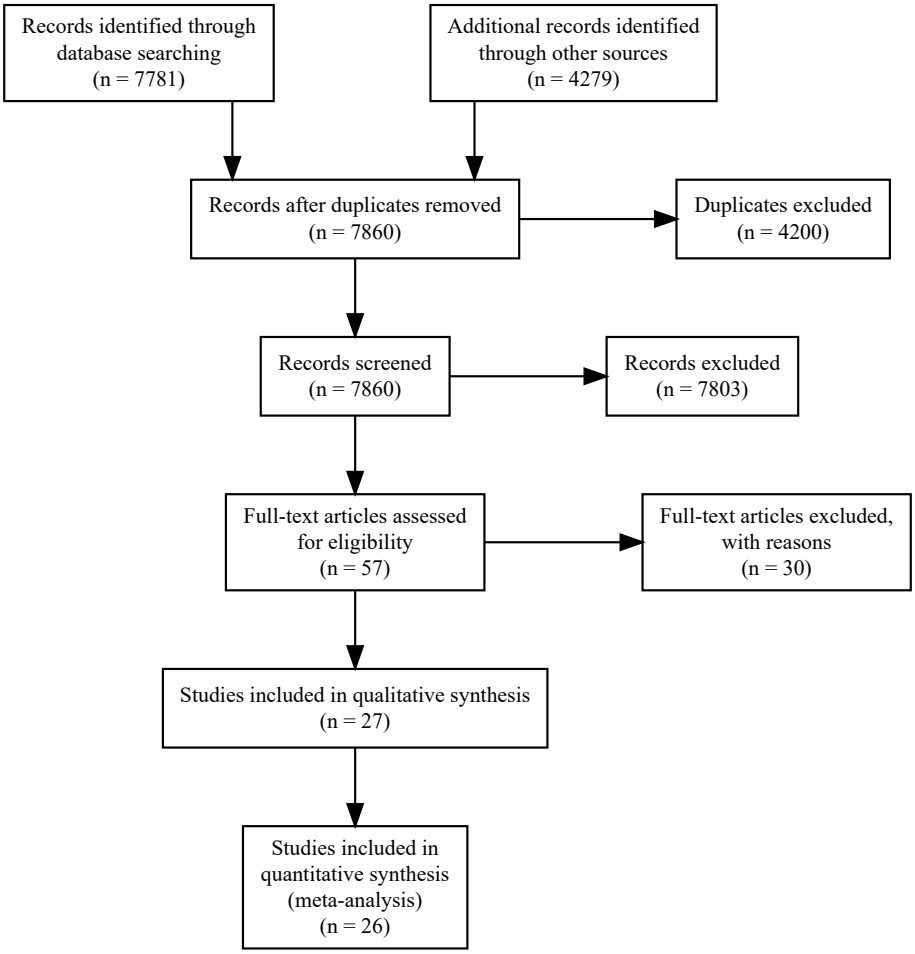
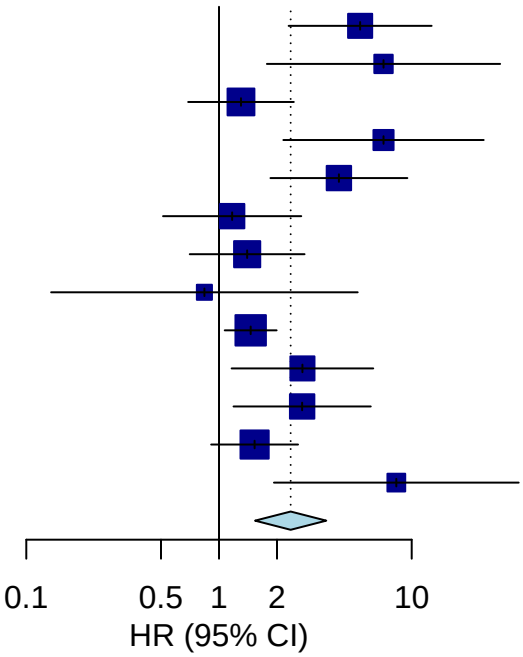


Figure 2.3: PRISMA flow chart

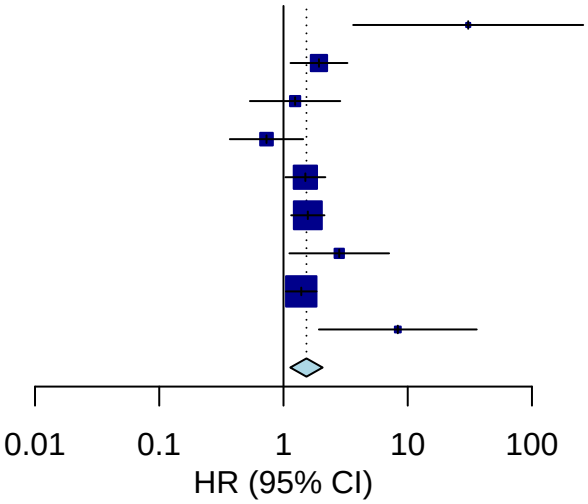
Source	HR (95% CI)
Arenillas 2008	5.40 [2.30; 12.69]
Arenillas 2003	7.14 [1.77; 28.77]
Benbir 2011	1.30 [0.69; 2.44]
Berezin 2014	7.14 [2.16; 23.63]
Corso 2009	4.19 [1.85; 9.49]
Cucchiara 2009	1.17 [0.51; 2.67]
Elkind 2014	1.40 [0.71; 2.78]
Idicula 2009	0.84 [0.13; 5.25]
Kitagawa 2017	1.46 [1.07; 1.99]
Purroy 2007	2.71 [1.16; 6.32]
Winbeck 2002	2.70 [1.19; 6.13]
Ye 2017	1.53 [0.91; 2.57]
Mengozzi 2020	8.32 [1.93; 35.91]
Total	2.35 [1.54; 3.60]



Heterogeneity: χ^2_{12} = 30.47 (P = .002), I^2 = 61%

Figure 2.4: Forest plot of effect sizes derived using cutoff values, for the outcome of CVE

