TEMPERATURE AND SEX AS PROGNOSTIC FACTORS IN STROKE

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Temperature and Sex as Prognostic Factors in Stroke

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bij herseninfarct en hersenbloeding

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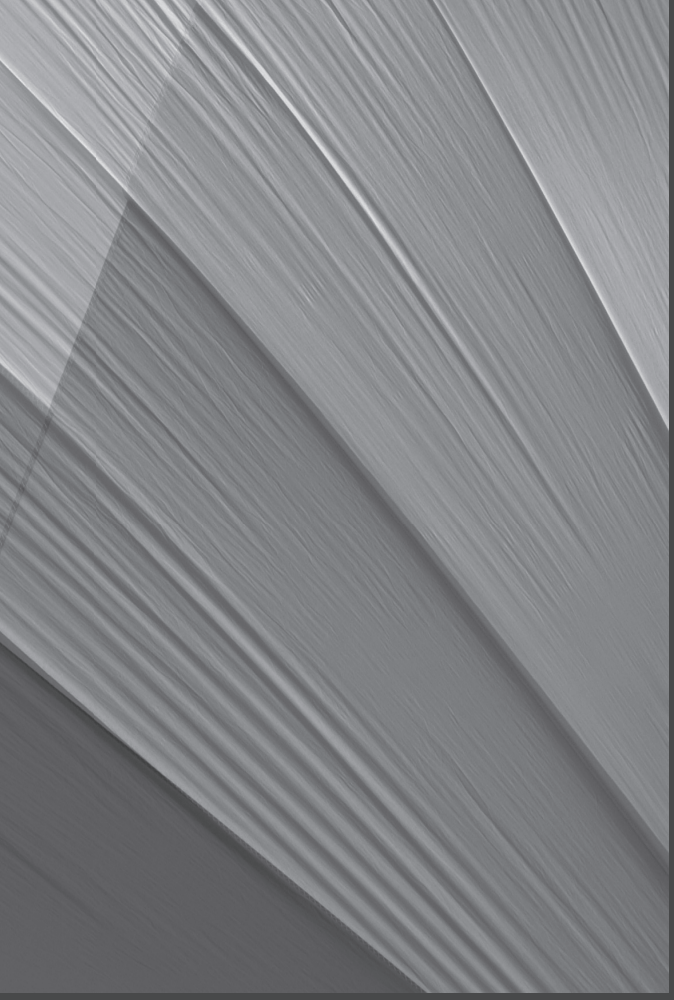

Chapter 1

General introduction

Temperature and sex as prognostic factors in stroke

Epidemiology and etiology of stroke

Anyone can suffer a stroke at any time and the consequences of stroke are often severe. Annually, almost 40 000 people in the Netherlands,[1] and 15 000 000 people worldwide suffer new or recurrent stroke.[2] The burden of stroke is high, with in the Netherlands an estimated 25 000 hospital admissions and 8 500 deaths per year. Also, 240 000 people are living with the consequences of stroke.[1] As the incidence of stroke rises with age, and as the population is aging, the burden of stroke is expected to increase further, especially among women.[3]

In the Netherlands, 12 754 men and 11 246 women were admitted because of stroke in 2012.[1] Mortality was higher in women: almost 2 000 more women died of stroke in 2012 compared with men (5 222 vs 3 302).[1] Women seem to have less favorable outcomes after stroke than men. For example, women have more physical impairments and limitations in activities of daily living (ADL), as measured by the Barthel index.[4, 5]

Strokes can be classified as ischemic stroke (IS) (87%), intracerebral hemorrhage (ICH) (10%) or subarachnoid hemorrhage (3%). The last is beyond the scope of this thesis. Imaging of the brain can distinguish between these types. Ischemic stroke is caused by a reduction of cerebral blood flow caused by occlusion of a cerebral artery or arteriole. The causes of ischemic stroke are diverse, and can be divided in large vessel disease, small vessel disease, cardioembolism and other causes.[6] Intracerebral hemorrhage is caused by rupture of a cerebral vessel. The most frequent risk factors are hypertension and amyloid angiopathy.[7]

Prognostic factors

Prognosis is a prediction of the course of a disease following its onset. It refers to all possible outcomes and the frequency with which these outcomes occur. Prognostic factors are characteristics that may be used to more accurately predict outcome, may improve clinical knowledge and management and may guide new therapies. Clinicians are often asked by patients or their family members to predict outcome after stroke. Hence, it is important to determine reliable prognostic factors. Comparing prognosis in patients with different characteristics may identify prognostic factors. In statistical analyses of clinical trials prognostic factors are important as they can act as confounders or effect modifiers. A confounder is a variable that correlates with both the dependent and independent variable. An effect modifier is a variable that modifies the effect of an intervention on outcome. At the same time, prognostic factors are important in clinical trials because they influence external validation and thereby determine to which patients the results of the trial apply.[8] Considering the foregoing, it remains an important challenge to find new prognostic factors and to further evaluate and explore the importance of known prognostic factors.

Known prognostic factors in stroke

A wide variety of factors are known to predict outcome after stroke, with age and stroke severity being the strongest. With age, chances of unfavorable functional outcome and mortality increase.[9, 10] Older ischemic stroke patients have increased change of dying or being discharged to nursing care facilities.[11] This factor affects women the most, as women are on average 3 years older at stroke onset than men (72 years compared with 69 years[1]). Stroke severity, measured with the National Institutes of Health Stroke Scale (NIHSS), is also a strong predictor of outcome. A higher score on the NIHSS is associated with a higher chance of poor functional outcome.[12] Other prognostic factors are infarct volume[13] and ICH volume,[14] intraventricular hemorrhage in case of ICH[15] and stroke etiology in case of IS.[16] Comorbidities such as atrial fibrillation,[17] migraine, diabetes mellitus[18] and dementia[19] may also play a role.

Temperature as prognostic factor in stroke

Subfebrile temperatures and fever are common in the first hours after stroke onset. Within the first day after stroke onset, a third of the patients has a body temperature higher than 37.5°C.[20] High body temperatures have been related to poor functional outcome and death. A previous study has shown an early (within 24 hours after onset of symptoms) rise in body temperature was associated with unfavorable outcome (aOR 1.30; 95% CI: 1.05 to 1.63) and death (aOR 1.51; 95% CI: 1.15 to 1.98).[21] Increased body temperature may either be a direct effect of stroke or of concurrent infections. Several mechanisms may account for the potential detrimental effect of high body temperature on clinical outcome. Animal studies have suggested that a rise in temperature results in increased ischemic damage through increased cerebral metabolic demands, increased blood-brain barrier permeability, acidosis, and an increased release of excitatory amino acids.[22] Vice versa, induced hypothermia reduced infarct volume and improved functional outcome in animals.[23] Lowering body temperature and prevention of fever are therefore likely candidates to improve functional outcome after stroke in humans as well.

Paracetamol is one of the most commonly prescribed antipyretic drugs. Its antipyretic properties are probably conferred by potent inhibition of prostaglandin production in the central nervous system.[24] Moreover, paracetamol is well tolerated by patients with acute stroke and has virtually no side effects. In patients admitted with acute ischemic stroke, it was shown to reduce body temperature by about 0.3°C within 4 hours after treatment onset when prescribed at a daily dose of six grams.[25] In the Paracetamol (Acetaminophen) in Stroke (PAIS) trial, patients treated with paracetamol showed more improvement on the modified Rankin Scale (mRS) at three-months than those treated with placebo, but this difference was just not statistically significant (adjusted odds ratio (OR) 1.21; 95% CI: 0.97 to 1.51).[26] Based on these findings, we started the Paracetamol (Acetaminophen) in Stroke 2 (PAIS 2) trial.

Sex as prognostic factor in stroke

Women differ from men in numerous ways, including differences in coagulation, immunity, hormone exposure, reproductive factors including pregnancy and childbirth, and social factors.[27] Stroke risk factors also differ between women and men, and may be unique for women, such as reproductive factors, or more common in women, for instance atrial fibrillation and migraine. Women live longer and the lifetime risk of stroke is therefore higher in women than in men (20% vs. 17%).[28] Regarding social factors are women more likely to be living alone and widowed before stroke, and have poorer prestroke functions and more comorbidities. A multitude of studies found that women with IS have worse functional outcome after stroke than men.[4, 5, 27, 29] However, due to a lack of consistency in the selection of confounders in these studies, it is unclear whether this difference is caused by their higher age, poorer prestroke functions and tendency to have more severe strokes or whether other factors, like differences in stroke presentation, acute management and effects of therapies, also play a role. As the burden of stroke is higher in women than men, these are important issues to be addressed. Results on mortality after ICH are conflicting. Women may have a higher mortality or there may be no difference at all.[30, 31] A recent study showed no differences in unfavorable outcome between women and men.[32]

Aims and outline of the thesis

The primary aim is of this thesis is to assess the effect of body temperature management on functional outcome in patients with acute stroke. The secondary aims are to investigate sex differences in the acute management of IS and sex differences in long-term functional outcome after ICH.

Chapter 2 describes the rationale, background, design and results of the Paracetamol (Acetaminophen) in Stroke 2 (PAIS 2) trial, a multicenter, randomized, double-blind, placebo-controlled clinical trial to assess the effect of high-dose paracetamol in patients with acute stroke and a body temperature of 36.5°C or above on functional outcome. In this chapter, I also describe the influence of baseline body temperature on the effect of alteplase on functional outcome. Chapter 3 describes sex-related differences in long-term functional outcome after ICH. I also describe differences between men and women in treatment with intravenous alteplase and sex-related differences in the effect of intra-arterial treatment for acute ischemic stroke. Chapter 4 and 5 provide a general discussion and summary of the results presented in this thesis.

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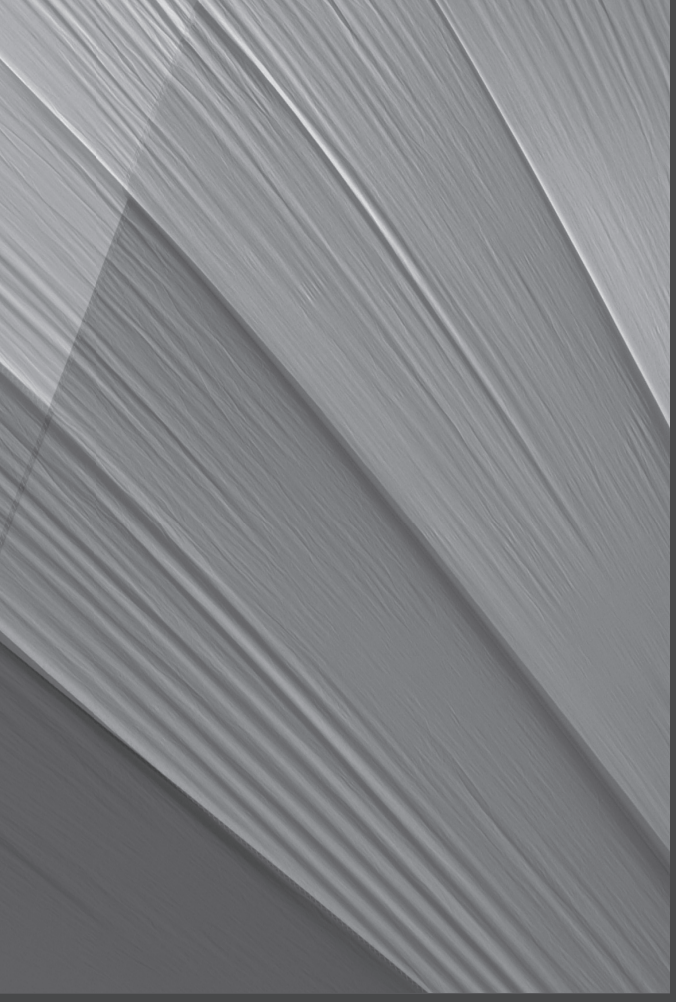
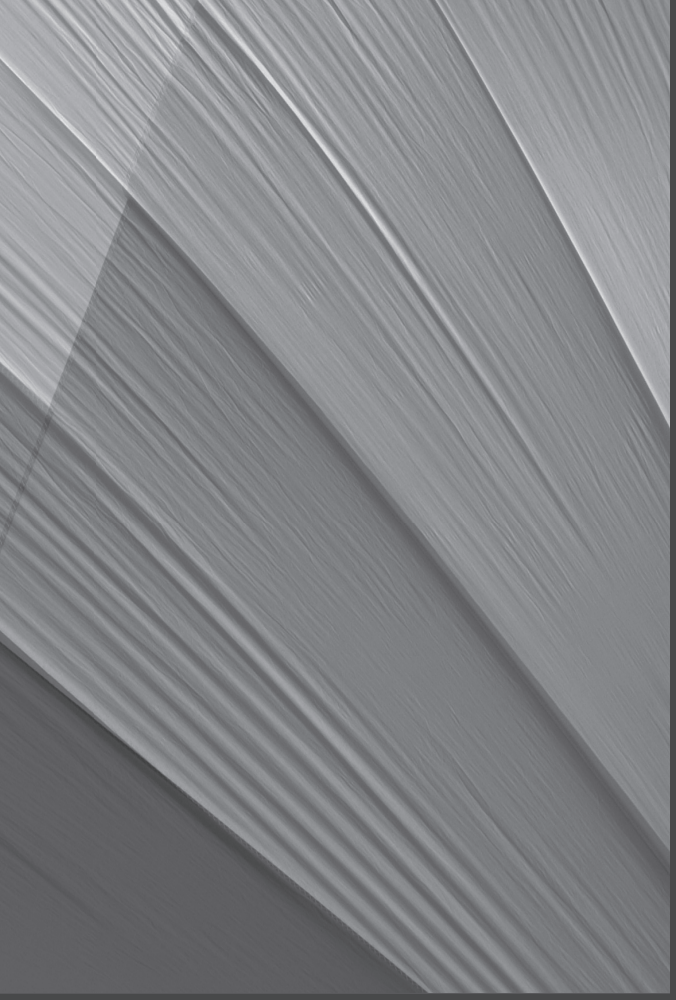


Chapter 2

Temperature as prognostic factor in stroke



Chapter 2.1

Paracetamol (Acetaminophen) in Stroke 2 (PAIS 2): Protocol for a randomized, double-blind placebo-controlled clinical trial

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Abstract

Rationale
In the first hours after stroke onset, subfebrile temperatures and fever have been associated with poor functional outcome. In the first Paracetamol (Acetaminophen) in Stroke (PAIS) trial, a randomized clinical trial of 1400 patients with acute stroke, patients who were treated with high-dose paracetamol showed more improvement on the modified Rankin Scale (mRS) at 3 months than patients treated with placebo, but this difference was not statistically significant. In the 661 patients with a baseline body temperature of 37.0°C or above, treatment with paracetamol increased the odds of functional improvement (odds ratio (OR) 1.43; 95% confidence interval (CI): 1.02-1.97). This relation was also found in the patients with a body temperature of 36.5 degrees Celsius or higher (OR 1.31; 95% CI 1.01-1.68). These findings need confirmation.

Aim
To assess the effect of high-dose paracetamol in patients with acute stroke and a body temperature of 36.5°C or above on functional outcome.

Design
The Paracetamol (Acetaminophen) In Stroke trial 2 (PAIS 2) is a multicenter, randomized, double-blind, placebo-controlled clinical trial. We use a power of 85% to detect a significant difference in the scores on the mRS of the paracetamol group compared with the placebo group at a level of significance of 0.05 and assume a treatment effect of 7%. Fifteen-hundred patients with acute ischemic stroke or intracerebral hemorrhage and a body temperature of 36.5°C or above will be included within 12 hours of symptom onset. Patients will be treated with paracetamol in a daily dose of 6 g or matching placebo for three consecutive days. PAIS 2 has been registered as NTR2365 in The Netherlands Trial Register.

Study outcomes
The primary outcome will be improvement on the mRS at 3 months as analyzed by ordinal logistic regression.

Discussion
If high-dose paracetamol will be proven effective, a simple, safe, and extremely cheap therapy will be available for many patients with acute stroke worldwide.

Introduction

Subfebrile temperatures and fever are common in the first hours after stroke onset. Within the first day after stroke onset, a third of the patients has a body temperature higher than 37.5°C. High body temperatures have been related to poor functional outcome and death.[1] In the Copenhagen study, the risk of poor outcome doubled with every degree Celsius increase in body temperature measured within 12 hours from stroke onset. In the Copenhagen study, the risk of poor outcome doubled with every degree Celsius increase in body temperature measured within 12 hours from stroke onset.[2] Increased body temperature may be either a direct effect of stroke or of concurrent infections. Several mechanisms may account for the potential detrimental effect of high body temperature on clinical outcome. Animal studies have suggested that a rise in temperature results in increased ischemic damage through increased cerebral metabolic demands, increased blood-brain barrier permeability, acidosis, and an increased release of excitatory amino acids.[3] Vice versa, induced hypothermia reduced infarct volume and improved functional outcome in animals.[4] Lowering body temperature and prevention of fever are therefore likely candidates to improve functional outcome after stroke in human beings as well.

Based on observational studies, guidelines for the treatment of patients with acute ischemic stroke[5, 6] or intracerebral hemorrhage[7, 8] advocate the use of antipyretic drugs, such as paracetamol (acetaminophen) in a daily dose of 4 grams, in patients with a body temperature higher than 37.5 or 38.0°C. However, routine prescription of antipyretics may lead to unrecognized infections. Paracetamol is one of the most commonly prescribed antipyretic drugs. Its antipyretic properties are probably conferred by potent inhibition of prostaglandin production in the central nervous system.[9] Moreover, paracetamol is well tolerated by patients with acute stroke and has virtually no side effects in doses up to 6 grams daily, although in patients with chronic liver failure this dose may lead to fatal complications.[10] In patients admitted with acute ischemic stroke, it was shown to reduce body temperature by about 0.3°C within 4 hours after treatment onset when prescribed at a daily dose of 6 grams.[10]

In the first Paracetamol (Acetaminophen) in Stroke (PAIS) trial, a double blind, placebo-controlled, randomized clinical trial of 1400 patients with acute stroke, paracetamol reduced body temperature at 24 hours after start of treatment by 0.26°C (95% CI: 0.18-0.31). Patients treated with paracetamol showed more improvement on the modified Rankin Scale (mRS) at three months than those treated with placebo, but this difference was not statistically significant (adjusted Odds Ratio (aOR) 1.21; 95% Confidence Interval (CI): 0.97-1.51).[11] In the 661 patients with a baseline body temperature between 37.0 and 39.0°C, paracetamol reduced body temperature more effectively than in those with a baseline temperature lower than 37.0°C (0.30°C vs. 0.19°C), increased the odds of improvement (OR 1.43; 95% CI: 1.02-1.97), and was associated with a 9% (95% CI: 1-16%; p=0.02) absolute decrease in the risk of poor outcome, defined as a score on the mRS$>$2. The relation between treatment with paracetamol and functional outcome was also found in the 1080 patients with a body temperature of 36.5°C or higher (OR, 1.31;

95% CI 1.01-1.68). The large majority of these patients had a baseline temperature between 36.5 and 37.5°C, and would not have received paracetamol according to the above-mentioned guidelines.

Although the observed benefit of paracetamol in patients with temperatures higher than 36.5°C is biologically plausible, this should be interpreted with caution, as this concerns a subgroup analysis within a randomized clinical trial in which no overall statistically significant beneficial effect could be demonstrated. Confirmation of this observation in an independent study is therefore needed.[12] For this reason, the aim of PAIS 2 is to assess the effect of high-dose paracetamol on functional outcome in patients with acute stroke and body temperature of 36.5°C or above in the first 12 hours after stroke onset.

Methods

Design

The Paracetamol (Acetaminophen) In Stroke 2 (PAIS 2) trial (Figure 2.1) is a multicenter, randomized, double-blind, placebo-controlled clinical trial of high-dose paracetamol.

Figure 2.1: The PAIS 2 trial logo.

Patients

Patients are eligible for inclusion if they have a diagnosis of ischemic stroke or intracerebral hemorrhage and a body temperature of 36.5°C or higher. Other inclusion and exclusion criteria are listed in Table 2.1.

Randomization

Patients are randomly allocated to paracetamol or an identical placebo. The study drug is provided in white paper boxes, with unique ascending numbers. Treatment allocation is based on a computer-generated list of random numbers with varying block size, linked to a unique treatment number.

Blinding

An independent trial statistician (HL), who is otherwise not involved in the study, will provide the list with allocated random numbers. The pharmacist of the coordinating study center will have a confidential list that indicates the treatment allocation for each randomized patient. Treatment assignment will be masked for all investigators, study personnel, and patients. They will remain blinded to the treatment assignments throughout the trial.

Intervention

Patients are treated with high-dose paracetamol (6 g daily) or matching placebo started within 12 hours after the onset of symptoms and continued for 72 hours or until discharge from hospital. The study medication (active compound or placebo) will be administered through identical suppositories or tablets. The suppositories may be used for patients with swallowing difficulties who do not have a nasogastric tube yet. They will therefore predominantly be used in the first 24 hours after admission, at the discretion of the treating physician and stroke nurse. To enhance compliance in patients who do not suffer from swallowing difficulties and do not need (continuation of) a nasogastric tube after the first 24 hours, treatment will be continued with tablets up to 72 hours of treatment. In patients with persisting swallowing deficits after the first 24 hours, tablets will be crushed and administered via the nasogastric tube. In the first 3 days of enrollment, concurrent treatment with open-label paracetamol is not allowed. For the treatment of pain, opioids are recommended. If fever occurs during treatment with trial medication, the source of fever should be ascertained and treated. If the treating physician thinks the fever should also be treated with open-label paracetamol, the trial medication should be stopped. The use of other antipyretic medication is allowed.

