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Jan-Dirk Vermeij

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Preventive antibiotic therapy in patients with acute stroke

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aan de Universiteit van Amsterdam

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ten overstaan van een door het College voor Promoties ingestelde commissie,

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Jan-Dirk Vermeij
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Contents

1

General introduction

General introduction

Stroke, or cerebrovascular disease, is characterised by a sudden or rapidly developing loss of cerebral functions by a vascular cause; which can include either the occlusion of a blood vessel (ischaemic stroke) or the leakage of blood from this vessel into the surrounding brain tissue (haemorrhagic stroke).[1] Its prevalence increases above 40 years of age, reaching the highest rate between 74 to 79 years. In 2015, there were globally 42 million prevalent cases.[2]

The most recent numbers from the Netherlands date from 2017 and show 477,800 prevalent cases (28.1 cases per 1,000 men; 27.7 cases per 1,000 women), of which 38,000 were new patients. Besides, there were 57,900 new patients with a 'transient ischaemic attack' (TIA): a temporary focal episode of neurological dysfunction, lasting less than 24 hours that clears without any residual symptoms. Stroke ranks second in 'disability adjusted life years' (DALYs), after coronary heart diseases. Almost 3% of the Dutch population above the age of 12 years has ever suffered from a stroke. These numbers are even believed to be an underestimation since patients living in nursing homes were not added to the statistics, and the absolute number of patients are still increasing due to the aging and growing population.[3]

Approximately 85% of all strokes are caused by an ischaemic event: the (partial) occlusion of a vessel supplying blood to the brain, causing a reduction in the cerebral blood flow (CBF). Reduction of CBF can often be compensated and does not necessarily cause neuronal dysfunction and/or the appearance of neurological deficits. Only if the reduction in CBF reaches less than 25% of normal, it causes metabolic failure and initiates a cascade of events leading to cell death and consequently the clinical features of stroke. Besides ischaemic events, 10% of all strokes are due to intracerebral haemorrhage, which may clinically present slightly different. Apart from a reduction in CBF, leakage of blood may also cause mass effect to the brain, as well as an increased intracranial pressure – also leading tot severe headache, vomiting or a sudden loss of consciousness. The final 5% of strokes are caused by other disorders, of which the rupture of an aneurysm ('subarachnoid haemorrhage') or the occlusion of a cerebral vein ('cerebral venous thrombosis') are the most common. This last category is beyond the scope of this thesis.

Despite the cause of neuronal dysfunction, there are three clinical features suggesting stroke[1] :

(1) a sudden onset of symptoms and signs (within seconds, or developing over a few minutes),

(2) focal symptoms and signs that are usually explained by one single lesion in the brain. These can include: motor symptoms (weakness or clumsiness of one side/ part of the body); sensory loss; speech-, reading- and/or writing deficits (either executing, or understanding); visual disturbances (neglect of one site, or blindness), or; symptoms that may suggest stroke only in the right context (swallowing difficulties, ataxia, diplopia, vertigo etc.).

(3) 'negative symptoms', suggesting loss of function.

To assess stroke severity, two scales are often used, both in clinical setting and in stroke studies: the National Institutes of Health Stroke Scale (NIHSS) is used to assess the level of neurological deficit (Appendix), and the Glasgow Coma Scale (GCS) is used to assess level of consciousness (Appendix).[4,5] Consciousness is most often impaired in haemorrhagic stroke, however, it may also be impaired in certain types of ischaemic stroke, such as severe occlusion of a large proximal artery or a basilar artery occlusion.

Regarding prognosis, a number as high as 15% of patients with ischaemic stroke have died after one month. After 1 year, this percentage has increased to 25%, compared to 55% of patients suffering from haemorrhagic stroke. Forty percent of the stroke survivors require (at least any) assistance in daily living between 1 month and 5 years after the event.[6] In stroke research, prognosis is usually assessed by either mortality rate or by functional outcome on the modified Rankin Scale (mRS) (Appendix).[7] The mRS runs from 0 to 6, indicating perfect health with no symptoms (mRS 0) to death (mRS 6). The six-point-scale is often dichotomised between 'favourable outcome' (mRS 0 to 2) – indicating that a patient only has slight disability, but does not need any assistance – or 'unfavourable outcome' (mRS 3 to 6) – indicating that a patient is in need of assistance or dead.

Over the years, different therapeutic strategies have been assessed to improve stroke outcome. These can be divided in three categories: acute therapy, the prevention of complications, and the prevention of stroke recurrences ('secondary prevention').

Acute stroke therapy is a field that has historically gained a lot of attention. It made great progression over the last years. In 1995, a large American double-blind study (the 'NINDS study') showed the beneficial effect of intravenous recombinant tissue plasminogen activator (rt-PA; also known as 'thrombolysis') on functional outcome in ischaemic stroke – despite a small increase in symptomatic intracerebral haemorrhages.[8] Treatment window and number of patients treated with rt-PA increased over time. Overall, for every 1,000 patients treated with thrombolysis, death or dependency is avoided in 41. If initiated within three hours after onset, there are even 95 fewer dead or dependent patients.[9] Despite the beneficial effect, only 12 to 15% of all ischaemic stroke patients are eligible to receive this therapy.[10] An additional acute stroke treatment became available in 2015, after multiple randomised trials showed the beneficial effect of intra-arterial thrombectomy (IAT).[11] IAT is an interventional procedure in which the blood clot is actively removed under image guidance, using a catheter that is introduced into the femoral artery in the groin and then moved upwards through the aorta and carotid artery towards the occluded artery in the brain. It can be used in addition to thrombolysis if therapy did not succeed, as well as in a group of patients that are unable to receive thrombolysis. For every 100 patients treated with IAT, 38 had a less disabled outcome compared with best medical management, and 20 more will achieve a favourable outcome with functional independence as result of the treatment. Risk of intracerebral haematoma and death is not higher than with best medical therapy alone.[11] Again, the percentage of patients that are eligible for IAT remains restricted: 3 to 5% of all ischaemic stroke patients, and 11 to 18% of all stroke patients that arrive to the emergency room within 6 hours.[12] Following intracerebral haematoma, no effective therapy to improve functional outcome has yet been found.

Although current possible acute treatment options show good results, the number of patients that meet the eligibility criteria is still limited. With the aim to further improve disease course in favour of all stroke patients (both ischaemic and haemorrhagic), other strategies are mandatory. The main focus should therefore not only be the treatment options in the very acute phase; we also need to investigate interventions on the longer run. On one hand, patients may benefit from the prevention of complications during hospital stay that might influence factors like length of admission, chance of rehabilitation and stroke outcome. Some examples of influenceable factors are: hyperglycaemia, hyperthermia, urinary retention, deep vein thrombosis, cerebral edema, and seizures. On the other hand, patients benefit from secondary prevention: reducing the chance of stroke recurrence in the future. This can be achieved by regular monitoring and (if necessary) treatment of vascular risk factors, like: hypertension, heart failure, diabetes mellitus, hypercholesterolaemia etc.[13]

Infections are amongst the variables that are a frequent complication in the acute phase after stroke. About 30% of all stroke patients develop any infection within the first days after onset. The most common are pneumonia and urinary tract infections, which both occur in 10% of the patients. If patients are admitted to the Intensive Care Unit, the chance of developing any infection even increases to 45%; 28% of patients develop pneumonia and 20% develop a urinary tract infection.[14]

Risk factors for the development of an infection might be related to both patient and stroke related factors. Women, for example, are more susceptible to urinary tract infections; this chance will increase after being admitted to the hospital and placing a urinary catheter. Patients with swallowing difficulties after stroke might have a bigger chance of developing pneumonia due to reduced coughing as well as an increased chance of choking. Mechanical ventilation after tube placement in the airway will even increase this risk. A bedridden state due to the neurologic deficits will increase the chance of any infection; diabetes mellitus, a common risk factor for the development of stroke, also decreases the immune system and consequently increases the chance of an infection even more; in summary, dozens of factors (co-)influence the chance of a post-stroke infection.[15]

Starting from 1998, several studies have investigated the effect of post-stroke infections on clinical outcome, of which many concluded that its occurrence is associated with a higher chance of poor functional outcome and higher mortality rates.[15] A very plausible hypothesis therefore is that by reducing the post-stroke infection rate, more patients will have a good functional outcome and survival will increase.

Prevention of infections can be achieved by the prophylactic administration of antibiotics. It seems obligatory to use an antibiotic from a spectrum that covers the most common causative bacteria of post-stroke infections. Besides, some antibiotics are also believed to have a neuroprotective action, leading to a reduction in the size of the damaged brain tissue.[15]

A promising antibiotic to improve stroke outcome seems to be the β-lactam antibiotic ceftriaxone. This off-patent medicine both provides a broad action against the most common causative bacteria of infection after stroke, but also showed to have neuroprotective properties in an animal model.[16] Preventive administration of ceftriaxone in the acute phase after stroke could therefore potentially have a beneficial effect for all patients suffering stroke – not only the relatively small amount of patients meeting specific eligibility criteria. On the other hand, increasing use of antibiotics might lead to increasing resistance rates and a potential benefit should therefore be carefully weighed.

Aim and outline of the thesis

The aim of this thesis is to investigate whether preventive antibiotic therapy in patients with acute ischaemic or haemorrhagic stroke is an effective and safe strategy to improve functional outcome.

In **Chapter 2** we describe the study protocol for a large randomised trial investigating the effect of preventive administration of ceftriaxone in the acute phase of stroke on functional outcome and infection rate. Results of the trial are presented in **Chapter 3**.

In **Chapter 4**, a post-hoc analysis is presented, aiming to gain more insight into the characteristics of patients who received intravenous thrombolysis: a patient group that was found to benefit from preventive ceftriaxone.

Chapter 5 provides a systematic review and meta-analysis of all randomised-controlled trials on preventive antibiotic therapy that were published until May 2017. Effect on functional outcome, mortality, infection rate, length of hospital stay and safety were assessed.

In **Chapter 6**, we provide a clinically oriented overview of current insights on post-stroke infections and the use of preventive antibiotic therapy in stroke. The first half of the chapter considers prevalence, risk factors and impact of post-stroke infections. In the second half, we focus on preventive antibiotics in stroke: from 'the early days', via current insights, to 'future directions'.

The last chapter of this thesis, **Chapter 7**, first provides a general introduction on current insights on the actual incidence of post-stroke infections and their impact on stroke outcome. Next, we summarize and discuss the previous chapters regarding the effect of preventive antibiotic therapy on stroke outcome, to end with implications for daily practice and future directions for research in this area.

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2.1

Update of the Preventive Antibiotics in Stroke Study (PASS): a randomised controlled phase 3 clinical trial

Willeke F Westendorp*, Jan-Dirk Vermeij*, Nan van Geloven, Diederik W J Dippel, Marcel G W Dijkgraaf, Tom van der Poll, Jan M Prins, Lodewijk Spanjaard, Frederique H Vermeij, Paul J Nederkoorn†, Diederik van de Beek†

* † These authors contributed equally

Trials 2014; 15: 133

Abstract

Background

Stroke is a leading cause of death worldwide. Infections after stroke occur in 30% of stroke patients and are strongly associated with unfavourable outcome. Preventive antibiotic therapy lowers infection rate in patients after stroke, however, the effect of preventive antibiotic treatment on functional outcome after stroke has not yet been investigated. The Preventive Antibiotics in Stroke Study (PASS) is an ongoing, multicentre, prospective, randomised, open-label, blinded end point trial of preventive antibiotic therapy in acute stroke. Patients are randomly assigned to either ceftriaxone at a dose of 2 g, given every 24 hours intravenously for four-days, in addition to stroke-unit care, or standard stroke-unit care without preventive antibiotic therapy. Aim of the study is to assess whether preventive antibiotic treatment improves functional outcome at three months by preventing infections.

Results

To date, 2,470 patients have been included in PASS. Median stroke severity of the first 2,133 patients (second interim analysis) is 5 (IQR 3 to 9) on the National Institutes of Health Stroke Scale (NIHSS). Due to the PROBE design, no outcome data are available yet. In the initial trial protocol we proposed a dichotomisation of the mRS as primary analysis of outcome and ordinal regression analysis as secondary analysis of primary outcome, requiring a sample size of 3,200 patients. However, ordinal analysis of outcome data is becoming increasingly more common in acute stroke trials, as it increases statistical power. For PASS, funding is insufficient for inclusion of 3,200 patients with the overall inclusion rate of 15 patients per week. Therefore we change the analysis of our primary outcome from dichotomisation to ordinal regression analysis on the mRS. Power analysis showed that with similar assumptions 2,550 patients are needed using ordinal regression analysis. We expect to complete follow-up in June 2014. A full statistical analysis plan will be submitted for publication before treatment allocation will be unblinded.

Conclusions

The data from PASS will establish whether preventive antibiotic therapy in acute stroke improves functional outcome by preventing infection. In this update, we changed our primary outcome analysis from dichotomisation to ordinal regression analysis.

Trial registration

Current controlled trials; ISRCTN66140176. Date of registration: 6 April 2010.

Update

Preventive Antibiotics in Stroke Study (PASS)

Stroke is a leading cause of death worldwide. Infections after stroke occur in 30% of stroke patients and are strongly associated with unfavourable outcome.[1,2] Preventive antibiotic therapy lowers infection rate in patients after stroke; however, the effect of preventive antibiotic treatment on functional outcome after stroke has not yet been investigated.[3,4]

The aim of PASS is to investigate whether preventive use of the antibiotic ceftriaxone improves functional outcome in patients with stroke. PASS is an ongoing, multicentre Prospective, Randomised, Open-label, Blinded End point trial (PROBE) of standard care with preventive ceftriaxone treatment which is compared with standard care without preventive ceftriaxone. Adult patients with stroke (both ischaemic and haemorrhagic) and a score ≥1 on the National Institutes of Health Stroke Scale will be included. Patients are randomly assigned to either ceftriaxone at a dose of 2 g, given every 24 hours intravenously for four-days, in addition to stroke-unit care, or standard stroke-unit care without preventive antibiotic therapy. All items from the World Health Organization Trial Registration Data Set are shown in Table 1. For description of the entire study protocol, including study procedures and data collection, assessment of infections and outcomes, allocation and blinding procedures, we refer to the initial trial protocol publication.[5] Changes to the protocol since the first version are shown in Table 2. Medical-ethical approval of the protocol and amendments was obtained by the medical ethical committee of the AMC. All participating centres are shown in Table 3.

The primary end point of the PASS is functional outcome at three-month follow-up on the modified Rankin Scale (mRS), a well-validated functional outcome scale in stroke patients.[6] In the protocol publication, the primary efficacy end point has been defined as the functional outcome at the three-month follow-up, as assessed by the mRS dichotomised as a favourable outcome (mRS 0 to 2) or as an unfavourable outcome (mRS 3 to 6). The proportional odds model was defined as the secondary analysis of the primary end point.[5] Secondary outcome measures were death rate at discharge and three-months, infection rate during hospital admission, length of hospital admission, volume of post-stroke care, use of antibiotics during follow-up, Quality-adjusted life years (QALYs); and costs. In this update publication of PASS, we change our primary outcome analysis from dichotomisation to ordinal regression analysis on the mRS. We also change the secondary outcome of use of antibiotics during follow-up into use of antibiotics during hospital stay.

Table 1. All items from the World Health Organization Trial Registration Data Set (SPIRIT checklist, item 2b)

Data category	Information
Primary registry and trial identifying number	Current controlled trials; http://www.controlled-trials.com; ISRCTN66140176
Date of registration in primary registry	6 April 2010
Secondary identifying numbers	-
Source(s) of monetary or material support	1. Netherlands Organisation for Health Research and Development (ZonMw) (Netherlands) (ref: 171002302) 2. Netherlands Heart Foundation (Nederlandse Hartstichting) (Netherlands) (ref: CD 300006)
Primary sponsor	Academic Medical Centre (AMC) (Netherlands)
Secondary sponsors	-
Contact for public queries	Paul J Nederkoorn; P.J.Nederkoorn@amc.uva.nl
Contact for scientific queries	Paul J Nederkoorn, Department of Neurology, Academic Medical Centre, PO box 22660, 1100 DD Amsterdam, The Netherlands
Public title	Preventive Antibiotics in Stroke Study
Scientific title	Preventive ceftriaxone to improve functional health in patients with stroke by preventing infection: a multicentre prospective randomised controlled trial
Countries of recruitment	The Netherlands
Health condition(s) or problem(s) studied	Stroke, infection
Intervention(s)	Optimal medical care and ceftriaxone 2,000 mg intravenously, once daily, for four days, versus optimal medical care without ceftriaxone.
Key inclusion and exclusion criteria	Inclusion criteria: aged greater than or equal to 18 years, either sex; stroke (ischaemic and haemorrhagic); any measurable neurological deficit defined as National Institutes of Health Stroke Scale (NIHSS) greater than 1; stroke onset less than 24 hours; admission.
	Exclusion criteria: symptoms or signs of infection on admission requiring antibiotic therapy; use of antibiotics less than 24 hours before admission; pregnancy; hypersensitivity for cephalosporin; previous anaphylaxis for penicillin or derivates; subarachnoid haemorrhage; death seems imminent.
Study type	Multicentre prospectrive randomized open-label blinded end point trial
Date of first enrolment	4 July 2010
Target sample size	2,550
Recruitment status	Recruiting
Primary outcome(s)	Functional health at three-month follow-up, as assessed by the modified Rankin Scale (mRS)
Key secondary outcomes	Death rate at discharge and three months, infection rate during hospital admission; length of hospital admission; volume of post-stroke care; use of antibiotics during hospital stay; Quality adjusted life years (QALYs); costs.

Table 2. Protocol revision chronology

Date	Protocol version and amandments
5 May 2010	Original protocol
15 August 2010	Protocol version 1.1. Amendments: exclusion criterion 'death seems imminent' added; compulsory urine analysis and culture on admission omitted.
9 December 2010	Protocol version 1.2. Amendments: new study centres with new estimations of included patients were added; paragraph 6.6 'drug-accountability': badge number of the administered ceftriaxone will be noted by the nurse administrating the medication into the 'drug accountability form' according to GCP-guidelines for pharmacies; paragraph 7.2 'randomisation, blinding and treatment allocation': randomisation will not be stratified according to stroke type, solely by study centre and stroke severity; assessment of blinded outcome is specified as performed by a person not involved in the trial team; performance of interim analyses is specified as performed by an independent statistician not involved in the trial team; paragraph 8.2 'adverse and serious adverse events': for each participating centre, a flowchart of serious adverse event/suspected unexpected serious adverse reactions (SAE/SUSAR) reporting will be provided in the local Investigator File; paragraph 8.5 'data monitoring': reference to the monitoring plan is added.
10 January 2014	Protocol version 1.3. Amendment: change in primary analysis of primary outcome from dichotomised analysis to ordinal regression analysis according to the proportional odds model.
Total course of study	Participating centres were added (all participating centres are shown in Table 3).

Change in primary analysis of primary outcome and adaptation of sample size

The modified Rankin Scale is a well-validated functional scale for assessing outcome after stroke. Analysis on a dichotomisation in favourable versus unfavourable outcome delivers easily comprehensible results. However, cut-off is arbitrarily and solely based on improvement beyond this one cut-off point. A secondary analysis including 55 datasets of stroke trials showed that statistical analysis based on the ordered nature of functional outcome data versus dichotomisation was more efficient and more likely to deliver reliable results.[7] Although there were some annotations regarding this publication, more and more studies are using ordinal regression analysis.[8-10]

In the design of PASS, both dichotomisation and ordinal regression analysis were described as analysis of the primary outcome.[5] We based our initial sample size calculation on the dichotomised outcome (favourable versus unfavourable outcome). Dichotomisation was chosen as primary analysis of efficacy because of the widespread use in stroke trials.[5,11] However, trial completion will take an unrealistically long time with excessive costs with the current inclusion rate of 15 patients per week. Therefore, we now propose a switch in primary analysis of the primary outcome using an ordinal outcome analysis. The primary outcome will remain to be assessed on the mRS. The primary outcome with dichotomisation will be presented as secondary analysis of primary outcome. Using ordinal regression analysis for PASS enables us to preserve the assumptions of the strength of the treatment effect with a lower total sample size.