Source	HR (95% CI)
Arenillas 2003	30.67 [3.64; 258.37]
Corso 2009	1.93 [1.14; 3.27]
Elkind 2014	1.24 [0.54; 2.86]
Elkind 2006	0.73 [0.37; 1.44]
Kitagawa 2017	1.50 [1.04; 2.17]
Li 2016	1.57 [1.15; 2.14]
Purroy 2007	2.81 [1.12; 7.07]
Woodward 2005	1.39 [1.05; 1.85]
Mengozzi 2020	8.32 [1.93; 35.91]
Total	1.53 [1.13; 2.07]



Heterogeneity: $\chi^2_8 = 20.44$ ($P = .009$), $I^2 = 61\%$

Figure 2.5: Forest plot of effect sizes for stroke recurrence according to noncontinuous hs-CRP

Table 2.6: Summary of included studies

1st author	Year	Study name or location	Study design	Age	% Male	N	blood sample drawn at:	Markers measured
Arenillas	2008	Barcelona, Spain. 2001-2004	prospective cohort	66.2	73.0%	75	3 months after qualifying event	CRP, E-selectin, ICAM-1, MCP-1, MMP, PAI-1 and Lp(a)
Arenillas	2003	Barcelona, Spain. 1999-2001	prospective cohort	67.4	57.7%	71	median of 8 months after stroke	hs-CRP
Benbir	2011	Turkey (ARDA study)	prospective multi-center cohort	64.7	55.4%	226	within 72 h after stroke	hs-CRP
Berezin	2014	Zaporozhye, Ukraine	prospective cohort	58.4	65.7%	102	at admission	hs-CRP
Castillo	2009	MITICO	prospective multi-center cohort	69.1	66.5%	780	at admission	hs-CRP, IL-6, IL-10, VCAM-1, ICAM-1, MMP-9, and c-Fn
Corso	2009	Cerebrovascular Aosta Registry	prospective cohort	73.9	48.5%	462	within 24h of stroke onset	hs-CRP
Coveney	2021	Dublin, Ireland (BIOSTROKETIA)	prospective multi-center cohort	70.0	62.5%	680	median 96 hrs (IQR 48-144) for stroke, 35 hrs (IQR 22-54) for TIA	serum cytokines, hs-CRP
Cucchiara	2009	UPenn/UMinnesota	prospective cohort	62.0	45.0%	167	mean of 26.2±12.7 hours after symptom onset	hs-CRP, LpPLA-A/M
DiNapoli	2002	Villa Pini stroke databank	prospective cohort	74.0	39.7%	473	within 24h after index stroke	D-dimer, fibrinogen, hs-CRP
Elkind	2014	LIMITS (ancillary to SPS3)	prospective multi-center nested cohort	63.3	63.4%	1244	$\geq$ 3 weeks after stroke	hs-CRP
Elkind	2006	Northern Manhattan Stroke Study	prospective cohort	68.9	45.4%	467	time of hospitalization or clinic visit	hs-CRP, Lp-PLA2
Greisenegger	2015	OXVASC	prospective cohort	74.0	49.0%	929	median 5 days	biomarkers related to inflammation and thrombosis
Idicula	2009	Bergen, Norway	prospective cohort	69.3	60.6%	498	within 24 hrs	hs-CRP
Kitagawa	2017	Tokyo, Japan. Substudy of J-Stars	prospective nested cohort	66.2	68.9%	1095	at randomization, after 2 and 6 months post randomization, at 2 and 5 years post randomization, at study completion	hs-CRP, LDL
Kuwashiro	2013	Fukuoka, Japan	prospective multi-center cohort	76.0	55.0%	425	within 24 hrs after admission	hs-CRP
Li	2016	CHANCE Substudy, Beijing, China	nested cohort	62.0	66.6%	3044	24±12 hrs after randomization	hs-CRP
Masotti	2006	Siena, Italy	retrospective observational	83.3	36.7%	196	within 12 hours from admission	CRP
Mengozzi	2020	Brighton, UK (ASIST)	prospective cohort	70.2	67.9%	78	within 14 days of TIA or lacunar stroke	hs-CRP, EPO, PRDX1

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1st author	Year	Study name or location	Study design	Age	% Male	N	blood sample drawn at:	Markers measured
Purroy	2007	Lleida, Spain. 10/2002-10/2003	prospective cohort	73.2	52.6%	135	<24 h after symptom onset	hs-CRP
Selvarajah	2011	NORTHSTAR Study. 06/2003-01/2006	prospective multi-center cohort	67.0	59.0%	711	at recruitment (6 weeks after symptom onset), median 12.5 days	CRP, IL-6, interleukin-1-receptor antagonist, fibrinogen, leucocyte counts, ESR
Wang	2014	Qilu Hospital, China	prospective cohort	67.1	56.6%	205	about 72 h after stroke onset.	PAPP-A, S100, hs-CRP
Whiteley	2011	Edinburgh Stroke Study	prospective cohort	71.4	52.8%	877	median of 2 days	hs-CRP, IL-6, Fibrinogen, WBC, glucose
Winbeck	2002	Munich, Germany	prospective cohort	65.0	58.3%	127	CRP1: immediately after admission CRP2: within 24 hours after symptom onset CRP3: 24 hours after CRP2	BI, mRS, DWI
Woodward	2005	PROGRESS	nested case-control	66.3	74.0%	1483	more than 1 month after event	fibrinogen, viscosity, tPA, D-dimer, CRP
Yan	2010	Hubei, China	prospective multi-center cohort	62.1	63.7%	291	not reported	tHcy and CRP
Ye	2017	Nanjing Stroke Registry	prospective cohort	60.0	73.3%	625	not reported	CRP, Hcy
Zhang	2017	Kunming, PRC	prospective cohort	63	52.4%	286	on first day of admission	CRP, fasting blood glucose, homocysteine