Study procedures

Before inclusion, the diagnosis of ischemic stroke or intracerebral hemorrhage will be confirmed by CT or MRI. In case of clinical suspicion of liver disease, liver enzymes will be measured and the patient will only be included if the lever enzymes are lower than twice the upper limit of normal values. The randomization procedure will be simple. Patients who meet the inclusion criteria and who have given written informed consent are assigned a box containing the study medication. The box is labeled with a unique study number. The local investigator will have to complete a short web-based form to include the patient into the study. The anonymous patient

data will be automatically added to the study database. An automatically updated log of the randomized patients will be available for each participating center.

Baseline data will include demographics, time of stroke onset, stroke severity (measured by the National Institutes of Health Stroke Scale (NIHSS)), body temperature at inclusion, pulse rate and blood pressure, results of CT scanning and vascular history and vascular risk factors. In selected centers, a blood sample will be taken on admission and 24 hours later, to assess markers of inflammation. During hospital stay (serious) adverse events will be reported to the trial office. Discharge data will include discharge destination, study compliance, body temperature and blood pressure at 24 hours, laboratory findings at day 2-3, stroke subtype and NIHSS at discharge. Follow-up will be carried out by the trial office and will be conducted through telephone interviews. Outcome assessments will be made through structured interviews by experienced research nurses of the trial center who will be stationed at the trial office of the neurovascular division of the department of neurology of the Erasmus MC University Medical Center Rotterdam (Figure 2.2). Patient data will be entered through a web-based form. Local investigators will be asked to fill out a one-page web-form at inclusion and a one-page web-form at one week or at discharge. All data are stored in central database, which is continuously updated.

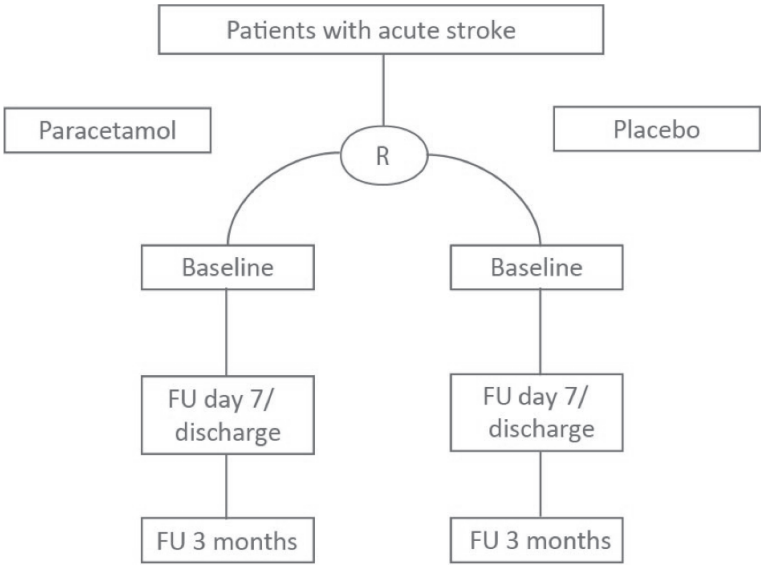

Figure 2.2: Study design.

Primary outcomes

The primary outcome will be the shift on the score on the modified Rankin Scale at three months. No dichotomization is necessary, as we will analyze shifts on the whole scale.

Secondary outcomes

Secondary outcome measures will include: poor outcome, defined as mRS>2 at three months; score on the Barthel index (BI), Telephone Interview for Cognitive Status (TICS) score and Euroqol 5D (EQ5D) score at three months, body temperature and markers of inflammation at 24 hours after start of treatment.

Data monitoring and safety committee

An independent data monitoring and safety committee (DMSC), consisting of two experienced neurologists and clinical investigators and an independent statistician, will meet at least annually. During the period of recruitment into the study, unblinded but strictly confidential interim analyses of in-hospital mortality and of any other information that is available on major outcome events, including serious adverse events, will be supplied every year to the chairman of the DMSC, along with any other analyses that the committee may request. In the light of these analyses, the DMSC will advise the chairman of the steering committee about continuation of the trial. These interim analyses will be prepared by the trial statistician, who is unblinded. However, the trial statistician is otherwise not involved with the execution of the trial as long as it runs.

Statistical analyses

Statistical analyses will be performed according to the intention-to-treat principle. The primary effect estimate will be the common odds ratio of improvement on the mRS assessed by means of multiple ordinal logistic regression, and be expressed as an odds ratio with 95% confidence interval. In order to increase the power of the study, adjustments will be made for chance imbalances between prognostic factors, including stroke severity (NIHSS), stroke type, ischaemic stroke subtype (lacunar versus non-lacunar), treatment with alteplase, previous stroke, atrial fibrillation, and diabetes mellitus.

We will test for heterogeneity of treatment effect across important clinical subgroups of patients: stroke type (ischemic versus hemorrhagic stroke), severity (dichotomized at the median NIHSS score), ischemic stroke subtype (lacunar versus non-lacunar ischemic stroke), time of onset of treatment (within 6 hours versus between 6 and 12 hours after onset of symptoms), treatment with rtPA and body temperature.[13, 14]

Sample size

The study will be powered to detect a statistically significant shift in the distribution of the scores on the mRS at three months, assuming an effect that leads to a 7% absolute increase in the cumulative proportion of patients with mRS between 0 and 2 in the paracetamol group, compared with patients on placebo. We base the expected distribution of outcome categories on the placebo arm of the PAIS trial.[11] Figure 2.3 shows the expected distributions of mRS categories. A total study size of 1410 patients (2x705 pts) allows for a power (1-beta) of 85% to

detect a significant difference in the scores on the mRS of the paracetamol group compared with the placebo group at a level of significance of 0.05.[15] The total study size that will be needed is rounded to 1500 patients.

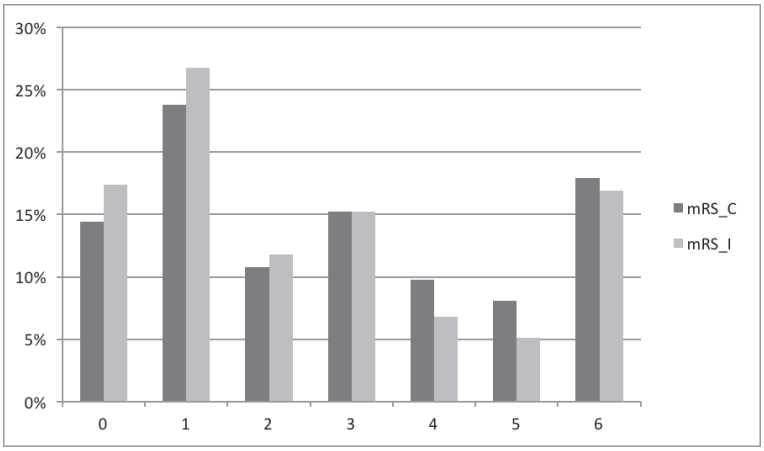

Figure 2.3: Distribution of scores on the modified Rankin Scale in the placebo group (mRS_C) based on data from PAIS (11) and estimated distribution of modified Rankin Scale scores in the intervention group (mRS_I) based on the assumption of a 7% shift in the cumulative probability of mRS 0-2.

Study organization and funding

PAIS 2 is an independent academic trial. This study is run by an executive committee that consists of members of the steering committee (SC) who are actually involved in carrying out the study on a daily basis (Table 2.2). The recruitment period will be 4.5 years. The central coordination is performed by the SC. They will meet at least once a year, and monitor the progress of the study. Decisions regarding continuation of the trial, amendments to the protocol, and publication of its results (taking into account the advice of the DMSC) will be taken by the SC. The SC strives for consensus decisions, but may have to settle for majority votes. The trial is funded by the Stichting Neurovasculair Onderzoek Rotterdam (Foundation for Neurovascular Research Rotterdam); additional support has been requested.

Public disclosure and publication policy

The investigators aim at public disclosure and publication of the research data in highly-ranked, international peer-reviewed, scientific journals. Results of this research project will be disclosed unreservedly. All authors will have to comply with the Vancouver protocol for authorship.

Discussion

Several observational studies have demonstrated a strong relationship between increased body temperature in the first hours after symptom onset and poor functional outcome after stroke.[1, 11] It remains unclear whether this relation is causal, and more importantly, whether lowering of body temperature and prevention of fever increases the likelihood of a favorable functional outcome. In the first PAIS trial, treatment with paracetamol increased the odds of improvement at 3 months in patients with a baseline body temperature between 37.0 and 39.0°C (OR 1.43; 95% CI: 1.02-1.97) and was associated with a 9% (95% CI: 1-16%, p=0.02) absolute decrease in the risk of unfavorable outcome. Because these findings are based on subgroup analyses, confirmation is needed. We updated a Cochrane systematic review of temperature-lowering therapy for acute ischemic stroke with the results of PAIS.[16] The results of the review are now dominated by the results of this study, and the estimate of the treatment effect leaves room for a substantial reduction in poor outcome (OR 0.93; 95% CI: 0.57-1.50). It should be noted however, that this systematic review combined physical and pharmacological strategies to reduce body temperature and included several phase 2 studies that had a long time window after symptom onset for the start of treatment. The last issue is crucial because the association of body temperature with clinical outcome is likely limited to the first 12-24 hours of stroke onset, and the amount of salvageable brain tissue will rapidly diminish over time.[1] We have previously shown that paracetamol reduces body temperature in patients with acute stroke within 4 hours after start of treatment.[17] It seems therefore rational to include patients up to 12 hours after onset of stroke symptoms.

Whether increased body temperature in acute stroke contributes to poor outcome or is just a marker of severe stroke remains unclear. Hyperthermia has been suggested to be an epiphenomenon of the extent of cerebral damage. Animal studies suggest that hyperthermia is associated with larger infarct size.[18] Body temperature may also be increased because of stroke-associated infections and central dysregulation.

Increased body temperature may also be an indicator of local cerebral inflammation and neuronal tissue injury. Cyclo-oxygenase-2 (COX-2) isoenzymes play an important role in temperature regulation at the level of the hypothalamus.[19] In animal studies, COX-2 mRNA and protein are upregulated 12 to 24 hours after the onset of focal cerebral ischemia.[20] COX-2 is expressed in neurons and vascular cells at the border of the ischemic territory.[21] COX-2 has also been found in the human brain after stroke.[22] COX-2 inhibition reduces neuronal damage in animal models of focal and global cerebral ischemia.[23] These observations raise the possibility that COX-2 reaction products, such as prostaglandin 2 (PGE2), contribute to cerebral ischemic injury, either directly, or through temperature elevation. It has been shown that paracetamol is effective in blocking cerebral COX-2 and lowering cerebral PGE2 production.[24] This indicates the possibility of a direct neuroprotective effect of paracetamol in case of ischemic stroke. Early after ICH also a strong pro-inflammatory response has been observed. Therefore, our hypothesis is that lowering cerebral COX-2 also has a neuro-protective effect in ICH.

With the PAIS trial, we showed that a large phase III trial with high-dose paracetamol is feasible in The Netherlands, although the recruitment rate was lower than expected. Fourteen hundred patients were included instead of the 2500 patients that were aimed for. Lessons learned from successful participating study centers in the PAIS trial were in particular that inclusion of patients has to be made as simple as possible. The PAIS 2 trial will therefore use a simple web-based randomization and data entry procedure. In addition, to enhance compliance, suppositories will be made available for rectal administration for the first 24 hours, after which time paracetamol will be administered orally. With oral administration, peak plasma levels are reached after 30 minutes to 1 hour; with rectal administration after 1 hour. Differences in pharmacokinetics between oral and rectal administration are therefore negligible. If oral administration is applied whenever possible, this reflects daily clinical practice best. This is the most important reason why intravenous administration of paracetamol has not been considered in this trial. In addition, intravenous administration is much more expensive and peak plasma levels are reached just slightly earlier (10-20 minutes after administration) when compared with oral administration.

We use ordinal logistic regression to estimate a treatment effect on the modified Rankin Scale. A common odds ratio for improvement on the scale will be estimated. Simulation studies have indicated that ordinal logistic regression is a more powerful method for analysis of trials with ordered categorical outcome data when compared with the more commonly used sliding dichotomy approach, and have proven its robustness against violations of the proportional odds assumption.[25]

The PAIS trial also showed that treatment with high-dose paracetamol is safe. The number of adverse events was similar in the treatment and placebo groups. We will closely monitor the adherence to inclusion and exclusion criteria in order to prevent inclusion of patients at increased risk of liver failure.

In conclusion, PAIS 2 will be a simple but highly relevant clinical trial. The treatment strategy tested is safe and inexpensive. If high-dose paracetamol will be proven beneficial, a simple, safe, and extremely cheap therapy will be available for many patients with acute stroke worldwide.

Table 2.1: Inclusion- and exclusion criteria.

Inclusion criteria

- a clinical diagnosis of ischemic stroke or intracerebral hemorrhage, confirmed by CT or MRI scan
- a measurable deficit on the National Institutes of Health Stroke Scale (NIHSS)
- the possibility to start treatment within 12 hours of symptom onset (for patients who noticed symptoms when awaking from sleep, the time last seen well is taken as the time of onset of symptoms
- body temperature of 36.5°C or higher
- age of 18 years or older
- signed informed consent

Exclusion criteria

- history of liver disease or alcohol abuse
- liver enzymes (ASAT, ALAT, AP or gamma-GT) increased above twice the upper limit of normal values
- allergy to paracetamol
- death appearing imminent at the time of inclusion
- any pre-stroke impairment that has led to dependency (modified Rankin Scale (mRS)>2) and therefore interferes with the assessment of functional outcome

Table 2.2: PAIS 2 study group. The steering committee consists of the principal investigators, local principal investigators, other study group members and the study coordinator. The executive committee consists of the principal investigators, study coordinator and trial manager. The core writing committee consists of the principal investigators and the study coordinator. The writing committee consists of the core writing committee, the local principal investigators and the other study group members.

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Data monitoring and safety committee (DMSC)	M. (Rien) Vermeulen, professor of neurology, Academic Medical Center Amsterdam, Amsterdam
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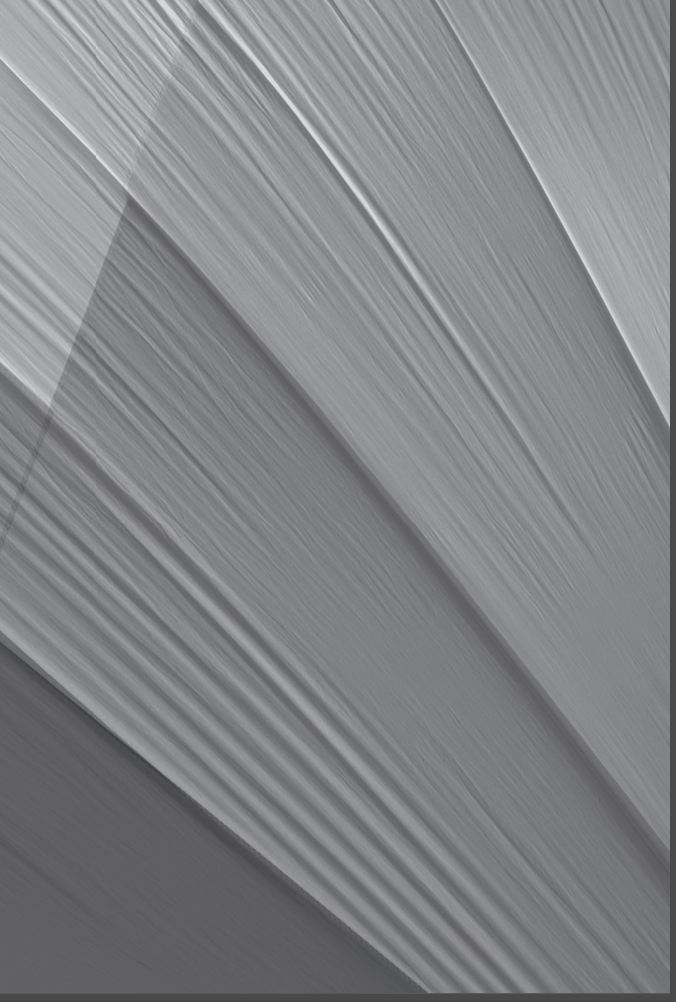

Chapter 2.2

Paracetamol (Acetaminophen) in Stroke 2 (PAIS 2): Results of a randomized, double-blind placebo-controlled clinical trial

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Abstract

Background
Subfebrile body temperature and fever in the first days after stroke are strongly associated with unfavorable outcome. A sub-group analysis of a previous trial suggested that early treatment with paracetamol may improve functional outcome in patients with acute stroke and a body temperature of 36.5°C or higher. In the present trial, we aimed to confirm this finding.

Methods
PAIS 2 was a multicenter, randomized, double-blind, placebo-controlled clinical trial. We aimed to include 1500 patients with acute ischemic stroke or intracerebral hemorrhage within 12 hours of symptom onset. Patients were treated with paracetamol in a daily dose of 6 g or matching placebo for 3 consecutive days. The primary outcome was functional outcome at 3 months, assessed with the modified Rankin Scale (mRS) and analyzed with multivariable ordinal logistic regression. Due to slow recruitment and lack of funding the study was stopped prematurely.

Results
Between December 2011 and October 2015, we included 256 patients, of whom 136 (53%) were allocated to paracetamol. In this small sample, paracetamol had no effect on functional outcome (acOR 1.15; 95% CI: 0.74-1.79). There was no difference in the number of serious adverse events (paracetamol n=35 (26%) versus placebo n=28 (24%)).
Conclusion
Treatment with high-dose paracetamol appeared safe. The effect of high-dose paracetamol on functional outcome remains uncertain. Therefore, a very large trial of early treatment with high dose paracetamol is still needed.

Background

High body temperatures after stroke are associated with unfavorable outcome.[1] The risk of poor outcome doubles with every degree Celsius increase in body temperature measured within the first 12 h from stroke onset.[2]

Animal studies have shown that high body temperature results in increased ischemic damage through increased cerebral metabolic demands, damage of the blood–brain barrier, acidosis, and an increased release of excitatory amino acids.[3] Lowering body temperature and prevention of fever are therefore simple and promising approaches to improve functional outcome after stroke.

There is currently no evidence that strategies to prevent or treat high body temperature reduce case fatality and improve functional outcome after stroke. US stroke guidelines recommend the use of antipyretic drugs when body temperature exceeds 38.0°C.[4] European (ESO) guidelines do not make any recommendation for treating hyperthermia as a means to improve outcome in patients with ischemic stroke (IS) and recommend further research.[5] A possible concern with routine prescription of antipyretic drugs is that this may delay the diagnosis of an infection.

Paracetamol is one of the most commonly prescribed antipyretic drugs. It blocks cerebral COX-2 and lowers cerebral PGE2 production.[6] In patients with acute stroke, treatment with high-dose paracetamol reduces body temperature about 0.3°C within 4 hours after start of treatment.[7] A post-hoc analysis of the Paracetamol (Acetaminophen) in Stroke (PAIS) trial suggested that treatment with high-dose paracetamol within 12 hours after stroke onset might improve functional outcome in patients with a body temperature of 36.5°C or higher (OR 1.31; 95% CI: 1.01–1.97).[8, 9] The large majority of these patients (n=1.022 (73%)) had a baseline temperature between 36.5°C and 38.0°C, and would not have received paracetamol according to the aforementioned guidelines. Although the observed benefit of paracetamol in patients with temperatures higher than 36.5°C is biologically plausible, this should be interpreted with caution, as this concerns a subgroup analysis within a randomized clinical trial in which no overall statistically significant beneficit could be demonstrated. Confirmation of this observation in an independent study is therefore needed.[10] Hence, the aim of PAIS 2 was to assess the effect of high-dose paracetamol on functional outcome in patients with acute stroke and a body temperature of 36.5°C and higher in the first 12 hours after stroke onset.

Methods

Study design and randomization

PAIS 2 was a multicenter, randomized, double-blind, placebo-controlled clinical trial. The study protocol has been published previously.[8] In short, patients were randomly allocated to treatment with high-dose paracetamol (intervention group) or placebo (control group) in a 1:1 allocation ratio. Treatment allocation was based on a computer-generated list of random numbers with varying block size, linked to a unique treatment number. The list was provided by the independent trial statistician.

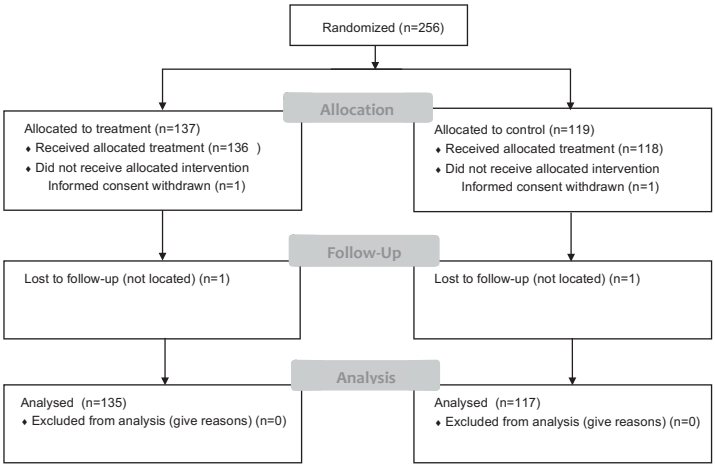

Figure 2.4: CONSORT 2010 Flow Diagram.