Table 3. Centres participating in the Preventive Antibiotics in Stroke Study (PASS) with local investigators

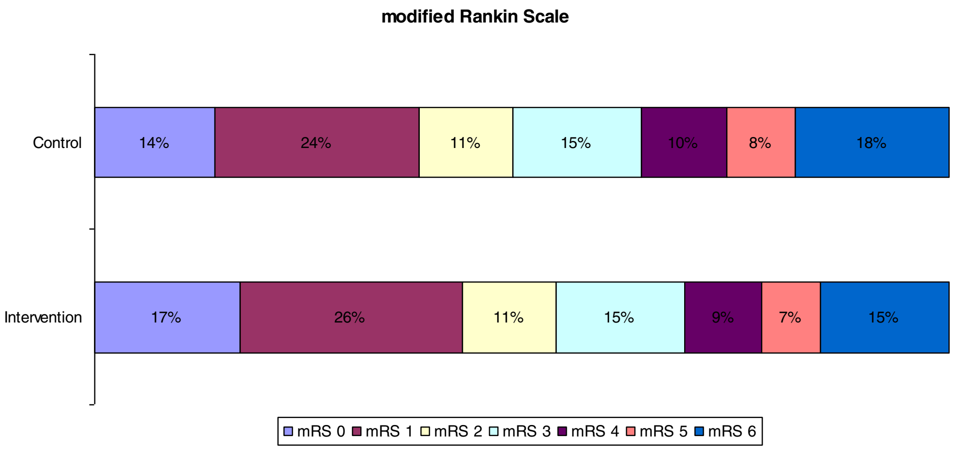
Centre	Local investigator
Academic Medical Centre, Amsterdam	D. van de Beek, P.J. Nederkoorn
Albert Schweitzer Hospital, Dordrecht	H. Kerkhoff
Amphia Hospital, Breda	M.J.M. Remmers
Amstelland Hospital, Amstelveen	D.S.M. Molenaar
Atrium Medical Centre, Heerlen	A. Schreuder
Boven-IJ Hospital, Amsterdam	M. Janmaat
Bronovo Hospital, The Hague	S.M. Manschot
Catharina Hospital, Eindhoven	K. Keizer
Erasmus Medical Centre, Rotterdam	D.W.J. Dippel
Groene Hart Hospital, Gouda	K. de Gans
HAGA Hospital, The Hague	S.F. de Bruijn
Kennemer Gasthuis, Haarlem	M. Weisfelt
Laurentius Hospital, Roermond	M.P.J. van Goor
Martini Hospital, Groningen	E.S. Schut
Medical Centre Haaglanden, The Hague	K. Jellema
Medical Centre Alkmaar, Alkmaar	R. ten Houten
Onze Lieve Vrouwe Gasthuis, Amsterdam	J.L.W. Bosboom
Orbis Medical Centre, Sittard	N. van Orshoven
Rijstate Hospital, Arnhem	S.E. Vermeer
Reinier de Graaf Hospital, Delft	L.A.M. Aerden
Slotervaart Hospital, Amsterdam	N.D. Kruyt
Spaarne Hospital, Hoofddorp	I.S.J. Merkies
Sint Franciscus Gasthuis, Rotterdam	F. Vermeij
University Medical Centre Radboud, Nijmegen	E.J. van Dijk
University Medical Centre Utrecht, Utrecht	H.B. van der Worp
VU Medical Centre, Amsterdam	M.C. Visser
Westfries Gasthuis Hoorn	T.C. van der Ree
IJselland Hospital, Capelle aan den IJssel	A.D. Wijnhoud
Zaans Medical Centre, Zaandam	R.M. van den Berg-Vos
ZGT, Almelo	L.J.A. Reichman

Sample size

We based our initial sample size calculation on the dichotomised outcome (favourable versus unfavourable outcome). With the assumption of reduction of unfavourable outcome of 5%, with a power of 80% and P-value of 0.05, we aimed to include 3,200 patients.

We now propose a new sample size of 2,550 patients, which is based on the ordinal regression analysis of the primary outcome. For this analysis we will use the 'proportional odds model', also known as the 'cumulative logit model'.[12] The assumption for the distribution on mRS in the control-arm is based on the control-arm in the Paracetamol (Acetaminophen) In Stroke (PAIS) trial, which had almost similar inclusion criteria as PASS.[13] We assumed a proportional odds ratio of 0.818 between all pairs of category groups, similar to the assumption in the original sample size calculation (odds ratio of 0.818 for mRS 0 to 2 versus mRS 3 to 6). Figure 1 shows the expected distribution of the two treatment arms. Using the method of Whitehead, with alpha 0.05 and power 80%, the desired sample size in the proportional odds model is estimated at a total of 2,410 patients.[14] Given an expected rate of patients lost to follow-up and/or patients with incomplete data of 5%, a conservative estimate for the new sample size with the primary end point analysed on all categories of the mRS is 2,531 patients. We will therefore adapt the sample size to 2,550 patients; a reduction of 650 patients compared to the original sample size estimate based on a dichotomous outcome on the mRS. This decision has been made by the researchers without any knowledge of outcome data per treatment group.

Figure 1. Expected distribution of the two treatment arms



The assumption for the distribution on mRS in the control-arm is based on the control-arm in the Paracetamol (Acetaminophen) In Stroke (PAIS) trial, which had almost similar inclusion criteria as PASS.[9]

Recruitment target

By 12 February 2014, 2,470 patients were included in the PASS. Up-to-date statistics can be found at http://www.passamc.nl. With a stable weekly inclusion rate of 15 patients, follow-up of the last included patient is expected in June 2014.

Definitions of infection

Infection rate during hospital admission will be assessed in two ways. First, clinical diagnosis according to the treating physician will be recorded. Second, diagnosis of infection will be judged by two experienced infectious diseases specialists, blinded for treatment allocation, using the modified criteria of the United States Centres for Disease Control and Prevention.[15] This will be done in all patients who developed fever or a new onset delirium during admission, in patients in whom there was suspicion of infection but no diagnostics were performed, and in patients in a palliative care setting. One important issue that needs to be addressed is the risk of performance and detection bias. Since the treating physician is aware of the treatment allocation, this could influence decisions on non-scheduled treatment. For the PASS, the most important issue to address is the detection and treatment of infection. A physician could be more or less likely to order investigations or start treatment for a possible infection depending on the treatment allocation. By giving recommendations for diagnostic procedures in the previously mentioned subgroups of patients, and by collecting results of these procedures in standardized case record forms, we try to limit this form of bias.

Monitoring of antibiotic resistance

One of the most important mechanisms of resistance against third generation cephalosporins is forming of extended-spectrum-β-lactamase (ESBL), an enzyme that renders antibiotics ineffective, in *Enterobacteriaceae*. In our study we monitor the prevalence of ESBL-producing bacteria in both treatment arms. We therefore collect stool specimens at admission and discharge in a subgroup of patients. To date, samples have been obtained in 300 patients.

Development of the statistical analysis plan

Currently, the statistical analysis plan is being finalised, without insight in to the unblinded data. It will be published before the randomisation code is broken in late 2014. The statistical analysis plan describes the analysis of primary outcome with ordinal regression analysis and a secondary dichotomised analysis into detail. It also describes a small number of prespecified subgroup analyses, and a larger number of exploratory secondary analyses, that will be performed, as well as treatment of missing values.

Discussion

The PASS aims to investigate whether preventive antibiotic therapy improves functional outcome by preventing infections. The results of a trial examining the effect of preventive antibiotic therapy on functional outcome are urgently warranted. Infection after stroke is common and infection has repeatedly been shown to worsen outcome.[1,2,16,17] Since previous studies on preventive antibiotic therapy were too small, heterogeneous, or did not investigate functional outcome, no sufficient information is available on the role of preventive antibiotic therapy in acute stroke.[4]

With this update of the protocol, we present a change in primary analysis of the primary outcome on the mRS from a dichotomised analysis to an ordinal regression analysis. Ordinal analysis of outcome data is increasingly common in acute stroke trials as well as in other trials, for example on traumatic brain injury.[7,18] Data is used more efficiently with ordinal analysis as compared to a dichotomised analysis. For example the ECASS-II trial failed to show an effect of treatment in the dichotomised approach, but did show an affect with ordinal shift analysis.[19]

Different approaches can be used for the analysis of ordinal outcome data. In the PASS we chose the ordinal regression analysis as primary analysis of outcome, which was already described as a secondary analysis of primary outcome in the original protocol. The proportional odds model provides additional information from ordinal outcome data, as it takes into account improvements at any point on the mRS.[18] This method is highly efficient when compared to a dichotomised approach, but also when compared to other ordinal approaches.[18] A possible disadvantage of this approach is the assumption of proportional odds across all groups. In PASS, we chose this method because we expected a similar effect of preventive antibiotic treatment across all outcomes, and therefore expect to meet the assumptions of the proportional odds model.

With the new sample size of 2,550 patients we expect completion of inclusion of patients in PASS in June 2014.

Acknowledgements

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Thirty Dutch hospitals participate in the PASS; all centres with local investigators are shown in Table 3.

The data safety monitoring committee (DSMB) is formed by: GJ Hankey, MD, PhD, Consultant Neurologist, Head of Stroke Unit, Department of Neurology, Royal Perth Hospital, Australia (chair); A Algra, MD, PhD, Clinical Epidemiologist, Julius Centre and Department of Neurology, UMC Utrecht, the Netherlands; MJM Bonten, MD, PhD, Microbiologist, Department of Medical Microbiology and Julius Centre, University Medical Centre Utrecht, Utrecht, the Netherlands. Advisory Board of the PASS consists of Professor M Vermeulen, Department of Neurology, AMC Amsterdam, and Professor RJ de Haan, Clinical Research Unit, AMC Amsterdam. We like to thank Professor P Sandercock, Department of Clinical Neurosciences, Western General Hospital, Edinburgh, for advising us with the design of the current protocol update.

Abbreviations

AMC: Academic Medical Centre
ESBL: extended-spectrum-β-lactamase
NIHSS: National Institutes of Health Stroke Scale
PASS: Preventive Antibiotics in Stroke Study
PROBE: Prospective, Randomised, Open-Label, Blinded End point
SAE: serious adverse event
SUSAR: suspected unexpected serious adverse reaction

Contribution of authors

WFW, JDV performed conception and design of the work and drafted the work. PJN and DvdB performed conception and design of the work and revised it critically for important intellectual content. NG performed the statistical analysis for this paper and revised it critically. DWJD, MGWD, TvdP, JMP, LS, FHV were involved in design of the work, and revised it critically for intellectual content. All authors approved the final version.

Declarations of interests

All authors declare not to have any competing interests.

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2.2

Update of the Preventive Antibiotics in Stroke Study (PASS): statistical analysis plan

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Trials 2014; 15: 382

Abstract

Background

Infections occur in 30% of stroke patients and are associated with unfavorable outcomes. Preventive antibiotic therapy lowers the infection rate after stroke, but the effect of preventive antibiotic treatment on functional outcome in patients with stroke is unknown. The PASS is a multicenter, prospective, phase three, randomized, open-label, blinded end-point (PROBE) trial of preventive antibiotic therapy in acute stroke. Patients are randomly assigned to either ceftriaxone at a dose of 2 g, given every 24 h intravenously for 4 days, in addition to standard stroke-unit care, or standard stroke-unit care without preventive antibiotic therapy. The aim of this study is to assess whether preventive antibiotic treatment improves functional outcome at 3 months by preventing infections. This paper presents in detail the statistical analysis plan (SAP) of the Preventive Antibiotics in Stroke Study (PASS) and was submitted while the investigators were still blinded for all outcomes.

Results

The primary outcome is the score on the modified Rankin Scale (mRS), assessed by ordinal logistic regression analysis according to a proportional odds model. Secondary analysis of the primary outcome is the score on the mRS dichotomized as a favorable outcome (mRS 0 to 2) versus unfavorable outcome (mRS 3 to 6). Secondary outcome measures are death rate at discharge and 3 months, infection rate during hospital admission, length of hospital admission, volume of post-stroke care, use of antibiotics during hospital stay, quality-adjusted life years and costs. Complications of treatment, serious adverse events (SAEs) and suspected unexpected serious adverse reactions (SUSARs) are reported as safety outcomes.

Conclusions

The data from PASS will establish whether preventive antibiotic therapy in acute stroke improves functional outcome by preventing infection and will be analyzed according to this pre-specified SAP.

Trial registration

Current controlled trials; ISRCTN66140176. Date of registration: 6 April 2010.

Update

Introduction

Stroke is a leading cause of death worldwide.[1] Infections occur in 30% of stroke patients and are associated with unfavorable outcomes.[2,3] Preventive antibiotic therapy lowers infection rate in patients after stroke, but the effect of preventive antibiotic treatment on functional outcome after stroke has not yet been investigated.[4,5] The Preventive Antibiotics in Stroke Study (PASS) is a phase three randomized clinical trial investigating whether the preventive use of the antibiotic ceftriaxone improves functional outcome in acute stroke patients by preventing infections. We previously published the trial protocol and an update of this protocol; we now present the statistical analysis plan (SAP).[6,7] This SAP was drafted without knowledge of any of the outcomes by the investigators and randomization code will not be broken before acceptance of the current paper for publication.

Summary study protocol

PASS is a multicenter prospective, randomized, phase III, open-label, blinded end-point superiority trial (PROBE) of standard care with preventive ceftriaxone treatment compared to standard care without preventive ceftriaxone. Adult patients with stroke (both ischemic and hemorrhagic), a score ≥1 on the National Institutes of Health Stroke Scale (NIHSS) and stroke onset within 24 hours were included.[8] Patients were excluded in case of infection at admission, use of antibiotics within 24 hours before admission, previous hypersensitivity or anaphylaxis to cephalosporins or penicillin, subarachnoid hemorrhage, pregnancy or when death seemed imminent. Patients were randomly assigned to either ceftriaxone at a dose of 2 g, given every 24 h intravenously for 4 days, in addition to stroke-unit care, or standard stroke-unit care without preventive antibiotic therapy. Randomization was performed through ALEA (online software for randomized trials; https://nl.tenalea.net/amc/ALEA/Login.aspx) and is based on a uniform distribution; weight of the arms is equal (1:1). Randomization is stratified according to study center (academic hospital, large non-academic hospital, or small non-academic hospital) and stroke severity (score on NIHSS 1 to 9 or >9) and performed by using random blocks with a maximum block size of 6; blocks of 2, 4 and 6 are made per stratum combination.[9] The study has a PROBE design, which implies that blinding is lost, but only as to treatment. Patient and physician were aware of treatment allocation; however, the assessors of outcome were not. Data were collected on admission, during hospital stay, and at 3 months by standardized case record forms. The primary outcome is functional outcome at 3 months follow-up, as assessed on the mRS during a structured telephone interview by a trained assessor blinded for treatment allocation. Secondary outcomes are death rate at discharge and at 3 months, infection rate during hospital admission, length of hospital admission, volume of post-stroke care, use of antibiotics during hospital stay, quality adjusted life years (QALYs) and costs. Safety outcomes are complications of treatment, Serious adverse events (SAEs) and suspected unexpected serious adverse reactions (SUSARs). In the initial trial protocol, we presented a binary logistic regression analysis on the dichotomized mRS (0 to 2 versus 3 to 6) as primary outcome, requiring a sample size of 3,200 patients, and a proportional odds model in a secondary analysis of the primary end point.[6] Blinded for any of the outcomes, we have changed the primary analysis in PASS from a binary logistic to an ordinal logistic regression on the original mRS, enhancing statistical power. The adapted power analysis showed that with identical assumptions on the clinical effect, using a 0.05 two-sided significance level and 80% study power, 2,550 patients were needed.[7] The analysis of dichotomized mRS data will now be the secondary analysis of the primary end point.

On 23 March 2014, all patients were included and the last follow-up is expected in June 2014. For the complete study protocol and update, we refer to previous publications.[6,7]

Protocol developments

PASS is registered at current controlled trials (http://www.controlled-trials.com; ISRCTN: 66140176; date of registration: 6 April 2010). The medical-ethical board of the Academic Medical Center, Amsterdam, approved the protocol on 5 May 2010, and 29 Dutch participating centers were added in the course of the study. Due to the change in primary analysis of primary outcome from a binary logistic approach to an ordinal logistic regression analysis and an expected rate of patients lost to follow-up and/or patients with incomplete data of 5%, the total sample size was reduced from 3,200 patients to 2,550 patients in 2014.[7] Importantly, no changes were made regarding the primary outcome measurement (that is, the assumed size of the effect on the mRS). This update of the protocol was recently published in this journal.[7]

Statistical analysis plan

General analysis principles

The code of the database will not be broken until all efficacy and safety data up to the last patient are included in the database, after data verification and validation are performed, and after the SAP has been accepted for publication. Analysis will be performed by the investigators of the PASS study group (see Acknowledgements section) assisted by a biostatistician of the Academic Medical Centre in Amsterdam.

Patient flow diagram

The flow of participants will be displayed in the Consolidated Standards of Reporting Trials (CONSORT) Flow diagram (Figure 1). Due to the pragmatic design of the study, the total number patients assessed for eligibility has not been assessed.

2.2

Definition of intention-to-treat and per-protocol population

Main analysis will be performed according to the intention to treat (ITT) principle. The safety analysis will be performed in a per protocol (PP) analysis. If a patient was by fault randomized more than once, the first randomization outcome was used. Patients who withdrew consent directly after randomization (that is, before treatment was initiated in those randomized for ceftriaxone in addition to standard care, or within 6 hours after randomization in those randomized for standard care) will be excluded from analysis. Patients with protocol deviations in eligibility are included in the ITT analysis and will be tabulated (Table 1). Patients not receiving their allocated treatment due to instantaneous crossover are considered protocol violations; these patients will be included in the ITT population. PP analysis will exclude patients for whom protocol deviations in treatment and eligibility were made (see protocol deviations in eligibility and protocol deviations in treatment).

Figure 1. Flow-chart of patients

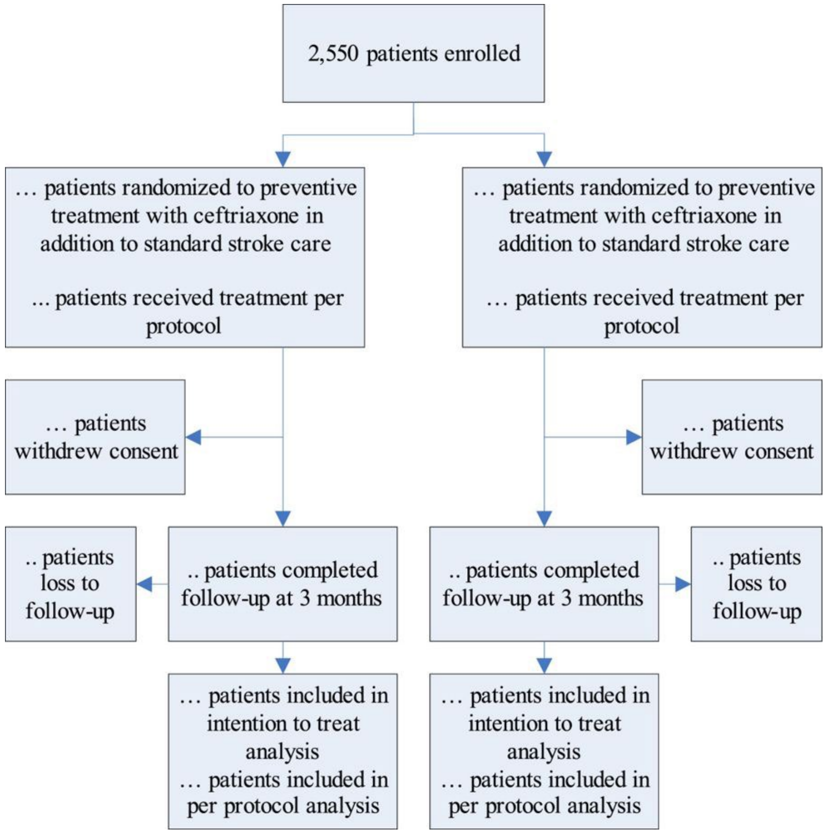


Table 1. Number and type of protocol violations in eligibility

Type of protocol violation in eligibility	Ceftriaxone + standard care (n = …)	Standard care (n = …)
Age < 18 years		
Stroke		
No neurological symptoms (NIHSS = 0)		
Onset of stroke > 24 hours ago		
Admission		
Infect at admission		
Use of antibiotics < 24 hours before admission		
Pregnancy		
Known hypersensitivity to cephalosporins		
Previous anaphylaxis for penicillin derivates		
Subarachnoidal hemorrhage		
Death is imminent		
Total number of protocol violations in eligibility		

Handling of missing data

If outcome data could not be obtained at the 3 month evaluation, we will first check the municipal council to ensure that the patient is not deceased. All other patients are considered lost to follow-up and will be tabulated, including the percentage of missing outcome data and the association with treatment. Missing outcome data will be obtained by imputation, using the coefficients of five rounds of imputation to obtain the final estimates. We will perform sensitivity analysis. First, we will use single imputation by last observation carried forward (LOCF). An observer blinded for treatment allocation will obtain the last observational score on the mRS using the medical charts and the letters of discharge of the stroke episode. All patients with LOCF will be tabulated with an explanation for the loss to follow-up (Table 2).