Table 2.7: Summary of included studies, additional characteristics

Author	Year	FU	Included patients	Excluded patients	Inf. excl.	Adjustment	Stroke etiology	Methods
Arenillas	2008	median 23 months	first-ever TIA/IS patients with symptomatic intracranial atherosclerosis (TCD+MRA/CTA)	presence of other potential causes of cerebral ischemia, nonatherosclerotic origin of intracranial stenoses, existence of conditions known to modify the levels of the studied molecules, impossibility to perform TCD long-term follow-up	Yes	age, sex, hypertension, DM, hypercholesterolemia, n of stenoses.	intracranial stenoses	Dade-Behring nephelometric assay system
Arenillas	2003	1 year	first-ever TIA or IS patients with intracranial stenoses detected by TCD, and confirmed by MRA or CTA	absence of angiographic confirmation; emboligenic cardiopathy; neoplasm; inflammatory conditions; use of immunosuppressants; nonatherosclerotic causes of intracranial stenosis, such as Sneddon syndrome, moyamoya disease	Yes	age, sex, vascular risk factors and variables showing P<0.1 in univariate testing (total cholesterol/HDL ratio, >2 risk factors)	IS or TIA attributable to intracranial stenoses	Dade-Behring nephelometric assay system
Benbir	2011	12 months	patients diagnosed with TIA or stroke of atherothrombotic etiology with significant (>50%) stenosis in intracranial/extracranial carotid or vertebral vasculature	patients with normal/minimal findings in doppler ultrasonography/angiography, age> 85 years, cerebrovascular event due to dissection or unapproved ischemic origin, cardioembolic stroke, small vessel disease, cerebral infarction secondary to a systemic disease or other etiologies, internal carotid artery occlusion or stenosis 70%, manifest heart failure, unstable angina pectoris or high-grade aortic or mitral valve stenosis	No	none	not reported	Dade-Behring nephelometric assay system

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Author	Year	FU	Included patients	Excluded patients	Inf. excl.	Adjustment	Stroke etiology	Methods
Berezin	2014	1 year	patients with acute IS classified according to the TOAST classification as cardioembolic infarction, large artery atherosclerosis, or lacunar infarction, with mild-to-moderate arterial hypertension, age older than 18 years, and sinus rhythm	symptomatic chronic heart failure; uncontrolled DM; severe kidney and liver diseases; malignancy, unstable angina, myocardial infarction within 30 days before study entry; brain injury within 3 months before enrollment; BMI >30 kg/m2 or <15 kg/m2; pulmonary edema; tachyarrhythmia; valvular heart disease; thyrotoxicosis; IS classified as undetermined or other determined etiology; ICH; acute infection; surgery, trauma, or any ischemic event during the previous 3 months; inflammatory conditions within 1 month; neoplasm; pregnancy; an implanted pacemaker; and refusal to participate or consent to this study	Yes	age, sex, type of IS, BI and mRS on admission and on the 21st day of hospitalization, side of weakness, vascular risk factors, severity of arterial hypertension on admission, DM, hypercholesterolemia	CE 11.7% LAA 2% SVD 86.3%	nephelometric technique using an AU640 Analyzer (Olympus Diagnostic Systems Group, Japan).
Castillo	2009	12 months	patients with IS between 1 and 3 months previously, with focal ischemic neurological deficit of >1 hour, with neuroimaging confirmation of IS (or neuroimaging exclusion of any other process), and with previous function <2mRS	use of anti-coagulants for any reason, acute adverse vascular episodes in the previous month, chronic inflammatory diseases, or treatments with pain killers for ≥ 1 week, neoplasia, major surgery, and acute inflammatory diseases in the 15 days prior to inclusion, or for more than 3 weeks during the follow-up period. repeated temperatures higher than 37.5 °C, potential signs of infection	Yes	age, previous TIA, carotid stenosis>50%, leukoaraiosis, cardiac disease, peripheral vascular disease, stroke subtype, leukocyte count at baseline, and treatment with cholesterol-lowering agents	LAA 31% SVD 20% Und 49%	Immulite 1000
Corso	2009	2.27 years	patients with a diagnosis of incident IS, a measurement of CRP within 24 h after stroke onset	all patients with recent infections monitored by medical history, chest X-ray, urine examination and physical examination, with body temperature >38.0°C at admission and with signs of acquired in-hospital infection	Yes	age, BMI, NIHSS, DM and hypercholesterolemia, admission blood glucose concentration, admission serum TC and LDL, history of smoking, ischemic heart disease, AF, hypertension, claudication, therapy at discharge	LAA 33.3% CE 24% SVD 30.1% Und 12.6%	Roche automated Modular P analyser, CRPLX test

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Author	Year	FU	Included patients	Excluded patients	Inf. excl.	Adjustment	Stroke etiology	Methods
Coveney	2021	1 year	first-ever or recurrent TIA (<48 hours of symptom onset) or non-severe ischaemic stroke (modified Rankin score [mRS] 0-3,<14 days after onset)	asymptomatic stroke on brain imaging, primary intracranial haemorrhage, pro-inflammatory co-morbidities (e.g., Infection, cancer, recent trauma<3 months, arthritis, myocardial infarction <4 weeks)	Yes	age, sex, carotid stenosis, smoking, prior TIA, hypertension, antiplatelets and statins	not reported	high-throughput immunotubridimetry
Cucchiara	2009	90 days	patients with suspected TIA evaluated within 48 hours of symptom onset, patients for whom there was sufficient clinical suspicion to justify diagnostic testing for a neurovascular cause	patients with severe or terminal illness likely to preclude full evaluation and follow-up; patients taking warfarin with an INR >=1.5	No	no adjustment due to non-significance	LAA 15% CE 8%	Hitachi 917 analyzer (Roche Diagnostics) assay using a turbidimetric immunoassay
DiNapoli	2002	2 years	a diagnosis of first-ever IS within 24 hours before enrollment	patients with history of recent clinical infection; concurrent major renal, hepatic, and cancerous disease; surgery or major trauma in the previous month; and obvious signs and clinical evidence of in-hospital–acquired infection	Yes	age, CNSS, triglyceride>180 mg/dl, alcohol abuse, coronary heart disease, pad, mitral/aortic valve disease, aspirin at discharge, ticlopidine at discharge, large infarct, brain swelling, cortical involvement>50%	LAA 40.5% CE 33.6% SVD 17% Und 8.9%	Dade-Behring nephelometric assay system
Elkind	2014	12 months	lacunar stroke syndromes, subcortical TIA with positive DWI on MRI, no cortical dysfunction, no ipsilateral cervical carotid stenosis, no major-risk cardioembolic sources	disabling stroke, previous ICH (excluding traumatic) or hemorrhagic stroke, age under 40 years, high risk of bleeding, anticipated requirement for long-term use of anticoagulants, prior cortical stroke/TIAm prior ipsilateral carotid endarterectomy, impaired renal function, intolerance or contraindications to aspirin or clopidogrel, a score < 24 MMSE	No	age, sex, race/ethnicity, region, hypertension, cardiac disease, DM, or smoking, BMI, HDL, LDL, statin use	lacunar	Dade-Behring nephelometric assay system
Elkind	2006	median of 4 years	first IS, 40 years and older, and in northern Manhattan for 3 months or longer in a household with a telephone	NA	No	age, sex, and race and ethnicity, history of coronary artery disease, DM, hypertension, hyperlipidemia, AF, and current smoking	LAA 16.5% SVD 23.3% CE 18.4% Und 41.7%	ELISA (Lp-PLA2: PLAC, diaDexus; hs-CRP: BioCheck)