Local investigators enrolled the patients and patients were assigned a box, labeled with a unique study number, containing study medication. Treatment allocation was masked for everyone except the trial statistician, throughout the trial. The study was approved by a central research ethics committee and the research board of each of the 11 participating centers. All patients or their legal representatives provided written informed consent. The trial was registered in the Netherlands Trial Register (NTR2365) and funded by the Foundation for Neurovascular Research Rotterdam.

Patients

Patients were eligible for inclusion if they had a diagnosis of ischemic stroke (IS) or intracerebral hemorrhage (ICH), a body temperature of 36.5°C or higher, were 18 years or older, and could be treated within 12 hours after stroke onset. Exclusion criteria were a history of liver disease or alcohol abuse, allergy to paracetamol, liver enzymes (ASAT, ALAT, AP, or gamma-GT) increased above twice the upper limit of normal values, death appearing imminent at the time of inclusion, and any pre-stroke impairment that has led to dependency (modified Rankin Scale > 2) and therefore interfering with the assessment of functional outcome.

Procedures

Before inclusion, the diagnosis of ischemic stroke or ICH was confirmed by computed tomography (CT) or magnetic resonance imaging (MRI). Patients were treated with high-dose paracetamol (6 g daily) or matching placebo, started within 12 hours after onset of symptoms and continued for 72 hours or until discharge from hospital if earlier. The study medication (active compound and placebo) was administered through identical tablets or suppositories. In the first 3 days of

enrollment, concurrent treatment with open-label paracetamol was not allowed. The use of other antipyretic medication was allowed. Baseline characteristics were collected, and at 3 months outcome scores and (serious) adverse events, including infections, were assessed by telephone interview by experienced research nurses of the trial office of the neurovascular division of the Erasmus MC University Medical Center Rotterdam. For more details, we refer to the study protocol.[8]

Outcome

The primary outcome was the shift on the modified Rankin scale (mRS) score at 90 days.[11] Secondary outcomes were body temperature at 24 hours after start of treatment, unfavorable outcome at 90 days, defined as a score on the mRS of 3 or more, activities of daily life, measured with the Barthel Index (BI)[12] and quality of life, measured with the EuroQol 5D 3L score at 90 days, estimated with the Dutch tariff.[13]

Sample size

We assumed an effect of paracetamol that leads to a 7% absolute increase in the cumulative proportion of patients with mRS between 0 and 2 in the paracetamol group, compared with placebo. We used a power (1-beta) of 0.85 to detect a significant difference in the scores on the mRS of the paracetamol group compared with the placebo group at a level of significance of 0.05. The total study size needed was calculated and rounded to 1500 patients.

Statistical analysis

Statistical analyses were performed according to the intention-to-treat principle. The primary effect estimate was the common Odds Ratio (cOR) of improvement on the mRS score assessed by means of multiple ordinal logistic regression analysis. A cOR larger than 1 would indicate a positive effect of treatment, with shift towards better functional outcome. Adjustments were made for age, NIHSS at admission and stroke type. For the continuous variables EuroQol 5D 3L and body temperature difference we used linear regression analysis. We tested for heterogeneity of treatment effect across important clinical subgroups of patients, as defined in the study protocol.[8] We also performed a systemic review of literature and updated a previous meta-analysis of paracetamol in acute stroke, both for all patients and for patients with a body temperature of 36.5°C or above (when data available).[14] Data were added to the meta-analysis at individual patient level. Favorable outcome in this meta-analysis was defined as an mRS 0-2. The effect estimate was Odds Ratio, with an OR larger than 1 indicating a positive effect of treatment. All analyses were performed with STATA 14 (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP.)

Results

Baseline characteristics

Recruitment started in December 2011 and inclusion was halted on October, 1st 2014 because of slow recruitment and lack of funding. The trial ended on January, 1st 2015. In total, 256 patients were randomized, of whom 136 (53%) were allocated to paracetamol (Figure 2.4). Two patients withdrew informed consent. Mean age was 69 years in both groups. Baseline characteristics did not differ between the treatment groups, except for sex (paracetamol 50% men; placebo 64% men) (Table 2.3). Two patients (0.4%) were lost to follow-up.

Table 2.3: Baseline characteristics by treatment allocation.

	Paracetamol (n=136)	**Placebo (n=118)**
Medical history		
Age, years (mean, SD)	69 (14)	69 (13)
Male sex	68 (50%)	76 (64%)
Hypertension	78 (57%)	65 (55%)
Atrial fibrillation	15 (11%)	17 (14%)
Diabetes mellitus	20 (15%)	20 (17%)
Previous stroke	33 (24%)	22 (19%)
Smoking	61 (45%)	55 (47%)
Pre mRS score (median, IQR)	0 (0 -0)	0 (0 -0)
Physical examination		
NIHSS at admission (median, IQR)	6 (3-8)	5 (2-8)
Systolic blood pressure at admission, mmHg (median, IQR)	154 (140-170)	160 (144-170)
Diastolic blood pressure at admission, mmHg (median, IQR)	81 (70-90)	82 (70-90)
Heart rate at admission, beats/min (median, IQR)	79 (69-89)	79 (70-89)
Body temperature at admission, °C (median, IQR)	36.9 (36.7-37.2)	36.8 (36.6-37.2)
CRP at admission, mg/L (median, IQR)	3 (1-8)	3 (1-7)
Stroke type		
Ischemic stroke	127 (93%)	107 (91%)
Ischemic stroke subtype*		
Large vessel disease	22 (17%)	19 (18%)
Cardiac embolism	21 (17%)	16 (15%)
Small vessel disease	26 (20%)	21 (20%)
Other	6 (5%)	11 (10%)

Unknown	52 (41%)	40 (37%)
Treatment characteristics		
Treatment with rt-PA	63 (47%)	53 (45%)
Time from onset to randomization, minutes (median, IQR)	363 (243-570)	390 (270-600)
Treatment with study medication within 6 hours after onset of symptoms	68 (50%)	63 (53%)

SD=standard deviation; mRS=modified Rankin Scale; IQR=interquartile range; NIHSS=National Institutes of Health Stroke Scale, CRP=C-reactive protein, rt-PA=recombinant tissue plasma activator.

*Based on definitions of the Trial of ORG10172 in Acute Stroke Therapy (TOAST) criteria.

Clinical outcomes

We found no difference in improvement on the mRS score between patients treated with paracetamol and those treated with placebo (cOR 1.02; 95% CI: 0.66 to 1.58) (Figure 2.5). Adjustment for age, NIHSS at admission and stroke type had no appreciable effect (acOR 1.15; (95% CI: 0.74 to 1.79). The treatment had no effect on secondary outcome measures (Table 2.4) except for body temperature at 24 hours, which was significantly lower in paracetamol-treated patients compared with control patients (median body temperature at 24 hours in paracetamol treated patients 36.8°C vs 37.0°C in placebo treated patients, mean temperature difference -0.27°C; 95% CI -0.44 to -0.11°C). We did not find a statistically significant effect of paracetamol on functional outcome in any pre-specified subgroup (Figure 2.6).

Safety outcomes

Mortality at 3 months did not differ between the paracetamol and placebo groups (n=11 (8%) vs. n=15 (13%) respectively). There were no cases of liver failure, and the rate of infections was similar in both groups (Table 2.5).

Meta-analysis

The overall OR in the meta-analysis of all controlled trials with paracetamol after acute stroke did not indicate a difference between paracetamol and placebo (OR 1.04; 95% CI: 0.87 to 1.25)(Figure 2.7). Figure 2.8 shows the meta-analysis of data from patients with a baseline temperature of 36.5°C and higher, with similar results (overall OR 1.08; 95% CI: 0.88-1.33).

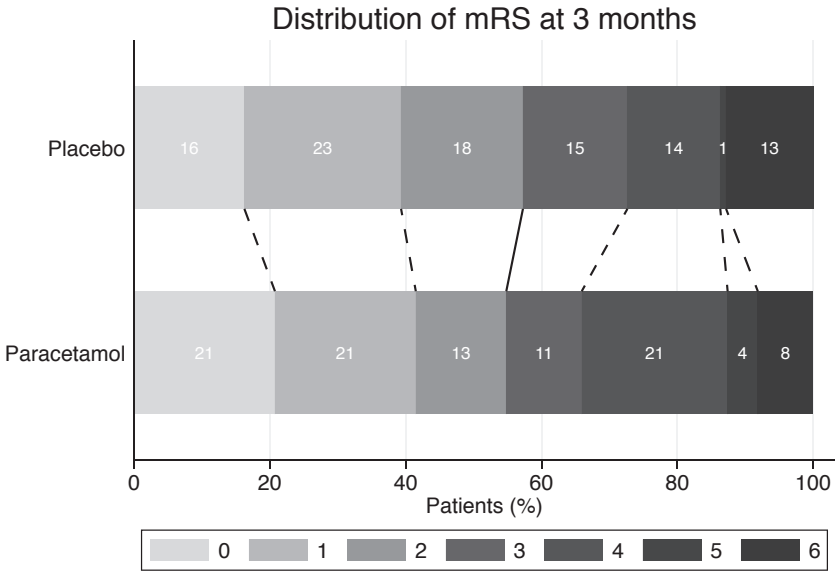

Figure 2.5: Effect of the intervention on primary outcome.

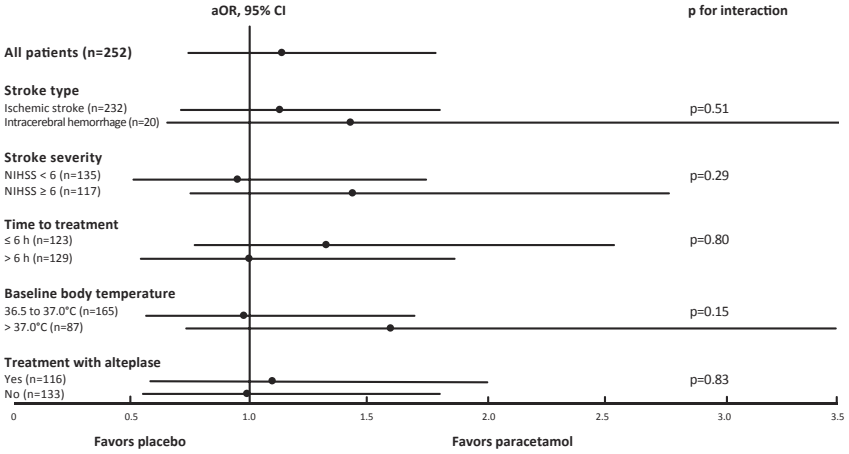

Figure 2.6: Subgroup Analysis. Adjusted Odds Ratio (aOR; black dots), 95% Confidence Interval (CI; horizontal lines), P values for the interaction between the treatment effect and any subgroup variable.

Table 2.4: Clinical and safety outcomes and treatment effects.

	Paracetamol (n=135)	Placebo (n=117)	Effect variable	Unadjusted value (95%CI)	Adjusted value (95%CI)
Primary outcome					
mRS at 90 days (median, IQR)	2 (1-4)	2(1-4)	Common odds ratio	1.02 (0.66 to 1.58)	1.15 (0.74 to 1.79)
Secondary outcome					
Favorable outcome at 90 days (mRS 0-2)	74 (54%)	67 (57%)	Odds ratio	0.91 (0.67 to 1.81)	1.01 (0.55 to 1.78)
Barthel index at 90 days (BI=100)	71 (52%)	64 (54%)	Odds ratio	0.92 (0.56 to 1.51)	1.02 (0.58 to 1.80)
EQ-5D at 90 days (median, IQR)	7 (5-9)	7 (5-9)	Beta	0.15 (-0.49 to 0.78)	-0.16 (-0.72 to 0.40)
Body temperature at 24 hours, °C (median, IQR)	36.8 (36.7-37.3)	37.0 (36.7-37.3)	Beta	-0.22 (-0.37 to -0.06)	-0.25 (-0.40 to -0.11)
Body temperature difference baseline-24 hours (mean, SD)	-0.09 (0.61)	0.18 (0.62)	Beta	-0.27 (-0.44 to -0.11)	-0.26 (-0.40 to -0.12)

IQR=interquartile range, mRS=modified Rankin Scale, EQ-5D=Euroqol 5D.

Adjustment were made for age, NIHSS at admission and stroke type.

Table 2.5: Safety outcomes.

	Paracetamol (n=135)	Placebo (n=117)
Safety variables		
Death within 7 days	5 (4%)	7 (6%)
Death within 90 days	11 (8%)	15 (13%)
Serious adverse events		
Any serious adverse event	35 (26%)	28 (24%)
Pneumonia	4 (3%)	3 (3%)
Urinary tract infection	3 (2%)	3 (2%)
Other infection	0 (0%)	1 (1%)
Liver failure	0 (0%)	0 (0%)
Gastro-intestinal hemorrhage	1 (1%)	0 (0%)

Discussion

In this prematurely terminated, small trial in patients with acute stroke and a body temperature of 36.5°C and higher, we found that treatment with high-dose paracetamol was safe, but did not improve functional outcome at 3 months. Treatment with paracetamol lowered body temperature by 0.3°C. Therapeutic hypothermia has been accepted as an effective treatment after cardiac arrest[15] and in hypoxic-ischemic encephalopathy in neonates.[16] An important issue in the ongoing discussion on cardiac arrest is the optimal target temperature. A recent study showed that a target temperature of 36.0°C may be non-inferior to therapeutic hypothermia aiming at a body temperature of 33.0-34.0°C.[17] In that study, patients who were maintained at a target of 36.0°C seemed to have less serious side effects, including hypokalemia, pneumonia and bleeding complications, but these differences were not statistically significant, except for hypokalemia (p=0.02).[17] Other subjects of debate are time to initiation of hypothermia, time to achieve target temperature, treatment duration and rewarming methods.[18] These issues are also applicable to therapeutic hypothermia in acute stroke.[19]

Whereas clinical studies assessing the effect of therapeutic hypothermia on outcome after acute stroke have so far been too small to demonstrate a benefit, pharmacological treatment aimed at maintaining normothermia may be an alternative. Six studies have been performed on pharmacological hypothermia in acute stroke. Five of these studies used paracetamol as antipyretic drug.[14] These studies, except for PAIS, were small and designed to test safety and feasibility. They showed no effect on outcome. In PAIS, more patients in the paracetamol group than in the placebo group improved beyond expectation, but this effect was just not statistically significant (aOR 1.20; 95% CI: 0.96–1.50). In a post-hoc analysis of a subgroup of patients with a body temperature of 37.0°C or above, treatment with paracetamol was associated with improved outcome. A previous meta-analysis using a dichotomized outcome showed a trend towards a favorable

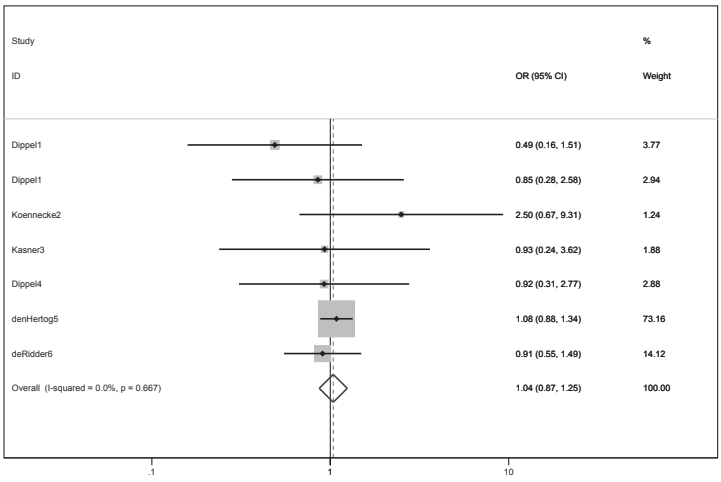

Figure 2.7: Updated meta-analysis of all studies with paracetamol after acute stroke in all patients. Favorable outcome is defined as an mRS 0-2. An OR larger than 1 indicates a positive effect of treatment.

References figure 2.7

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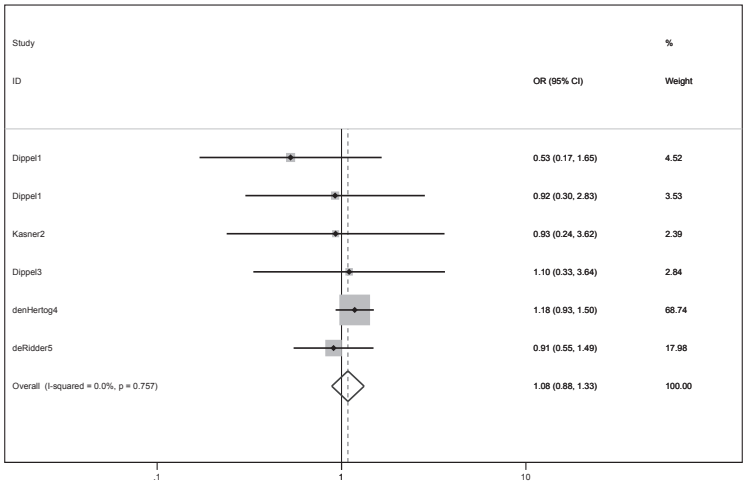

Figure 2.8: Updated meta-analysis of all studies with paracetamol after acute stroke in patients with a body temperature of 36.5 degrees and higher. Favorable outcome is defined as an mRS 0-2. An OR larger than 1 indicates a positive effect of treatment.

References figure 2.8

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effect of temperature-lowering treatment.[14] Furthermore, the QASC trial showed that early detection and management of fever (body temperature > 37.5°C), swallowing difficulties and hyperglycaemia led to a significantly higher chance of being alive or independent during follow up.[20] This trial was however not designed to determine which of these interventions made the difference.

The results of PAIS 2 are neutral. However, because of serious lack of power we cannot exclude an alternative result. We updated the previous meta-analysis with the results of PAIS 2 (Figure 2.7) and found no significant difference between temperature-lowering treatment and control in the proportion of patients who were alive and independent (mRS2)(overall OR 1.04; 95% CI: 0.87 to 1.25). We also performed a meta-analysis of data of all patients with a baseline temperature of 36.5°C or above (Figure 2.8), which showed comparable results (overall OR 1.08; 95% CI: 0.88-1.33). However, these analyses were binary and did not have the advantage of ordinal regression analysis.

The main limitation of this study is that it was preliminary stopped and therefore the study is strongly underpowered to detect a difference in functional outcome after treatment with high-dose paracetamol in acute stroke. Patient recruitment was low, which was most likely caused by the low intensity of trial coordination activities, such as creating awareness for the study, providing information, feedback and support to the participating centers. This was a consequence of the low budget. The simultaneous conduct of several large randomized clinical trials of acute stroke treatment in the Netherlands also added to the low recuitment rate. A strength of the study is that it allowed us to update the meta-analysis with the results of this study.

The results of PAIS 2 provide no evidence for the routine use of high dose paracetamol in acute stroke. Although the relative effect may be low, an absolute risk reduction in unfavorable outcome of 5% may be clinically relevant, considering the large number of patients involved and the simple, safe, and cheap nature of the therapy. Taking into account the results of the updated meta-analysis, such a moderate effect could not be excluded. Further large clinical trials are necessary to further explore this finding. This will be assessed in the trial PRECIOUS trial: Prevention of Complications to Improve Outcome in elderly patients with Stroke (www.precious-trial.eu).

Conclusion

Treatment with paracetamol after acute stroke appears safe. However, the effect of treatment with high-dose paracetamol after acute stroke remains unclear. A very large trial of early treatment with paracetamol after acute stroke is needed to answer this question.

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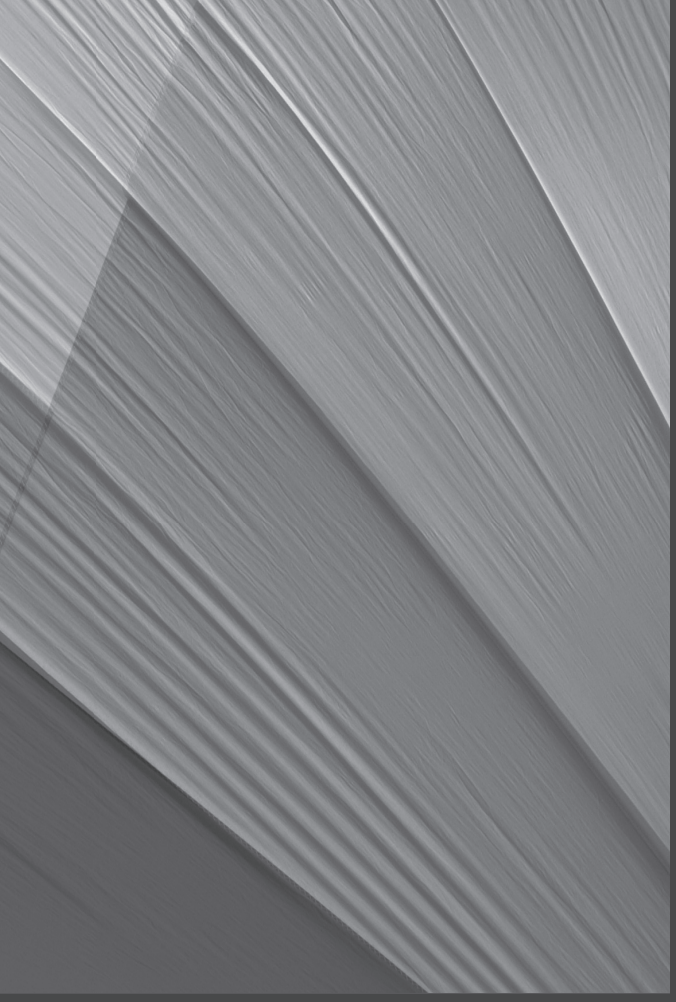

Chapter 2.3

Increased benefit of alteplase in patients with ischemic stroke and a high body temperature

Inger de Ridder, Heleen den Hertog, Maarten van Gemert, Diederik Dippel, and Bart van der Worp, for the PAIS investigators.