Table 2. Assessment of follow-up by LOCF according to treatment allocation

Patient number	Explanation	Treatment allocation

We will also perform a sensitivity analysis of baseline characteristics of the group of patients not lost-to-follow-up versus all patients included in PASS. In addition, we will also perform a joint model analysis of the loss to follow-up and the mRS change during follow-up.[10] Missing values of baseline characteristics will not be included or imputed in the display of baseline characteristics. When values are missing for dichotomous variables, the actual denominator will be stated. In case of continuous variables, a footnote will be added to show the number of patients for whom the variable was missing.

Protocol deviations in eligibility, consent procedure, treatment

When a patient was randomized but did not adhere to inclusion or exclusion criteria, this was considered a protocol deviation regarding eligibility. Patients with protocol deviations in eligibility were included in the ITT analysis, but excluded from PP analysis. In each center, the local investigator obtained written informed consent from the patient or representative according to the PASS study protocol. Patients who withdrew consent directly after randomization were excluded from further analysis. The flow of patients is displayed in the CONSORT flowchart (Figure 1).

Treatment allocation was regarded as carried out according to the study protocol when a patient randomized for ceftriaxone in addition to standard care received ceftriaxone 2 gram each 24 hours for 4 days. Patients were also considered as treated PP when treatment was terminated within 4 days due to discharge, death, a palliative care policy, an allergic reaction without anaphylaxis or a previous allergic reaction in medical history (see inclusion and exclusion criteria and protocol deviation in eligibility), other side effects of treatment, or when treatment with ceftriaxone was changed into treatment with another antibiotic because of an infection because these situations and what to do were all defined and described in the initial protocol.[6] In patients allocated to standard care, treatment was carried out according to the study protocol when patients did not receive preventive antibiotic therapy.

Baseline characteristics

Baseline characteristics of all patients will be outlined per treatment allocation in a baseline table describing the following variables: age, sex, medical history (atrial fibrillation/flutter, stroke, hypercholesterolemia, hypertension, myocardial infarction, cardiac valve insufficiency/stenosis/replacement, peripheral vascular disease, obstructive pulmonary disease, and immunocompromised), current smoking, specific medication (anticoagulants, antiplatelet, statin, ACE inhibitor, ß-blocker, and proton pump inhibitor) prior to stroke, disability prior to stroke on mRS, stroke severity on NIHSS, performance of a screening test for swallowing function, dysphagia, acute treatment (IV thrombolysis and anticoagulant antagonist therapy) and diagnosis at discharge (infarct, haemorrhage, TIA, or other). Outline of the table is displayed in Table 3. All variables will be presented categorized by treatment arm. Dichotomous variables will be displayed in percentage with the number of patients divided by the total number of evaluated patients. Continuous variables will be reported as means with standard deviations when normally distributed and in medians with interquartile ranges when they do not meet the criterion of being normally distributed, as assessed by the Kolmogorov-Smirnov test. For continuous variables, the number of patients evaluated will be presented in a footnote of Table 3.

Table 3. Baseline characteristics

Baseline characteristics	Ceftriaxone + standard care (n = …)	Standard care (n = …)
Age - years		
Male sex - % n/N		
Medical history - % n/N Atrial fibrillation/flutter Stroke Hypercholesterolemia Hypertension Myocardial infarction Cardiac valve insufficiency/stenosis/ replacement Peripheral vascular disease Obstructive pulmonary disease Immunocompromised		
Current smoker - % n/N		
Medication prior to stroke - % n/N Anticoagulants Antiplatelet Statin ACE inhibitor Bèta-blocker Proton pump inhibitors		
Disability prior to stroke – mRS *		
Stroke severity – NIHSS **		
Swallowing screening performed - % n/N		
Dysphagic patients - % n/N		
Acute treatment - % n/N IV thrombolysis Coagulant therapy		
Diagnosis at discharge - % n/N Infarction Haemorrhage Transient ischaemic attack (TIA) Other		

* mRS, denotes modified Rankin Scale

** NIHSS denots National Institutes of Health Stroke Scale

Assessment of primary outcome

A structured telephone interview with each patient was held at 3 months by one of three trained research nurses, blinded for treatment allocation, to assess the primary outcome on the mRS. This structured telephone interview was validated in an earlier study.[11]

Assessment of secondary outcomes

The assessment of secondary outcomes will be performed as described below, for each outcome separately:

Infection rate during hospital admission

The total number of patients diagnosed with one or more infection(s) during hospital admission will be reported, as well as the total number of infections. Infections will be reported according to subtypes pneumonia, urinary tract infection and other infection. Infection will be assessed in two ways. First, the infection will be diagnosed in the clinical setting by the treating physician and registered as pneumonia, urinary tract infection or other infection. The clinical diagnosis of infection will be used for the primary analysis. Suspected infections without diagnostics being performed are also recorded and reported as such (for example, in a patient with a palliative care policy). Second, infection will be categorized by two infectious disease specialists who are blinded for treatment allocation, using the modified criteria of the Centers for Disease Control and Prevention (CDC criteria).[12] For this second categorization, patients with fever, new onset delirium or clinical diagnosis of infection during hospital admission will be reviewed. For this purpose, data on the diagnostic procedures during admission as recorded in the Case Record Form (CRF) will be used. For the diagnosis of pneumonia and urinary tract infection prespecified algorithms will be used based on the CDC-criteria (Figures 2 and 3). Patients with a positive blood culture or a positive culture from the presumed site of infection, other than the lungs or urine, with a clinically relevant pathogen will be diagnosed as 'other infection.' Patients will be categorized as having confirmed pneumonia, urinary tract infection, or other infection. Only bacterial infections will be assessed since preventive antibiotic therapy aims to reduce these infections. Infection with Clostridium difficile is reported as a treatment complication. Case definition of this infection is diarrhea plus a positive C. difficile toxin test. Clostridium infection was diagnosed by the treating physician and was reviewed by the expert panel.

Figure 2. Diagnosis of pneumonia

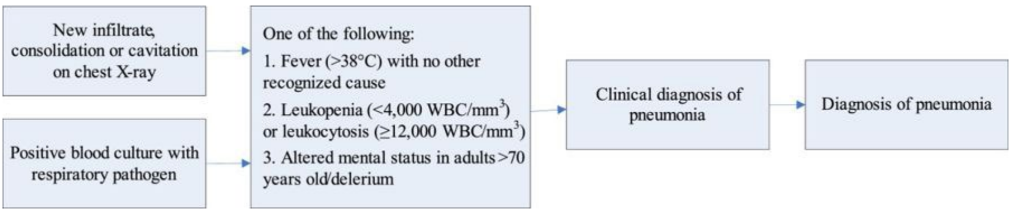
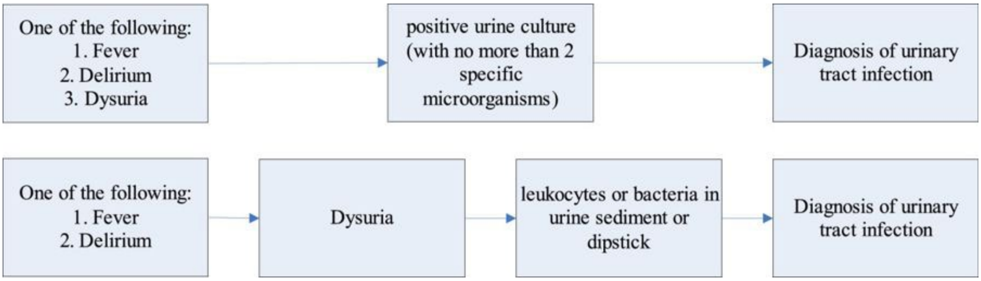


Figure 3. Diagnosis of urinary tract infection



Death rate at discharge and at 3 months

Death during hospital admission was recorded in the CRF by the treating physician and notified as an SAE to the trial office. Death was also registered at the 3 months follow-up. If needed, survival status at 3 months was evaluated through contact with general practitioners and the municipality register.

Length of hospital stay

The day of admission and discharge was recorded in the CRF by the treating physician. Length of hospital admission is measured in days.

Total use of antibiotics during hospital stay

The use of antibiotics other than preventive antibiotic therapy will be recorded in the case record form. Total antibiotic use will be recorded in units of the 'defined daily dose' (DDD) and the number of days of use. For definitions of the DDD, classification according to the World Health Organization (WHO) will be used for each antibiotic.[13]

Volume of post-stroke care, cost-effectiveness analysis

The cost-effectiveness will be measured by an economic analysis conducted alongside the study. This analysis is not included in the publication to which this analysis plan applies.

Assessment of safety outcomes

Safety outcomes are complications of treatment, SAEs and SUSARs. All SAEs and SUSARs during the hospital stay are recorded in case record forms by the treating physician and reported to the trial office. SAEs and SUSARs occurring after discharge are recorded during the follow-up interview at 3 months. The physician records treatment complications in the CRF (diarrhea caused by C. difficile, allergic reaction that caused cessation of ceftriaxone, infection with ceftriaxone resistant microorganism, phlebitis at place of IV-catheter, elevation of liver enzymes, oliguria or elevation of serum creatinine).

Cause of death will be reviewed by two independent observers. They will use information from the hospital discharge letter or the medical correspondence received by the general practitioner in case the patient died after discharge. Discrepancies will be reviewed in a consensus meeting in the presence of a third investigator. Outcome parameters were derived from three recent cardiovascular trials and were modified for expected outcomes in our study.[14-16] A distinction will be made among a cardiovascular cause (brain infarction, brain hemorrhage, myocardial- or pulmonary embolism. or another cardiovascular cause), an infection (pneumonia, sepsis or another infection), death by any type of malignancy, death by any other cause (for example, traffic accident), withdrawal of treatment due to a poor prognosis or unknown cause of death.

Analysis of primary outcome

An ordinal regression model on the total range of the mRS will be performed as the first analysis of primary outcome, under the assumption of proportional odds.[7] The distribution of primary outcome (for example, functional outcome on the mRS) in both treatment groups will be expressed in a histogram (Figure 4). Both adjusted and unadjusted analyses will be performed and reported. In clinical trials, adjusting for prognostic covariates improves statistical power, can correct for imbalances in baseline prognostic variables and can reduce variability in data[17,18]. The choice of prognostic covariates is mostly based on imbalances across treatment groups, prognostic factors that are related to the primary outcome, or a combination of both.[17] As the investigators are blinded for all outcome data until the statistical analysis plan is accepted for publication, we chose to use the most important prognostic factors for outcome after stroke: age, stroke severity on the NIHSS, history of stroke, history of diabetes, prior disability as defined on mRS, and stroke type.[19] Stratification of randomization was performed according to both study center and stroke severity, so we will also include study center as a covariate. The second analysis of the primary endpoint, that is, the dichotomized score on the mRS (for example, favorable versus unfavorable, mRS 0 to 2 versus mRS 3 to 6), will be expressed as OR with 95% confidence intervals (CI; Table 4). Results of the dichotomized approach will be compared to the results of the primary analysis of primary outcome.

Figure 4. Graphic display of primary outcome

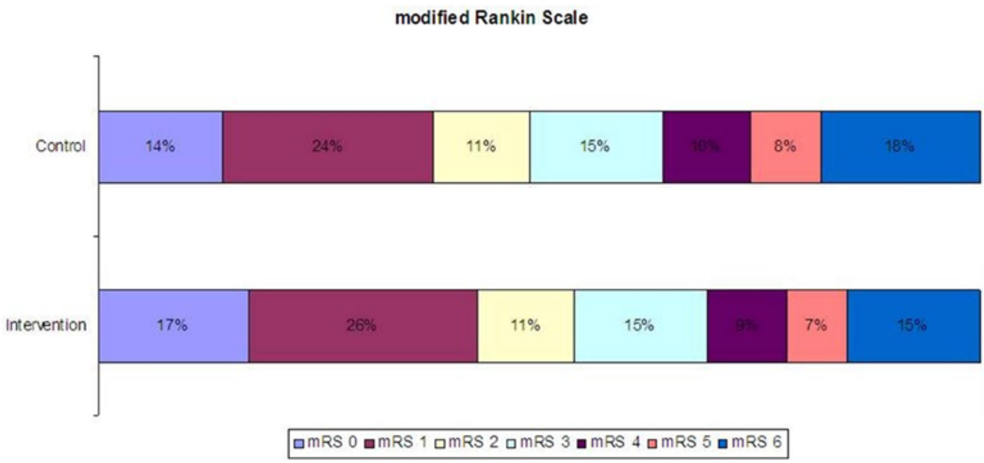


Table 4. Secondary outcomes

	Ceftriaxone + standard care (n = …)	Standard care (n = …)	p	OR 95% CI
Secondary analysis of primary outcome				
Favorable outcome - % n/N				
Secondary outcomes:				
Diagnosis of infection during admission Pneumonia Urinary tract infection Other				
Diagnosis of infection based on expert panel Pneumonia Urinary tract infection Other				
Mortality - % n/N At discharge At 3 months				
Length of hospital stay - days				NA

NA, not applicable.

Analysis of secondary outcomes

The number of patients with one or more in-hospital post-stroke infection(s) will be presented as numbers with event of numbers evaluated and analyzed using the chi-square test, and OR estimates with 95% CI. Infection rates will be reported as 'judged by treating physicians' and 'infectious diseases panel.' Death rate at discharge and at 3 months will also be analyzed using the chi-squared test and presented as OR estimates and 95% CI. Use of antibiotics in defined daily doses and length of hospital admission will be analyzed using the two group t-test or Mann- Whitney test where appropriate (Table 4). The analysis of volume of post-stroke care, use of antibiotics during 3 months follow-up and the cost-effectiveness analysis will be analyzed using a separate analysis protocol and presented in a subsequent paper and is, therefore, not discussed here.

Safety outcomes

Complications of treatment, SAE's and SUSAR's per patient will be tabulated according to treatment group, and analyzed using the chi-squared test (Table 5 and Table 6).

Table 5. Number and type of serious adverse events (SAEs)

Type of SAE – % n/N	Ceftriaxone + standard care (n = …)	Standard care (n = …)	p
Death			
Life-threatening event			
New hospitalization			
Prolongation of existing hospitalization			
Persistence of significant disability or incapacity			
Total number of SAEs			

Table 6. Complications of treatment

Adverse reaction – % n/N	Ceftriaxone + standard care (n = …)	Standard care (n = …)	p
Diarrhea caused by *C. difficile*			
Allergic reaction that caused cessation of ceftriaxone			
Infection with ceftriaxone-resistant microorganism			
Phlebitis at place of IV-catheter			
Elevation of liver enzymes			
Oliguria or elevation of serum creatinine			
Total number of adverse reactions – number %			

Subgroup analysis of primary and secondary outcomes

We will perform the following sub-group analyses for the primary outcome: stroke type (infarction or hemorrhage), stroke severity (NIHSS 1 to 9 or NIHSS 10 to 30), time between stroke symptoms and start of the antibiotic treatment (0 to 12 h versus 12 to 24 h) and age. For the subgroup analysis of primary analysis of primary outcome, the single OR from the proportional odds model will be calculated for each subgroup separately.

For the subgroup analysis of secondary analysis of primary outcome, we will tabulate the results and analyze them using the chi-squared test and presented as OR and 95% CI (Table 7: subgroup analysis of primary outcome). In addition to these predefined subgroup analyses, we will perform a larger set of exploratory additional analyses. For secondary outcomes, we will perform all the previous mentioned subgroup analyses (stroke type, severity, time to treatment, and age). In addition we will perform analysis on presence of a swallowing disorder, respiratory tract infections, and a urinary catheter.

Table 7. Subgroup analysis of primary outcome

	Ceftriaxone + standard care (n = …)	Standard care (n = …)	p	OR 95% CI
Favorable outcome (mRS* 0 to 2) - % n/N				
Ischemic stroke				
Hemorrhagic stroke				
Transient ischemic attack (TIA)				
Other				
Favorable outcome (mRS 0 to 2) - % n/N				
NIHSS** 1 to 0				
NIHSS 10 to 30				
Favorable outcome (mRS 0 to 2) - % n/N				
Time to treatment 0 to 6 h				
Time to treatment 6 to 12 h				
Time to treatment 12 to 24 h				

* mRS, denotes modified Rankin Scale

** NIHSS denots National Institutes of Health Stroke Scale

Authorship

Two PhD students (WFW and J-DV) of this project will share first authorship; the two principle investigators (PIs) of this project will share last authorship (DvdB and PJN; DvdB corresponding author); local investigators who included at least 100 patients will be co-author; PASS study group members and physicians in expert panels for outcome-scoring will be co-author; and all local investigators who included less than 100 patients in PASS will be explicitly listed in the PASS investigators list.

Discussion

The aim of our study is to investigate whether preventive antibiotic therapy improves functional outcome by reducing the number of infections in acute stroke patients. With this SAP, we present the analyses that will be published in the primary publication. By publishing the statistical analysis plan before knowledge of any outcome, we stimulate transparency of scientific conduct and allow others to add timely suggestions for additional analyses.

Patients in the acute phase of stroke are at risk for infections. A systematic review and meta-analysis of 87 studies showed that infections complicate stroke in 30% of all stroke patients. Pneumonia was associated with mortality with an OR of 3.62 (95% CI 2.80 to 4.68).[3] The effect of preventive antibiotic therapy on outcome in stroke patients has been investigated in few studies. Two meta-analyses of these studies showed that preventive antibiotic therapy reduced the number of infections.[4,5] The proportion of patients who died and the number of disabled patients were not significantly reduced, but numbers of included patients were small.

The PROBE design with open-label preventive antibiotics might introduce detection bias for infection. Physicians are aware of the treatment allocation, which potentially influences decisions on nonscheduled treatment (that is, the detection and treatment of patients with infection). This might influence the outcome measure of infection rate. To control for this bias, we will provide a secondary judgement of infection diagnosis by a blinded expert panel, according to CDC criteria. The CDC criteria are restrictive and use ancillary investigations such as blood tests, chest X-rays and culture results to confirm the diagnosis of infection. In clinical practice, for a stroke patient with fever, a cough and abnormalities on auscultation, a physician will often not wait for culture results or refrain from treating pneumonia when a chest X-ray does not (yet) show a consolidation.

Preventive treatment with ceftriaxone after stroke might improve outcome by preventing infections. A potential beneficial effect on functional outcome might be caused by a direct effect of prevention of infections in patients after stroke, most commonly pneumonia, but also by the result of decreased length of stay on the stroke unit of even in the hospital. A recent study of individual patient data in a meta-analysis of randomized trials of ventilator-associated pneumonia prevention showed that an overall attributable mortality of ventilator-associated pneumonia is 13%, which was mainly caused by prolonged exposure to the risk of dying due to increased length of ICU stay.[20] Ceftriaxone also has neuroprotective properties, at least in animal studies of stroke, which may be mediated by increased expression and activity of the glutamate transporter.[21]

Antibiotics may induce overgrowth of antibiotic resistant pathogens in individual patients.[22] In the general population, selective antibiotic pressure is an important determinant of emergence and dissemination of antibiotic resistance.[23,24] Previous clinical trials on preventive antibiotic therapy in stroke, antibiotic resistance patterns of bacteria cultured from patients with or without preventive antibiotics were similar, but numbers of patients were low.[25] Previous work has showed that implementation of preventive antibiotics in the ICU did not increase resistance rates in an environment with low levels of antibiotic resistance.[26] We will compare total antibiotic use in both treatment groups during hospital stay and collect stool specimens in a nested case control study that includes 300 patients.

During the course of the study we changed the analysis of primary outcome on the mRS from a dichotomized analysis toward an ordinal regression analysis. The ordinal regression analysis is increasingly used in stroke trials because of its higher efficiency[27]. Importantly, our primary outcome (for example, functional outcome on the mRS) was not changed, and the assumptions used in the initial sample size calculation were maintained. By using ordinal regression analysis, the total sample size was lowered from 3,200 patients to 2,550 patients. Using this method enables us to reduce the number of patients without changing the assumptions on the magnitude of the effect on the primary outcome scale from the original sample size calculation.

Acknowledgements

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Abbreviations

CRF:	Case Record Form
CONSORT:	Consolidated Standards of Reporting Trials
DDD:	defined daily dose
ITT:	intention to treat
LOCF:	last observation carried forward
mRS:	modified Rankin Scale
NIHSS:	National Institutes of Health Stroke Scale
OR:	odds ratio
PASS:	Preventive Antibiotics in Stroke Study
PP:	per protocol
QALYS:	quality adjusted life years
SAE:	serious adverse event
SAP:	statistical analysis plan
SUSAR:	suspected unexpected serious adverse reaction
TIA:	transient ischemic attack
VAP:	ventilator-associated pneumonia.

Contribution of authors

WFW, JDV performed conception and design of the work and drafted the work. PJN and DvdB performed conception and design of the work and revised it critically for important intellectual content. DWJD, MGWD, TvdP, JMP, FHV, YBWEMR, MCB, AHZ were involved in design of the work, and revised it critically for intellectual content. All authors approved the final version.