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Author	Year	FU	Included patients	Excluded patients	Inf. excl.	Adjustment	Stroke etiology	Methods
Greisen-egger	2015	median 6.4 years	TIA or minor stroke	NA	No	age, sex, hypertension, DM, previous MI, previous IS, previous PAD, current smoker, AF, hyperlipidaemia, previous antiplatelet therapy, previous antihypertensive therapy, previous therapy with statins	CE: 16%, LAA 12% SVD 17% Und: 45% Unk: 6% Multiple 14% Other: 3%	Biochip multiple immunoassay system (Randox Laboratories Ltd), Cerebral Array II
Idicula	2009	2.5 years	IS patients, blood sampling within 24 hours of symptom onset	clinical and radiological findings not consistent with stroke	No	age, sex, NIHSS, pre-existing DM and intravenous thrombolysis	NA LACI: 25% TACI:17% PACI: 41% POCI: 17%	Tina-quant latex method using Modular P (Roche Diagnostics)
Kitagawa	2017	5 years	patients aged 45–80 years with a history of non-cardiogenic IS within the preceding 1 month to 3 years, previously diagnosed with hyperlipidemia and demonstrated stable serum total cholesterol levels at 180–240 mg/dL	cerebral infarction of determined rare etiology, (e.g., vertebral artery dissection, fibromuscular dysplasia, or moyamoya disease), infarction associated with catheterization or surgery, and preferred use of statins for the treatment of comorbid coronary artery disease	No	stroke subtypes, hypertension, and DM, age, sex, BMI, HDL-C, TG, FBG, smoking and statin use	noncardioembolic	Special Reference Laboratory, Inc. (Tokyo)
Kuwashiro	2013	1 year	IS patients with cardioembolic etiology	IS patients with other etiologies (LAA, SVD, undetermined)	No	sex, age, pneumonia and urinary tract infections, hypertension, BMI, DBP on admission, hematocrit	cardioembolic	NA
Li	2016	1 year	Acute minor stroke (NIHSS<=3) or high risk TIA ABCD2>=4	major nonischemic brain disease (hemorrhage, vascular malformation, tumor, abscess), clear indication for anticoagulation, and exclusion criteria of CHANCE trial	No	age, BMI, sex, medical histories of myocardial infarction, hypertension and DM, baseline NIHSS score, baseline leukocyte count, randomized treatment of aspirin monotherapy or dual antiplatelet therapy, and use of antihypertension agents	not reported	Roche Modular P800

Continued on next page

Author	Year	FU	Included patients	Excluded patients	Inf. excl.	Adjustment	Stroke etiology	Methods
Masotti	2006	12 months	patients aged>75, with IS diagnosis	patients with cancer diagnosis, chronic inflammatory disorders, recent infectious diseases, chronic liver/renal failure, elevated ESR	Yes	none	LAA 34.7% SVD 19.4% CE 45.9%	Dade-Behring nephelometric assay system
Mengozzi	2020	42 months	patients with a diagnosis of lacunar stroke or TIA confirmed by a stroke physician	Hs-CRP>50 mg/l, WBC>10000/ml	Yes	previous stroke, previous TIA, age, sex, smoking status, DM, BMI, AF, diagnosis of HTN, average systolic BP, and average diastolic BP	TIAs or lacunar strokes	ELISA (DRG Instruments GmbH, Oxford Biosystems)
Purroy	2007	1 year	TIA patients (reversible episode, completely resolved deficit within 24h)	NA	No	age, sex, vascular risk factors, CHD, carotid territory, large-artery occlusive disease, extra/intracranial stenoses, chronic infarct in CT	LAA 23.7% SVD 5.2% CE 28.9% Und 41.5%	Dade-Behring nephelometric assay system
Selvarajah	2011	3 months	patients with a diagnosis of TIA or minor stroke confirmed by a consultant stroke physician or neurologist, symptom onset within the preceding 6 weeks, age >18 year, mRS <1	cognitive impairment sufficient to interfere with independent daily living, significant comorbidity limiting participation in the study	No	none	LAA 22% SVD 23% CE 13% Other 2% Und 40%	competitive ELISA
Wang	2014	6 months	patients diagnosed with the first-ever acute IS based on both clinical information and radiological information	patients with autoimmune diseases, cancer, infections, severe heart, liver, kidney or other potential fatal diseases, patients with heparin administration before the blood sampling, patients with incomplete follow-up data	Yes	age, sex, NIHSS score, history of arterial hypertension, DM and coronary syndromes	not reported	immunoassay method on a Roche 2010 analyzer
Whiteley	2011	mean of 2.12 years	stroke patients admitted to the hospital or seen as outpatients, with a first-ever-in-a-lifetime clinically evident stroke, demonstrated by brain imaging (CT or MRI) to be ischemic	primary hemorrhage	No	age, cardiac failure, AF (current or past), or previous stroke, TIA, peripheral vascular disease, or MI	LAA 8.2% SVD 20.3% CE 13.3% Mixed 6.6% Und 56.3%	Dade-Behring nephelometric assay system