Cerebrovascular Diseases. (2013);35:60-63.

Abstract

Background
In observational studies, high body temperature has been associated with unfavorable outcome. In in vitro studies, the fibrinolytic activity of alteplase decreased 5% per degree Celsius reduction in temperature. The modifying effect of body temperature on treatment with alteplase in patients with acute ischemic stroke is unclear. We assessed the influence of baseline body temperature on the effect of alteplase on functional outcome in patients with acute ischemic stroke, included in the Paracetamol (Acetaminophen) in Stroke (PAIS) trial.

Methods
PAIS was a randomized, double-blind clinical trial to assess the effect of high-dose paracetamol on functional outcome in patients with acute stroke. For the present study, we selected all patients with ischemic stroke and randomization within 6 hours of symptom onset. We estimated the effect of treatment with alteplase on the modified Rankin Scale score at 3 months with ordinal logistic regression, stratified by baseline body temperature. We made adjustments for confounding factors and expressed associations as adjusted odds ratios (aOR) with 95% confidence intervals (CI). We also tested for interaction between treatment with alteplase and body temperature.

Results
Of the 1400 patients in PAIS, we included 647 in the present study. Treatment with alteplase was associated with improved functional outcome at 3 months (aOR, 1.51; 95%CI, 1.09 to 2.08). In the 286 patients (44%) with a baseline body temperature of 37.0°C or higher, alteplase was associated with a larger effect (aOR, 2.13; 95%CI, 1.28 to 3.45) than in patients with a temperature below 37.0°C (aOR, 1.11; 95%CI, 0.71 to 1.69). A test for interaction between body temperature and alteplase did not reach statistical significance (p=0.18).

Conclusion
Patients with ischemic stroke and a high body temperature may have a larger benefit of treatment with alteplase than patients with lower body temperatures. These findings are in line with those from in vitro studies, in which lowering temperature decreased the fibrinolytic activity of the enzyme alteplase. This interaction should be explored further in randomized clinical trials of thrombolytic therapy or modification of body temperature. Trials of therapeutic hypothermia should be controlled for treatment with thrombolytics, and trials of thrombolytic treatment should consider body temperature as a potential effect modifier.

Introduction

Treatment with intravenous alteplase is of proven benefit for patients with acute ischemic stroke presenting within 4.5 hours after onset of symptoms.[1] In observational studies, high body temperature has been associated with unfavorable outcome.[2] Physical cooling or prophylactic administration of antipyretic drugs therefore appear promising treatment strategies.[3] In in vitro studies, the fibrinolytic activity of alteplase decreased 5% per degree Celsius reduction in temperature,[4] but the modifying effect of body temperature on thrombolytic treatment with alteplase in patients with acute ischemic stroke is unclear.[5] We assessed the influence of baseline body temperature on the effect of intravenous alteplase on functional outcome in patients with acute ischemic stroke, by analyzing data from the Paracetamol (Acetaminophen) in Stroke (PAIS) trial.[6]

Methods

Patients

PAIS was a multicenter, randomized, double-blind, placebo-controlled trial to assess the effect of high-dose paracetamol on functional outcome in patient with acute stroke. The trial design and main results have been published elsewhere.[6] In short, patients were eligible for inclusion in PAIS if they had ischemic stroke or intracerebral hemorrhage and if start of treatment was possible within 12 hours after onset of symptoms. Demographic data, medical history, score on the National Institutes of Health Stroke Scale (NIHSS), rectal or tympanic temperature, treatment with intravenous alteplase, time from onset of symptoms to start of treatment, and functional outcome at 3 months, measured by the modified Rankin Scale (mRS), were recorded. For the present analysis, we selected all patients with acute ischemic stroke and randomization within 6 hours from onset, to take any delays between treatment with alteplase and randomization into account.

Statistical analysis

We assessed the effect of treatment with alteplase on the score on the mRS at 3 months with multiple ordinal logistic regression analysis. We adjusted for age, sex, baseline NIHSS score, previous stroke, atrial fibrillation, smoking, and diabetes mellitus. We stratified for body temperature ($<$37.0°C vs.$\geq$ 37.0°C). We also tested for interaction between treatment with alteplase and body temperature. To estimate the effect of treatment with paracetamol on the effect of alteplase, we used the same type of analysis. We expressed associations as adjusted odds ratios (aOR) with 95% confidence intervals (CI).

Results

Of the 1400 patients enrolled in PAIS, we included 647 (46%) in the present study. The patients who were excluded from the present study had been included in PAIS later than 6 hours after symptom onset (642 (46%)) or had intracerebral

hemorrhage (111 (8%)). Of the 647 included patients, 215 (33%) had been treated with intravenous alteplase. These patients had on average more severe strokes, more non-lacunar infarcts and less often atrial fibrillation and other co-morbidities than patients who were not treated with alteplase (Table 2.6). In the group of 361 patients (55%) with a body temperature of 37.0°C or higher, 117 (32%) were treated with intravenous alteplase. Of the 286 patients with a body temperature below 37.0°C, this number was 98 (34%). There were no differences in baseline characteristics between these groups. After adjustment for confounders, treatment with alteplase was associated with improved functional outcome at 3 months (aOR, 1.51; 95% CI, 1.09 to 2.08) (Figure 2.9). In the patients with a baseline body temperature of 37.0°C or higher, alteplase was associated with a larger benefit (aOR, 2.13; 95%CI, 1.28 to 3.45) than in patients with a temperature below 37.0°C (aOR, 1.11; 95%CI, 0.71 to 1.69). A test for interaction between body temperature and alteplase did not reach statistical significance (p=0.18). The effect of alteplase was similar in patients on high-dose paracetamol (332 patients) and those on placebo (315 patients) (Figure 2.9).

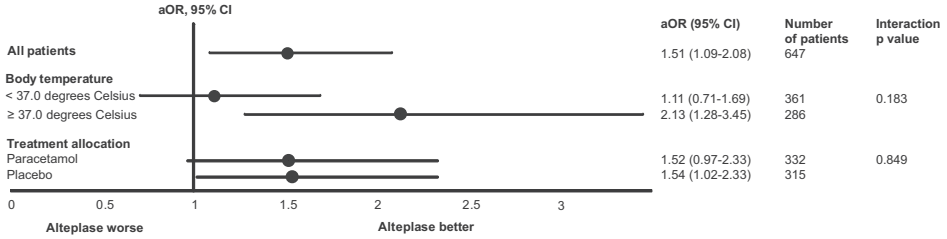

Figure 2.9: Association between treatment with alteplase and functional outcome at 3 months for all patients and different subgroups (adjusted Odds Ratio (aOR) with 95% Confidence Interval (CI)).

Discussion

This study suggests that in patients with ischemic stroke, the effect of treatment with alteplase may be larger with higher body temperatures. Our results should be interpreted with caution, as a formal (but not very sensitive) test for interaction between body temperature and alteplase was not significant.

Our findings are in line with those from in vitro studies, in which lowering temperature decreased the fibrinolytic activity of the enzyme alteplase. A pooled analysis of these in vitro studies has suggested a 5% reduction in fibrinolytic activity per degree Celsius decrease in temperature.[4]

The effect modification by body temperature of the effect of alteplase on functional outcome in patients has remained unclear. In contrast to the commonly observed inverse relation between body temperature and outcome after stroke, high body temperature was associated with favorable outcome in an observational study of 111 patients treated with alteplase.[5, 7] In a study of 5586 patients from

the VISTA database, no influence of body temperature on the effect of treatment with alteplase could be demonstrated, although the point estimates of the effect by temperature categories suggested an attenuation of the benefit of alteplase in patients with either a high (>37.5°C) or a low (<35.5°C) body temperature.[7] The effect of body temperature has not been assessed in randomized trials of alteplase or in pooled analyses of these trials.

Our results suggest effect modification of the benefit of alteplase by body temperature. An important question is whether this modification, if real, is relevant, especially in planned and ongoing trials that aim at reducing body temperature. In these trials, physical cooling or antipyretic treatment will be started after administration of alteplase. The plasma half-life of alteplase is very short (5 minutes), but the half-life of the resulting alteplase-plasmine-fibrinogen complex may be quite prolonged, and its activity may also be affected by body temperature.[8] Antipyretic treatment with paracetamol lowers body temperature with only 0.26°C and is therefore unlikely to reduce the benefit of alteplase.[6] We have confirmed this hypothesis in our data, where we observed no interaction between paracetamol and the effect of alteplase. We recently started a second multicenter, randomized trial to confirm the benefit of high- dose paracetamol on functional outcome in patients with a baseline body temperature of 36.5°C or higher,[9] observed in a subgroup analysis of PAIS.

The present study has limitations. PAIS was not designed to assess the effects of treatment with intravenous alteplase on functional outcome, but of early antipyretic treatment with high-dose paracetamol. Baseline characteristics were therefore not balanced between the groups. However, our analyses were adjusted for imbalances in baseline factors.

In conclusion, our study suggests that in patients with ischemic stroke, the benefit of treatment with alteplase may depend on body temperature. This interaction should be explored further in randomized clinical trials of thrombolytic therapy or modification of body temperature. Trials of therapeutic hypothermia should be controlled for treatment with thrombolytics, and trials of thrombolytic treatment should consider body temperature as a potential effect modifier.

Table 2.6: Baseline characteristics by treatment with intravenous alteplase.

	Patients treated with alteplase	**Patients not treated with alteplase**	**P-value**
Number of patients	215	432	
Age, years (mean, SD)	67.1 (14.5)	72.3 (12.2)	0.00
Sex (male)	127 (59.1%)	247 (57.27%)	0.65
Body temperature on admission (mean, SD)	36.8 (0.6)	36.9 (0.6)	0.36
Body temperature on admission <37.0°C	117 (54.4%)	244 (56.5%)	0.62
NIHSS on admission (median, IQR)	10 (5-16)	5 (3-10)	0.00

Hypertension	86 (40.0%)	214 (49.5%)	0.06
Atrial fibrillation	25 (11.6%)	83 (19.2%)	0.02
Diabetes mellitus	20 (9.3%)	72 (16.7%)	0.01
Current smoking	80 (37.2%)	113 (26.2%)	0.06
Previous stroke	33 (15.4%)	93 (21.5%)	0.06
Previous myocardial infarction	28 (13.0%)	55 (12.8%)	0.46
Peripheral artery disease	19 (8.8%)	58 (13.4%)	0.10
Stroke subtype (lacunar infarct)	16 (7.4%)	88 (20.4%)	0.00

SD=standard deviation; NIHSS=National Institute of Health Stroke Scale;

IQR=interquartile range

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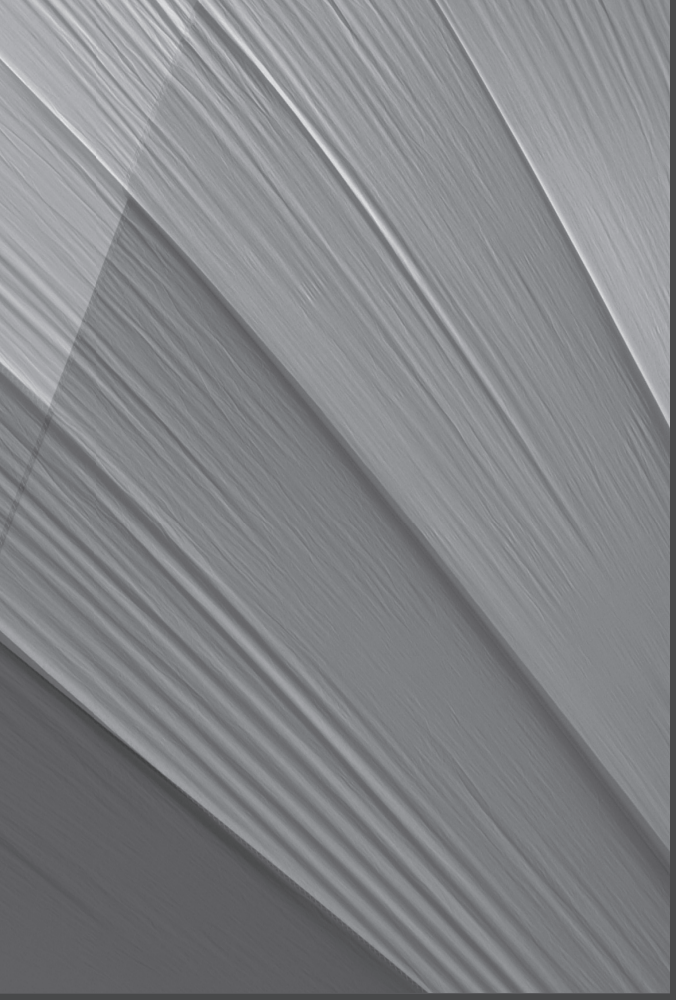
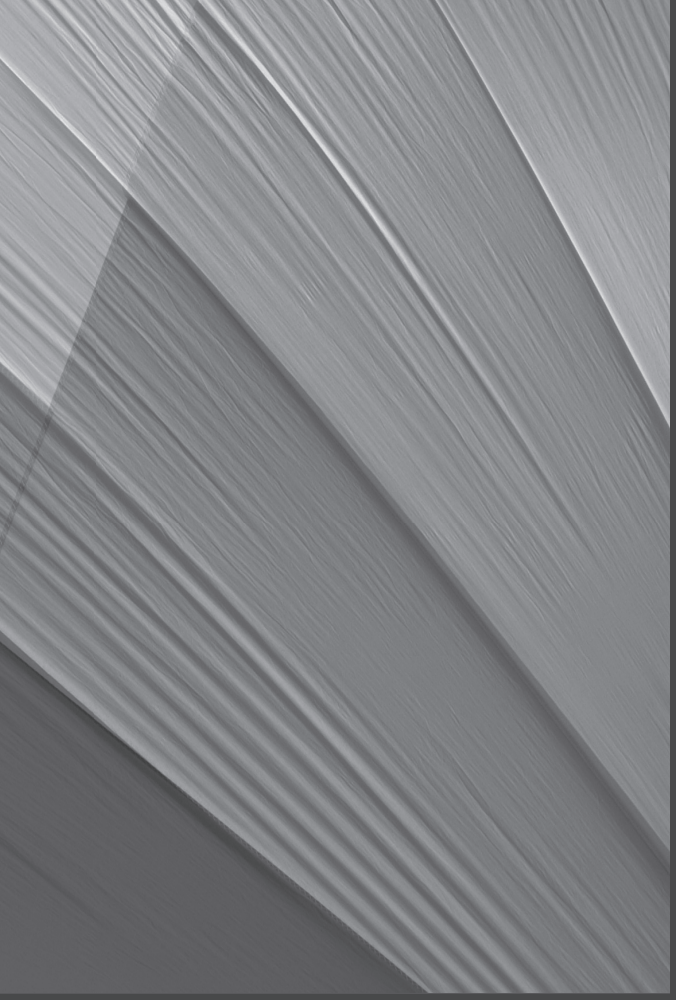


Chapter 3

Sex as prognostic factor in stroke



Chapter 3.1

Unequal access to treatment with intravenous alteplase for women with acute ischemic stroke

Inger de Ridder, Maaike Dirks, Louis Niessen, and Diederik Dippel, for the PRACTISE investigators.

Stroke. (2013);44:2610-12.

Abstract

Background and purpose
A recent meta-analysis showed that women with acute ischemic stroke are less likely to receive treatment with intravenous alteplase than men. The aim of this study is to assess sex differences in treatment with intravenous alteplase, and to explore the reasons for these differences.

Methods
We analyzed data from the PRACTISE study. We applied a multiple logistic regression model, and expressed the association between sex and treatment with an age-adjusted odds ratio (aOR) with 95% confidence interval (CI).

Results
In total, 5515 patients were included in PRACTISE. Women were on average 4 years older than men. The median NIHSS score was 6 in women and 5 in men. Fewer women were treated with intravenous alteplase (11% vs. 14%, aOR 0.8; 95% CI: 0.7 to 1.0). However, fewer women arrived within four hours after onset (27% vs. 33%, aOR 0.8; 95% CI: 0.7 to 0.9).

Conclusions
Fewer women present themselves within four hours from stroke onset than men and consequently receive less often thrombolytic treatment. This difference may be caused by the on average older age of women and consequently women more often living alone.

Introduction

A recent meta-analysis of 18 studies showed that women with acute ischemic stroke are less likely to receive treatment with intravenous alteplase than men.[1] As women account for at least 60% of all deaths due to stroke, sex differences in treatment of acute stroke are important.[2] There are several hypotheses about this sex disparity.[1] Women are generally older. Although age is not a contraindication for treatment,[3] physicians may be less inclined to treat older patients. Women may more often have contraindications for thrombolysis, such as use of oral anticoagulants.

So far, the lower treatment rate in women has not yet been explained. The aim of the present study is to assess the differences between men and women in treatment with intravenous alteplase, and to study reasons for this difference.

Methods

We analyzed data from the PRACTISE study; all individuals aged 18 and older with acute stroke presenting within 24 hours of symptom onset were registered in 12 hospitals in the Netherlands between 2003 and 2005. Patients presenting within 4 hours were assessed in more detail. Stroke severity, assessed with the National Institutes of Health Stroke Scale (NIHSS), was available in all patients with an ischemic stroke presenting within 4 hours. The study protocol and main results have been published elsewhere.[4, 5]

We defined outcome as treatment with rtPA in the total stroke population and in the subgroup of patients with ischemic stroke presenting within 4 hours, and onset-to-door time as the time from onset of symptoms to presentation and registration at the emergency department (ED). We used a multivariable logistic regression model with adjustment for age and expressed associations as adjusted odds ratios with 95% confidence intervals. We explored the effect of onset to door time, intervention of a GP, and contraindications for treatment on the likelihood of being treated.

Results

A total of 5515 patients were included in the PRACTISE study, 2778 (50,4%) women and 2737 men. On average, women were four years older than men. The median NIHSS score was 6 in women and 5 in men (Table 3.1). Considering the total population, fewer women were treated with intravenous alteplase (11% vs. 14%, OR 0.8; 95% CI: 0.7 to 0.9). Adjustment for age did not affect this association (aOR 0.8; 95% CI: 0.7 to 1.0). In the 1657 ischemic stroke patients presenting within 4 hours of stroke onset, the median NIHSS score was 6 in women and 5 in men (Table 3.1). Within this subgroup 41.6% women versus 42.4% men were treated with intravenous alteplase (OR 1.0; 95% CI: 0.8 to 1.2) (Table 3.2).

Fewer women presented at the ED within 4 hours (27% vs. 33%, OR 0.8; 95% CI: 0.7 to 0.9). After adjustment for age, the onset to door time in women was on average 27 minutes longer (95% CI: 9 to 47 minutes). We found no differences in sex-distribution in occurrence of hemorrhagic stroke, patients who called a GP or

Table 3.1: Baseline characteristics by sex.

	Women	Men
All patients (n=5515)	**n=2778 (50%)**	**n=2737 (50%)**
Age, years (mean, SD)	74 (13)	70 (12)
Age 80 years or above	1140 (41%)	605 (22%)
Patients with ischemic stroke presenting within 4 hours from onset (n=1657)	**n=755 (46%)**	**n=902 (54%)**
Age, years (mean, SD)	74 (13)	70 (12)
Hypertension	414 (55%)	434 (48%)
Atrial fibrillation	144 (19%)	153 (17%)
Diabetes mellitus	134 (18%)	140 (16%)
Hypercholesterolemia	285 (38%)	353 (39%)
Current smoking	138 (18%)	260 (29%)
Previous ischemic stroke	139 (18%)	193 (21%)
Previous myocardial infarction	63 (8%)	164 (18%)
NIHSS at admission (median, IQR)	6 (3-13)	5 (3-10)

SD=standard deviation; NIHSS=National Institute of Health Stroke Scale; IQR= interquartile range

were visited by the GP, or had contraindications for treatment with alteplase (Table 3.2).

Discussion

Our study showed that women are treated just as often with thrombolytic agents as men in the Netherlands, once they arrived in time for treatment. However, if we consider the complete stroke care pathway, fewer women presented at the ED within 4 hours of stroke onset. So far, this lower treatment rate in women has not yet been clarified.[1] Our results suggest that this difference could be caused by delayed presentation to the ED. This is also confirmed in other studies.[6] Also in myocardial infarction there is an underutilization of thrombolytic therapy in women, and a delay in care seeking.[7] Possible reasons for this delay are differences in presenting symptoms and the on average older age of women. Older people more often live alone [8] and suffer an unwitnessed stroke. Living alone makes care seeking difficult and also delays the referral and diagnostic process.