Declarations of interests

The authors declare that they have no competing interests.

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The Preventive Antibiotics in Stroke Study (PASS): a pragmatic randomised open-label masked endpoint clinical trial

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Abstract

Background

In adults with acute stroke, infections occur commonly and are associated with an unfavourable functional outcome. In the Preventive Antibiotics in Stroke Study (PASS) we aimed to establish whether or not preventive antimicrobial therapy with a third-generation cephalosporin, ceftriaxone, improves functional outcome in patients with acute stroke.

Methods

In this multicenter, randomised, open-label trial with masked endpoint assessment, patients with acute stroke were randomly assigned to intravenous ceftriaxone at a dose of 2 g, given every 24 h intravenously for 4 days, in addition to stroke unit care, or standard stroke unit care without preventive antimicrobial therapy; assignments were made within 24 h after symptom onset. The primary endpoint was functional outcome at 3 months, defined according to the modified Rankin Scale and analysed by intention to treat. The primary analysis was by ordinal regression of the primary outcome. Secondary outcomes included death, infection rates, antimicrobial use, and length of hospital stay. Participants and caregivers were aware of treatment allocation but assessors of outcome were masked to group assignment. This trial is registered with controlled-trials.com, number ISRCTN66140176.

Findings

Between July 6, 2010, and March 23, 2014, a total of 2,550 patients from 30 sites in the Netherlands, including academic and non-academic medical centers, were randomly assigned to the two treatment groups: 1,275 patients to ceftriaxone and 1,275 patients to standard treatment (control group). 12 patients (seven in the ceftriaxone group and five in the control group) withdrew consent immediately after randomisation, leaving 2,538 patients available for the intention-to-treat-analysis (1,268 in the ceftriaxone group and 1,270 in the control group). 2,514 (99%) of 2,538 patients (1,257 in each group) completed 3-month follow-up. Preventive ceftriaxone did not affect the distribution of functional outcome scores on the modified Rankin Scale at 3 months (adjusted common odds ratio 0.95 [95%CI 0.82–1.09], $p = 0.46$). Preventive ceftriaxone did not result in an increased occurrence of adverse events. Overgrowth infection with *Clostridium difficile* occurred in two patients (<1%) in the ceftriaxone group and none in the control group. Interpretation Preventive ceftriaxone does not improve functional outcome at 3 months in adults with acute stroke. The results of our trial do not support the use of preventive antibiotics in adults with acute stroke.

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Introduction

Infection is a common complication in the acute phase after stroke.[1] In a meta-analysis of 87 studies, the pooled infection rate after stroke was 30%, most commonly pneumonia and urinary tract infections.[2] About half of pneumonia cases occur within the first 48 h after stroke onset.[3] Many studies have shown that the occurrence of infections after stroke is associated with poor functional outcome and mortality[3,4] , although other investigators have reported that infection after stroke is merely a marker of stroke severity without an independent outcome effect when it is treated promptly.[5]

Five randomised studies have assessed the preventive use of antimicrobials in patients with acute stroke, with conflicting results.[6-10] A Cochrane meta-analysis including 506 patients concluded that although studies differed in populations analysed, type of antimicrobial used, and definition of infection, overall antimicrobial prophylaxis reduced the infection rate from 36% to 22% (relative risk 0.58 [95%CI 0.43–0.79]) without major adverse effects.[11] However, whether or not preventive antimicrobials reduce the risk of poor functional outcome after stroke remains uncertain.[11] Existing guidelines for management of acute stroke state that preventive use of antibiotics is not indicated because it has not been proven effective.[12] We undertook this study to establish whether or not preventive antimicrobial therapy with a third-generation cephalosporin, ceftriaxone, improves functional outcome in patients with acute stroke.

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Methods

Study design and participants

In this multicentre, prospective, randomised, open label, masked endpoint trial — the Preventive Antibiotics in Stroke Study (PASS) — we randomly assigned patients from 30 sites, including academic and non-academic medical centers, in the Netherlands in a 1:1 ratio to treatment with ceftriaxone in addition to stroke unit care, or to standard stroke unit care without preventive antimicrobial therapy.

Patients were enrolled by their treating physicians. Patients were eligible for inclusion if they were aged 18 years or older, had clinical symptoms of a stroke (ischaemic or haemorrhagic), an onset of symptoms less than 24 h, and a score of 1 or more on the National Institutes of Health Stroke Scale (NIHSS). Exclusion criteria were clinical signs of infection on hospital admission requiring antibiotic therapy, use of antimicrobials less than 24 h before admission, pregnancy, hypersensitivity for cephalosporins, previous anaphylaxis for penicillin derivatives, subarachnoid haemorrhage, and imminent death.

The trial protocol and its updates, detailed procedures of randomisation, and the statistical analysis plan have been published.[13-15] The institutional review board of the Academic Medical Center (Amsterdam, Netherlands) approved the study protocol. The study was undertaken according to Good Clinical Practice standards and was independently monitored by the Clinical Research Unit of the Academic Medical Center, University of Amsterdam. All patients or their legal representatives provided written informed consent. An independent data and safety monitoring board periodically reviewed data.

Randomisation and masking

Randomisation was done with an online tool within 24 h after symptom onset, and was stratified according to study center (university hospital, large non-university hospital, or small non-university hospital) and stroke severity (score on NIHSS of 1–9 vs a score of 10 or more), using permuted blocks of varying block size (with a maximum block size of 6). Local investigators and patients were not masked, but the research nurses who did the follow-up interviews were masked to treatment allocation. Trained and masked research nurses based at the Academic Medical Center assessed functional outcome at 3 months using a validated structured telephone interview.[16] The statistical analysis plan was written without knowledge of outcome data.

Procedures

Preventive antibiotics were initiated within 24 h after the onset of symptoms. The study medication was ceftriaxone 2 g, given intravenously once daily for 4 days. Ceftriaxone was discontinued if patients were discharged or active treatment was withdrawn. The treating clinician could decide whether or not to treat a patient with suspected infection with (additional) antimicrobials. Study procedures with respect to antimicrobial treatment are detailed in the study protocol.[13-15]

Outcomes

The primary endpoint was functional outcome at 3 months, defined by the modified Rankin Scale (mRS), which ranges from 0 (no symptoms) to 6 (death). Secondary endpoints were death at discharge and 3 months after randomisation, infection rate, total antimicrobial use, length of hospital stay, volume of poststroke care, and quality-adjusted life-years and costs. Cost-effectiveness analyses will be presented separately. Infections were categorised as diagnosed by the clinician, and as judged by an independent adjudication committee (masked to treatment allocation) according to modified Centers for Disease Control and Prevention criteria.[17] The scoring algorithms for infections used by this committee have been described previously.[14] *Clostridium difficile* infection was defined as diarrhoea in combination with a positive *C difficile* toxin test. Antimicrobial use during hospital stay was converted to units of defined daily doses according to the classification of the WHO Anatomical Therapeutic Chemical Classification System with Defined Daily Doses Index.[18]

Statistical analysis

We based our initial sample size calculation on the dichotomised outcome on the mRS at 3 months (a score of 0–2 vs a score of 3–6). We assumed that ceftriaxone would reduce the proportion of patients with an unfavourable outcome from 50% to 45%[13] , at a power of 80% and p value of 0.05, and aimed to include 3,200 patients in the trial. On Feb 24, 2014, we changed the analysis of our primary outcome from dichotomisation to ordinal regression analysis on the mRS in a protocol amendment without any knowledge of any of the outcomes.[14,15] The main reason for this change was insufficient funding for PASS.[15] Ordinal analysis of outcome data is often used in stroke trials and increases statistical power.[15] We assumed a proportional odds ratio (OR) of 0.818 between all pairs of category groups, similar to the assumption in the original sample size calculation (assumed distribution of mRS scores in control group: score of 0, 14%; 1, 24%; 2, 11%; 3, 15%; 4, 10%; 5, 8%; 6, 18%). With use of Whitehead's method[19] , with alpha 0.05 and power 80%, the desired sample size in the proportional odds model was estimated to be a total of 2,410 patients. In view of an expected 5% rate of patients lost to follow-up or with incomplete data, an estimate for the new sample size with the primary endpoint analysed on all categories of the mRS was 2,531 patients; we therefore set the sample size as 2,550 patients. Our power analyses are detailed in the protocol update and statistical analysis plan.[14,15]

The primary analysis was by ordinal regression of the entire range (0–6) of the primary outcome measure: the mRS score. We assumed the treatment OR between one level and the next to be constant, so a single parameter (a common OR) summarises the shift in outcome distribution between the treatment and control groups (parallel lines test in the analysis, p = 0.43). We did unadjusted and adjusted analyses. The adjusted analyses were defined beforehand and included age, stroke severity as defined as NIHSS score, history of stroke, history of diabetes, previous disability as defined on the mRS, stroke type, and categorisation of study center. A secondary analysis of the mRS score at 3 months was dichotomised into a score of 0–2 indicating a favourable outcome, versus a score of 3–6 indicating an unfavourable outcome. Predefined subgroup analyses were age, stroke type, stroke severity, and time between stroke onset and treatment. The analysis of subgroups was assessed by an interaction test, for which we used the dichotomized outcome on the mRS. Results for primary and secondary endpoints refer to the intention-to-treat population assessed 3 months after stroke. For the safety analysis, we analysed all patients who actually started the first dose of ceftriaxone.

In case of missing outcomes at the 3-month assessment, we checked the municipal council register to see whether or not these patients had died. Other patients (ie, those with missing outcomes at 3 months who were not registered as deceased on the register) were judged to be lost to follow-up. Missing outcome data in the primary outcome analysis were replaced by imputation with five rounds of imputations. Final estimates of the coefficients

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in the regression models were obtained by averaging with use of Rubin's rule.[20] Variables in the imputation model were stratification factors for randomisation (hospital, and minor or major stroke) and strong prognostic variables for outcome in acute stroke (age, mRS at baseline, history of diabetes or stroke, and stroke severity). We did sensitivity analyses with single imputation of the last observed functional score carried forward by an observer masked to treatment allocation based on medical charts and the letters of discharge of the stroke episode. Characteristics of patients with missing outcomes at the 3-month assessment and findings of sensitivity analyses are reported in the appendices (Appendix 2 and Appendix 4).

Summary statistics are reported as crude statistics. Group comparisons are based on the intention-to-treat population. We did tests of group differences using standard methods depending on the type of variable. We compared normal and non-normal distributed variables with the Student's t test and the Mann-Whitney U test. We judged two-tailed p values less than 0.05 to indicate statistical significance.

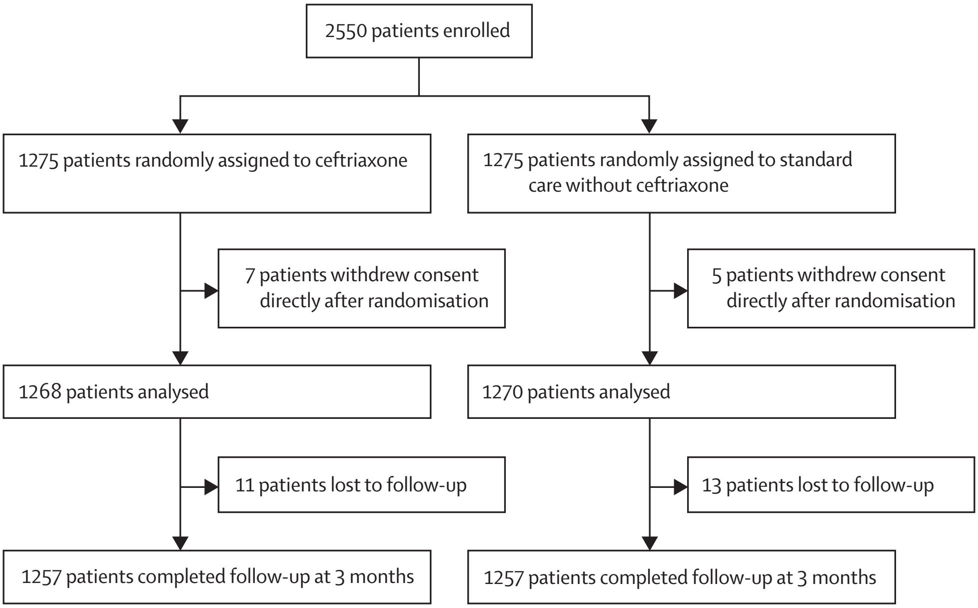
Role of the funding source

The PASS group designed the study. This study received no commercial support. The funders had no role in the study design; collection, analysis, or interpretation of the data; or in writing of the report. The two principal investigators (DvdB and PJN) had full access to all the data and vouch for the completeness and accuracy of the data. DvdB had final responsibility for the decision to submit for publication.

Results

Between July 6, 2010, and March 23, 2014, 2,550 adult patients from 30 Dutch sites were enrolled and randomly assigned in a 1:1 ratio to the two study groups: 1,275 patients to receive ceftriaxone and 1,275 patients to receive standard stroke unit treatment without ceftriaxone (the control group) (Figure 1). 12 patients (seven in the ceftriaxone group and five in the control group) withdrew consent immediately after randomisation, leaving 2,538 patients available for the intention-to-treat-analysis (1,268 in the ceftriaxone group and 1,270 in the control group). Preventive antimicrobial treatment was started in 1,242 (98%) of 1,268 patients in the ceftriaxone group; 117 patients received one dose, 176 patients two doses, 173 three doses, and 776 four doses of ceftriaxone. 3 months after randomisation, the score on the mRS was known for 2,514 (99%) of 2,538 patients (1,257 in each group). 24 patients were lost to follow-up (11 in the ceftriaxone group and 13 in the control group; Appendix 2).

Figure 1. Trial profile



The baseline demographic and clinical characteristics of the two groups were similar (Table 1). The definite diagnosis was assessed at discharge in 2,538 patients: 2,125 (84%) had cerebral infarction, 93 (4%) had a transient ischaemic attack, 269 (11%) had cerebral haemorrhage, and 51 (2%) had an alternative diagnosis (21 had functional neurological symptoms, four epileptic seizures, and three migraine; other diagnoses are listed in Appendix 3). Intravenous thrombolytic therapy was given to 836 (33%) of 2,538 patients.

Table 1. Baseline characteristics

	Ceftriaxone group (n = 1268)	Control group (n = 1270)
Age, years	73 (63–81)	74 (63–81)
Male sex	719 (57%)	725 (57%)
History		
Atrial fibrillation/flutter	184/1265 (15%)	207/1269 (16%)
Stroke	406/1266 (32%)	421/1270 (33%)
Hypercholesterolaemia	332/1260 (26%)	333/1258 (27%)
Hypertension	694/1266 (55%)	706/1268 (56%)
Myocardial infarction	172/1266 (14%)	159/1270 (13%)
Cardiac valve disease †	95/1265 (8%)	78/1270 (6%)
Peripheral vascular disease	91/1262 (7%)	99/1267 (8%)
Obstructive pulmonary disease	115/1267 (9%)	93/1266 (7%)
Diabetes mellitus	251/1268 (20%)	251/1270 (20%)
Alcoholism	58/1268 (5%)	64/1270 (5%)
Malignancy	112/1268 (9%)	122/1270 (10%)
Immunocompromised ‡	53/1268 (4%)	31/1270 (2%)
Current smoker	319/1253 (26%)	301/1256 (24%)
Previous medication		
Anticoagulants	142/1267 (11%)	141/1270 (11%)
Antiplatelet therapy	514/1267 (41%)	504/1269 (40%)
Statins	473/1266 (37%)	476/1270 (38%)
Angiotensin-converting enzyme inhibitors	347/1264 (28%)	297/1268 (23%)
β blockers	428/1266 (34%)	457/1267 (36%)
Proton pump inhibitors	327/1265 (26%)	328/1266 (26%)
Modified Rankin Scale score before stroke symptoms §	0 (0–1)	0 (0–1)
National Institutes of Health Stroke Scale score ¶	5 (3–9)	5 (3–9)
Dysphagia	307/1178 (26%)	316/1193 (27%)
Acute stroke treatment		
Intravenous thrombolysis	437/1268 (35%)	399/1270 (31%)
Coagulant therapy	26/143 (18%)	19/125 (15%)
Discharge diagnosis		
Cerebral infarction	1058 (83%)	1067 (84%)
Transient ischaemic attack	44 (4%)	49 (4%)
Cerebral haemorrhage	143 (11%)	126 (10%)
Other	23 (2%)	28 (2%)

Data are median (IQR) or n/N (%).

† Cardiac valve disease was defined as cardiac valve insufficiency, stenosis, or replacement.

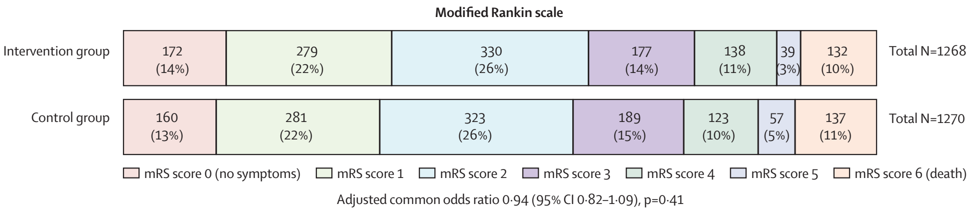
‡ Immunocompromised was defined as changed immune status, diabetes mellitus, alcoholism, malignancy, or immunosuppressive medication.

§ Scores on the modified Rankin Scale range from 0 to 6, with 6 indicating death; modified Rankin Scale scores before onset of stroke symptoms were assessed in 2,538 patients (1,268 in the ceftriaxone group and 1,270 in the control group).

¶ Scores on the National Institutes of Health Stroke Scale range from 0 to 30, with 30 indicating highest degree of stroke severity; these scores were assessed in 2,538 patients (1,268 in the ceftriaxone group and 1,270 in the standard treatment group).

Preventive ceftriaxone was not associated with a shift in the distribution of scores on the mRS (adjusted common OR 0.94 [95%CI 0.82–1.09], p = 0.41; Figure 2). Sensitivity analysis in which last observation carried forward was used to predict the 24 missing outcomes showed similar results (Appendix 4). In the dichotomised analysis of the mRS, the percentages of patients with an unfavourable outcome in the two groups were similar (38% in the ceftriaxone group vs 40% in the control group; OR 0.94 [95%CI 0.80–1.11], p = 0.49) (Table 2).

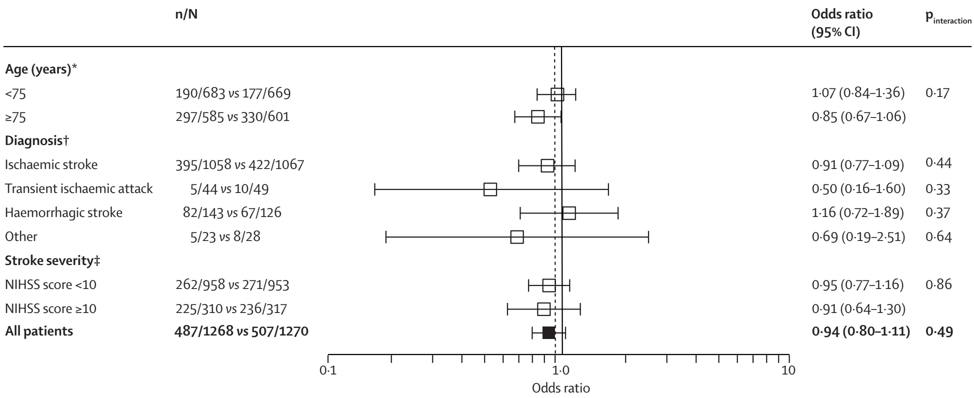
Figure 2. Distribution of modified Rankin Scale scores 3 months after randomisation.



mRS = modified Rankin Scale. Scores on the scale range from 0 to 6, with 0 indicating no symptoms and 6 indicating death. Numbers in the intervention group do not add up to the total number of patients in this group because of the used imputation techniques of outcome in 24 patients.

The treatment effect did not differ significantly in the predefined subgroups: age (patients aged 75 years or older), stroke type (infarction, haemorrhage, transient ischaemic attack, or other), or stroke severity (patients presenting with NIHSS score of 10 or more; Figure 3). In the ceftriaxone group, we recorded no association between time to start of the prophylactic treatment (categories: 0–3 h, 3–6 h, 6–12 h, and 12–24 h after symptom onset) and outcome (data not shown).

Figure 3. Subgroup analyses on unfavourable outcome 3 months after randomization



Subgroups are age, stroke type, and stroke severity.

* Age was dichotomised at 75 years; the median age was 73 years (IQR 63–81) in the ceftriaxone group and 74 years (IQR 63–81) in the control group.