Continued on next page

Author	Year	FU	Included patients	Excluded patients	Inf. excl.	Adjustment	Stroke etiology	Methods
Winbeck	2002	1 year	proven first-ever stroke no more than 12 hours after symptom onset, without thrombolysis	history of recent infection as outpatients, surgery or trauma in the previous month, obvious signs of acquired in-hospital infection before onset of the index stroke, or an initial CRP level >10 mg/dL	Yes	age, Barthel Index at admission, incidence of hypertension, CHD, DM, and hypercholesterolemia	not reported	Tina-quant CRP (latex) highly sensitive assay (Roche)
Woodward	2005	3.9 years	patients with a history of stroke or TIA within the previous 5 years	indication or contraindication for ACE inhibitors	No	age, sex, treatment and therapy allocated, region, and most recent qualifying event, systolic blood pressure, smoking, peripheral artery disease prevalence, statin use, and antiplatelet use	within IS subset: LAA 13.6% SVD 24.2%	Dade-Behring nephelometric assay system
Yan	2010	5 years	first-ever ischemic or hemorrhagic stroke	other types of stroke, including transient ischemic attack, embolic brain infarction, subarachnoid hemorrhage, brain tumors, and cerebrovascular malformation, and severe systemic diseases, inflammation, liver, neoplastic, or renal disease	Yes	sex, age, BMI, blood pressure, blood cholesterol and history of DM, hypertension and smoking	ischemic and hemorrhagic stroke	enzyme-linked immunosorbent assay
Ye	2017	1 year	patients with first-ever IS, blood drawn within 2 weeks of symptom onset, aged 18 years or older, stroke subtype was determined according to TOAST as LAA	history of coronary heart disease, transient ischemic attack (TIA), or ischemic or hemorrhagic stroke, recent clinical infection, concurrent cancerous disease or liver or kidney failure, surgery or major trauma in the previous month	Yes	adjusted for age, sex, hypertension, dyslipidemia, and DM.	LAA only	nephelometry (IMMAGE 800 Immunochemistry System, Beckman Coulter, Inc., CA, USA)
Zhang	2017	1 year	patients with ischemic stroke diagnosis validated by MRI and/or CT, presenting within 24 hrs of symptom onset	patients with malignant tumor, head trauma, severe edema, renal insufficiency (creatinine > 1.5 mg/dl), acute or chronic inflammatory disease, and autoimmune diseases	Yes	age, sex, BMI, infarct volume, time of blood sampling, stroke syndrome, stroke etiology, vascular risk factors, NIHSS score, prestroke and acute treatment, HCY, FBG	LAA 22.0% SVD 20.3% CE 32.9% other 12.2% Und 12.6%	MINDRAY BS800M

Table 2.8: Quality assessment of included studies

First author	Year	Study participation	Study attrition	Prognostic factor measurement	Study confounding	Outcome measurement	Statistical analysis and reporting
Arenillas	2008	Low	High	Low	Low	Moderate	Low
Arenillas	2003	Low	Low	Low	Low	Low	Low
Benbir	2011	Low	Moderate	Low	Moderate	Low	Moderate
Berezin	2014	Low	Moderate	Low	Low	Low	Low
Castillo	2009	Low	Low	Low	Low	Low	Moderate
Corso	2009	Low	Moderate	Low	Low	Low	Moderate
Cucchiara	2009	Low	Low	Low	Moderate	Low	High
DiNapoli	2002	Low	Low	Low	Low	Low	Low
Elkind	2014	Low	Low	Low	Low	Low	Low
Elkind	2006	Low	Moderate	Low	Low	Low	Low
Greisenegger	2015	Low	Low	Low	Moderate	Low	Low
Idicula	2009	Low	Moderate	Low	Low	Low	Moderate
Kitagawa	2017	Low	Low	Low	Moderate	Low	Low
Kuwashiro	2013	Low	Moderate	Moderate	Low	Low	Moderate
Li	2016	Low	Low	Low	Low	Low	Low
Masotti	2006	Low	Moderate	Low	Moderate	Moderate	High
Purroy	2007	Low	Moderate	Low	Low	Low	Moderate
Selvarajah	2011	Low	Low	Low	Moderate	Low	Moderate
Wang	2014	Low	Moderate	Low	Low	Low	Low
Whiteley	2011	Low	Moderate	Low	Low	Low	Low
Winbeck	2002	Low	Low	Low	Low	Low	Low
Woodward	2005	Low	Low	Low	Low	Low	Low
Yan	2010	Moderate	Moderate	Low	Low	Low	Moderate
Ye	2017	Low	Low	Low	Low	Low	Low
Coveney	2021	Low	Low	Low	Low	Low	Low
Mengozzi	2020	Low	Moderate	Low	Low	Low	Low
Zhang	2017	Low	Low	Low	Low	Low	Low

2.2.6 Test for publication bias

Figure 2.7 shows funnel plots for defined subgroups of effect sizes, as well as for all collected effect sizes. Effect sizes that lie outside the funnel in Figure 2.7d show asymmetry, which is not as present in defined subgroups, implying that the asymmetry is due to heterogeneity.