This study has some limitations. Limited demographic details of the included patients were available, therefore no assumptions on the influence of specific symptoms on onset-to-door time could be made. Also the data are collected in 2003-2005, and therefore can be a little outdated. The strength of the present study is that all patients in the participating centers and the intervention of the

GP's were registered and therefore provides a good representation of the whole population. The participating hospitals were representative in size, geographic distribution and frequency of procedures.

Finally, the impact of sex differences in treatment of acute stroke is huge: almost 25% more women should be treated with alteplase in order to abolish the difference and this number is increasing with the aging population. Therefore further research is needed to understand the reasons why fewer women with acute stroke are admitted in time for treatment to be able to deal with this inequality.

Table 3.2: Associations between sex and various characteristics, adjusted for age.

	Women	**Men**	**aOR (95% CI)**
All patients (n=5515)	**n=2778 (50%)**	**n=2737 (50%)**	
Treatment with alteplase	314 (11%)	382 (14%)	0.8 (0.7 to 1.0)
GP called	1418 (51%)	1341 (49%)	1.0 (0.9 to 1.1)
GP visited	1209 (44%)	1124 (42%)	1.0 (0.9 to 1.1)
Onset to door time, min (median, IQR)	427 (414-440)	391 (378-404)	0.7 (0.3 to 1.5)
Patients with ischemic stroke presenting within 4 hours from onset (n=1657)	**n=755 (46%)**	**n=902 (54%)**	
Treatment with alteplase	314 (42%)	382 (42%)	1.0 (0.8 to 1.2)
Contraindications for treatment with alteplase	369 (49%)	449 (50%)	0.9 (0.8 to 1.1)

aOR=adjusted Odds Ratio; CI=confidence interval; GP=general practisioner;

IQR=interquartile range

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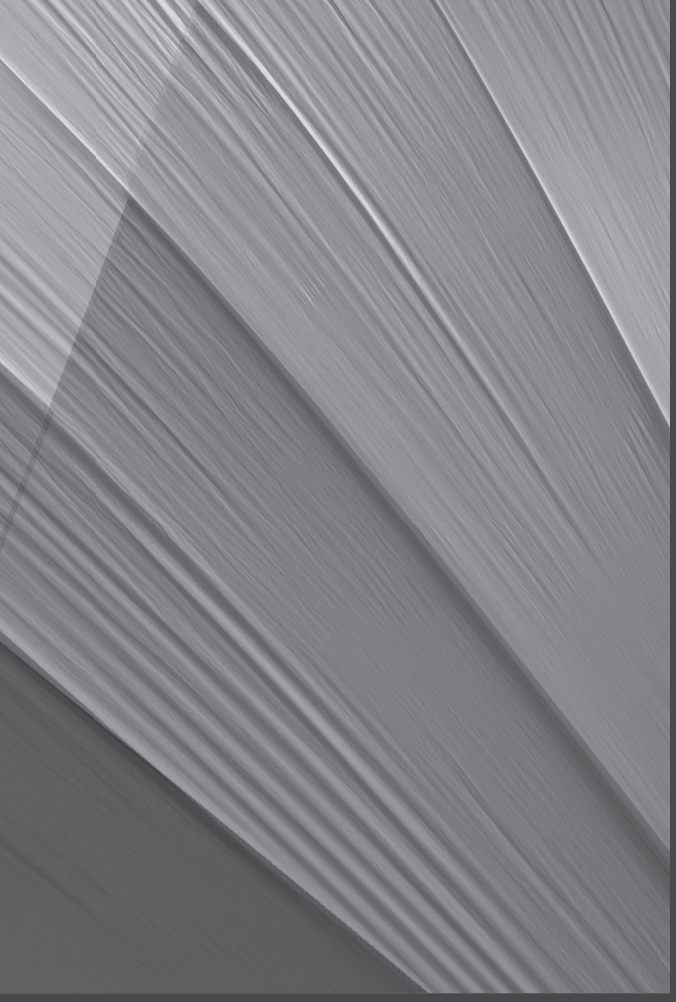

Chapter 3.2

Is intra-arterial treatment for acute ischemic stroke less effective in women than in men?

Inger R de Ridder, Puck SS Fransen, Debbie Beumer, Olvert A Berkhemer, Lucie A van den Berg, Marieke J Wermer, Hester Lingsma, Wim H van Zwam, Yvo B Roos, Robert J van Oostenbrugge, Charles B Majoie, Aad van der Lugt, and Diederik WJ Dippel, for the MR CLEAN investigators.

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Abstract

Introduction
Stroke etiology and outcome after ischemic stroke differ between men and women. We examined if sex modifies the effect of intra-arterial treatment (IAT) in a randomized clinical trial of IAT for acute ischemic stroke in the Netherlands (MR CLEAN).

Patients and methods
Primary outcome was the score on the modified Rankin Scale at 90 days. We tested for interaction between sex and treatment and estimated treatment effect by sex with multiple ordinal logistic regression, with adjustment for prognostic factors.

Results
All 500 patients were included in the analysis, 292 (58.4%) were men. Treatment effect (adjusted common Odds Ratio) was 2.39 (95% Confidence Interval (CI) 1.55-3.68) in men and 0.99 (95%CI 0.60-1.66) in women (pinteraction=0.016). In women, mortality was higher in the intervention group than in the control group (24% vs 15%, p=0.07). Serious adverse events (SAE) occurred more often in women than in men undergoing intervention. There were no differences in neuroimaging outcomes.

Discussion and conclusion
Contrary to other studies, we found a significant interaction between sex and treatment effect in the MR CLEAN trial. Pooled analyses of all published thrombectomy trials did not confirm this finding. In MR CLEAN, women seem to have a slightly more unfavorable profile, causing higher mortality and more SAEs, but insufficient to explain the absence of an overall effect. This suggests a play of chance and makes it clear that IAT should not be withheld in women.

Introduction

Women in high-income countries have a higher lifetime stroke risk and higher stroke mortality than men. Poor outcome after stroke is more frequent in women, which may add to the anticipated increase in burden of stroke.[1] Female stroke survivors are more likely to be disabled and single after stroke and are institutionalized 3.5 times more often than male survivors.[2] The sex-specific effects on stroke incidence and outcome might be contributed to female physiology, which differs from men in terms of coagulation, immunity and hormone exposure.[2] Recently, intra-arterial treatment (IAT) with stent thrombectomy devices for acute ischemic stroke (AIS) has been proven safe and effective.[3, 4, 5, 6, 7] In this study we aim to assess if sex modifies the effect of IAT in AIS.

Methods

Detailed methods of the MR CLEAN trial have been described earlier.[3, 8] In short, MR CLEAN was a multi-center randomized-controlled trial of IAT for AIS. Patients were randomized to IAT plus usual care, or usual care alone. Eligible patients had a proximal arterial occlusion in the anterior cerebral circulation that was confirmed on vessel imaging and could be treated within 6 hours after symptom onset. Approval was obtained from a central medical ethics committee and the research board of each participating center, and all participants (or legal representatives) provided written informed consent.

Outcome

The primary outcome measure was the score on the modified Rankin Scale (mRS) at 90 days. Secondary outcome measures included 90-day functional independence (mRS 0-2) and mortality. Radiological outcome measures included arterial recanalization measured with the modified Thrombolysis in Cerebral Infarction (mTICI) score on Digital Subtraction Angiography (DSA) and final infarct volume on non-contrast CT (NCCT) at 5-7 days.[9] Safety parameters included hemorrhagic complications, progression of ischemic stroke, recurrent ischemic stroke and death. Symptomatic intracranial hemorrhage was defined as neurological deterioration of 4 or more points on the National Institute of Health Stroke Scale (NIHSS) with confirmed intracranial hemorrhage on neuroimaging.

Statistical analysis

The primary effect parameter was the adjusted common odds ratio (acOR) for a shift in the direction of better outcome on the mRS, which was estimated with multivariable ordinal logistic regression. We looked for interaction between sex and treatment effect by adding a multiplicative term in the multivariable ordinal logistic regression model. The acOR and all secondary effect variables were adjusted for pre-specified potential imbalances in major prognostic variables between intervention and control group: age, NIHSS at baseline, time from onset to randomization, previous stroke, atrial fibrillation, diabetes mellitus and internal carotid

artery terminus (ICA-T) occlusion, and reported with 95% confidence intervals (CI). All statistical analyses were performed with Stata/SE 13.1 (StataCorp, Texas, USA).

Table 3.3: Baseline characteristics by sex.

	Women (n=208)	**Men (n=292)**
Age, years (median, IQR)	68 (55-79)	64 (56-74)
Pre-stroke mRS 0	157 (75%)	247 (85%)
Pre-stroke mRS 1-2	38 (18%)	37 (13%)
Atrial fibrillation	60 (29%)	75 (26%)
NIHSS (median, IQR)	17 (14-21)	18 (15-22)
ICA-T occlusion	50 (24%)	84 (29%)
Time from onset to randomization, min (median, IQR)	197 (149-258)	204 (153-262)

IQR=interquartile range; mRS=modified Rankin Scale; NIHSS=National Institute of Health Stroke Scale; ICA-T=internal carotid artery terminus

Results

All 500 patients were included in the analysis, 292 (58.4%) were men. Women were on average 4 years older than men and had a lower percentage mRS 0 before randomization and slightly more atrial fibrillation (Table 3.3). The overall effect of intervention on shift on the mRS at 90 days was acOR 1.67 (95% CI; 1.21 to 2.30). The acOR was 0.99 (95%CI; 0.60-1.66) in women and 2.39 (95% CI; 1.55-3.68) in men (Figure 3.1). P-value for interaction was 0.016. In men, mortality in the intervention group was lower than in the control group (15% vs 21% p=0.17), whereas in women mortality was higher in the intervention group than in the control group (24% vs 15%, p=0.07). Functional independence at 90 days was reached in 36% men and 29% women (aOR in men 2.19 (95% CI; 1.24-3.87), in women 2.05 (95% CI; 0.96-4.35). For women, this results in violation of the proportional odds assumption and a neutral common odds ratio. There were no differences in revascularization (mTICI score) between women and men (both 59% mTICI 2B or 3). In men, the difference in infarct volume was statistically significant (27ml, p=0.01), whereas in women this was not (12ml, p=0.34). Women in the intervention group experienced more serious adverse events, including recurrent stroke, than controls (46% vs 36%, p=0.16; Table 3.4).

Discussion

We studied the interaction between sex and IAT and showed that men experienced a major benefit from IAT in MR CLEAN, whereas we did not find a beneficial

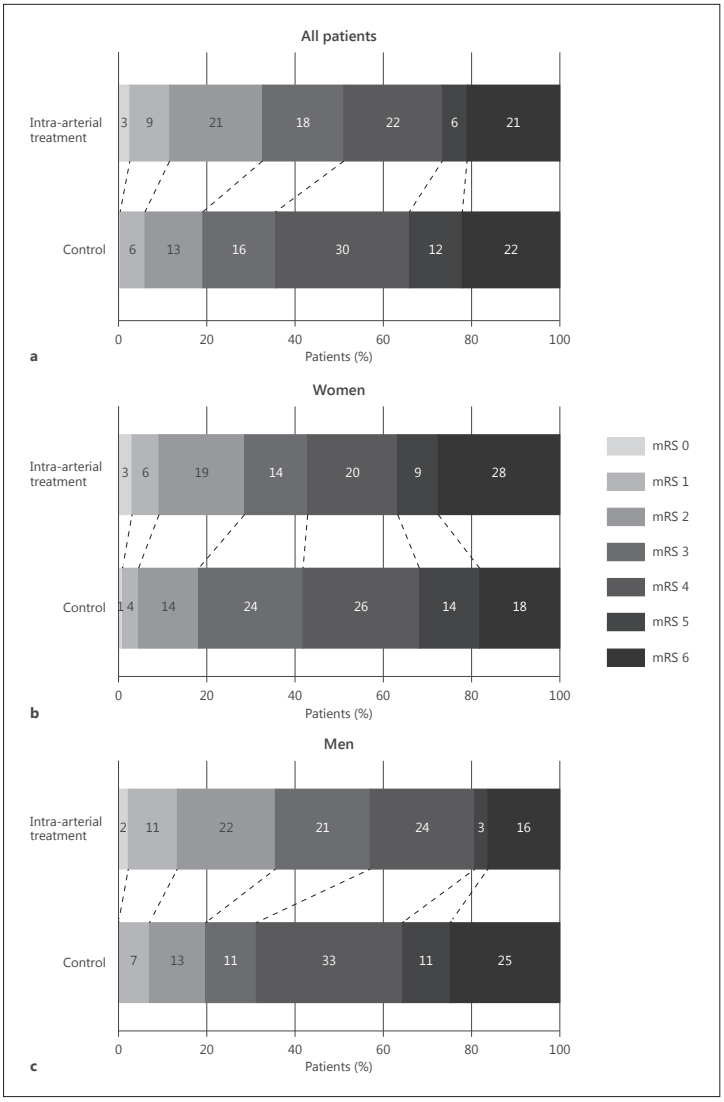

Figure 3.1: Effects of the intervention on the primary outcome in all patients **(a)**, women **(b)** and men **(c)**.

treatment effect in women. In women, the proportionality assumption required for ordinal analysis was not met and the effect on reaching independence was offset by an increase in mortality and severe disability. Moreover, women had a more unfavorable risk profile with a lower percentage mRS 0 before randomization and slightly more atrial fibrillation, and they experienced more serious adverse events in the intervention group compared to men. However, this does not totally explain the large difference in treatment effect, which suggests a play of chance.

Several studies have assessed sex differences in outcome recanalization and functional outcome after IAT for AIS.[5, 7, 10, 11] However, these studies did not report baseline characteristics by sex. Therefore we could not compare the baseline characteristics of women in our study to the baseline profile of women in other studies. None of these studies found differences in recanalization rate and favorable outcome between women and men. Pooled analyses of all published thrombectomy trials also showed no difference between men and women in favorable outcome after IAT.[12] The increased mortality and poor outcome among women compared to men in MR CLEAN has no firm clinical or biological basis. These considerations suggest that a play of chance is the plausible explanation for our findings, and make it clear that IAT should not be withheld in women.

Table 3.4: Safety outcomes by sex and treatment allocation.

	Women			**Men**		
Treatment allocation	**IAT (n=98)**	**Control (n=110)**	**p-value**	**IAT (n=135)**	**Control (n=157)**	**p-value**
Death within 7 days	16 (16%)	6 (5%)	0.01	17 (13%)	21 (13%)	0.84
Death within 30 days	24 (24%)	16 (15%)	0.07	20 (15%)	33 (21%)	0.17
Any SAE	45 (46%)	40 (36%)	0.16	65 (48%)	73 (47%)	0.78
Hemicraniectomy	5 (5%)	3 (3%)	0.37	9 (7%)	6 (6%)	0.92
sICH	9 (9%)	4 (4%)	0.10	9 (7%)	13 (8%)	0.60
Recurrent stroke	6 (6%)	1 (1%)	0.04	7 (5%)	0 (0)	0.00
Progression of stroke	20 (20%)	18 (16%)	0.45	26 (19%)	29 (19%)	0.64

IAT=intra-arterial treatment; SAE=serious adverse event; sICH=symptomatic intracerebral hemorrhage

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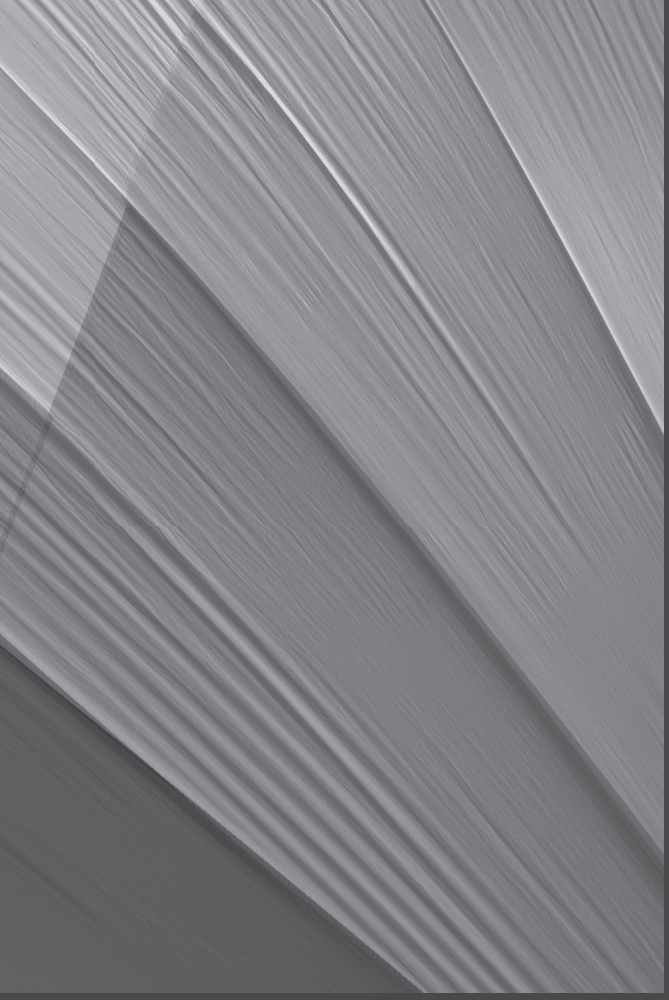

Chapter 3.3

No sex differences in long-term functional outcome after intracerebral hemorrhage

Inger R de Ridder, Joji B Kuramatsu, Stefan Gerner, Dominik Madzar, Hannes Lücking, Stefan Kloska, Diederik WJ Dippel, Stefan Schwab, and Hagen B Huttner.

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Abstract

Background
There is conflicting evidence about the influence of sex on outcome after spontaneous intracerebral hemorrhage (sICH) and the majority of the research focused on mortality and short-term outcome only. We investigated sex differences in long-term functional outcome after sICH.

Methods
We used data from a prospective hospital registry and included all consecutive patients with ICH admitted to our institution between January 2006 until July 2014. Functional outcome was assessed by modified Rankin Scale evaluated 3 and 12 months after ICH. We explored the influence of sex on long-term functional outcome using multivariable regression models and additionally by means of propensity score matching.

Results
We analyzed 823 patients, of whom 380 (46%) women. Women were on average 3 years older ($p<0.001$), men had more often deep hematomas ($p=0.01$). Unadjusted mortality rates were significantly increased in women at 3 months (42% vs.35%; OR 1.35; 95%CI 1.02-1.80). After adjusting for baseline prognostic factors there were no differences between men and women in short- and long term mortality (OR 1.01; 95%CI 0.66-1.54 and OR 1.04; 95%CI 0.69-1.57, respectively) and short and long term unfavorable outcome (OR 1.02; 95%CI 0.67-1.55 and OR 0.96; 95%CI 0.62-1.48, respectively).

Conclusion
We found no sex-related differences in long-term functional outcome in patients with sICH. The apparently worse functional outcome in women can be explained by differences in age.

Introduction

The rates of disability and mortality after spontaneous intracerebral hemorrhage (sICH) are high. Although age, use of oral anticoagulation (OAC), hematoma volume, sICH location and intraventricular hemorrhage (IVH) are known prognostic factors,[1] evidence is conflicting about the influence of sex on outcome after sICH.[2] Existing analyses showed varying results regarding sex differences in outcome, some reporting a lower mortality in men,[3, 4] other an increased survival rate in women[5, 6, 7] and there are also neutral findings.[8, 9] As sex-related differences in patients with sICH may possibly confound clinical management and prognostication of outcome, we for investigated sex differences in long-term functional outcome after sICH.

Methods

We used data from a prospective hospital registry. Consecutive patients aged > 18 years with sICH admitted to the Department of Neurology of the University of Erlangen-Nuremberg, Erlangen, Germany, from January 2006 until July 2014 were included. We excluded patients with secondary etiologies (e.g. ICH related to tumor, vascular malformations including aneurysms, trauma). The ethics committee at the University of Erlangen-Nuremberg approved the study. Informed consent was obtained from all patients, legal representatives, or closest relatives.

We retrieved data on demographics, prior comorbidities, stroke severity, and treatment (including do not treat orders, defined as withhold or withdrawal of care within 24 hours after diagnosis) from institutional electronic databases. ICH was diagnosed using brain computed tomography (CT). Two neuroradiologists blinded to clinical data assessed all CT-scans independently. Hematoma site was defined as lobar, deep, or posterior fossa. Hematoma volume was measured using the ABC/2 or ABC/3 formula, as appropriate.[10] Hematoma enlargement was defined as described previously.[11]

As described previously,[11] we assessed functional outcome, defined as the score on the mRS, and mortality by mailed questionnaires and semi-quantitative telephone interviews 3 months and 1 year after ictus. We defined favorable outcome as mRS=0-3 and clinical improvement during follow-up as mRS at 1 year less than mRS at 3 months.

Statistics

All statistical analyses were performed using SPSS version 20.0. We used multivariable regression models to adjust for the pre-specified prognostic factors age, pre-stroke mRS score, use of OAC, Glasgow Coma Scale (GCS) score at admission, hematoma volume and IVH and expressed associations as (adjusted) odds ratios (aOR) with 95% confidence intervals (CIs). In addition, we performed a propensity score matching using a balanced, parallel, 1:1 ratio nearest-neighbor approach.[11] The propensity score was calculated to balance parameters showing relevant($p<0.1$) sex-related differences.