†Stroke type was defined as the discharge diagnosis.

‡Stroke severity was dichotomised on the National Institutes of Health Stroke Scale, between a score of 1–9 versus a score of 10 or more. Scores on the National Institutes of Health Stroke Scale range from 0 to 30, with 30 indicating the highest degree of stroke severity.

During hospital stay, physicians diagnosed an infection in 348 (14%) of 2,538 patients: urinary tract infection in 173 (7%) patients, pneumonia in 159 (6%) patients, and other infections in 50 (2%) patients. The adjudication committee (expert panel) diagnosed infection in 129 (5%) of 2,538 patients: urinary tract infection in 76 patients (3%), pneumonia in 57 patients (2%), and other infections in nine patients (<1%). One infection episode occurred in 306 patients as scored by physicians (clinical diagnosis) and in 116 patients as scored by the expert panel (using strict and predefined definitions); two episodes in 29 and eight patients, respectively; three episodes in 11 and four patients, respectively; and four episodes in two and one patient, respectively. Kaplan-Meier curves on occurrence of infection for all patients and for patients with severe stroke, as defined as those presenting with an NIHSS score of 10 or more, are available in Appendix 10.

In multiple regression analyses, adjusted ORs for unfavourable outcome for clinical diagnosis of infection was 3.48 (95% CI 2.53–4.77, p <0.0001) and that for expert panel diagnosis of infection was 4.37 (2.51–7.59, p <0.0001). Occurrence of each category of infection (urinary tract infections and pneumonia) was associated with outcome. For clinically diagnosed infections, in a multiple regression analysis including age, stroke severity as defined by NIHSS score, history of stroke, history of diabetes, previous disability as defined by mRS score, stroke type, and categorisation of study centre, pneumonia was associated with unfavourable outcome (adjusted OR 9.64 [95%CI 5.06–18.42], p <0.0001), as was urinary tract infection (1.86 [1.24–2.79], p = 0.003).

Prophylactic ceftriaxone prevented infections (clinical diagnosis OR 0.55 [95%CI 0.44–0.70], p <0.0001; expert panel diagnosis OR 0.44 [0.30–0.65], p <0.0001) (Table 2). This result was mainly driven by lower rates of urinary tract infections in the ceftriaxone group than in the control group (Table 2). Rates of pneumonia between the ceftriaxone and control group were similar (Table 2). Patients with higher stroke severity were at increased risk of infection: 72 (23%) of 310 patients in the ceftriaxone group admitted with an NIHSS score of 10 or more developed an infection, compared with 122 (38%) of 317 patients in the control group, and 58 (6%) of 958 patients in the ceftriaxone group admitted with a NIHSS score of 9 or less developed an infection compared with 96 (10%) of 953 patients in the control group (Appendix 11). The median number of days between randomisation and diagnosis of first infection for patients included in the ceftriaxone and control groups was similar (clinical diagnosis 3 days [IQR 1–7] vs 3 days [IQR 2–6; p = 0.80]; expert panel diagnosis 4 days [IQR 2–9] vs 3 days [IQR1–6; p = 0.23]). The total antimicrobial use during hospital stay, as measured by defined daily doses, was higher in the ceftriaxone group than in the control group (4,979 defined daily doses vs 2,120 defined daily doses). In the ceftriaxone group, 4,075 defined daily doses were study medication and 904 were “rescue” medication or antibiotics prescribed after the period of the study medication. The number of deaths and length of hospital stay did not differ between the groups (Table 2).

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Table 2. Outcomes

	Ceftriaxone group (n = 1,268)	Control group (n = 1,270)	Odds ratio (95% CI)	p value
Unfavourable outcome*				
All patients	487/1268 (38%)	507/1270 (40%)	0.94 (0.80–1.11)	0.49
Ischaemic stroke	395/1058 (37%)	421/1067 (39%)	0.91 (0.77–1.09)	0.32
Transient ischaemic attack	5/44 (11%)	10/49 (20%)	0.51 (0.16–1.64)	0.26
Haemorrhagic stroke	82/143 (57%)	67/126 (54%)	1.13 (0.69–1.84)	0.63
Other	5/23 (22%)	8/28 (29%)	1.14 (0.35–3.73)	0.83
Mortality				
At discharge	57/1257 (5%)	61/1257 (5%)	0.93 (0.65–1.35)	0.77
At 3 months	131/1257 (10%)	136/1257 (11%)	0.96 (0.74–1.24)	0.80
Diagnosis of infection during admission				
All infections	130/1268 (10%)	218/1270 (17%)	0.55 (0.44–0.70)	<0.0001
Pneumonia	71/1268 (6%)	88/1270 (7%)	0.80 (0.58–1.10)	0.19
Urinary tract infection	46/1268 (4%)	127/1270 (10%)	0.34 (0.24–0.48)	<0.0001
Other infections	25/1268 (2%)	25/1270 (2%)	1.00 (0.57–1.75)	0.99
Diagnosis of infection based on expert panel				
All infections	40/1268 (3%)	89/1270 (7%)	0.44 (0.30–0.65)	<0.0001
Pneumonia	23/1268 (2%)	34/1270 (3%)	0.67 (0.39–1.15)	0.18
Urinary tract infection	16/1268 (1%)	60/1270 (5%)	0.26 (0.15–0.45)	<0.0001
Other infections	5/1268 (0.4%)	4/1270 (0.3%)	1.25 (0.34–4.76)	0.75
Length of hospital stay, days †	6 (3–10)	6 (3–11)	NA	0.35

Data are n/N (%) or median (IQR). NA=not applicable.

* Unfavourable outcome was defined on the modified Rankin Scale: a score of 0–2, indicating a favourable outcome, versus a score of 3–6, indicating an unfavourable outcome. Unfavourable outcome at 3 months was assessed in 1,257 patients in the ceftriaxone group and in 1,257 in the control group; 24 outcomes were imputed according to the protocol.

† Length of hospital stay was assessed in 1,267 patients in the ceftriaxone group and in 1,268 patients in the control group.

Post-hoc analyses showed that in the patients who received intravenous thrombolysis with alteplase unfavourable outcomes occurred in 144 (33%) of the 437 patients who received ceftriaxone compared with 160 (40%) of 399 in the control group (adjusted OR 0.77 [95% CI 0.61–0.99], p = 0.04). The p value for the interaction between intravenous thrombolysis with alteplase and preventive ceftriaxone on functional outcome was 0.03 (other data not shown). Baseline demographic and clinical characteristics of the 437 thrombolysis-treated patients in the ceftriaxone group and the 399 thrombolysis-treated patients in the control group were similar (Appendix 5).

Preventive ceftriaxone treatment did not result in an increased occurrence of adverse events (Table 3). Seven patients in the ceftriaxone group developed an allergic reaction to the drug during treatment: four patients developed skin rash; one patient had pruritus without skin rash; one patient developed a swollen tongue (with concomitant intravenous alteplase); and one patient developed sweating, dizziness, and vomiting. Ceftriaxone was discontinued in all seven patients. *C difficile* infection occurred in two patients in the ceftriaxone group and none in the control group.

Table 3. Adverse events

	Ceftriaxone group (n=1,242)	Control group (n=1,270)	p-value
Diarrhoea caused by *Clostridium difficile* *	2 (<0.2%)	0	-
Allergic reaction causing cessation of ceftriaxone	7 (0.6%)	0	-
Infection by a ceftriaxone resistant microorganism			
Reported by clinicians †	6 (0.5%)	5 (0.4%)	0.77
As scored by the expert panel ‡	1 (<0.1%)	0	-
Phlebitis at intravenous catheter	15 (1.2%)	9 (0.7%)	0.22
Raised liver enzymes §	152 (12%)	129 (10%)	0.06
Oliguria or raised plasma creatinine ¶	101 (8%)	112 (9%)	0.62

Data are n (%). For the safety analysis, we analysed all patients who actually started the first dose of ceftriaxone (n=1246).

* Diarrhoea caused by *Clostridium difficile* was defined as the clinical diagnosis of diarrhoea in combination with a positive *C difficile* toxin test.

† Infections by a ceftriaxone-resistant microorganism were not further defined in the case record form but were assessed by the expert panel.

‡ One urinary tract infection by a ceftriaxone-resistant microorganism occurred in the ceftriaxone group.

§ Elevation of liver enzymes was defined as aspartate aminotransferase or alanine transaminase concentrations higher than 30 U/L or alkaline phosphatase concentrations higher than 90 U/L.

¶ Oliguria or elevation of plasma creatinine was defined as a concentration higher than 110 µmol/L for men or higher than 100 µmol/L for women.

Discussion

Our study did not show improved functional outcome by preventive antimicrobial therapy in patients with acute stroke, nor did it shorten length of hospital stay or reduce in-hospital or follow-up mortality. One possible reason for these findings could be the high-quality care provided at the stroke units participating in the trial. Prophylaxis with ceftriaxone prevented infections and had a favourable safety profile, but was not associated with a shift in the distribution of scores on the mRS.

Ceftriaxone significantly and safely prevented infections. Treatment with ceftriaxone reduced the proportion of patients with post-stroke infection from 18% to 10%. The most common infections were urinary tract infections and pneumonia, which is in line with previous reports (panel).[1,2,21] Ceftriaxone, a third-generation cephalosporin, has broad action against infection-causing bacteria after acute stroke.[21] The occurrence of an overgrowth infection with *C difficile* in ceftriaxone-treated patients was rare (< 1%).

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The proportion of patients with infection in our study was quite low. Infection rates in previous trials of preventive antimicrobials in stroke ranged from 19% to 90%.[6-11] This difference could well be explained by study population and the definitions used for infection.[11] Study populations of previous studies were often limited to patients with severe stroke. We also included patients with mild strokes; the median score on the NIHSS of patients in our study was 5. For infection we used two definitions: on one hand, we used a pragmatic approach in which clinicians diagnosed and treated infections in the clinical setting, and on the other hand a strict approach in which an expert panel used predefined criteria.[14,17]

Infections were associated with decreased functional outcome. Because ceftriaxone prevented infections, the question arises as to why we did not record a significant effect on functional outcome. Infections could be just a marker or bystander of poor functional outcome, and antimicrobial prophylaxis might not change the course of disease. Another explanation could be that preventive ceftriaxone is not superior to optimum stroke unit care, which implies early treatment with antimicrobials in case of suspected infection.[12] In our study, pneumonia was strongly associated with unfavourable outcome and preventive ceftriaxone did not significantly prevent pneumonia. One could argue that ceftriaxone might prevent pneumonia suboptimally, such as in cases of staphylococcal infection, which is a common cause of post-stroke pneumonia.[2] However, the preventive effect of ceftriaxone on infections was similar, or even superior, to that of other preventive antimicrobials in patients with stroke.[11] Our findings could imply that post-stroke pneumonia can be viewed as a post-stroke respiratory syndrome, rather than solely a bacterial infection.

We recorded no significant differences in the treatment effect in predefined subgroups. Further exploration showed that ceftriaxone decreased unfavourable outcomes in the patients who received intravenous alteplase. Alteplase is an effective treatment for patients with acute ischaemic stroke within a timeframe of 4.5 h of stroke onset.[22] The effect of ceftriaxone in this subgroup might imply that ceftriaxone is beneficial if administered early. However, we recorded no association between timing of prophylaxis and outcome within the ceftriaxone group. In rodents with cerebral infarction, ceftriaxone also has a neuroprotective action by reducing amounts of pro-inflammatory cytokines and matrix metalloproteinases.[23,24] Analogous to the presumed neuroprotective action of the tetracycline antibiotic minocycline,[25] ceftriaxone might offer neuroprotection in patients treated with alteplase. An interaction might also exist between ceftriaxone and thrombolytic therapy, rather than an early or neuroprotective effect that might benefit these patients with stroke. Whether or not early ceftriaxone therapy can benefit patients with ischaemic stroke treated with intravenous thrombolysis remains to be confirmed.

Our pragmatic study has some limitations. First, the possibility of risk of performance and detection bias was a concern. Our study was an open-label masked endpoint study. Physicians were aware of the treatment allocation, which could have potentially affected decisions about diagnostics and non-scheduled treatment. The diagnosis of infections was confirmed or refuted by two masked infectious specialists who also had access to clinical data and the results of diagnostic tests. However, this adjudication panel assessed only those patients previously classified as having a clinical infection by the unmasked physician. This situation might have introduced detection bias in our secondary outcome infections. Importantly, the primary outcome was assessed masked to the treatment allocation. Second, the low infection rate might cause an underestimation of the effect of preventive antibiotics. We also did not show a benefit of preventive ceftriaxone in those with severe stroke, although the numbers of patients in this subgroup were low.

In conclusion, the results of our trial do not support the use of preventive antibiotics in adults with acute stroke. Although preventive ceftriaxone did reduce the infection rate, it did not improve functional outcome in patients with acute stroke, nor did it shorten length of hospital stay or reduce in-hospital or follow-up mortality. Subgroup analysis suggested that ceftriaxone might improve functional outcome in patients with ischaemic stroke who received intravenous thrombolysis, but this idea needs further confirmation.

Panel. Research into context

Systematic review

We searched PubMed between Jan 1, 1950, and Dec 14, 2014; Embase between Jan 1, 1988, and Dec 14, 2014; the Cochrane Central Register of Controlled Trials between Jan 1, 1993 and Dec 14, 2014; ClinicalTrials.gov until Dec 14, 2014; and ISRCTN.org until Dec 14, 2014, for randomised controlled trials of preventive antibiotic therapy versus control (placebo or open control) in patients with acute ischaemic or haemorrhagic stroke, and scanned the reference lists of the articles we found. Our search terms were: "(stroke OR cerebrovascular disorders OR brain ischemia OR brain infarction OR cerebral ischemia OR cerebral infarction) OR ((cerebral or intracerebral or intracranial or brain or cerebellar or subarachnoid) AND (haemorrhage or haemorrhage or haematoma or hematoma or bleeding or aneurysm)) AND (antibiotic OR anti-bacterial agents OR amoxicillin OR cephalosporin OR doxycycline OR erythromycin OR fluoroquinolone OR gentamicin OR minocycline OR penicillin OR streptomycin OR tetracycline OR vancomycin) AND (infection OR pneumonia OR sepsis OR bacteraemia OR fever OR inflammation) AND (prophylaxis OR prevention)." We did not use any language restrictions in our search. In 2012, a Cochrane systematic review and meta-analysis was published and pooled data from five studies including 506 patients.[11] Since this Cochrane review, no new studies about preventive antibiotic therapy in acute stroke have been published. We identified two ongoing trials: STROKE-INF Study, a cluster randomised trial of different strategies of antibiotic use to reduce the incidence and consequences of chest infection in acute stroke patients with swallowing problems (n=1,200; ISRCTN37118456); and STRAWINSKI, a multicentre, randomised, controlled trial with masked assessment of outcome comparing procalcitonin ultrasensitive-guided antibiotic treatment with standard care (n=200; NCT01264549).

Interpretation

The studies done so far were analysed in the previously described Cochrane meta-analysis[11] ; included studies were small and heterogeneous in study population, type of antibiotic, and definition of infection. Based on the studies in this review, preventive antibiotic therapy seemed to reduce the risk of infection but did not reduce the number of dependent or deceased patients. However, because studies were small and heterogeneous, no conclusions could be drawn regarding the effect of preventive antibiotic therapy on functional outcome. The results of our study show that prophylactic ceftriaxone does not improve functional outcome at 3 months in adults who have had a stroke despite preventing infection. Preventive antibiotic therapy should not be included in standard care for all patients with acute stroke.

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Contribution of authors

DvdB and PJN contributed equally — they acquired funding; designed the study; collected, analysed, and interpreted the data; and wrote the report. WFW and J-DV also contributed equally — they designed the study; collected, analysed, and interpreted the data; and wrote the report. IJH contributed to study design, data collection, and trial management. EZ, NDK, HJLWB, VIHK, MW, MJMR, RtH, AHCMS, SEV, EJvD, RPK, and HK contributed to data collection, data interpretation, and writing the report. DWJD, MGWD, LS, MV, TvdP, and FHV contributed to study design, data collection, data interpretation, and writing the report. JMP and YBWEMR contributed to study design, data collection, the outcome committee, data interpretation, and writing the report. MCB contributed to study design, data collection, the outcome committee, data analysis, data interpretation, and writing the report. AHZ contributed to study design, statistical advice, data analysis, data interpretation, and writing the report.

Declarations of interests

We declare no competing interests.

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Appendix 1.

Centres and investigators participating in the 'Preventive Antibiotics in Stroke Study'

The following centres and investigators participated in the Preventive Antibiotics in Stroke Study:
Academisch Medisch Centrum, Amsterdam: D. van de Beek, P.J. Nederkoorn, W.F. Westendorp, J-D. Vermeij; Albert Schweitzer Ziekenhuis, Dordrecht: H. Kerkhoff, Elles Zock, Ruud P. Kleyweg; Onze Lieve Vrouwe Gasthuis, Amsterdam: J.L.W. Bosboom, V.I.H. Kwa; Kennemer Gasthuis, Haarlem: M. Weisfelt; Slotervaartziekenhuis, Amsterdam: N.D. Kruyt; Amphia Ziekenhuis, Breda: M.J.M. Remmers; Radboud Universitair Medisch Centrum, Nijmegen: E.J. van Dijk; Sint Franciscus Gasthuis, Rotterdam: F. Vermeij; Atrium Medisch Centrum, Heerlen: A. Schreuder; Ziekenhuis Rijnstate, Arnhem: S.E. Vermeer; Medisch Centrum Alkmaar, Alkmaar: R. ten Houten; Erasmus MC, Rotterdam: D.W.J. Dippel; Universitair Medisch Centrum Utrecht, Utrecht: L.J. Kappelle, H.B. van der Worp; Spaarne Ziekenhuis, Hoofddorp: I.S.J. Merkies; HagaZiekenhuis, Den Haag: S.F.T.M. de Bruijn, K.F. de Laat; Medisch Centrum Haaglanden, Den Haag: K. Jellema; Catharina Ziekenhuis, Eindhoven: K. Keizer, M.C. de Rijk, A.J. Vermeij; VU Medisch Centrum, Amsterdam: M.C. Visser; Reinier de Graaf Groep, Delft: L.A.M. Aerden; Martini Ziekenhuis, Groningen: E.S. Schut; Zorg Groep Twente, Almelo: L.J.A. Reichman; Groene Hart Ziekenhuis, Gouda: K. de Gans; Zaans Medisch Centrum, Zaandam: R.M. van den Berg-Vos; Laurentius Ziekenhuis, Roermond: M.P.J. van Goor; IJselland Ziekenhuis, Capelle aan den IJssel: A.D. Wijnhoud; Westfriesgasthuis, Hoorn: T.C. van der Ree; BovenIJ Ziekenhuis, Amsterdam: M. Janmaat; Orbis Medisch Centrum, Sittard: N. van Orshoven; Bronovo Ziekenhuis, Den Haag: S.M. Manschot. **Study group**: D. van de Beek, P.J. Nederkoorn, D.W.J. Dippel, M.G.W. Dijkgraaf, T. van der Poll, J.M. Prins, L. Spanjaard, F.H. Vermeij.

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Appendix 2.

Patients loss-to-follow-up

PASS no.	Reason	Follow-up based on
04-043	Patient and representative could not be reached	Report of visit outclinic Neurology
04-100	Patient refused participation in follow-up interview	Report of visit outclinic Neurology
05-016	Patient refused participation in follow-up interview	Report of visit outclinic Neurology
05-053	Patient withdrew from study	Report of visit outclinic Neurology
05-061	Patient and representative could not be reached	Report of visit outclinic Neurology
11-099	Representative could not be reached	Report of visit outclinic Neurology
19-086	Patient and representative could not be reached	Report of visit outclinic Neurology
21-028	Patient and representative could not be reached	Report of visit outclinic Neurology
50-036	Patient withdrew from study	Report of visit outclinic Neurology
50-053	Patient and representative could not be reached	Report of visit outclinic Neurology
11-002	Patient withdrew from study	Report of visit outclinic Neurology
12-111	Patient withdrew from study	Discharge letter nursing home
14-033	Patient and representative could not be reached	Discharge letter hospital
14-020	Patient withdrew from study	Discharge letter hospital
14-065	Patient withdrew from study	Chart review and discharge letter
14-221	Patient withdrew from study	Report of visit community health service
14-265	Patient withdrew from study	Chart review and discharge letter
12-051	Patient withdrew from study	Discharge letter hospital
58-002	Patient refused participation in follow-up interview	Case Record Forms PASS
14-464	Patient refused participation in follow-up interview	Report of visit outclinic Neurology
14-448	Patient and representative could not be reached	Report of visit outclinic Neurology
21-024	Patient refused participation in follow-up interview	Report of revalidation clinic, discharge letter
04-135	Patient and representative could not be reached	Discharge letter hospital
16-058	Patient withdrew from study	Discharge letter of readmission

Appendix 3.