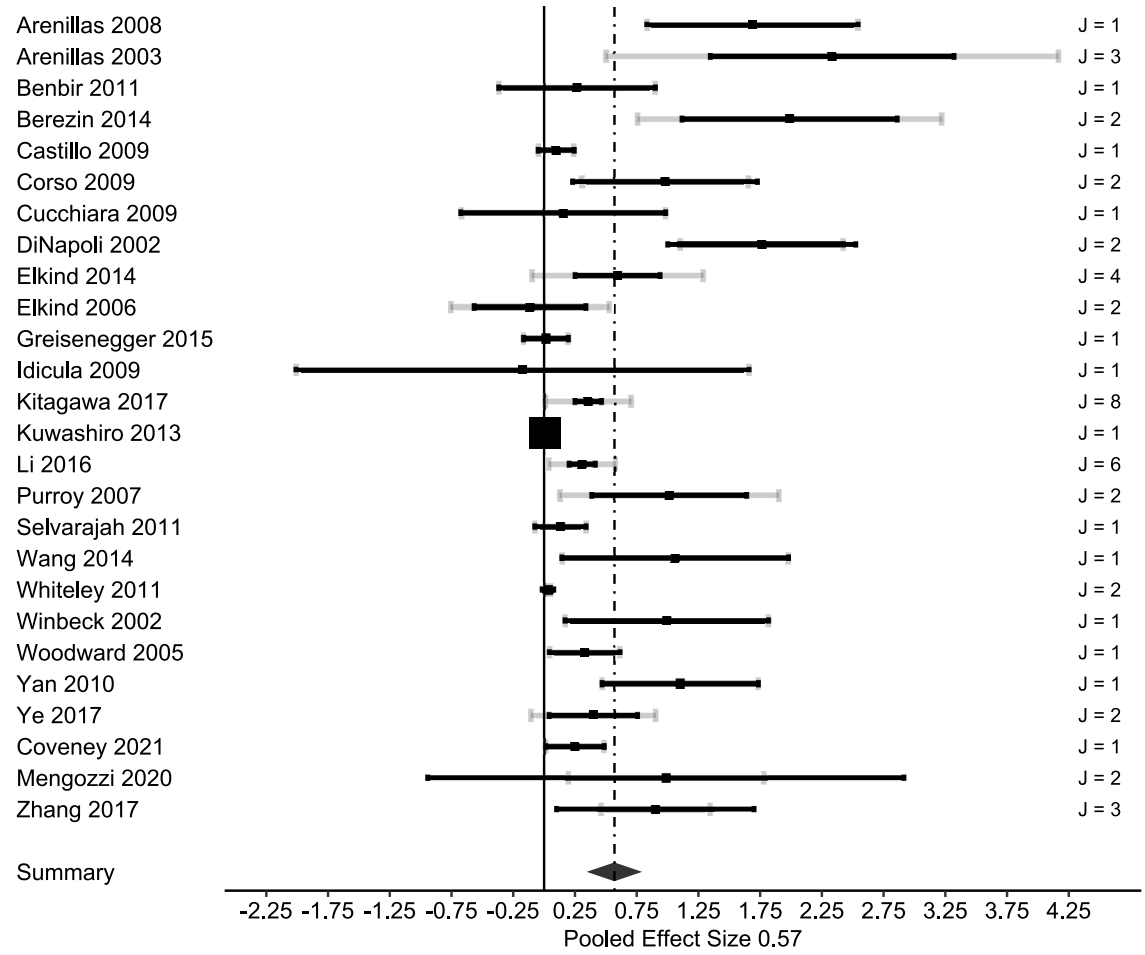
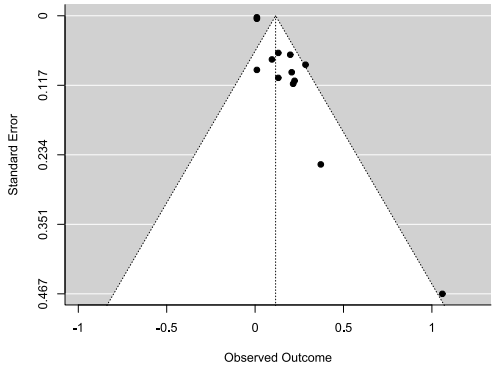
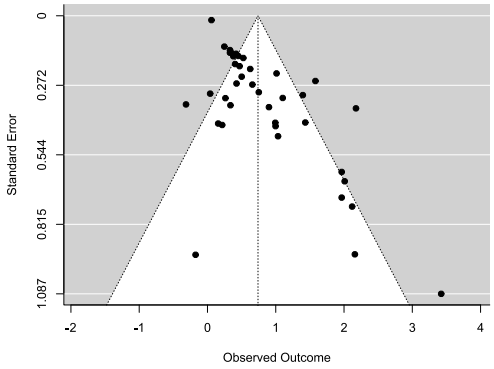


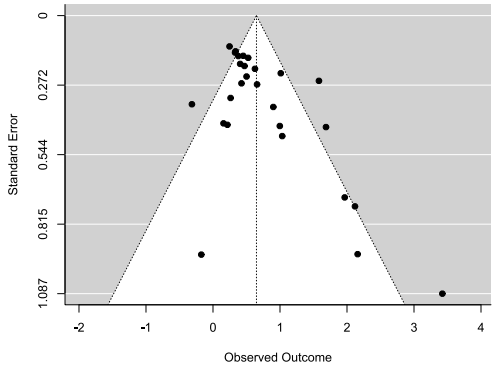
Figure 2.6: Forest plot of multi-level meta-analysis including all available effect sizes. The thickness of the grey confidence intervals is proportional to the number of effect sizes reported within studies. Effect size and confidence intervals are represented on the natural log scale



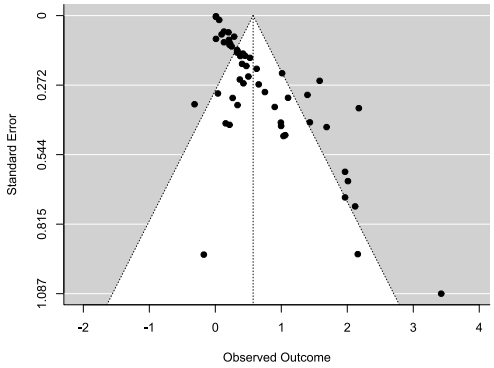
(a) All ES - continuous



(b) All ES - noncontinuous



(c) ES for recurrent vascular events - noncontinuous



(d) All ES

Figure 2.7: Funnel plots of subgroups of effect sizes. (a) Subgroup of effect sizes calculated according to continuous CRP (either log, or per SD). (b) All effect sizes calculated using either hs-CRP cutoff or highest vs. lowest N-tile. (c) Of the effect sizes in (b), those that looked at recurrent vascular events. (d) Funnel plot of all extracted effect sizes.

3 Discussion

3.1 Interpretation of results

Evidence in the literature and in our cohort study show that there is a statistically significant correlation between recurrence rates of vascular events and levels of CRP measured after incident stroke. This is in line with numerous meta-analyses that were previously published 27, 56, 62, 60. The advantages of measuring CRP include its ubiquitous availability and affordability of measurement, the abundance of studies that evaluate its association with vascular risk, and the existence of potential therapies targeted at its reduction. With the role of inflammation firmly established in the pathophysiology of atherosclerosis, it appears reasonable to monitor the level of inflammation to assess vascular risk.