Results

We included 931 patients in this study. 108 patients had incomplete follow-up data or refused participation. These patients were significantly younger and had less severe strokes, however did not differ in the proportion of male sex. Of the remaining 823 patients, 380(46%) were women. Clinical baseline characteristics differed significantly between women and men (Table 3.5). Women were older than men ($p<0.001$) and were less frequently on OAC ($p=0.02$). Men more often had a history of arterial hypertension ($p=0.05$) and deep ICH ($p=0.01$). Women tended to have more do not treat orders ($p=0.07$). After adjustment for age, this difference disappeared (aOR=1.36;95%CI=0.93-2.01).

Mortality rates were increased in women at 3 months and 12 months (42%vs.49% in women and 35%vs.42% in men; OR 1.35; 95%CI: 1.02-1.80 and OR 1.32; 95%CI 1.00-1.74, respectively (Table 3.6)). Functional outcome at 3 months and 1 year did not show sex-specific differences. Women had a 4% (95%CI -3%-11%) higher risk of unfavorable outcome at 1 year than men.

After adjustment for age, pre-stroke mRS score, use of OAC, GCS score at admission, hematoma volume and IVH, the differences between men and women in short- and long-term mortality disappeared (aOR 1.01; 95%CI 0.66-1.54 and aOR 1.04; 95%CI 0.69-1.57, respectively). There were no differences in short- and long-term unfavorable outcome (aOR 1.02; 95%CI 0.67-1.55 and aOR 0.96; 95%CI 0.62-1.48, respectively). In addition, we performed a propensity-score matching and re-assessed long-term mortality and functional outcome. Both mortality and long-term functional outcome of the propensity-matched cohort is displayed in Figure 3.2 without evidence of significant differences between women and men.

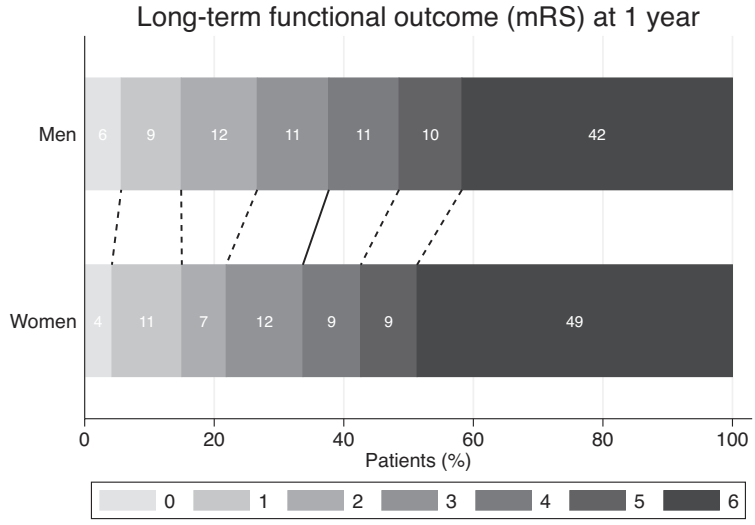

Figure 3.2: Distribution of functional outcome (mRS) of the propensity-score matched cohort at 1 year.

Discussion

This study investigated sex-related differences in long-term functional outcome in a large cohort of sICH. We found that women with ICH are on average older than men, whereas men more often had arterial hypertension and deep hemorrhages. We found no sex-related differences in long-term functional outcome and mortality in patients with sICH.

We confirmed previous studies suggesting men suffered more often from deep ICH [12] and women were on average older than men.[13] As both age and IVH are established parameters affecting outcome,[14] it is notable that a careful statistical approach accounting for these prognostic factors has not been undertaken in previous observational studies.[3, 4] When analyzing outcome, it is important to control for do not treat orders. As shown in other studies,[13] women in our study seem to have more often do not treat orders than men. In our study, this difference could be fully explained by women's higher age.

The results of this analysis are based on a single-center, hospital-based database. Also, patients with incomplete follow-up or who refused participation were on average younger and had less severe strokes. Yet, the other baseline characteristics of these patients did not differ compared with patients included in the study. Despite these shortcomings the present study revealed sufficient evidence that there are no outcome differences between women and men after sICH.

Conclusion

We found no sex-related differences in long-term functional outcome in patients with sICH. The apparently worse functional outcome in women can be explained by differences in age. Irrespective of possible future sex-specific treatments, it appears that both men and women will be likely to benefit from further improvements in clinical management and neither sex should be afflicted with worse prognosis after sICH.

Table 3.5: Baseline characteristics by sex.

	Women (n=380)	**Men (n=443)**	**P-value**
Clinical characteristics			
Age, years (mean, SD)	73 (12)	70 (12)	<0.001
Pre mRS (median, IQR)	1 (0-2)	0 (0-2)	0.55
Hypertension	296 (78%)	369 (83%)	0.05
Atrial fibrillation	74 (19%)	106 (24%)	0.12
Use of oral anticoagulants	71(19%)	112(25%)	0.02
Previous stroke	65 (23%)	127 (29%)	0.49
GCS at admission (median, IQR)	12 (5-15)	13 (5-15)	0.37
NIHSS at admission (median, IQR)	15 (6-28)	12 (5-26)	0.06
Hemorrhage characteristics			

Deep hemorrhage	143 (38%)	205 (46%)	0.01
Lobar hemorrhage	191 (50%)	198 (45%)	0.11
Posterior fossa hemorrhage	41 (11%)	34 (8%)	0.12
IVH only	5 (1%)	6 (1%)	0.96
ICH volume, ml (mean, SD)	14 (4)	12 (5)	0.47
IVH	196 (52%)	228 (52%)	0.99
GRAEB score (median, IQR)	1 (0-4)	0 (0-4)	0.88
Hematoma enlargement (n=602)	32 (10%)	48 (13%)	0.32
Treatment characteristics			
Extra ventricular drainage	92 (24%)	132 (30%)	0.07
Ventilation	151 (40%)	202 (47%)	0.07
Hematoma evacuation	17 (5%)	28 (7%)	0.26
Do not treat orders	74 (19%)	56 (13%)	0.07

SD=standard deviation; mRS=modified Rankin Scale; IQR=interquartile range; GCS=Glasgow Coma Scale; IVH=intraventricular hemorrhage; ICH=intracerebral hemorrhage.

Table 3.6: Univariable and multivariable outcome analysis.

	Women (n=380)	**Men (n=443)**	**Univariable analysis, OR(95%CI)**	**Multivariable analysis, OR(95%CI)**
In hospital mortality	116 (31%)	111 (25%)	1.31 (0.97-1.79)	1.14 (0.73-1.79)
Functional outcome at 3 months, mRS (median, IQR)	5 (3-6)	5 (3-6)	1.27 (1.00-1.63)	1.04 (0.77-1.41)
Unfavorable outcome at 3 months (mRS 4-6)	263 (70%)	285 (65%)	1.26 (0.94-1.69)	1.02 (0.67-1.55)
Mortality at 3 months	158 (42%)	153 (35%)	1.35 (1.02-1.80)	1.01 (0.66-1.54)
Functional outcome at 1 year, mRS (median, IQR)	5 (3-6)	5 (2-6)	1.26 (0.98-1.62)	0.94 (0.68-1.30)
Unfavorable outcome at 1 year (mRS 4-6)	252 (66%)	276 (62%)	1.19 (0.89-1.59)	0.96 (0.62-1.48)
Mortality at 1 year	185 (49%)	185 (42%)	1.32 (1.00-1.74)	1.04 (0.69-1.57)
Improvement on mRS at 1 year	71 (19%)	88 (20%)	0.93 (0.65-1.31)	1.25 (0.81-1.92)

OR=Odds Ratio; CI=Confidence Interval; IQR=interquartile range; mRS=modified Rankin Scale.

Adjustments are made for age, pre-stroke mRS score, OAC, GCS score at admission, hematoma volume and IVH.

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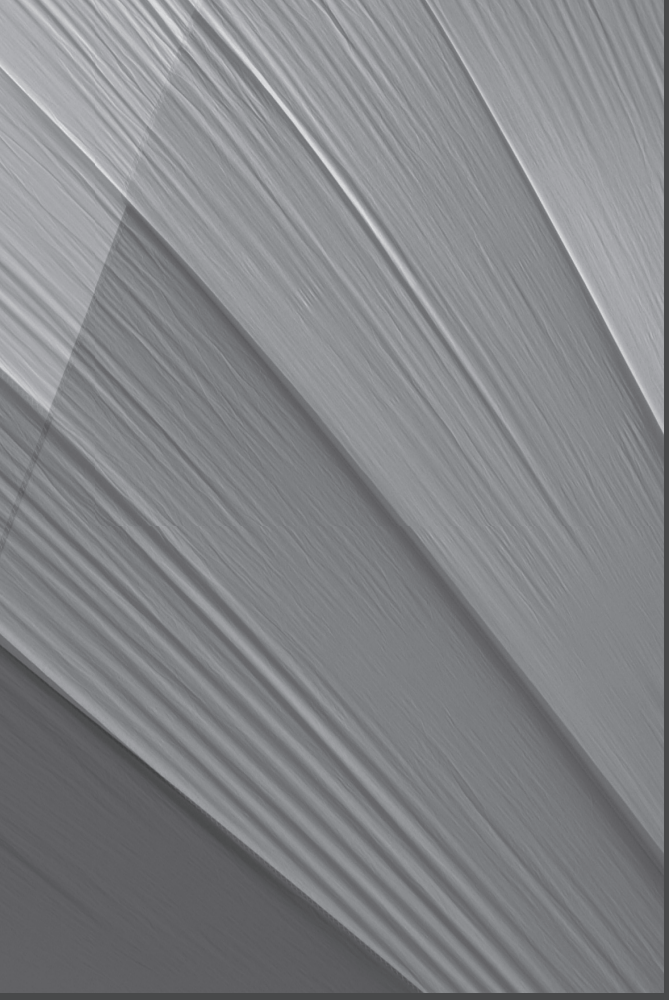

Chapter 4

General discussion

Introduction

In the past decades, acute stroke treatment has seen major improvements with the introduction of standardized stroke-unit care and treatment with intravenous alteplase (IVT) within 4,5 hours after ischemic stroke (IS).[1] As a result, case fatality rates dropped and stroke mortality declined.[2] Recently, intra-arterial treatment (IAT) by means of stent-thrombectomy for a proximal intracranial arterial occlusion in patients with IS has been proven highly effective.[3] However, stroke continues to be a major contributor to disability worldwide. It also forms a sizable economic burden and the development of new treatment strategies remains crucial. Prognostic factors are characteristics that may be used to more accurately predict outcome, improve clinical knowledge and management and guide the development of new treatments. Hence, gaining better knowledge of prognostic factors may improve decision making, but it may also guide the development of new treatments. This has been the topic of this thesis.

The primary aim of this thesis was to assess the effect of body temperature management on functional outcome in patients with acute stroke. The secondary aims were to investigate sex differences in acute management after ischemic stroke (IS) and sex differences in long-term functional outcome after intracerebral hemorrhage (ICH). In this chapter, I will summarize and explain the main findings of my studies, put them in the context of existing literature, and discuss clinical implications and provide recommendations for further research.

Body temperature as prognostic factor in stroke: the effect of body temperature management on functional outcome in patients with acute stroke

Main results of the PAIS 2 trial

The primary aim of this thesis was to assess the effect of body temperature management on functional outcome in patients with acute stroke. To answer this question, we performed the Paracetamol (Acetaminophen) in Stroke 2 (PAIS 2) trial. PAIS 2 was a multicenter, randomized, double-blind, placebo-controlled clinical trial which aimed to assess the effect of treatment with high-dose paracetamol on functional outcome in patients with acute stroke and a body temperature of 36.5°C and above. Patients with ischemic stroke or intracerebral hemorrhage who could be treated within 12 hours after symptom onset were treated with paracetamol 6 grams per day or matching placebo for 3 consecutive days. Primary outcome was defined as the score on the modified Rankin Scale (mRS) at 3 months. Inclusion started in December 2011 and the trial was preliminary ended on January 1st 2015 due to slow recruitment and lack of funding. We included a total number of 256 patients of whom 136 (53%) were allocated to paracetamol. Treatment with paracetamol did not influence functional outcome at 3 months (OR 1.02; 95% CI: 0.7-1.6). After adjustment for age, stroke severity (NIHSS at admission) and stroke type this finding did not change. There were no cases of liver failure and infections were evenly balanced between both groups. We concluded that treatment with parac-

etamol after acute stroke is safe. However, the effect of treatment with high-dose paracetamol on functional outcome after acute stroke remains unclear. We updated a meta-analysis of all studies with paracetamol after acute stroke, but adding the results of the PAIS 2 trial did not provide the final answer.

PAIS 2 trial in the context of other studies

Therapeutic hypothermia (TH) is an intentional reduction of body temperature and has been assessed in several diseases, such as neonatal hypoxic-ischemic encephalopathy, traumatic brain injury, myocardial infarction and cardiac arrest, with varying results. TH reduces mortality without increasing major disability in term and late preterm newborns with hypoxic ischemic encephalopathy.[4] In traumatic brain injury, TH did not lead to lower mortality and unfavorable outcome rates at 3 or 6 months.[5] In myocardial infarction, TH did not result in smaller infarct sizes or a reduction of major adverse cardiac events.[6] One study found a significant reduction in the incidence of heart failure,[7] but this finding needs confirmation in other studies. TH has been accepted as an effective treatment after cardiac arrest.[8] The American Heart Association Guideline recommends to maintain a constant temperature between 32.0 and 36.0°C for at least 24 hours in patients who remain comatose after return of spontaneous circulation.[9] This recommendation is based on 3 randomized controlled trials. Two of these trials, published in 2002, reported increased survival and functional recovery with induced hypothermia to 32.0 to 34.0°C.[8, 10] These studies were nevertheless not accepted by the whole field, due to a small sample size and the risk of bias. One of the topics of the following debate was the optimal target temperature, and a more recent study found no difference in mortality and neurological outcome between patients treated with target temperature of 33.0°C and patient treated with a target temperature of 36.0°C.[11] Unfortunately, statistical testing for equivalence was not performed.

Methods for body temperature-lowering management can be categorized as physical and pharmacological. Two types of physical cooling are surface cooling and endovascular cooling. In case of surface cooling the skin is cooled with air or fluids.[12] Endovascular cooling uses intravascular catheters to lower body temperature. Endovascular cooling is more reliable and induction rates are quick, but it is also more invasive and time consuming.[12] Prevention of shivering also requires sedation protocols, which may lead to unwanted side effects as pneumonia. Physical cooling to improve functional outcome after ischemic stroke has been assessed in several trials. One of these trials is the recently published ICTus 2 Trial.[13] The ICTus 2 Trial was stopped early because of the approval of IAT for ischemic stroke and the subsequent changes in standard ischemic stroke care. The researchers found a trend toward more pneumonia in hypothermia treated patients, the trial was however not designed and too small to assess an effect on functional outcome. Further research is ongoing.

Six studies have been performed on pharmacological temperature reduction in acute stroke. Five of these studies used paracetamol as antipyretic drug.[14] These studies were, except for PAIS, small and designed to test safety and feasibility. They showed no effect on outcome. In the PAIS trial, 260 of 697 (37%) patients

receiving paracetamol and 232 of 703 (33%) receiving placebo improved beyond expectation (adjusted OR 1.20; 95% CI: 0.96-1.50). In a post-hoc analysis of patients with a baseline body temperature between 37.0 and 39.0C treatment with paracetamol was associated with improved functional outcome (adjusted OR 1.43; 95% CI: 1.02-1.97).[15] A previous meta-analysis using a dichotomized outcome showed a trend towards a favorable effect of temperature-lowering treatment.[14] The results of PAIS 2 can be considered neutral. However, because of serious lack of power we cannot exclude an alternative result.

Current American AHA/ASA guidelines recommend the use of antipyretic drugs when body temperature exceeds 38.0°C in patients with IS.[16] European (ESO) guidelines do not make any recommendation for treating hyperthermia as a means to improve outcome in patients with IS.[17] For patients with ICH there is insufficient evidence from randomized controlled trials to make strong recommendations on whether, when, and for whom preventive or early fever treatment should be given after acute ICH.[18] The differences in American and European recommendations may result from different objectives. The AHA/ASA guidelines aim for lowering body temperature and relief of symptoms often accompanying fever, such as pain and discomfort, whereas the ESO guidelines aim for better functional outcome.

Preliminary ending of the PAIS 2 trial

The PAIS 2 trial was preliminary ended, mainly because of slow recruitment of patients. This was most likely caused by the low intensity of trial coordination activities - a consequence of the low budget - and the simultaneous conduct of several large randomized clinical trials of acute stroke treatment in the Netherlands. The trial was started in anticipation of upcoming funding, which never appeared. Stopping a trial may cause harm to several people involved.[19] Firstly, it may cause physical and emotional disadvantage to enrolled study participants.[19] In our trial, after inclusion was stopped, 3 month follow-up was completed. Physical disadvantage due to preliminary stopping of the trial is therefore unlikely. However, emotional disadvantage may have been caused to the study participants. They may feel that they have been put at risk by participating in a trial that due to preliminary stopping was underpowered to answer the research question. Secondly, preliminary stopping a trial may cause harm to the researchers.[19] They lose the time, money and efforts already invested in the trial. The researchers may be disappointed to have lost the chance to help patients and gain positive trial results, which can be published in a high end journal. They also may lose faith from participating clinicians. However, stopping a trial may also result in benefit for the reseachers.[19] They may have gained experience in developing and conducting a clinical trial, which gives further clinical trials a higher success rate. Thirdly, preliminary stopping a trial may cause harm to society.[19] One of the primary goals of a clinical trial is to add knowledge to society. When a trial is ended early, the results often remain unpublished. In our case, we presented the results at an international stroke congress and will publish them shortly. In the end, every piece of evidence from randomized trials will add to the general knowledge about the particular treatment effect, and should therefore not be discarded.

Implications of PAIS 2

With the PAIS 2 trial we confirmed that treatment with paracetamol is safe. There were no cases of liver failure; infections and other adverse events were evenly balanced between both groups. However, a small chance of liver failure still exists and may offset the possible treatment effect of paracetamol. The results of the trial could not confirm our hypothesis. Also, adding our results to the latest meta-analysis of all paracetamol trials could not answer the research question definitively. The question is how to proceed after PAIS 2. There is insufficient evidence to suggest routine use of high-dose paracetamol in patients with acute stroke and a body temperature of 36.5°C and above. As more severely affected patients may profit more from treatment with paracetamol, and this finding is biological plausible,[20] the PRECIOUS (Prevention of Complications to Improve Outcome in elderly patients with Stroke) trial has just started. This study will assess the effect of preventive treatment with paracetamol, metoclopramide and ceftriaxone on a comprehensive array of relevant health outcomes in a factorial design. The study will focus on elderly patients with a score on the NIHSS of 6 or higher.

Recommendations for further research

Further research on body temperature-lowering management is warranted. Important factors that influence the neuroprotective effect of temperature-lowering management include the time window before treatment is initiated, the duration of treatment, and depth of body temperature-lowering management.[21] The time window between stroke onset and initiation of treatment should be as short as possible. The central concept in the processes taking place in ischemic brain tissue is that of the ischemic penumbra. This part of the ischemic brain tissue suffers from restricted blood supply, but is still capable of survival when reperfusion is achieved in a certain period of time.[22] Reperfusion is therefore crucial to protect the brain from permanent damage and should be achieved as soon as possible, as other forms of neuroprotection only can achieve success in not permanently damaged brain tissue. Consensus in stroke literature is that most of the permanent brain damage arising from focal ischemia occurs within a few hours time period. This consensus is based on the narrow therapeutic window of a few hours of neuroprotective drugs shown in rodent models, the rapid progression of brain damage seen on CT or MRI, and the rapid reduction with time of the efficacy of alteplase- and trombectomy-induced reperfusion.[21] The optimum duration of body temperature lowering management is unknown. Previous studies suggested that a longer duration confers better neuroprotection.[21] However, a longer duration may be associated with a higher rate of pneumonia.[23] Finally, the optimal depth of body temperature-lowering management is also unknown. Animal studies have shown that cooling to 27.0°C showed less reduction in total infarct volume than cooling to a temperature of 32.0°C,[24] suggesting that cooling may not be too deep. Moreover, improved functional outcome was found in animals with a target temperature of 33.0-34.0°C compared with other temperatures.[25] In humans the optimal depth of body temperature-lowering management is however unknown.

Analog with TH in cardiac arrest it seems rational to aim for a temperature of 33.0-34.0°C. Temperatures of 36.0-36.5°C may be still as effective.[11]

To shorten the time window from onset of stroke symptoms and initiation of treatment, pre-hospital administration of neuroprotective agents may be an interesting approach. Paracetamol is an attractive candidate drug for this approach, because both patients with IS and ICH may profit from treatment with paracetamol. Radiological assessment preceding treatment is not necessary and patients could be given paracetamol during transportation to the hospital.[26]

The effect of pharmacological temperature reduction with paracetamol is small and might be combined with other neuroprotective agents to increase the neuroprotective effects. The processes taking place in the human brain are extremely complicated and multiple pathways are involved, for example the release of excitatory amino acids, formation of free radicals, increased metabolic demands, activation of the immune system and increased blood-brain barrier permeability.[20] It seems therefore reasonable to combine multiple drugs targeting different pathways and processes. An example of this strategy might be a combination of paracetamol and metoclopramide, a gastric prokinetic drug,[27] or metformin, which has anti-inflammatory effects.[28] The combination of first two drugs will be assessed in the PRECIOUS trial. It should be noted that assessing interactions in a factorial design results in the need of a very large study population.