Other diagnosis according to treatment allocation

Diagnosis	Ceftriaxone group (n = 20)	Control group (n = 29)
Functional neurologic symptoms	8	13
Delirium	1	0
Epilepsy	2	2
Encephalopathy	0	1
Hypoperfusion due to carotid artery stenosis	0	1
Hypoglycaemia	1	0
Migraine	3	0
Multiple sclerosis	0	1
Myelopathy	1	0
Peripheral neuropathy	1	1
Peripheral vascular disease	1	0
Posterior reversible encephalopathy syndrome	1	0
Retinal infarction	0	1
Rhombencephalitis	0	2
Subdural haemorrhage	0	1
Uncertain	1	3
Vestibular neuritis	0	1
Vestibulopathy	0	2

Appendix 4.

Sensitivity analysis including follow-up of 24 patients lost-to-follow-up according to last-observation carried-forward (LOCF) outcome assessment

Primary analysis of primary outcome

Result of the primary analysis of primary outcome: adjusted common odds ratio, 0.95; 95%CI 0.82 – 1.09; p = 0.434.

Secondary analysis of primary outcome

	Ceftriaxone group (n = 1,268)	Control group (n = 1,270)	Odds Ratio	95 % confidence interval	p value
Unfavourable outcome*					
All patients †	489 (39)	508 (40)	0.94	0.80 – 1.10	0.47
Ischemic stroke	395 (38)	419 (40)	0.82	0.77 – 1.09	0.35
Transient ischemic attack	5 (11)	10 (20)	0.49	0.15 – 1.56	0.27
Haemorrhagic stroke	81 (57)	68 (54)	1.11	0.69 – 1.81	0.71
Other	8	12	0.92	0.32 – 2.65	1.00
Mortality ‡					
Discharge	57 (5)	61 (5)	0.93	0.65 – 1.35	0.78
3 months	131 (10)	136 (11)	0.96	0.75 – 1.24	0.80

* Unfavourable outcome at 3 months was evaluated in 1,257 patients in the ceftriaxone group and 1,257 patients in the control group; 24 outcomes were imputed according to protocol.

† No. of patients with characteristic (percentage).

‡ Mortality was evaluated at discharge in 1,267 patients in the ceftriaxone group and 1,269 patients in the control group; mortality at 3 months was evaluated in 1,268 patients in the ceftriaxone group and 1,270 patients in the control group.

Appendix 5.

Baseline characteristics of thrombolysed patients

Characteristic	Ceftriaxone group (n = 437)	Control group (n = 399)
Age – year (interquartile range)	72 (62-80)	73 (61-80)
Male sex – no. (%)	252/437 (58)	234/399 (59)
History – no. (%)		
Atrial fibrillation/flutter	37/436 (9)	51/399 (13)
Stroke	150/437 (34)	140/399 (35)
Hypercholesterolemia	117/435 (27)	105/394 (27)
Hypertension	218/436 (50)	209 (52)
Myocardial infarction	62/437 (14)	64/399 (16)
Cardiac valve disease§	16/436 (4)	22/399 (6)
Peripheral vascular disease	27/437 (6)	30/398 (8)
Obstructive pulmonary disease	28/437 (6)	21/398 (5)
Immunocompromise	23/437 (5)	12/399 (3)
Current smoker – no. (%)	96/433 (22)	104/395 (26)
Prior medication – no. (%)		
Anticoagulants	12/437 (3)	12/399 (3)
Antiplatelet	209/437 (48)	186/399 (47)
Statin	165/437 (38)	145/399 (36)
ACE-inhibitor	118/437 (27)	79/399 (20)
Beta-blocker	148/437 (34)	136/399 (34)
Protonpompinhibitor	110/437 (25)	99/399 (25)
Modified Rankin Scale score		
Median	0	0
Range	0-5	0-4
Mean	0.56	0.53
National Institutes of Health Stroke Scale score		
Median	6	6
Range	0-26	1-29
Mean	8	8
Dysphagia – no. (%)	103/403 (26)	107/376 (29)
Discharge diagnosis – no. (%)		
Cerebral infarction	426 (98)	380 (95)
Transient ischemic attack	3 (1)	7 (2)
Cerebral haemorrhage	0	0
Other	8 (1)	12 (3)

ACE denotes Angiotensin-converting enzyme.

Appendix 6.

Patients with allergic reaction as noted by physician that caused cessation of ceftriaxone

PASS no.	Allergic reaction
01-091	Skin rash during 2nd dose ceftriaxone
10-111	Skin rash after 2nd dose ceftriaxone
12-063	Dizziness, chest pain, sweating, nausea and vomiting, no cardiac cause was found
12-064	Skin rash during 1st dose ceftriaxone
14-237	Itching without skin rash during 2nd dose ceftriaxone
15-039	Swelling of tongue after ceftriaxone, possibly also due to thrombolysis
20-058	Cessation after 1st dose ceftriaxone because of allergic reaction to penicillin in medical history which was not noted at time of inclusion
59-038	Skin rash after 2nd dose ceftriaxone

Appendix 7.

Infections with ceftriaxone resistant organisms

PASS no.	Infection
02-001	Urinary tract infection (by physician and panel) by *Escherichia coli* and *Proteus mirabilis* after 2 days of treatment with ceftriaxone therapy changed to sulfamethoxazole/trimethoprim 2dd 960 mg 5 days.
05-069	Physician: pneumonia. Panel: urinary tract infection. Urinary culture positive for *Staphylococcus epidermidis*, resistant to oxacilline, sensitive to vancomycin and rifampicin. Tracheal cultures showed *Pseudomonas aeruginosa* sensitive to penicillin
12-005	Diagnosis urinary tract infection and pneumonia by physician and panel. ESBL producing E. coli cultured from urine, no symptoms, no treatment initiated.
13-025	Other infection by physician, no other infection by panel
14-195	Other infection by physician and panel: sepsis by candida. No antibiotic resistance reported.
14-386	Urinary tract infection by physician, not by panel
14-439	Urinary tract infection and pneumonia by physician and panel. As treatment for pneumonia ceftriaxone continued (no resistant micro-organisms reported in discharge letter) and urinary tract infection treated with nitrofurantoin (no resistant MO reported)
18-003	Pneumonia by physician, not by panel. In discharge letter no resistant micro-organism reported and treatment with amoxicillin/clavulanate potassium was started
19-111	Pneumonia by physician and panel. No resistant microorganism reported.
20-037	Urinary tract infection by physician during treatment with ceftriaxone, treatment with gentamycin and tobramycin was started (not scored as infection by panel)
20-049	Pneumonia by physician, urinary tract infection by panel. No data on resistant micro-organisms reported

Appendix 8.

Protocol violations in eligibility

Diagnosis	Ceftriaxone group	Control group
Score of 0 on NIHSS	2	1
Infection at admission	2	0
Randomisation in another intervention trial	2	0
Use of antibiotics < 24 hours of admission	1	0
Onset of stroke > 24 hours ago	2	0

Appendix 9.

Explanation of protocol violations in eligibility

PASS no.	Explanation of protocol violation in eligibility
11-160	Score of 0 on NIHSS and diagnosis subdural hematoma
12-050	Score of 0 on NIHSS
12-136	Infection and use of antibiotic at admission
14-038	Score of 0 on NIHSS
14-105	Patient was already randomized in another intervention trial
14-221	Use of antibiotics < 24 hours of admission
15-057	Onset of stroke > 24 hours ago
16-020	Onset of stroke > 24 hours ago
16-046	Infection at admission
19-105	Patient was already randomised in another intervention trial

Appendix 10.

Subanalysis on infections in patients with severe stroke (NIHSS >9)

	Ceftriaxone group (n = 310)	Control group (n = 317)	OR (95%CI)
As defined by physician			
All infections	72 (23)	122 (38)	0.48 (0.34 – 0.68)
Pneumonia	46 (15)	56 (18)	0.81 (0.53 – 1.24)
Urinary tract infection	21 (7)	67 (21)	0.27 (0.16 – 0.46)
As defined by expert panel			
Infection	27 (10)	45 (14)	0.58 (0.34 – 0.96)
Pneumonia	15 (6)	20 (6)	0.76 (0.40 – 1.50)
Urinary tract infection	11 (4)	27 (9)	0.40 (0.19 – 0.81)

Appendix 11.

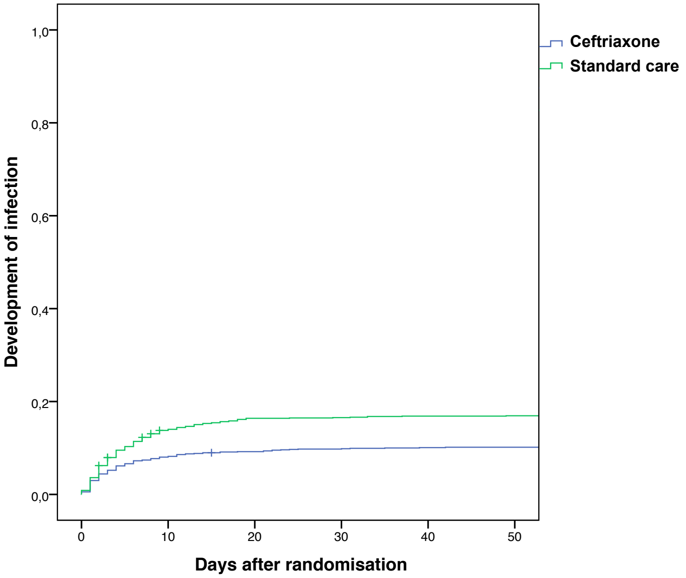
Subanalysis on infections in patients with mild stroke (NIHSS <=9)

	Ceftriaxone group (n = 958)	Control group (n = 953)	OR (95%CI)
As defined by physician			
All infections	58 (6)	96 (10)	0.58 (0.41 – 0.81)
Pneumonia	25 (3)	32 (3)	0.77 (0.45 – 1.31)
Urinary tract infection	25 (3)	60 (6)	0.40 (0.25 – 0.64)
As defined by expert panel			
Infection	13 (1)	44 (5)	0.28 (0.15 – 0.53)
Pneumonia	8 (1)	14 (1)	0.56 (0.24 – 1.35)
Urinary tract infection	5 (1)	33 (3)	0.15 (0.06 – 0.38)

Appendix 12.

Kaplan-Meier curves for the occurrence of infection: infections defined by the physician in ceftriaxone (blue line) and control group (green line). Panel A shows curves for all patients, Panel B shows curves for patients with severe stroke, as defined as of NIHSS score of 10 or more on admission.

A



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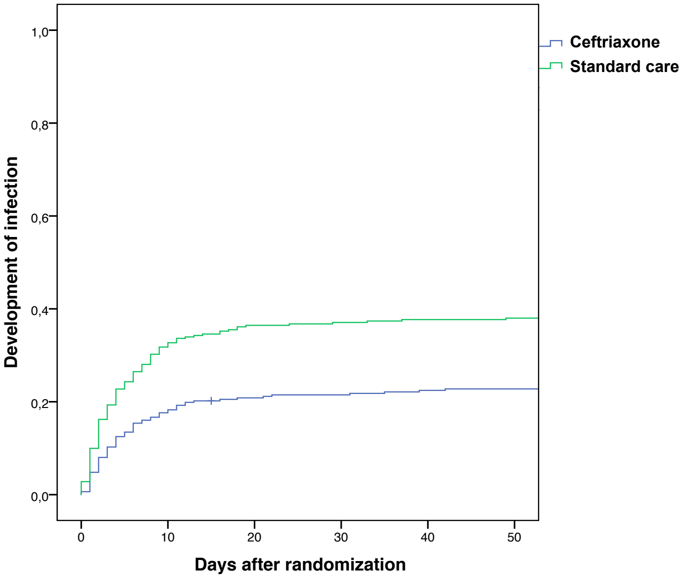

4

Preventive ceftriaxone in patients with stroke treated with intravenous thrombolysis: post hoc analysis of the Preventive Antibiotics in Stroke Study (PASS)

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Abstract

Background
The Preventive Antibiotics in Stroke Study (PASS), a randomized open-label masked endpoint trial, showed that preventive ceftriaxone did not improve functional outcome at 3 months in patients with acute stroke (adjusted common odds OR 0.95; 95%CI 0.82–1.09). Post-hoc analyses showed that in patients who received intravenous thrombolysis (IVT) favorable outcomes occurred significantly more often in patients who received ceftriaxone compared with the control group. This study aimed to gain more insight in the characteristics of these patients.

Methods
In PASS, 2,550 patients were randomly assigned to preventive antibiotic treatment with ceftriaxone or standard care. In current post hoc analysis 836 patients who received IVT were included. Primary outcome included functional status on the modified Rankin Scale, analyzed with adjusted ordinal regression. Secondary outcomes included infection rate and symptomatic intracerebral hemorrhage (sICH) rate.

Results
For all patients in PASS, the p-value for the interaction between IVT and preventive ceftriaxone regarding functional outcome was 0.03. Of the 836 IVT-treated patients, 437 were administered ceftriaxone and 399 were allocated to the control group. Baseline characteristics were similar. In the IVT subgroup, preventive ceftriaxone was associated with a significant reduction of unfavorable outcome (adjusted common OR 0.77; 95%CI 0.61–0.99; $p = 0.04$). Mortality at three months was similar (OR 0.75; 95%CI 0.48–1.18). Preventive ceftriaxone was associated with a reduction in infections (OR 0.43; 95%CI 0.28–0.66) and a trend towards an increased risk for sICH (OR 3.09; 95%CI 0.85–11.31). Timing of administration of ceftriaxone did not influence outcome (aOR 1.00; 95%CI 0.98–1.03; $P=0.85$).

Conclusions
According to the post-hoc analysis of PASS, preventive ceftriaxone may improve the functional outcome in IVT-treated patients, despite a trend towards an increased rate of post-IVT-sICH.

Introduction

Infection is a common complication in the acute phase after stroke.[1] In a recent meta-analysis of 87 studies, the pooled infection rate after stroke was 30%; most commonly pneumonia and urinary tract infections.[2] Many studies have shown that the occurrence of infections after stroke is associated with a poor functional outcome and mortality[3] there are some reports suggesting that an infection after stroke is merely a marker of stroke severity without an independent effect on outcome when it is treated promptly.[4]

Previously, 6 randomized studies had assessed the preventive use of antimicrobials in patients with acute stroke, with conflicting results.[5-10] A Cochrane meta-analysis including 506 patients from 5 studies concluded that although studies differed in populations analyzed, type of antimicrobial used, and definition of infection, overall antimicrobial prophylaxis reduced the infection rate from 36% to 22% (relative risk 0.58; 95%CI 0.43–0.79) without any major adverse effects. Whether or not preventive antimicrobials reduced the risk of poor functional outcome after stroke remained uncertain: the overall mortality rate was non-significantly reduced from 15% in the placebo group to 13% (95%CI 0.47–1.51) and the number of dependent patients was reduced from 61% to 47% (95%CI 0.32 – 1.43).[3]

Recently, results of the Preventive Antibiotics in Stroke Study (PASS) were published, a pragmatic randomized open-label masked endpoint clinical trial investigating the efficacy and safety of the preventive use of the β-lactam antibiotic ceftriaxone in patients with the acute stroke.[10-12] A total of 2,550 patients from 30 sites in the Netherlands, including academic and non-academic medical centers, were randomly assigned to two treatment groups: 1,275 patients to ceftriaxone and 1,275 patients to standard treatment (control group). Preventive ceftriaxone did not improve functional outcome at 3 months after stroke (adjusted common OR 0.95; 95%CI 0.82–1.09). Post-hoc analyses showed that in the patients who received intravenous thrombolysis (IVT) with alteplase, unfavorable outcomes occurred significantly less frequent in patients who received ceftriaxone as compared with the control group. This study aimed to gain more insight into the characteristics of the patients treated with IVT.

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Methods

We performed a post hoc analysis of the patients in PASS who received IVT with alteplase (IVT-patients). In PASS, patients were randomly assigned in a 1:1 ratio for treatment with ceftriaxone in addition to stroke unit care, or to standard stroke unit care without preventive antimicrobial therapy. Details on study design, procedures, outcome measurements and statistical analysis have been published.[11-13] The study was registered with controlled-trials.com, number ISRCTN66140176. Participating centers are mentioned in Appendix 1.

Patients were eligible for inclusion if they were aged 18 years or older, had clinical symptoms of a stroke (ischemic or hemorrhagic), an onset of symptoms less than 24h, and a score of 1 or more on the National Institutes of Health Stroke Scale (NIHSS). Exclusion criteria included clinical signs of infection on hospital admission requiring antibiotic therapy, use of antimicrobials less than 24 h before admission, pregnancy, hypersensitivity for cephalosporins, previous anaphylaxis for penicillin derivatives, subarachnoid hemorrhage, and imminent death.

Preventive antibiotics were initiated within 24h after the onset of symptoms. The study medication was ceftriaxone 2g, given intravenously once daily for 4 days. Ceftriaxone was discontinued if patients were either discharged or in whom active treatment was withdrawn. The treating clinician could decide whether or not to treat a patient with suspected infection with (additional) antimicrobials. Study procedures with respect to antimicrobial treatment are detailed in the study protocol.[11,12]

The primary outcome was functional status at 3 months, defined by the modified Rankin Scale (mRS), which ranges from 0 (no symptoms) to 6 (death). Secondary outcomes were death at discharge and 3 months after randomization, infection rate, total antimicrobial use, length of hospital stay, volume of post-stroke care, and quality-adjusted life-years and costs. Infections were categorized as diagnosed by the clinician, and as judged by an independent adjudication committee (masked to treatment allocation) according to modified Centers for Disease Control and Prevention criteria.

In the current post-hoc analysis, an interaction analysis was comparing thrombolysis with study allocation (ceftriaxone vs standard care), was performed first. Functional status was assessed by comparing IVT patients with non-IVT-patients (patients in PASS 'who potentially could have been treated with IVT, given their diagnosis: 'infarct', 'TIA' or 'other'; a total of 1,433 patients').

Next, among the IVT patients, the functional status was assessed according to study allocation, both by ordinal regression of the entire range (0-6) of the mRS score at 3 months and by a dichotomized outcome (favorable (mRS 0-2) vs unfavorable (mRS 3-6)). Secondary outcomes were death at discharge and at 3 months after randomization, infection rate during admission and symptomatic intracerebral hemorrhage (sICH) rate as a safety outcome. Cause of death during admission was retrieved from the hospital discharge letters and patient records. Causes were categorized according to the most frequently observed etiology.

To investigate if earlier timing of administration of ceftriaxone in IVT patients was associated with outcome, the time between the onset of symptoms and the administration of the first gift of ceftriaxone and its effect on functional outcome was calculated.

Tests of group differences were performed by using standard methods depending on the type of variable. Normal and non-normal distributed variables were compared with the Student's t test and the Mann-Whitney U test. For the ordinal regression model, both unadjusted and adjusted analyses were performed. Adjustment variables were defined beforehand and included age, stroke severity as defined as NIHSS score, history of stroke, history of diabetes, previous disability as defined on the mRS, stroke type and categorization of study center. Two-tailed p values < 0.05 were identified to indicate statistical significance.

Results

Interaction analysis

In PASS, unplanned post-hoc dichotomous analyses showed that in the patients who received IVT with alteplase (IVT-group) unfavorable outcomes occurred in 144 (33%) of the 437 patients who received ceftriaxone compared with 160 (40%) of 399 in the control group (aOR 0.77; 95%CI 0.61–0.99; p = 0.04).[10] Including all patients, the p value for the interaction between IVT with alteplase and preventive ceftriaxone on functional outcome was 0.03.

The proportion of patients with a history of atrial fibrillation, hypertension, cardiac valve disease or obstructive pulmonary disease was higher in the non-IVT group as compared to the IVT group (Appendix 2), as was the case when comparing 'ceftriaxone and IVT-treated' with 'ceftriaxone and non-IVT-treated' patients (Appendix 3)

There were no differences in functional outcome or infection rate except for urinary tract infections, which was diagnosed more often in non-thrombolysed patients when comparing the IVT group with the non-IVT group (Table 1).