3.2 Difficulties in interpreting observed data

The correlations observed in included studies are likely dependent on methodological aspects that were highly heterogeneous. Some studies included all-cause mortality in the combined vascular endpoint, possibly adding the association between elevated CRP and death from nonvascular causes such as cancer to the effect size. Some studies failed to exclude patients with high CRP due to inflammatory conditions, such as acute infections or existing autoimmune diseases. More significant effect sizes could be calculated by using different comparison groups, and the highest could be calculated by comparing the highest against the lowest quartile or quintile. While CRP is frequently measured in ischemic stroke cohorts, the results are more likely to be published when the analysis yields significant correlations, which might lead to publication bias. Furthermore, even if the results are significant after adjustment, a causal inference cannot

be made based on these results, as they are based on purely observational data.

There was a high variability in the timepoints at which blood samples were drawn for analysis, ranging from within 12 hours of stroke onset, to as much as 5 years after the qualifying event. CRP levels are influenced by stroke severity for as long as a year after symptom onset [8], which makes the interpretation of the observed association fraught with pitfalls. Are we seeing an effect, which is actually based on the association between stroke severity and vascular risk? Most of the studies performed blood sampling in the acute phase of stroke, presumably as logistically, it is the most convenient time. Can the association between hs-CRP measured in the acute phase and vascular risk be considered in the same way as when measured in the chronic phase?

Another difficulty in analysis is the dependent censoring through the endpoint of death. When analyzing the association between risk factors and recurrent vascular events without death, one comes across the problem that the censoring of deceased patients is dependent on multiple factors, including vascular risk factors. As a result, when performing survival analysis of events excluding death, the number of person-years of, in particular, those who are at high risk is selectively reduced. Using survival analysis methods that take into account dependent censoring or correlated endpoints [10, 9] might yield a more correct interpretation of survival data.

3.3 Previously published meta-analyses

Studies have consistently shown a correlation between CRP measured shortly after onset of ischemic stroke and functional outcome as well as mortality. VanGilder et al. [56] performed a systematic review in 2014 and found 5 studies meeting their inclusion criteria, with the majority of them reporting a positive correlation between worse functional outcome and higher CRP levels measured within 24 hours of ischemic stroke onset. Another more recent meta-analysis similarly found a significant association between hs-CRP levels and unfavorable functional outcome in ischemic stroke patients [18]. Yu et al. 2019 found 8 studies with 3604 ischemic stroke patients that showed an overall hazard ratio of 2.07 (95% CI:1.60–2.68) for all-cause mortality when CRP categories were compared, with no evidence of significant publication bias in the Egger's test ($P = 0.293$) [60]. Another meta-analysis published in 2021 reported similar results [21].

Evidence also supports the relationship between CRP measured after a stroke and the risk of recurrent vascular events. In the general population, Zhou et al. 2016 found an overall risk ratio of 1.46 (95% CI:1.27–1.67) for the occurrence of ischemic stroke during follow-up, but not for hemorrhagic stroke (RR 1.23, 95% CI: 0.997–1.51) [62]. In a meta-analysis looking at

3 inflammatory biomarkers (CRP, fibrinogen, IL-6) in ischemic stroke patients, McCabe et al. 2021 found a pooled hazard ratio of 1.14 (95% CI:1.06–1.22, $P < 0.01$) for recurrent stroke and 1.21 (95% CI:1.10–1.34, $P < 0.01$) for major vascular events, per one standard deviation increase in logCRP 27.

3.4 Meta-analytic methods

In this study, alternative methods of synthesizing meta-analytic data were applied, that allowed the taking into account of dependent effect sizes in one comprehensive analysis. This yielded a pooled effect size that was similar to that obtained by synthesizing only independent effect sizes. There still remained significant heterogeneity, which could be partly explained by moderator variables. A meta-regression may help to elucidate which factors influence effect sizes. The moderators could be of methodological nature, such as how hs-CRP groups were compared, whether adjustment for potential confounders was performed, or which laboratory method was used; or they may provide insight into the inherent nature of the association between vascular risk and hs-CRP, e.g., by differentiating risks according to stroke etiology.

Though advances in meta-analysis methodology make such elaborate analyses possible, no further information is gained when the primary studies are of low quality, or provide such heterogeneous results, that quantitative synthesis is meaningless. These limitations are entrenched in the meta-analysis of observational studies examining CRP in vascular disease. Often, the studies are based on cohorts that were not designed ad hoc with CRP in mind, thus potentially leaving out important aspects in the study design, such as excluding patients who may have high CRP due to other diseases, which are difficult to correct post hoc. As mentioned above, in such cohorts, if CRP did not yield statistically significant associations, the results would probably not have been published, leading to even more publication bias. Limitations such as these are elaborated in a methods paper by Riley et al. 40, using precisely CRP in coronary disease as an example.

It is undeniable, however, that there is an abundance of studies published reporting a significant association between CRP and vascular risk, and that meta-analyses more often than not report statistically significant pooled effect sizes. It is also hard to ignore the increasingly recognized role of inflammation in atherogenesis. These two factors combined point to the relevance of assessing the level of inflammation in a patient with vascular disease. And even if CRP is too unspecific a marker, an alternative that is more specific to vascular disease and is as well-established clinically, has yet to be found.

3.5 Genetic studies linking inflammation and vascular risk

Studies using genetic data, such as genome-wide association studies, have the potential to provide an unbiased way to identify molecular pathways involved in atheroprogression and stroke. Mauersberger et al. 2021 [19] summarize the results of such studies so far, with respect to inflammation-related genes. Several risk variants of genes involved in immune and inflammatory response were identified, though CRP has been conclusively shown to not influence the development of atherosclerosis [61]. Risk variants of interleukin-6 receptor (IL6-R), ARHGEF26 (rho guanine nucleotide exchange factor 26 or SGEF, involved in the formation of intercellular adhesion molecule-1 (ICAM-1)-induced docking structures in the endothelium), interleukin 5, SVEP1 (sushi, von Willebrand factor type A, EGF, and pentraxin domain-containing protein 1), CXCL12, SH2B adaptor protein 3 (SH2B3), and platelet endothelial cell adhesion molecule 1 (PECAM-1) are among those thus far identified.