A promising new physical cooling method is intra-arterial cooling in case of succesfull IAT.[29] This method combines IAT with local intra-arterial infusion of a cold saline bolus into the cerebral arteries. The safety and feasibility has been shown in a previous study.[29] Randomized clinical trials are needed to investigate the efficacy of this therapy.

Sex as prognostic factor in stroke: sex differences in acute management after ischemic stroke and long term functional outcome after ICH

Main results

The secondary aims of this thesis were to investigate sex differences in acute management of IS and functional outcome after ICH. We showed that women receive less often IVT due to a later presentation to the emergency department.[30] In addition, we found a significant interaction between sex and treatment effect of intra-arterial treatment (IAT) in the MR CLEAN trial.[31] Pooled analyses of all published thrombectomy trials did not confirm this finding.[32] Finally, we detected no sex-related differences in long term functional outcome after ICH.[33]

Sex differences in stroke

The role of sex in stroke is complicated. The interaction between characteristics that differ between women and men (sex hormones, coagulation, immune system), risk factors more common in women (atrial fibrillation, metabolic syndrome, migraine), risk factors unique for women (use of contraceptives, hormonal changes,

pregnancy, pregnancy-induced hypertension, child birth, menopause) and social factors is very complex.[34] All these factors act together to cause stroke. Women live longer than men, resulting in a higher life time risk of stroke in women than in men (20% vs. 17%).[34] Women also experience other stroke types, with subarachnoid hemorrhage and cerebral venous thrombosis being more common in women; and other ischemic stroke subtypes, with large vessel strokes being more common in women.[35] Moreover, sex may influence treatment. Both the efficacy and utilization of treatment may differ between women and men.[36] Finally, all the above-mentioned factors determine outcome after stroke. Women have poorer functional outcomes, are more often institutionalized, more often have depression and a lower quality of life after stroke.[34, 35, 37, 38] It is unclear whether this difference is caused by women's higher age, poorer prestroke functions and tendency to have more severe strokes or whether other factors, like differences in stroke presentation, acute management, effects of therapies and social factors as not being able to perform care-giver activities, also play a role. As the burden of stroke is higher in women than in men, these are important issues to be addressed. I will discuss some of these factors in detail below.

Estrogens

When discussing sex differences in stroke, the role of estrogens must be addressed. Estrogen is the primary female sex hormone. It is responsible for the development and regulation of the female reproductive system and secondary sex characteristics. Estrogens diffuse easily through the plasma membrane and form complexes with estrogen receptors.[39] These receptors are present in both men and women and are widely distributed in many organs, including the cardiovascular system. In the cardiovascular system they cause the production of endothelial factors, such as nitric oxide, opening of potassium channels and activation of numerous signal transduction cascades, leading to rapid vasodilation. With this, estrogens play a role in blood pressure regulation and appear to be an important factor in the development of hypertension and cardiovascular diseases.[39] Hormonal levels change after menopause and the synthesis of estrogens is decreased and stroke risk seems to increase after menopause.[40] In observational studies, hormone replacement therapy (HRT) was reported to reduce the risk of arterial vascular events. A systematic review found however that HRT is associated with an increased risk of cardiovascular disease, stroke and venous thromboembolism.[41] The reasons for these unexpected findings have been discussed comprehensively. Factors that might have contributed are the age of the patients, preexisting cardiovascular diseases, age of HRT initiation, type of HRT (mono or combination therapy) and dosage.[39] Prospective studies on the influence of HRT on blood pressure in postmenopausal women were so far inconclusive. In spite of the potential beneficial effects of estrogens, their mechanisms of action and interaction seem to be more complex when it comes to therapy applications and therefore additional research is needed. Knowledge of these mechanisms in the cardiovascular system may contribute to the development of new therapies with sex hormones.

Stroke symptoms

Stroke symptoms may differ between men and women. Women are more likely to have impaired consciousness,[42, 43, 44] aphasia[42, 44] and visual field disturbances.[42] These symptoms, in combination with women's higher age and social situation (more often living alone), make care seeking difficult and lead to pre-hospital delays and fewer treatment possibilities. Women may also experience more nonspecific symptoms as pain, headache, behavioral changes and vision changes.[45] Women, caregivers and health care providers could be trained to recognize these differences. A real-life example for this is aphasia. Aphasia may be easily misdiagnosed as confusion. A simple screening test is able to distinguish between both, is easy to implement and may lead to a faster recognition of stroke in women. Sex differences in presenting symptoms are also found in patients with acute myocardial infarction. Compared to men, women present less often with the typical symptoms chest pain and arrhythmia and wait longer before seeking treatment.[46] The explanation for these differences in presenting symptoms in both stroke and myocardial infarction is unknown and further research is needed.

Utilization of treatment

Women seem to be treated less often with IVT than men.[36, 47] A meta-analysis of 16 studies found that women are 25% less likely to be treated with IVT than men, although a significant between-study variation existed. More recent studies showed limited sex difference in the utilization of treatment, with again variable findings.[36] We found that women receive IVT less often because they present less often to the emergency department within 4 hours from stroke onset.[30] This delay could be explained by differences in presenting symptoms (as described above) and women's older age. Women live longer than men and are on average older when having a stroke.[2] Older people more often live alone and experience unwitnessed stroke. Living alone makes care seeking difficult and also delays the referral and diagnostic process. Unfortunately, these factors are not easily addressed. Solutions may be found in personal devices that send alarms through the internet when needed.

Efficacy of treatment

Women seem to benefit more from treatment with IVT than men.[36] A pooled analysis of 3 randomized controlled trials of IVT showed that the proportion of men and women having a favorable outcome following treatment with IVT was similar (38.5% vs. 40,5%). However, among the placebo group men had significantly better outcomes (favorable outcome in men 36.7%, women 30.3%). These data suggest a significant interaction between sex and IVT, with women having a substantial greater benefit from IVT than men.[48] There are several possible explanations for the interaction between sex and IVT response. The first explanation is that women have an increased recanalization rate after following IVT. This is believed to be due to a reduced arterial size,[49] resulting in smaller clots, and estrogen-related differences in levels of clotting factors.[48] Women also have cardio-embolic stroke

more frequently, which clots seem to be richer in fibrin. The exact mechanisms of this interaction are however unknown. An interaction between sex hormones and IVT could also be one of the reasons. Concerning women's lower likelihood of receiving IVT, there is a huge challenge for health care providers and the health care system to increase the proportion of women treated with IVT.

In the MR CLEAN trial, a multicenter randomized-controlled trial of intra-arterial treatment for IS, we found a significant interaction between sex and treatment effect, with men receiving a greater benefit from IAT than women.[31] This finding may be explained by a more unfavorable risk profile with a lower percentage mRS 0 before randomization and slightly more atrial fibrillation in women in MR CLEAN, and more serious adverse events in women in the intervention group compared to men. The increased mortality and poor outcome among women compared to men in the MR CLEAN trial has no firm clinical or biological basis. Pooled analyses of all published thrombectomy trials did not confirm this sex difference.[32] These considerations suggest that a play of chance is the most plausible explanation for our findings and make it clear that IAT should not be withheld from women.

Implications

The role of sex in stroke remains very complex. Over the past years, attention for sex-specific differences in stroke has grown. The 2014 World Stroke Day theme was "I am woman: stroke affects me". Aim of this day was to raise awareness among women about stroke and stroke symptoms. Women have a higher life time risk of stroke than men, but are also the main caregivers in the majority of patients. The Women Initiative for Stroke in Europe (WISE) is a European Stroke Organization (ESO) Working Group to guarantee better stroke care for women. WISE promotes scientific initiatives to improve women's stroke care. Further research is needed on many topics. I will discuss some of them in the section below. Women are less likely to be treated for risk factors than men.[34] To reduce this gap, sex-specific stroke guidelines have been published in recent years. As an example, the American Heart Association/ American Stroke Association published the "guideline for the prevention of stroke in women."[34] This guideline focuses on risk factors unique for women, such as reproductive factors, hormonal conception, and pregnancy, and gives sex-specific recommendations. We performed two studies that focused on differences in acute treatment of IS and showed that once in hospital, women are treated as often as men with IVT and that there are no reasons to withhold IAT in women. Health care providers should be aware of sex-specific differences in presentation, however they do not have to keep in mind sex-specific differences in acute treatment.

Recommendations for further research

Further research on sex-specific differences in stroke is required. To start with basic scientific research, I would recommend to test all interventions in both male and female animal models. Usually most of the animals are male and the translation from animal models to patients often fails. This may be caused by sex-related

differences and therefore it may be rational to use both male and female animal models in basic scientific research. If interventions fail in one sex but are effective in the other, it would be interesting to determine the nature of the difference at molecular levels. Another interesting field would be to assess the neuroprotective effects of sex steroids. Estrogens are thought to cause vasodilatation and have other neuroprotective effects. However, hormone replacement therapy (HRT) does not reduce stroke risk. By contrast, it raises stroke risk. The use of oral contraceptives is associated with a 2-fold increase in IS risk.[50] This increase may be caused a high estrogen dose, but even more by the addition of progresteron.[50] This suggests a more complex interaction between sex steroids. Research on the neuroprotective effects of sex steroids may focus on different stroke subtopics, such as coagulation, the vascular system, the immune system and genetics. This research could take place in animals, but probably the best categories are women with the highest hormone levels, such as patients on HRT and pregnant women.

In clinical research, women need to be included in clinical trials in sufficient numbers to provide power for preplanned subgroup analysis. Unfortunately, women and other minorities are less likely to consent to participate in research studies. As discussed earlier, women may suffer from aphasia and a lower level of consciousness more often, making asking for consent more difficult. In many cases a proxy consent is needed. Families tend to fear about making the right decision, because they are in charge of someone's life.[51] It would be interesting to assess if trained researchers achieve higher consent rates. Finally, it would be interesting to develop a sex-specific prediction model to predict outcome after acute stroke. Most likely, a female and male model will be needed to incorporate all important prognostic factors. This model could be used to inform patients and proxies about prognosis, to plan discharge destination and to select patients for new treatment strategies.

Conclusion

In this thesis I have focused on temperature and sex as prognostic factors in stroke. Reducing body temperature after acute stroke seems an promising therapeutic approach and may be performed in different ways. Patients with more severe strokes may profit most from this therapy and further research will focus on that subgroup of acute stroke patients. The role of sex in the prognosis of acute stroke is very complex. Social factors rather than biological factors may explain sex differences in acute stroke. As stroke continues to be a major contributor to disability and economic burden of disease, it is extremely important to improve existing management strategies and therapies and to find new approaches to solve sex disparities. And that is vital, as anyone can suffer a stroke at any time.

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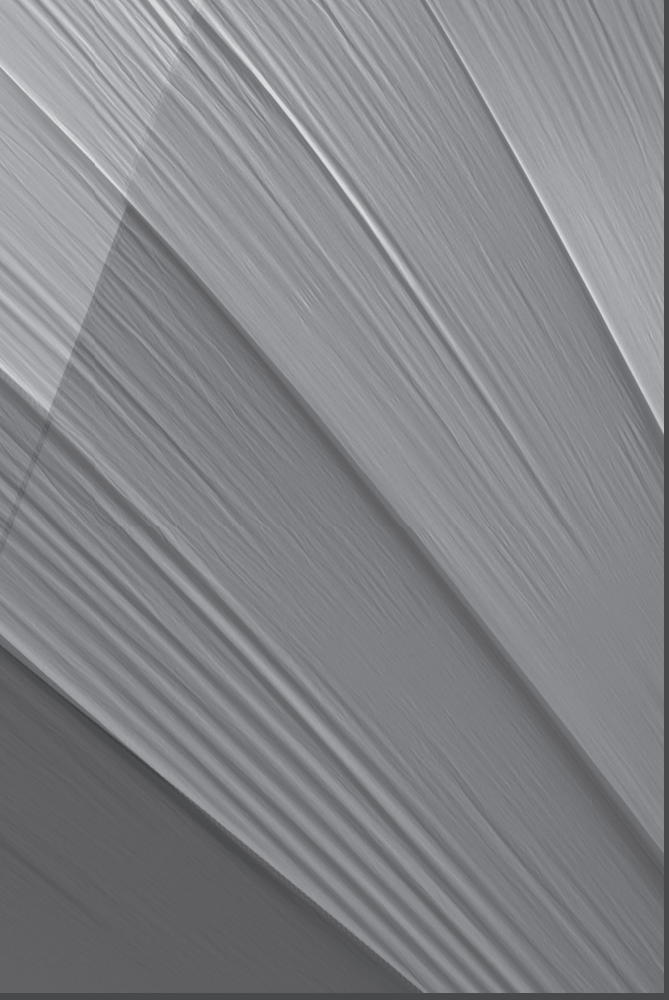


Chapter 5

Summary/samenvatting

English summary

Anyone can suffer a stroke at any time and the consequences of stroke are often severe. As the incidence of stroke rises with age, and as population is aging, the burden of stroke is expected to increase further, especially among elderly women. Women also seem to have a higher chance to die from stroke and to have less favorable outcomes. Prognostic factors are characteristics that may be used to more accurately predict outcome, may improve clinical knowledge and management and may guide new therapies. It remains an important challenge to find new prognostic factors and to further evaluate and explore known prognostic factors. This thesis focusses on the effect of body temperature lowering therapy in acute stroke. The secondary aims are to investigate sex differences in acute management of ischemic stroke (IS) and sex differences in long term functional outcome after intracerebral hemorrhage (ICH).

Chapter 2 describes body temperature as prognostic factor in stroke. In Chapter 2.1 and 2.1 I describe the rationale, background, design and results of the Paracetamol (Acetaminophen) in Stroke 2 (PAIS 2) trial. Subfebrile body temperature and fever in the first days after stroke are strongly associated with unfavorable outcome. Lowering body temperature and prevention of fever are therefore interesting approaches to improve functional outcome after stroke. Paracetamol is one the most commonly prescribed antipyretic drugs. Treatment with high-dose paracetamol induces a reduction in body temperature of about 0.3 degrees Celsius in patients without fever. The Paracetamol (Acetaminophen) in Stroke (PAIS) trial suggested that early treatment with high-dose paracetamol might improve functional outcome in patients with a body temperature of 36.5°C and higher (OR 1.31; 95% CI: 1.01–1.97). As this result was based on a posthoc analysis, confirmation in an independent study was needed. Hence, the aim of PAIS 2 was to assess the effect of high-dose paracetamol on functional outcome in patients with acute stroke and a body temperature of 36.5°C and above in the first 12 hours after stroke onset.

PAIS 2 was a multicenter, randomized, double blind, placebo-controlled clinical trial. We aimed to include 1500 patients with acute ischemic stroke or intracerebral hemorrhage within 12 hours of symptom onset. Patients were treated with paracetamol in a daily dose of 6 g or matching placebo for 3 consecutive days. The primary outcome was improvement on the modified Rankin Scale score at 3 months, assessed with multivariable ordinal logistic regression. Due to slow recruitment and lack of funding the study was stopped prematurely. Between December 2011 and October 2015, we included 256 patients, of whom 136 (53%) were allocated to paracetamol. We found no significant effect of paracetamol on the shift of scores on the mRS (acOR 1.02; 95% CI: 0.7-1.6). There was no difference in serious adverse events (paracetamol n=35 (26%) versus placebo n=28 (24%)). We concluded that treatment with paracetamol after acute stroke is safe. The effect of treatment with high-dose paracetamol on functional outcome after acute stroke remains however uncertain. A very large trial of early treatment with paracetamol after acute stroke is needed to provide more precise effect estimates.

In Chapter 2.3, I assess the influence of baseline body temperature on the effect of alteplase on functional outcome in patients with acute ischemic stroke. In in vitro studies, the fibrinolytic activity of alteplase decreased 5% per degree Celsius reduction in temperature, but the modifying effect of body temperature on thrombolytic treatment with alteplase in patients with acute ischemic stroke was unclear. I found that the effect of treatment with alteplase may be larger with higher body temperatures. As a consequence, trials of therapeutic hypothermia should be controlled for treatment with alteplase, and trials of thrombolytic treatment should consider body temperature as a potential effect modifier.

Chapter 3 describes sex as prognostic factor in stroke. Chapter 3.1 discusses sex disparities in utilization of intravenous trombolytic therapy (IVT). We found that women are treated just as often with IVT as men in the Netherlands, once they arrived in time for treatment. However, if we consider the complete stroke care pathway, fewer women present themselves within four hours from stroke onset than men and consequently receive less often IVT. This difference may be caused by the on average older age of women and consequently women more often living alone.

Chapter 3.2 describes sex differences in functional outcome after intra-arterial treatment (IAT) for IS. We showed that men experienced a major benefit from IAT in MR CLEAN, whereas we did not find a beneficial treatment effect in women. This finding has no firm clinical or biological basis. Women seemed to have a slightly more unfavorable profile, causing higher mortality and more serious adverse events. Pooled analyses of all published thrombectomy trials showed indeed no difference between men and women in favorable outcome after IAT. These considerations suggest that a play of chance is the plausible explanation for our findings, and make it clear that IAT should not be withheld in women.

In Chapter 3.3, I investigate sex differences in long-term functional outcome after spontaneous ICH (sICH). We analyzed data from 823 patients from a prospective hospital registry and found that women with ICH are on average older than men, whereas men more often had arterial hypertension and deep hemorrhages. There were no sex-related differences in long-term functional outcome and mortality in patients with sICH. The apparently worse functional outcome in women can be explained by differences in age. Irrespective of possible future sex-specific treatments, it appears that both men and women will be likely to benefit from further improvements in clinical management and neither sex should be afflicted with worse prognosis after sICH.

In chapter 4, I summarize and explain the main findings of my studies described in this thesis, discuss clinical implications and provide recommendations for further research. I start with a discussion about the results and implications of the PAIS 2 trial. I discuss the various forms of hypothermia and results of previous studies. Next I discuss sex as prognostic factor in stroke, describing the role of estrogens and sex differences in stroke symptoms, utilization and efficacy of IVT. I end with the conclusion that further research on both subjects is needed in order to develop new treatment strategies that reduce health and economic burden of stroke. And that is vital, as anyone can suffer a stroke at any time.

Nederlandse samenvatting

Iedereen kan op elk moment een beroerte krijgen, en de gevolgen van een beroerte zijn vaak ernstig. Doordat de incidentie van beroertes toeneemt met de leeftijd en de bevolking steeds ouder wordt, nemen de gevolgen van beroertes steeds verder toe, voornamelijk onder vrouwen. Dit komt doordat de mortaliteit van beroertes hoger is bij vrouwen dan bij mannen en doordat vrouwen een grotere kans hebben op een slechte functionele uitkomst. Prognostische factoren zijn factoren die gebruikt kunnen worden om uitkomst te voorspellen, tevens kunnen ze behandelingen verbeteren en richting geven aan de ontwikkeling van nieuwe therapieën. Het doel van dit proefschrift is het effect van lichaamstemperatuur verlagende therapie bij patiënten met een acute beroerte te onderzoeken. Subdoelen zijn het onderzoeken van sekseverschillen in de behandeling van herseninfarcten en in lange termijn uitkomst na een hersenbloeding.

Hoofdstuk 2 beschrijft lichaamstemperatuur als prognostische factor bij beroertes. In paragraaf 2.1 en 2.2 beschrijf ik de rationale, achtergrond, het design en de resultaten van het Paracetamol (Acetaminophen) in Stroke 2 (PAIS 2) onderzoek. Subfebriele lichaamstemperatuur en koorts in de eerste dagen na een beroerte zijn sterk geassocieerd met slechte functionele uitkomst. Het verlagen van de lichaamstemperatuur en het voorkomen van koorts zouden daarom het functioneel herstel na een beroerte kunnen verbeteren. Paracetamol is het meest voorgeschreven middel bij koorts. Uit eerder onderzoek bij mensen zonder koorts weten we dat paracetamol de lichaamstemperatuur kan verlagen met 0.3 graden Celsius. Het eerste Paracetamol (Acetaminophen) in Stroke (PAIS) onderzoek vond dat vroege behandeling met hoge doseringen paracetamol de functionele uitkomst na een beroerte mogelijk verbetert bij patiënten met een lichaamstemperatuur van 36.5°C en hoger (OR 1.31; 95% CI: 1.01–1.97). Deze resultaten waren gebaseerd op een posthoc gedefinieerde subgroep analyse, en daarom was bevestiging van de resultaten in een onafhankelijk onderzoek nodig. Het doel van PAIS 2 was te onderzoeken of behandeling met hoge dosering paracetamol effect heeft op functionele uitkomst bij patiënten met een acute beroerte en een lichaamstemperatuur van 36.5°C en hoger.