Table 1. Outcomes of thrombolysed patients in the PASS (IVT group) as compared with non-thrombolysed patients (non-IVT group) *, **

	IVT-group (n = 836)	Non-IVT group (n = 1,433)	OR	95% CI
Ordinal regression on mRS				
Adjusted †	NA	NA	1.38	0.39 – 4.96
Unfavorable outcome §	304/833 (37)	535/1416 (38)	0.95	0.79 – 1.13
Mortality				
Discharge	44/834 (5)	52/1433 (4)	1.48	0.98 – 2.32
3 months	85/833 (10)	137/1416 (10)	1.06	0.80 – 1.41
Diagnosis of infection during admission				
All infections	100/836 (12)	194/1433 (14)	0.87	0.67 – 1.12
Pneumonia	46/836 (6)	78/1433 (5)	1.01	0.70 – 1.47
Urinary tract infection	39/836 (5)	105/1433 (7)	0.62	0.42 – 0.90
Other infections	22/836 (3)	31/1433 (2)	1.22	0.70 – 2.13
Diagnosis of infection based on expert panel				
All infections	38/836 (5)	64/1433 (5)	1.02	0.68 – 1.54
Pneumonia	18/836 (2)	27/1433 (2)	1.15	0.63 – 2.03
Urinary tract infection	22/836 (3)	38/1433 (3)	0.99	0.58 – 1.69
Other infections	3/836 (0)	3/1433 (0)	1.72	0.35 – 8.53

*Data are n/N (%) or median (IQR). NA = not applicable.

** Non-IVT-group is considered the population in PASS that could potentially have been treated with IVT, given their diagnosis: 'infarct', 'TIA' or 'other'.

† Predefined adjustment included age, stroke severity as defined as NIHSS score, history of stroke, history of diabetes, previous disability as defined on the mRS, stroke type, and categorization of study center.

§ Unfavorable outcome was defined on the mRS: a score of 0–2, indicating a favorable outcome, versus a score of 3–6, indicating an unfavorable outcome.

When adjusting for the observed differences in baseline characteristics, no differences were found when comparing 'ceftriaxone and IVT-treated' with 'ceftriaxone and non-IVT-treated' patients. As expected, a better outcome was observed in IVT-treated patients as compared with non-IVT treated patients (Appendix 4).

Baseline characteristics and stroke outcome

The baseline demographic and clinical characteristics according to study allocation within the IVT-group were similar, although in the ceftriaxone group more patients were on angiotensin converting enzyme inhibitors and the median systolic blood pressure at presentation was slightly lower (Table 2). The definite diagnosis was a cerebral infarction in 806 cases (96%), TIA in 10 patients (1%), and an alternative diagnosis in 20 patients (2%). Alternative diagnoses were functional neurological symptoms in 10, multiple sclerosis in 3, and migraine, epilepsy, vestibulopathy, cervical myelopathy each in one; in 3 patients the definitive diagnosis was lacking due to diagnostic uncertainty by the neurologist.

Table 2. Baseline characteristics of IVT patients in PASS according to treatment allocation *

Characteristic	Ceftriaxone group (n = 437)	Control group (n = 399)
Age, years	71 (61-80)	72 (60-79)
Male sex	252 (58)	234 (59)
History		
Atrial fibrillation/flutter	37/436 (9)	51/399 (13)
Stroke	150/437 (34)	140/399 (35)
Hypercholesterolemia	117/435 (27)	105/394 (27)
Hypertension	218/436 (50)	209/399 (52)
Myocardial infarction	62/437 (14)	64/399 (16)
Cardiac valve disease †	16/436 (4)	22/399 (6)
Peripheral vascular disease	27/437 (6)	30/398 (8)
Obstructive pulmonary disease	28/437 (6)	21/398 (5)
Immunocompromise ‡	23/437 (5)	12/399 (3)
Current smoker	96/433 (22)	104/395 (26)
Prior medication		
Anticoagulants	12/437 (3)	12/399 (3)
Antiplatelet	209/437 (48)	186/399 (47)
Statin	165/436 (38)	145/399 (36)
ACE-inhibitor	118/436 (27)	79/399 (20)
Beta-blocker	148/436 (34)	136/398 (34)
Protonpompinhibitor	110/436 (25)	99/398 (25)
Systolic blood pressure at presentation		
Median (mmHg)	155	160
Range (mmHg)	95 – 230	83 - 230
Diastolic blood pressure at presentation		
Median (mmHg)	82	85
Range (mmHg)	30 - 152	36 - 144
Modified Rankin Scale score §		
Median	0	0
Range	0-5	0-4
National Institutes of Health Stroke Scale score ¶		
Median	6	6
Range	0-26	1-29
Dysphagia	103/403 (26)	107/376 (29)
Discharge diagnosis		
Cerebral infarction	426/437 (98)	380/399 (95)
Transient ischemic attack	3/437 (1)	7/399 (2)
Cerebral hemorrhage	0	0
Other	8/437 (1)	12/399 (3)

* Data are n/N (%) or median (IQR). NA = not applicable.

† Cardiac valve disease was defined as cardiac valve insufficiency, stenosis, or replacement.

‡Immunocompromised was defined as changed immune status, diabetes mellitus, alcoholism, malignancy, or immunosuppressive medication.

§ Scores on the mRS range from 0 to 6, with 6 indicating death

¶ Scores on the NIHSS range from 0 to 30, with 30 indicating highest degree of stroke severity

Preventive ceftriaxone was associated with a significant shift towards a better functional outcome at 3 months (unadjusted common OR 0.75; 95%CI 0.59–0.96; p = 0.02). After adjustment for stroke severity, age, history of diabetes or previous stroke, mRS on baseline and type of hospital, the beneficial effect remained, as was mentioned earlier (Figure 1). In the dichotomized analysis of the mRS, preventive ceftriaxone was associated with a significant reduction in the risk of an unfavorable outcome (144 (33%) vs 160 (40%); OR 0.73; 95%CI 0.55–0.97). There was no significant difference in mortality between the two treatment groups after discharge (21 (5%) vs 23 (6%); OR 0.83; 95%CI 0.45–1.52) or after three months (39 (9%) vs 46 (12%); OR 0.75; 95%CI 0.48–1.18) (Table 3).

Table 3. Outcomes of IVT patients in the PASS according to treatment allocation *

	Ceftriaxone group (n = 437)	Control group (n = 399)	OR	95%CI
Ordinal regression on mRS				
Not adjusted	NA	NA	0.75	0.59 – 0.96
Predefined adjustment †	NA	NA	0.77	0.61 – 0.99
Post hoc adjustment ‡	NA	NA	0.78	0.61 – 0.99
Unfavorable outcome §	144/436 (33)	160/397 (40)	0.73	0.55 – 0.97
Mortality				
Discharge	21/436 (5)	23/398 (6)	0.83	0.45 – 1.52
3 months	39/436 (9)	46/397 (12)	0.75	0.48 – 1.18
Diagnosis of infection during admission				
All infections	34/437 (8)	66/399 (17)	0.43	0.28 – 0.66
Pneumonia	19/437 (4)	27/399 (7)	0.63	0.34 – 1.15
Urinary tract infection	11/437 (3)	28/399 (7)	0.34	0.17 – 0.70
Other infections	7/437 (2)	15/399 (4)	0.42	0.17 – 1.03
Diagnosis of infection based on expert panel				
All infections	13/437 (3)	25/399 (6)	0.46	0.23 – 0.91
Pneumonia	9/437 (2)	9/399 (2)	0.91	0.36 – 2.32
Urinary tract infection	5/437 (1)	17/399 (4)	0.26	0.10 – 0.71
Other infections	1/437 (0)	2/439 (1)	0.46	0.04 – 5.04

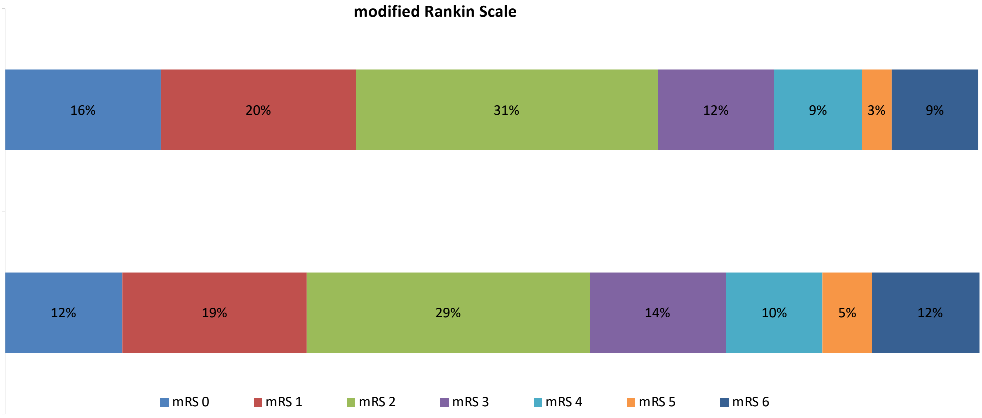
* Data are n/N (%) or median (IQR). NA=not applicable.

† Predefined adjustment included age, stroke severity as defined as NIHSS score, history of stroke, history of diabetes, previous disability as defined on the mRS, stroke type, and categorisation of study center.

‡ Post hoc adjustment included predefined adjustment parameters plus prior using of an ACE inhibitor.

§ Unfavorable outcome was defined on the mRS a score of 0–2, indicating a favorable outcome, versus a score of 3–6, indicating an unfavorable outcome.

Figure 1. Distribution of mRS scores 3 months after randomization



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After adjustment for stroke severity, age, history of diabetes or previous stroke, mRS on baseline and type of hospital, preventive ceftriaxone remained associated with a significant shift towards a better functional outcome at 3 months (aOR 0.77; 95%CI 0.61–0.99; p = 0.04). Scores on the modified Rankin scale range from 0 to 6, with 0 indicating no symptoms and 6 indicating death.

Symptomatic intracerebral hemorrhage

sICH developed in 15 of 836 (1.8%) IVT patients during hospital admission, of which 13 within 48 hours after the onset of symptoms (defined as post-IVT-sICH). Although non-significantly, preventive ceftriaxone was associated with the development of post-IVT-symptomatic intracerebral hemorrhage (10 of 437 (2.3%) vs 3 of 399 (0.8%); OR 3.09; 95%CI 0.85–11.31).

As expected, post-IVT-sICH was associated with unfavorable outcome (10 of 13 (77%) vs 294 of 820 (36%); OR 5.96; 95%CI 1.63–21.84).

Cause of death

The cause of death was categorized for all 44 IVT patients who died within follow-up time of the study. Median time between admission and death was 5 days in both the intervention and the control groups. Patients in the ceftriaxone group tended to die significantly more often of post-IVT-sICH (7 of 21 (33%) vs 2 of 23 (9%); p = 0.06), whereas patients in the control group died more often of infection (9 of 23 (39%) vs 0 patients; p = 0.002). Other causes of death were more or less equally distributed between both groups (Table 4).

Table 4. Cause of death during initial admission according to treatment allocation within the IVT group

	Ceftriaxone group (n = 21)	Control group (n = 23)
post-IVT-sICH	7	2
sICH, not IVT related	1	0
Cerebral edema	8	5
Cardiopulmonary complications	3	2
Infection	0	9
Palliative care after no post-IVT improvement	2	3
Death after unknown deterioration	0	1
Other (ileus)	0	1

Effect of early treatment with ceftriaxone on stroke outcome

IVT patients received ceftriaxone earlier compared with non-IVT patients (median 8 vs 12 hours). Considering the current 'treatment window', a timeframe of 6 hours after onset of symptoms, 171 of 414 (41%) IVT-patients received ceftriaxone vs 122 of 648 (19%) non-IVT patients ($p < 0.001$). The timing of administration of ceftriaxone in thrombolysed vs non-thrombolysed patients did not influence stroke outcome after three months in an adjusted ordinal regression model (aOR 1.00; 95%CI 0.98–1.03; $p = 0.85$).

Discussion

This post-hoc analysis of the PASS suggests that preventive antimicrobial therapy with ceftriaxone in IVT patients with acute stroke diminishes the number of infections and improves the functional outcome despite a trend towards an increased rate of post-IVT-sICH. Whether or not treatment with preventive ceftriaxone provides benefit in patients with acute ischemic stroke treated with intravenous thrombolysis remains to be confirmed.[14]

Overall, in the entire study cohort of 2,550 patients in PASS, preventive antimicrobial therapy did neither improve functional outcome, nor was the length of hospital stay shortened or was the in-hospital or follow-up mortality reduced. Prophylaxis with ceftriaxone, however, did significantly decrease the infection-rate and had a favorable safety profile. The only subgroup in which an effect on functional outcome could be shown, - in a post-hoc analysis-, was the group of patients with acute ischemic stroke who were treated with IVT.

We would like to emphasize that the patient population in the current analysis slightly differs from the population of the entire PASS. Of the 2,538 patients used in the original intention-to-treat analysis, 269 patients suffering from cerebral hemorrhage were excluded. The remaining 2,269 patients could potentially have been treated with IVT, given their diagnosis: 'infarct', 'TIA' or 'other'. Since risk factors between intracerebral infarction and hemorrhage differ, slight differences in baseline characteristics between the original population and current subgroup analysis are possible. These differences could indeed be observed when comparing IVT-treated patients (n = 836) with non-IVT-treated-patients (n = 1,433) (Appendix 2) and when comparing 'ceftriaxone and IVT-treated patients' (n = 437) with 'ceftriaxone and non-IVT-treated' patients (n = 688) (Appendix 3): IVT-treated patients more often had a history of atrial fibrillation, hypertension, cardiac valve disease or obstructive pulmonary disease. These differences are considered a finding by chance, since prior history is not considered when making the decision for thrombolysis in the acute phase in the emergency room. These differences in baseline characteristics did not influence the functional outcome (Appendix 4).

For the observed beneficial effect of ceftriaxone in IVT patients with acute stroke, several possible explanations are available.

First, we realize the effect could have been due to chance. Post-hoc observations should be interpreted with care irrespective of their statistical significance, and the current study-design therefore remains the most important limitation of the study, as will be discussed later.[14] However, there are several reasons to believe the observed effect could be real.

Despite the finding that, as compared with the total population in PASS (n = 2,550), the absolute risk reduction of infection when comparing 'preventive antimicrobial therapy' with 'no antimicrobial therapy', is slightly higher within the IVT-treated subgroup (n = 836), it seems unlikely that only this would explain the observed treatment effect since differences are small and more subgroup analyses in PASS would likely have shown the treatment effect. Whilst in in the intervention arm in PASS ceftriaxone reduced the proportion of patients with post-stroke infection from 18% to 10%, current reduction ranged from 17% to 8%. This effect is also more pronounced for the incidence of pneumonia, which in PASS was reduced from 7% to 6%, as compared with a reduction from 7% to 4% in IVT patients. As a local inflammatory response within the central nervous system is implicated in secondary growth of the ischemic brain lesion and systemic immune alterations are rapidly induced by ischemic stroke, infections in the immediate post-stroke period are associated with increased morbidity and mortality.[15] However, if only the prevention of infection during admission and the subsequent decreased inflammatory response would explain the observed treatment effect; very likely more subgroup analyses within PASS, for example, the comparison of IVT vs non-IVT patients, would have shown beneficial effects of preventive ceftriaxone.

Another possible explanation for the observed treatment effect is a possible interaction between ceftriaxone and alteplase that might enlarge the effect of both drugs. The observed trend towards an increased rate of post-IVT-sICH in ceftriaxone-treated patients might in this case be an indication. As for pharmacokinetics, a dose-dependent effect of alteplase on stroke outcome has been shown in different studies with significant higher rates of ICH rate with doses above 0.95 mg/kg, which has led to a licensed dose for administration of 0.9 mg/kg body weight.[16] The drug is predominantly metabolized by the liver and is eliminated via urine[16] , as is ceftriaxone.[17] However, to date no data comparing both pharmacokinetic profiles has been published. As for pharmacodynamics, in polymerized ampicillin, another beta lactam antibiotic, a dose-dependent inhibition of the fibrinolytic activity of plasminogen has been shown in one in vitro study.[18] Ceftriaxone might also interact directly with coagulation: the antibiotic showed weak compatibility with platelets, as revealed by enhanced platelet aggregation and thromboxane A_2 production in an in vitro study; prostacyclin production and t-PA release were inhibited.[19]

Ceftriaxone also has neuroprotective properties that might explain a beneficial effect on stroke outcome.[20] However, this would not explain why the beneficial effect is limited to IVT-treated patients; except when ceftriaxone and alteplase again show an interaction. In vitro and rodent studies have shown that the beta-lactam antibiotic appears to increase the glutamate transporter activity (glutamate transporter 1, GLT-1) that is normally reduced following stroke, leading to the release of (neurotoxic) glutamate into the extracellular space and eventually neuronal loss by excitotoxicity.[21-24] Alteplase itself can also influence the glutamate transporter activity. A rodent model for stroke showed that t-PA amplifies the downregulation of the glutamate transporter, independent of cerebral ischemia.[25] Another hypothesis for neuroprotection could be through matrix metalloproteinases (MMPs).[26-28] For the tetracycline antibiotic minocycline, a reduction in MMPs has been shown, both in vitro and in vivo, with a decrease in infarct size and a 2-fold decrease in post-IVT-sICH.[26-30] To date, however, little is known about possible interactions between ceftriaxone and MMPs since current experiments are mainly on rodent models of meningitis experimenting the effect of other MMP inhibitors (e.g. Ro 32-3555 or GM6001) on neuronal loss or learning abilities with ceftriaxone only as comedication.[31-37] Since studies on minocycline show a 2-fold decrease-, whereas ceftriaxone in current analysis showed a 3-fold increase in post-IVT-sICH rate, an identical effect of ceftriaxone on MMPs seems unlikely.

As mentioned earlier, the most important limitation of this study is the fact that current findings are based upon an unplanned post-hoc analysis. The observed treatment effect might, therefore, be purely based on chance[38] : with increasing numbers of subgroup analyses with a p value of 0.05, the probability increases that one or more findings by chance arise that may consequently be misinterpreted as clinically directive (false positive result).[39] However, despite the consideration that post-hoc analysis may lead to overstated and misleading results[40] , current observed treatment effect remains present after adjustment for multiple possible confounders and might therefore be genuinely caused by a treatment effect.

Since our findings are based on a post-hoc analysis, further research needs to be done before implying preventive ceftriaxone after thrombolysis in everyday practice. In the recently published 'Antibiotics to Prevent Infections in Stroke (STROKE-INF)' study, an open-label cluster-randomised controlled trial investigating the effect of preventive antibiotics (as chosen by the intervention center) in 1,217 patients with dysphagia after stroke, no interaction was observed between preventive antibiotics and IVT for functional outcome at 90 days (aOR 0.60; 95%CI 0.33–1.09; $p = 0.097$).[41] A pooled-analysis is planned combining both studies, with particular emphasis on predefinded subgroups, among which are IVT-treated patients. Simultaneously, more preclinical trials are required to gain more insight in the pharmacokinetic and –dynamic effects of ceftriaxone on alteplase.

Summary

This post hoc analysis of PASS suggests that preventive ceftriaxone may improve the functional outcome in IVT-treated patients with acute stroke, despite a trend towards an increased rate of post-IVT-sICH.

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Contribution of authors

Mr. Jan-Dirk Vermeij performed data analysis and prepared the first draft of the manuscript. Mrs. Willeke F. Westendorp performed data analysis and reviewed the manuscript. Mr. Yvo B. Roos and Mr. Matthijs C. Brouwer reviewed the manuscript. Prof.Dr. Diederik van de Beek and Dr. Paul J. Nederkoorn were involved in study design, data collection, data analysis; review and in completing the final version of the manuscript.

Declarations of interest

All authors have no conflict of interest.

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Appendix 1.

Participating centers and investigators in the PASS

Study center, city	Principal investigators
Academisch Medisch Centrum, Amsterdam	D. van de Beek, P.J. Nederkoorn, W.F. Westendorp, J-D. Vermeij
Albert Schweitzer Ziekenhuis, Dordrecht	H. Kerkhoff, E. Zock, R.P. Kleyweg
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Amstelland Ziekenhuis, Amstelveen	-
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Medisch Centrum Haaglanden, Den Haag	K. Jellema
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Universitair Medisch Centrum Utrecht, Utrecht	L.J. Kappelle, H.B. van der Worp
VU Medisch Centrum, Amsterdam	M.C. Visser
Westfriesgasthuis, Hoorn	T.C. van der Ree
Zaans Medisch Centrum, Zaandam	R.M. van den Berg-Vos
Ziekenhuis Rijnstate, Arnhem	S.E. Vermeer
Ziekenhuisgroep Twente, Almelo	L.J.A. Reichman

Appendix 2.