Malik et al. 2018 [26] reported on a total of 32 loci associated with stroke risk, and their related vascular traits. Referencing published GWAS studies on other vascular diseases, they showed that the largest genetic correlation existed with hypertension, as well as with venous thromboembolism and cardiac mechanisms. Novel loci were also found that were consistent with atherosclerotic mechanisms (e.g. EDNRA), which was specifically associated with large-artery atherosclerotic stroke. Overall, the genetic landscape of cerebrovascular disease is distinct from that of cardiovascular disease, and reflects the presence of multiple etiologies. We can conclude that investigations of stroke risk prediction, whether genetic or epidemiological, deserve dedicated studies separate from cardiovascular ones, like the Framingham cohort study. Subsuming stroke risk under general "major adverse vascular event" risk may ignore other potentially treatable risk factors specific to stroke.

3.6 Alternative causal pathways

Recent evidence suggests that it is possible that CRP after an ischemic stroke confers additional predictive value owing to a separate mechanism. One study in a mouse model showed that an ischemic stroke itself leads to the activation of systemic endothelial inflammation and the progression of atherosclerosis via the release of high mobility group box 1 (HMBG1), which binds to and activates the receptor of advanced glycation end-products (RAGE) pathway [43]. Blockade of the HMBG1-RAGE pathway in turn leads to a reduction of atheroprogression after stroke. CRP increases slowly after a stroke, reaching its maximum at about day 5-7 post-stroke [8]. HMBG1 rises much more quickly, with maximal values being measured within 24 hours of stroke onset [46]. Depending on their genetic predisposition and severity of incident stroke, individuals may have varying levels of inflammatory markers, which, through the aforementioned

pathway, may compound atheroprogression and risk of vascular events. This may also explain why we observe increased risk of recurrence in patients with higher CRP in the acute phase of stroke. It may be even more worthwhile to measure CRP on days 5–7 - something that was done in studies out of convenience - to capture the additional predictive value of CRP that reflects systemic inflammation triggered by the inciting event.

3.7 Conclusion

Finally, it is not the intention of this study to convince readers that CRP is causally related to vascular events, nor that lowering it should be the goal of secondary prevention. Rather, the aim is to show the quantitative relationship between CRP levels and vascular risk in the special population of ischemic stroke patients, as observed in our cohort study, and in literature. It is most likely that CRP has no causal relationship whatsoever in atheroprogression and vascular events, and is instead a "bystander" [30] molecule that reflects the intensity of a process that leads to atherosclerosis. This should not, however, discredit the observed association, or its potential usefulness in identifying patients, barring those who have another obvious reason for high CRP where we can expect a higher risk for recurrent vascular events or death from any cause.

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Eidesstattliche Versicherung

Ich, Ja Bin Hong, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: High sensitivity C reactive protein in the ischemic stroke cohort (Hochsensitives C-reaktives Protein in Schlaganfallkohorten), selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren/innen beruhen, sind als solche in korrekter Zitierung kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) werden von mir verantwortet.

Ich versichere ferner, dass ich die in Zusammenarbeit mit anderen Personen generierten Daten, Datenauswertungen und Schlussfolgerungen korrekt gekennzeichnet und meinen eigenen Beitrag sowie die Beiträge anderer Personen korrekt kenntlich gemacht habe (siehe Anteilserklärung). Texte oder Textteile, die gemeinsam mit anderen erstellt oder verwendet wurden, habe ich korrekt kenntlich gemacht.

Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem Erstbetreuer, angegeben sind. Für sämtliche im Rahmen der Dissertation entstandenen Publikationen wurden die Richtlinien des ICMJE (International Committee of Medical Journal Editors; www.icmje.og) zur Autorenschaft eingehalten. Ich erkläre ferner, dass ich mich zur Einhaltung der Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis verpflichte.

Weiterhin versichere ich, dass ich diese Dissertation weder in gleicher noch in ähnlicher Form bereits an einer anderen Fakultät eingereicht habe.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§§156, 161 des Strafgesetzbuches) sind mir bekannt und bewusst.

Datum Unterschrift

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Publikationsliste

1. Hamann T, **Hong** JB, Lange KS, Overeem LH, Triller P, Rimmele F, Jürgens TP, Kropp P, Reuter U, and Raffaelli B. Perception of typical migraine images on the internet: Comparison between a metropolis and a smaller rural city in Germany. PLOS ONE 2023;18. Ed. by Sohail MT:e0290318.

2. **Hong** JB, Lange KS, Fitzek M, Overeem LH, Triller P, Siebert A, Reuter U, and Raffaelli B. Impact of a reimbursement policy change on treatment with erenumab in migraine – a real-world experience from Germany. The Journal of Headache and Pain 2023;24:144.

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Bescheinigung

Hiermit bescheinige ich, dass Frau Ja Bin Hong innerhalb der Service Unit Biometrie des Instituts für Biometrie und klinische Epidemiologie (iBikE) bei mir eine statistische Beratung zu einem Promotionsvorhaben wahrgenommen hat. Folgende Beratungstermine wurden wahrgenommen:

- Termin 1: 16.06.2022

Folgende wesentliche Ratschläge hinsichtlich einer sinnvollen Auswertung und Interpretation der Daten wurden während der Beratung erteilt:

- Time-to-Event Analyse: Angabe von Hazard Ratio und 95% Konfidenzintervall
- Berichten des ‚crude' und adjustierten Hazard Ratio
- Einschluss der Variablen ins Modell anhand von Literatur und deskriptiver Statistik (nicht nur Einschluss signifikanter Variablen)

Diese Bescheinigung garantiert nicht die richtige Umsetzung der in der Beratung gemachten Vorschläge, die korrekte Durchführung der empfohlenen statistischen Verfahren und die richtige Darstellung und Interpretation der Ergebnisse. Die Verantwortung hierfür obliegt allein dem Promovierenden. Das Institut für Biometrie und klinische Epidemiologie übernimmt hierfür keine Haftung.

Datum: 16.06.2022

Name des Beraters/der Beraterin: Alice Schneider

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