PAIS 2 was een multicenter, gerandomiseerd, dubbelblind, placebogecontroleerd onderzoek. Het doel was het includeren van 1500 patiënten met een acuut herseninfarct of hersenbloeding binnen 12 uur na het ontstaan van de symptomen. Patiënten werden behandeld met 6 gram paracetamol per dag of placebo gedurende 3 opeenvolgende dagen. De primaire uitkomstmaat was verbetering op de modified Rankin Scale (mRS) score gemeten na 3 maanden en geanalyseerd met multivariabele ordinale regressie. Het onderzoek werd vroegtijdig afgebroken in verband met langzame inclusie van patiënten en een tekort aan financiële middelen. Van december 2011 tot en met oktober 2015 werden 256 patiënten geïncludeerd, 136 patiënten (53%) werden behandeld met paracetamol. We vonden geen significant effect van behandeling met paracetamol op de verschuiving van de mRS score (acOR 1.02; 95% CI: 0.7-1.6). Er was geen verschil in ernstige, ongewenste voorvallen (paracetamol n=35 (26%) versus placebo n=28

(24%)). We concludeerden dat behandeling met paracetamol veilig is. Het effect van behandeling met hoge dosering paracetamol op functionele uitkomst na een doorgemaakte beroerte blijft echter onzeker. Er is een zeer groot onderzoek naar vroege behandeling met paracetamol na een acute beroerte nodig om een meer precieze schatting te geven van het behandeleffect.

In hoofdstuk 2.3 onderzoek ik de invloed van baseline lichaamstemperatuur op het effect van behandeling met alteplase op functionele uitkomst bij patiënten met een acuut herseninfarct. In in vitro onderzoeken nam de fibrinolytische activiteit van alteplase per graad Celsius verlaging van de temperatuur af met 5%. Het effect van lichaamstemperatuur op behandeling met alteplase in patiënten met een acuut herseninfarct was echter onduidelijk. Ik vond dat het effect van behandeling met alteplase groter zou kunnen zijn bij hogere lichaamstemperaturen. Bij het onderzoeken van therapeutische hypothermie na een acute beroerte is het daarom van belang te controleren voor behandeling met alteplase, en onderzoeken naar behandeling met alteplase zouden kunnen overwegen lichaamstemperatuur als potentiele effect modificator te beschouwen.

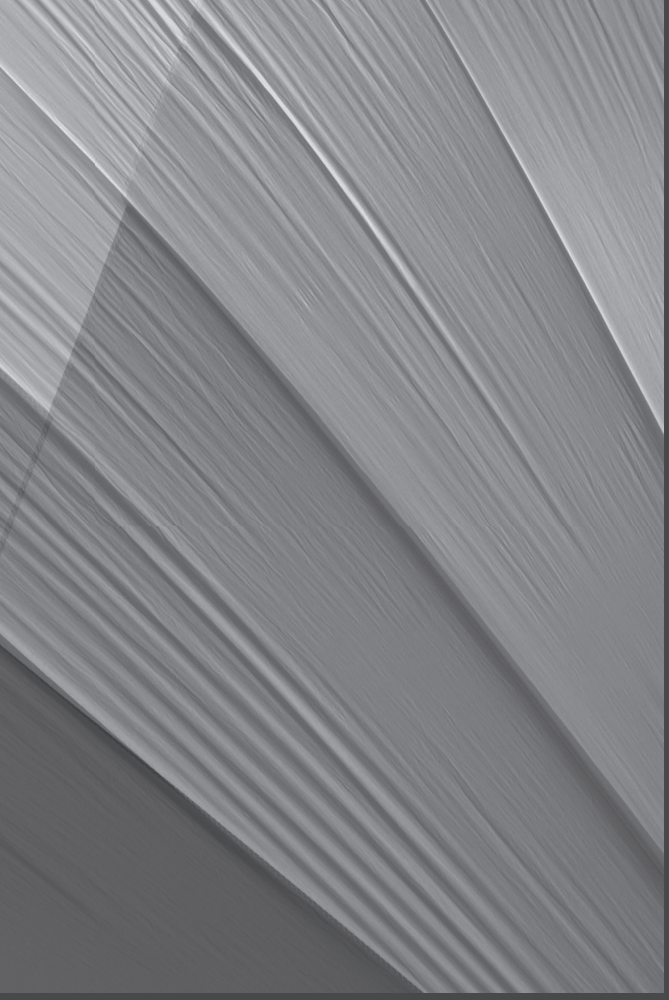
Hoofdstuk 3 beschrijft geslacht als prognostische factor bij beroertes. Hoofdstuk 3.1 bespreekt geslachtsongelijkheid in het krijgen van intraveneuze alteplase behandeling. We vonden dat vrouwen even vaak worden behandeld met intraveneuze alteplase als mannen wanneer ze op tijd voor behandeling in het ziekenhuis aankomen. Echter wanneer we de hele weg, die patiënten met een beroerte afleggen, onderzoeken, blijkt dat minder vrouwen binnen 4 uur het ziekenhuis bereiken dan mannen en daarom minder vaak met intraveneuze alteplase worden behandeld. Dit verschil zou verklaard kunnen worden door de gemiddeld hogere leeftijd waarop vrouwen een beroerte krijgen, waardoor ze vaker alleenstaand zijn.

Hoofdstuk 3.2 beschrijft geslachtsverschillen in functionele uitkomst na intra-arteriële behandeling bij een acuut herseninfarct. We toonden in het MR CLEAN onderzoek aan dat mannen veel baat hadden van intra-arteriële behandeling, terwijl we bij vrouwen geen gunstig effect vonden van intra-arteriële behandeling. Deze bevinding heeft geen solide biologische verklaring. Vrouwen leken een licht ongunstiger profiel te hebben, met als gevolg een hogere mortaliteit en meer ernstige, ongewenste voorvallen. Statische analyse van alle onderzoeken naar intra-arteriële behandeling liet inderdaad geen verschil zien tussen mannen en vrouwen in functionele uitkomst na intra-arteriële behandeling. Deze afwegingen suggereren dat kans de plausibele verklaring is voor onze bevindingen en maken duidelijk dat vrouwen intra-arteriële behandeling niet onthouden moet worden.

In hoofdstuk 3.3 onderzoek ik geslachtsverschillen in lange termijn functionele uitkomst bij patiënten met een spontane hersenbloeding. Voor dit onderzoek analyseerden we gegevens van 823 patiënten uit een prospectieve ziekenhuis registratie. We vonden dat vrouwen gemiddeld ouder zijn dan mannen wanneer zij een hersenbloeding krijgen. Mannen daarentegen hadden vaker hypertensie en diep gelegen bloedingen. Er waren geen verschillen in lange termijn functionele uitkomst en mortaliteit tussen mannen en vrouwen. De ongecorrigeerd slechtere functionele uitkomst bij vrouwen lijkt te kunnen worden verklaard door het leeftijdsverschil. Mannen en vrouwen lijken dus beiden te gaan profiteren van nieuwe

ontwikkelingen in de behandeling van hersenbloedingen.

In hoofdstuk 4 vat ik de belangrijkste bevindingen van mijn onderzoek beschreven in dit proefschrift samen en geef ik aanbevelingen voor verder onderzoek. Ik begin met de bespreking en interpretatie van de resultaten van PAIS 2. Tevens bespreek ik de verschillende vormen van hypothermie en de resultaten van eerder onderzoek. Daarna bediscussieer ik geslacht als prognostische factor bij beroertes. Ik beschrijf de rol van oestrogenen bij beroerte, geslachtsverschillen in symptomen van beroerte en het gebruik en het effect van behandeling met intraveneuze alteplase. Ik eindig met de aanbeveling dat het zeer belangrijk is bestaande behandelingen voor beroertes te verbeteren en nieuwe therapieën te ontwikkelen, omdat iedereen op elk moment een beroerte kan krijgen.



Chapter 6

Epilogue
Dankwoord
About the author
PhD portfolio
List of publications

Dankwoord

Nu dit proefschrift is afgerond, kan ik terugkijken op een uitdagende en leerzame onderzoeksperiode. Tijdens deze periode coördineerde ik een nationaal multicenter onderzoek; dit bracht veel sociale contacten zich mee en leerde mij veel over samenwerking en communicatie. Het was ook een periode van doorzetten, want het was niet altijd makkelijk om onderzoek doen te combineren met de opleiding tot neuroloog. Daarbij was de hulp en steun van anderen onmisbaar. Het proefschrift dat nu voor u ligt, is dan ook het resultaat van samenwerken met vele anderen. Deze mensen wil ik graag bedanken voor hun inzet. Een aantal van hen wil ik graag speciaal noemen.

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Het belangrijkste gedeelte van dit proefschrift bestaat uit het PAIS 2 onderzoek, een Nederlands multicenter onderzoek. Dit onderzoek had niet kunnen plaatsvinden zonder de inzet van vele mensen, die ik hiervoor wil bedanken. Ik wil de leden van de stuurgroep van PAIS 2, Hester Lingsma, Tobien Schreuder, Annemieke Ruitenberg, Ritu Saxena, Lisette Maasland, Jordie van Tuijl, Ben Jansen, Renske Van den Berg-Vos, Frederique Vermeij, Peter Koudstaal, Jaap Kappelle en Ale Algra bedanken. Het is jammer dat we met elkaar de 1500 patiënten niet hebben mogen halen, maar het resultaat mag er desondanks zijn. Een speciaal woord van dank gaat hierbij uit naar Heleen den Hertog. Beste Heleen, als voorganger bij PAIS en voorbeeld als AIOS en neuroloog ben ik je veel dank verschuldigd. Je hebt me erg geholpen bij het afronden van het PAIS 2 onderzoek en ik waardeer je input en ideeën altijd zeer. Jammer dat je, om begrijpelijke reden overigens, niet in de commissie kunt plaatsnemen. Graag wil ik hier ook Maarten van Gemert noemen. Beste Maarten, je was al topincludeerder bij PAIS en ook bij PAIS 2 heb je de meeste patiënten weten te motiveren voor het onderzoek. Je praktische kijk hield ons met de beide benen op de grond. Hartelijk dank daarvoor.
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Working on the neuro-intensive care ward from the University Hospital of Erlangen has been a special experience and crucial for my further professional and personal development. I would like to thank professor Stefan Schwab for giving me the opportunity and my colleagues Natalia, Claudia, Julia, Kosmas, Katrin, Jasmin, Matthias, Christina, Klemenz, Juliane, Kristin, Marina, Stefan, Tobias, Bastian, Antje and Vanessa for the warm welcome and cooperation. I would like to address a special thanks to professor Hagen Huttner and Joji Kuramatsu. Dear Hagen and Joji, thank you for giving me the opportunity to work with your data on ICH and write a paper together. I am looking forward to our next project.

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Het schrijven van een proefschrift is niet mogelijk zonder ontspanning op z’n tijd. Ik dank mijn bestuursgenootjes van het 38ste bestuur van de MFVR voor de gezellige avondjes met meestal net iets te veel wijn. Lieve Floor, ik denk met veel plezier terug aan onze vrijdagse lunches in de stad en onze saunabezoeken. In alle ontspanning hadden we openhartige gesprekken die mij menig inzicht in mezelf hebben gegeven. Gaan we snel weer bubbels drinken en naar de sauna?

Mijn paranimfen, Susanne Fonville en Willemijn Idema, ontzettend leuk en bijzonder dat jullie op deze belangrijke dag naast me willen staan. Lieve Suus, a.k.a. pietje Posterior, met je aanstekelijke lach heb je me door moeilijke momenten heen geholpen én voor onvergetelijke momenten gezorgd. We groeiden in onze rol als onderzoeker en arts-assistent tijdens vele congressen, Biemonds en de ESO Summer School in Debrecen, Hongarije. Daarnaast genoten we van het leven onder het genot van lekker eten en drinken. Ik waardeer jouw organisatietalent, optimisme en gezelligheid. Ik hoop je lach nog vaak te mogen horen.
Lieve Wil, sinds we samen in het 38ste bestuur van de MFVR hebben gezeten zijn we vriendinnen. We kregen tegelijkertijd onze doctoraalbul en je was erbij toen ik de belofte van Hippocrates aflegde. Nu sta je naast me op deze bijzondere dag. Je bent er altijd voor me, tijdens moeilijke én mooie momenten. Je bent zeer sociaal en oprecht en weet de juiste vragen te stellen om me aan het denken te zetten. Samen kunnen we ook erg van het leven genieten. Zo kijk ik met plezier op onze gezamenlijke vakanties met giechelwijn en oesters. Wat wordt onze volgende bestemming?

Lieve schoonfamilie, Arie en Margot, Wieke en Tom, en Frederike, dank voor jullie interesse, steun, afleiding, en, in de laatste periode, zorg voor Bente wanneer ik weer eens druk was met de afronding van dit proefschrift en de voorbereiding op de verdediging. Fijn dat jullie er altijd voor ons zijn.

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mijn hart. Lieve mama, wat had je deze vreugde graag gedeeld met papa, net als de vreugde die Bente je geeft. Ik heb bewondering voor de veerkracht die je hebt en de vastberadenheid waarmee je het leven zonder papa opnieuw vormgeeft. Ik hoop nog vele bijzondere herinneringen met je te mogen maken.

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About the author

Inger Rebecca de Ridder was born April 6th, 1982 in Almelo, the Netherlands and raised in Rijssen. She attended secondary school at Het Noordik in Almelo, from which she graduated in 2000. The same year she started to study business administration at the Radboud University in Nijmegen. In 2001, she proceeded to study medicine at the Erasmus University in Rotterdam. During her studies, she was an active member of the Rotterdam Medical Student Association (MFVR) and full-time board member in 2004-2005. In 2008, she wrote her master thesis titled "Homocysteine and the risk of ischemic stroke and TIA" under supervision of prof. dr. P.J. Koudstaal and obtained her medical degree later that year.
The same year she started to work as resident neurology at the Fransciscus Gasthuis in Rotterdam. In 2010, she started her training as neurologist at the Erasmus MC in Rotterdam (head prof. dr. P.A.E. Sillevis Smitt). She combined her training with research underlying this thesis at the department of neurology of the Erasmus MC under supervision of prof. dr. D.W.J. Dippel and dr. H.B. van der Worp (University Medical Center Utrecht). She was trial coordinator of the Paracetamol (Acetaminophen) in Stroke 2 (PAIS 2) trial. As part of her research training she attended summer school in neurovascular diseases at the University of Debrecen, Hungary in 2011. As part of her residency she worked from April to October 2015 on the neuro-intensive care ward of the University Hospital of Erlangen, Germany (head prof. S. Schwab). She lives with her boyfriend Willem Nugteren and daughter Bente in Rotterdam.

PhD portfolio

Name PhD student	Inger Rebecca de Ridder
Erasmus MC department	Neurology
Research school	Cardiovascular Research School Erasmus University Rotterdam (COEUR)
PhD period	January 2011-January 2017
Promotor	Prof. dr. D.W.J. Dippel
Co-promotor	Dr. H.B. van der Worp

	Year	Workload (ECTS)
General courses		
Biostatistics for clinicians (NIHES, Rotterdam, The Netherlands)	2011	1.0
Regression analysis for clinicians (NIHES, Rotterdam, The Netherlands)	2011	1.9
Basiscursus Regelgeving en Organisatie voor Klinisch Onderzoekers (Erasmus MC, Rotterdam, The Netherlands)	2011	1.0
Intervention research and clinical trials (NIHES, Rotterdam, The Netherlands)	2012	0.9
Quantitative Methods (COEUR seminar, Rotterdam, The Netherlands)	2015	0.2
In depth courses		
Dutch neurovascular network scientific meeting (NNW, Amsterdam, The Netherlands)	2011, 2012	0.4
Cardiovascular Medicine (COEUR Course, Rotterdam, The Netherlands)	2011	1.5
Endothelin in the picture (COEUR Seminar, Rotterdam, The Netherlands)	2011	0.2
Summer school neurovascular diseases (ESO, Debrecen, Hungary)	2012	2.0
Fellowship intensive care neurology (University Hospital Erlangen, Erlangen, Germany)	2015	5.0
Sex and gender differences in metabolism (COEUR Seminar, Rotterdam, The Netherlands)	2015	0.2
Neuro-intensive Care (COEUR Seminar, Rotterdam, The Netherlands)	2017	0.2

	Year	Workload (ECTS)
(Inter)National conferences		
European Stroke Conference (Hamburg, Germany)	2011	1.0
European Stroke Conference (Lisbon, Portugal)	2012	1.0
Dutch society for neurologist scientific meeting (Nunspeet, The Netherlands)	2012	0.4
European Stroke Conference (London, England)	2013	1.0
European Stroke Conference (Nice, France)	2014	1.0
European Stroke Organisation Conference (Barcelona, Spain)	2016	1.0
Oral presentations		
Gender differences in treatment with alteplase in acute stroke		
European Stroke Conference (Hamburg, Germany)	2011	1.0
Gender differences in presenting symptoms of acute stroke		
European Stroke Conference (Hamburg, Germany)	2011	1.0
Paracetamol (Acetaminophen) in Stroke 2 (PAIS 2)		
Dutch neurovascular network scientific meeting (Amsterdam, The Netherlands)	2011	1.0
Update PAIS 2		
Dutch neurovascular network scientific meeting (Amsterdam, The Netherlands)	2012	1.0
Prediction of disability and functional outcome in patients with ischemic stroke for efficient discharge planning		
European Stroke Conference (London, England)	2013	1.0
Richtlijn intracerebral hemorrhage		
Department of neurology, Erasmus MC, Rotterdam, The Netherlands	2015	1.0
Results from the PAIS 2 study		
European Stroke Organisation Conference (Barcelona, Spain)	2016	1.0
PAIS 2: resultaten van een multicenter, gerandomiseerde, placebo gecontroleerde studie		
Department of neurology, Erasmus MC, Rotterdam, The Netherlands	2016	1.0
Poster presentations		
Paracetamol (Acetaminophen) in Stroke 2 (PAIS 2): ongoing trial		

	Year	Workload (ECTS)
European Stroke Conference (Hamburg, Germany)	2011	0.5
High body temperature: larger benefit of alteplase in acute ischemic stroke?		
European Stroke Conference (Lisbon, Portugal)	2012	0.5
Course of body temperature in the first 24 hours after admission for acute stroke		
European Stroke Conference (Lisbon, Portugal)	2012	0.5
Paracetamol (Acetaminophen) in Stroke 2 (PAIS 2): ongoing trial		
European Stroke Conference (Lisbon, Portugal)	2012	0.5
Paracetamol (Acetaminophen) in Stroke 2 (PAIS 2): ongoing trial		
European Stroke Conference (London, England)	2013	0.5
Teaching activities		
Supervising research projects	2012-2014	2.2
Other		
Peer review for scientific journals	2015-present	1.0

List of publications

de Ridder IR, van der Worp HB, van Gemert HM, Schreuder AH, Ruitenberg A, Maasland EL, et al. Does paracetamol improve recovery after stroke?. *Ned Tijdschr Geneeskd.* 2011;155:A4169

de Ridder I, Dirks M, Niessen L, Dippel D. Unequal access to treatment with intravenous alteplase for women with acute ischemic stroke. *Stroke.* 2013;44:2610-2612

Dirks M, **de Ridder IR,** Dippel DWJ. Verschil in behandeling met trombolyse met intraveneuze alteplase tussen mannen en vrouwen: gegevens uit het PRACTISE onderzoek. In: Koopman C, van Dis I, Visseren FLJ, Vaartjes I, Bots ML. Hart- en vaatziekten in Nederland 2012, cijfers over risicofactoren, ziekte en sterfte. Den Haag: Hartstichting, 2012.

de Ridder I, den Hertog H, van Gemert M, Dippel D, van der Worp B. Increased benefit of alteplase in patients with ischemic stroke and a high body temperature. *Cerebrovasc Dis.* 2013;35:60-63

de Ridder IR, de Jong FJ, den Hertog HM, Lingsma HF, van Gemert HM, Schreuder AH, et al. Paracetamol (acetaminophen) in stroke 2 (pais 2): Protocol for a randomized, placebo-controlled, double-blind clinical trial to assess the effect of high-dose paracetamol on functional outcome in patients with acute stroke and a body temperature of 36.5 degrees C or above. *Int J Stroke.* 2015;10:457-462

de Ridder I, Kuramatsu J, Gerner S, Madzar D, Lucking H, Kloska S, et al. No sex differences in long-term functional outcome after intracerebral hemorrhage. *Int J Stroke.* 2016 epub ahead of print

de Ridder IR, Fransen PS, Beumer D, Berkhemer OA, van den Berg LA, Wermer MJ, et al. Is intra-arterial treatment for acute ischemic stroke less effective in women than in men? *Interv Neurol.* 2016;5:174-178

de Ridder IR, den Hertog HM, van Gemert HM, Schreuder AH, Ruitenberg A, Maasland EL, et al. PAIS 2 (Paracetamol [Acetaminophen] in Stroke 2): Results of a Randomized, Double-Blind Placebo-Controlled Clinical Trial. *Stroke.* 2017;48:977-982

TEMPERATURE AND SEX AS PROGNOSTIC FACTORS IN STROKE

Anyone can suffer a stroke at any time and the consequences of stroke are often severe. It therefore remains an important challenge to find new prognostic factors and to further evaluate the importance of known prognostic factors, as this may improve clinical knowledge and management, and may guide new therapies. This thesis focusses on the influence of the prognostic factors temperature and sex on treatment and outcome of patients with acute stroke. We aimed to assess the effect of body temperature management on functional outcome in patients with acute stroke by performing the Paracetamol (Acetaminophen) in Stroke 2 (PAIS 2) trial. In addition, we investigated sex differences in the acute management of ischemic stroke and sex differences in long-term functional outcome after intracerebral hemorrhage.

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