Baseline characteristics of thrombolysed patients in the PASS (IVT-group) as compared with non-thrombolysed patients (Non-IVT-group)* **

Characteristic	IVT-group (n = 836)	Non-IVT-group (n = 1,433)
Age – year	72 (61-80)	74 (63-81)
Male sex	486 (58)	821 (57)
History		
Atrial fibrillation/flutter	88/853 (11)	259/1430 (18)
Stroke	290/836 (35)	466/431 (33)
Hypercholesterolemia	222/829 (27)	386/1421 (27)
Hypertension	427/835 (51)	818/1430 (57)
Myocardial infarction	126/836 (15)	176/1431 (12)
Cardiac valve disease †	38/835 (5)	122/1431 (9)
Peripheral vascular disease	57/835 (7)	117/1308 (8)
Obstructive pulmonary disease	49/835 (6)	137/1429 (10)
Immunocompromise ‡	35/836 (4)	42/1433 (3)
Current smoker	200/828 (24)	371/1417 (26)
Prior medication		
Anticoagulants	24/836 (3)	213/1432 (15)
Antiplatelet	395/836 (47)	539/1431 (38)
Statin	310/835 (37)	552/1432 (39)
ACE-inhibitor	197/835 (24)	385/1429 (27)
Beta-blocker	284/834 (34)	525/1431 (37)
Protonpompinhibitor	209/834 (25)	388/1429 (27)
Modified Rankin Scale score §		
Median	0	0
Range	0-5	0-4
National Institutes of Health Stroke Scale score ¶		
Median	6	4
Range	0-29	0-30
Dysphagia	210/779 (27)	309/1357 (23)
Discharge diagnosis		
Cerebral infarction	806 (96)	1319 (92)
Transient ischemic attack	10 (1)	83 (6)
Other	20 (2)	31 (2)

* Data are n/N (%) or median (IQR). NA = not applicable.

** Non-IVT-group is considered the population in PASS that could potentially have been treated with IVT, given their diagnosis: 'infarct', 'TIA' or 'other'.

† Cardiac valve disease was defined as cardiac valve insufficiency, stenosis, or replacement.

‡ Immunocompromised was defined as changed immune status, diabetes mellitus, alcoholism, malignancy, or immunosuppressive medication.

§ Scores on the modified Rankin Scale range from 0 to 6, with 6 indicating death

¶ Scores on the National Institutes of Health Stroke Scale range from 0 to 30, with 30 indicating highest degree of stroke severity

Appendix 3.

Baseline characteristics of ceftriaxone treated patients, comparing thrombolysed patients (Ceftriaxone IVT-group) with non-thrombolysed patients (Ceftriaxone Non-IVT-group)* **

Characteristic	Ceftriaxone IVT-group (n = 437)	Ceftriaxone Non-IVT-group (n = 688)
Age – year	72 (61-80)	73 (63-81)
Male sex	486 (58)	395 (57)
History		
Atrial fibrillation/flutter	37/436 (9)	123/686 (18)
Stroke	150/437 (34)	213/686 (31)
Hypercholesterolemia	117/435 (27)	181/683 (27)
Hypertension	218/436 (50)	389/687 (57)
Myocardial infarction	62/437 (14)	96/686 (14)
Cardiac valve disease †	16/436 (4)	71/686 (10)
Peripheral vascular disease	27/437 (6)	59/682 (9)
Obstructive pulmonary disease	28/437 (6)	76/687 (11)
Immunocompromise ‡	23/437 (5)	25/688 (4)
Current smoker	96/433 (22)	196/681 (29)
Prior medication		
Anticoagulants	12/437 (3)	102/687 (15)
Antiplatelet	209/437 (48)	253/687 (37)
Statin	165/436 (38)	255/687 (37)
ACE-inhibitor	118/436 (27)	197/685 (29)
Beta-blocker	148/436 (34)	242/687 (35)
Protonpompinhibitor	110/436 (25)	183/686 (27)
Modified Rankin Scale score §		
Median	0	0
Range	0-5	0-4
National Institutes of Health Stroke Scale score ¶		
Median	6	4
Range	0-26	0-30
Dysphagia	103/403 (26)	147/647 (23)
Discharge diagnosis		
Cerebral infarction	426/437 (98)	632/688 (92)
Transient ischemic attack	3/437 (1)	41/688 (6)
Other	8/437 (2)	15/688 (2)

* Data are n/N (%) or median (IQR). NA = not applicable.

** Non-IVT-group is considered the population in PASS that could potentially have been treated with IVT, given their diagnosis: 'infarct', 'TIA' or 'other'.

† Cardiac valve disease was defined as cardiac valve insufficiency, stenosis, or replacement.

‡ Immunocompromised was defined as changed immune status, diabetes mellitus, alcoholism, malignancy, or immunosuppressive medication.

§ Scores on the modified Rankin Scale range from 0 to 6, with 6 indicating death

¶ Scores on the National Institutes of Health Stroke Scale range from 0 to 30, with 30 indicating highest degree of stroke severity

Appendix 4.

Outcomes of ceftriaxone treated patients, comparing thrombolysed patients (Ceftriaxone IVT-group) with non-thrombolysed patients (Ceftriaxone Non-IVT-group)* **

	Ceftriaxone IVT-group (n = 437)	Ceftriaxone Non-IVT-group (n = 688)	OR	95%CI
Ordinal regression on modified Ranking Scale				
Not adjusted	NA	NA	0.92	0.75 – 1.14
Predefined adjustment †	NA	NA	0.69	0.55 – 0.86
Adjustment for baseline ††	NA	NA	0.98	0.79 – 1.23
Unfavorable outcome §	144/436 (33)	259/679 (38)	0.80	0.62 – 1.03
Mortality				
Discharge	21/436 (5)	24/688 (4)	1.40	0.77 – 2.55
3 months	39/436 (9)	67/679 (10)	0.90	0.59 – 1.36
Diagnosis of infection during admission				
All infections	34/437 (8)	73/688 (11)	0.70	0.46 – 1.07
Pneumonia	19/437 (4)	36/688 (5)	0.82	0.47 – 1.46
Urinary tract infection	11/437 (3)	31/688 (5)	0.55	0.27 – 1.10
Other infections	7/437 (2)	15/688 (2)	0.73	0.30 – 1.80
Diagnosis of infection based on expert panel				
All infections	13/437 (3)	17/688 (3)	1.21	0.58 – 2.52
Pneumonia	9/437 (2)	9/688 (1)	1.59	0.63 – 4.03
Urinary tract infection	5/437 (1)	7/688 (1)	1.13	0.37 – 3.57
Other infections	1/437 (0)	2/688 (0)	0.79	0.07 – 8.70

* Data are n/N (%) or median (IQR). NA=not applicable.

** Non-IVT-group is considered the population in PASS that could potentially have been treated with IVT, given their diagnosis: 'infarct', 'TIA' or 'other'.

† Predefined adjustment included age, stroke severity as defined as NIHSS score, history of stroke, history of diabetes, previous disability as defined on the mRS, stroke type, and categorisation of study center.

†† Adjusted for differences in baseline characteristics: atrial fibrillation, hypertension, cardiac valve disease, obstructive pulmonary disease and prior use of anticoagulants and antiplatelets.

§ Unfavorable outcome was defined on the mRS a score of 0–2, indicating a favorable outcome, versus a score of 3–6, indicating an unfavorable outcome.

5

Antibiotic therapy for preventing infections in people with acute stroke

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Cochrane Database of Systematic Reviews. 2018;1:CD008530

Abstract

Background

Stroke is the main cause of disability in high-income countries and ranks second as a cause of death worldwide. Infections occur frequently after stroke and may adversely affect outcome. Preventive antibiotic therapy in the acute phase of stroke may reduce the incidence of infections and improve outcome. In the previous version of this Cochrane Review, published in 2012, we found that antibiotics did reduce the risk of infection but did not reduce the number of dependent or deceased patients. However, included studies were small and heterogeneous. In 2015, two large clinical trials were published, warranting an update of this Review.

Objectives

To assess the effectiveness and safety of preventive antibiotic therapy in people with ischaemic or haemorrhagic stroke. We wished to determine whether preventive antibiotic therapy in people with acute stroke:

- reduces the risk of a poor functional outcome (dependency and/or death) at follow-up;
- reduces the occurrence of infections in the acute phase of stroke;
- reduces the occurrence of elevated body temperature (temperature≥38° C) in the acute phase of stroke;
- reduces length of hospital stay; or
- leads to an increased rate of serious adverse events, such as anaphylactic shock, skin rash, or colonisation with antibiotic-resistant micro-organisms.

Search methods

We searched the Cochrane Stroke Group Trials Register (25 June 2017); the Cochrane Central Register of Controlled Trials (CENTRAL; 2017, Issue 5; 25 June 2017) in the Cochrane Library; MEDLINE Ovid (1950 to 11 May 2017), and Embase Ovid (1980 to 11 May 2017). In an effort to identify further published, unpublished, and ongoing trials, we searched trials and research registers, scanned reference lists, and contacted trial authors, colleagues, and researchers in the field.

Selection criteria

Randomised controlled trials (RCTs) of preventive antibiotic therapy versus control (placebo or open control) in people with acute ischaemic or haemorrhagic stroke.

Data collection and analysis

Two review authors independently selected articles and extracted data; we discussed and resolved discrepancies at a consensus meeting with a third review author. We contacted study authors to obtain missing data when required. An independent review author assessed risk of bias using the Cochrane 'Risk of bias' tool. We calculated risk ratios (RRs) for dichotomous outcomes, assessed heterogeneity amongst included studies, and performed subgroup analyses on study quality.

Main results

We included eight studies involving 4488 participants. Regarding quality of evidence, trials showed differences in study population, study design, type of antibiotic, and definition of infection; however, primary outcomes among the included studies were consistent. Mortality rate in the preventive antibiotic group was not significantly different from that in the control group (373/2208 (17%) vs 360/2214 (16%); RR 1.03, 95% confidence interval (CI) 0.87 to 1.21; high-quality evidence). The number of participants with a poor functional outcome (death or dependency) in the preventive antibiotic therapy group was also not significantly different from that in the control group (1158/2168 (53%) vs 1182/2164 (55%); RR 0.99, 95% CI 0.89 to 1.10; moderate-quality evidence). However, preventive antibiotic therapy did significantly reduce the incidence of 'overall' infections in participants with acute stroke from 26% to 19% (408/2161 (19%) vs 558/2156 (26%); RR 0.71, 95% CI 0.58 to 0.88; high-quality evidence). This finding was highly significant for urinary tract infections (81/2131 (4%) vs 204/2126 (10%); RR 0.40, 95% CI 0.32 to 0.51; high-quality evidence), whereas no preventive effect for pneumonia was found (222/2131 (10%) vs 235/2126 (11%); RR 0.95, 95% CI 0.80 to 1.13; high-quality evidence). No major side effects of preventive antibiotic therapy were reported. Only two studies qualitatively assessed the occurrence of elevated body temperature; therefore, these results could not be pooled. Only one study reported length of hospital stay.

Authors' conclusions

Preventive antibiotics had no effect on functional outcome or mortality, but significantly reduced the risk of 'overall' infections. This reduction was driven mainly by prevention of urinary tract infection; no effect for pneumonia was found.

Plain language summary

Antibiotic therapy for preventing infections in people with acute stroke

Review question

Does preventive antibiotic therapy in people with acute stroke reduce the risk of dependency and death at follow-up, and does it reduce the infection rate?

Background

Stroke is the main cause of disability in high-income countries and ranks second as a cause of death worldwide. It is often followed by complications, especially infections, which occur in approximately 30% of people who have had a stroke. The occurrence of an infection may adversely affect clinical outcome after stroke. Preventive antibiotic therapy may reduce the number of infections, thereby improving stroke outcome.

Search date

This review is current to May 2017.

Study characteristics

We included eight studies on preventive antibiotic therapy, with a total of 4488 people with stroke: 2230 participants were randomised to preventive antibiotic therapy, and 2258 to control. The mean age of participants in the preventive antibiotics group was 74.2 years, and in the control group 74.8 years. In both groups, the percentage of men was 52%. Study interventions differed in all eight studies; in two studies, trialists selected the (type of) antibiotic according to local antibiotic policy, with the aim of treating pneumonia.

Key results

Preventive antibiotic treatment did not reduce the risk of dependency or death.
However, preventive antibiotic therapy did significantly reduce the occurrence of 'overall' infections from 26% to 19%. Regarding type of infection, findings were highly significant for urinary tract infections (4% vs 10%) but showed no effect on pneumonia (10% vs 11%). No major side effects of preventive antibiotic therapy were reported.

Quality of evidence

It has become possible to draw first 'overall' conclusions on the net effect of preventive antibiotic therapy in stroke; however, the decision of whether to use preventive antibiotic therapy in acute stroke should be reached with care. Studies were heterogeneous, and despite the large numbers of participants, results from a total of eight studies are limited. In two of these studies, risk of bias was considered to be high for three out of six criteria. Overall, reviewers considered the quality of evidence for the main outcomes of this review – looking at 'any' preventive antibiotic therapy, in 'any' dose, at any length of treatment – as high to moderate.

Summary of findings for the main comparison

Preventive antibiotic therapy compared with placebo and/or conventional management in acute stroke

Patient or population: patients with acute ischemic or haemorrhagic stroke
Setting: acute stroke management
Intervention: preventive antibiotic therapy for systemic use, at any dose or length of treatment
Comparison: placebo and/or conventional acute stroke management

Outcomes	Absolute risk*		Relative effect (95% CI)	No. of participants (studies)	Quality of evidence (GRADE)
	Risk with placebo and/or conventional management	**Risk with preventive antibiotic treatment**			
Case fatality at the end of follow-up	Study population		RR 1.03 (0.87 to 1.21)	4422 (8)	⊕⊕⊕⊕ **high**[a,b]
	163 per 1000	**169 per 1000**			
Poor functional outcome at the end of follow-up	Study population		RR 0.99 (0.89 to 1.10)	4332 (7)	⊕⊕⊕⊕ **moderate**[a,b,c,d,e]
	547 per 1000	**535 per 1000**			
Number of infections at the end of follow-up	Study population		RR 0.71 (0.58 to 0.88)	4317 (7)	⊕⊕⊕⊕ **high**[a,b,f]
	259 per 1000	**189 per 1000**			
Number of UTIs at the end of follow-up	Study population		RR 0.40 (0.32 to 0.51)	4257 (6)	⊕⊕⊕⊕ **high**[a,b]
	96 per 1000	**39 per 1000**			
Number of pneumonias at the end of follow-up	Study population		RR 0.95 (0.80 to 1.10)	5257 (6)	⊕⊕⊕⊕ **high**[a,b]
	111 per 1000	**105 per 1000**			
Occurrence of elevated body temperature	Insufficient data. Assessed qualitatively in only 2 studies				
Rate of serious adverse events	No major side effects of preventive antibiotic therapy were reported				

* The absolute risk is calculated using the absolute number of events in both study arms.
CI: confidence interval; RR: risk ratio; UTIs: urinary tract infections

GRADE Working Group grades of evidence.
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[a] Large number of included studies, large number of participants, and small confidence interval (ultimately low risk of bias). Good applicability in clinical practice.

[b] Limited publication bias cannot be excluded, as funnel plots for primary outcomes were skewed at the base, towards good outcomes.

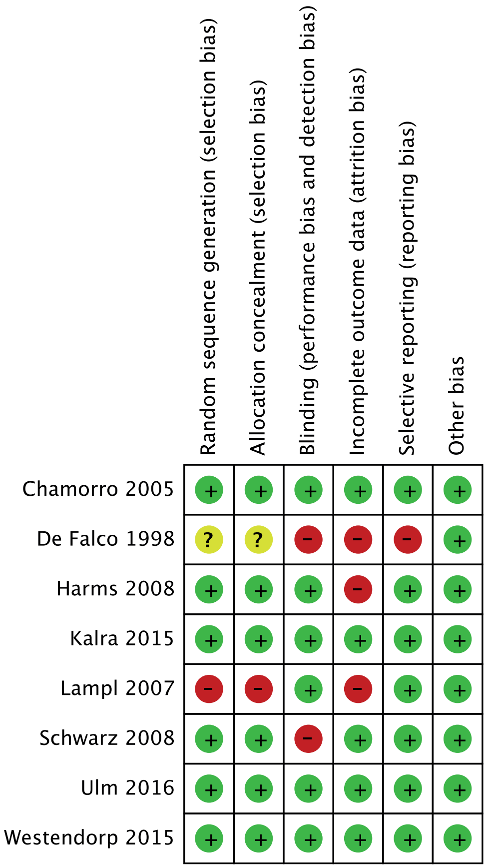
[c] Regarding risk of bias of individual included studies, more than two of the included studies scored at least one criterion of 'unclear' and/or 'high' risk of bias on the Cochrane 'Risk of bias' summary (Figure 1). However, the effect on primary outcomes was consistent among studies with 'low' risk of bias.

[d] Regarding consistency of effect, heterogeneity was substantial (I^2 = 79%). However, overall effect estimates were precise. Stratifying for included studies with 'low risk of bias' resulted in loss of heterogeneity (I^2 = 4%) and did not affect outcomes. Therefore, we did not downgrade the quality of evidence.

[e] Downgraded owing to multiple remarks on GRADE considerations, despite the fact that all remarks can be explained and rectified.

[f] Regarding consistency of effect, heterogeneity was moderate (I^2 = 56%). However, overall effect estimates were precise. Therefore, we did not downgrade the quality of evidence.

Figure 1. Risk of bias summary.



Review authors' judgements about each risk of bias item for each included study, using the Cochrane 'Risk of bias' tool. '+' is defined as low risk of bias, '-' as high risk of bias, '?' as unclear risk of bias.

Background

Stroke is a main cause of disability and death worldwide in both high-income and developing countries. Infection is a common complication in the acute phase after stroke, affecting approximately 30% of these patients; in particular pneumonia and urinary tract infections.[1]

The increased risk of infection can be attributed to different factors. First, infections are associated with a person's clinical condition. Older people with more severe stroke experience infections more frequently. Also, people with swallowing disturbances with subsequent aspiration are at increased risk of pneumonia.[2-5] Second, use of invasive procedures, such as urinary catheterisation or mechanical ventilation, is associated with the occurrence of infections.[6,7] In addition, acute stroke may lead to stroke-induced immunodepression - a systemic anti-inflammatory response that is thought to increase vulnerability to infection amongst people in the acute phase of stroke.[8-10]

The effect of infection on stroke outcome remains uncertain. Several studies showed that infection was associated with poor functional outcome and mortality[11-13], whereas other studies found that infections were merely a marker of stroke severity that had no effect on clinical outcome.[14]

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Different interventions have been proposed to prevent infection after stroke, such as use of a protocol by trained nurses for man-aging people with dysphagia[15], as well as avoidance of urinary catheters. Most of these are only partially effective. In a previous Cochrane meta-analysis, we showed that the incidence of infection could be significantly reduced (from 36% to 22%) by preventive antibiotics in the acute phase after stroke.[16] However, effects on stroke outcome remained unclear because included studies were small and heterogeneous. To establish with certainty whether preventive antibiotic therapy has a place in the treatment of acute stroke, large randomised controlled trials (RCTs) were needed.

Two large clinical trials published in 2015 warranted an update of this review.[17,18] This meta-analysis aims to assess current knowledge on the effect of preventive antibiotic therapy on functional outcome after stroke, incidence of infections, and length of hospital stay, along with the number of adverse events.

Description of the condition

Acute ischaemic or haemorrhagic stroke.

Description of the intervention

Oral or parenteral preventive antibiotic treatment, of any duration, started after onset of stroke symptoms in people without infection at presentation.

How the intervention might work

Infections occurring within seven days after stroke are thought to be associated with poor outcome, independent of stroke severity and other prognostic factors.[11-13,16] Preventive antibiotic therapy may prevent infections in people with acute stroke, resulting in a better functional outcome and lower case fatality rates.

Preventive antibiotic treatment may also produce adverse effects such as anaphylactic shock, skin rash, gastrointestinal complications, neurotoxicity (epileptic seizures), ototoxicity (damage to the ear by a toxin), and nephrotoxicity (the poisonous effect of some substances on the kidneys). Besides, antibiotic treatment may lead to colonisation with antibiotic-resistant micro-organisms. As a consequence, patients may develop infections that are difficult to treat.

Why it is important to do this review

A previous meta-analysis showed that preventive antibiotic use resulted in a significant reduction in infections.[16] Several studies have suggested an association between the occurrence of an infection after stroke and poor outcome. Therefore, preventive use of antibiotic therapy could potentially improve stroke outcome.

Objectives

To assess the effectiveness and safety of preventive antibiotic therapy for people with ischaemic or haemorrhagic stroke. We wished to determine whether preventive antibiotic therapy for people with acute stroke:

- reduces the risk of a poor functional outcome (dependency and/or death) at follow-up;
- reduces the occurrence of infections in the acute phase of stroke;
- reduces the occurrence of elevated body temperature (temperature ≥ 38°C) in the acute phase of stroke;
- reduces length of hospital stay; or
- leads to an increased rate of serious adverse events, such as anaphylactic shock, skin rash, or colonisation with antibiotic-resistant micro-organisms.

Methods

Criteria for considering studies for this review

Types of studies

We searched for all randomised controlled trials (RCTs) of preventive antibiotic therapy versus control (placebo or open control).

Types of participants

People with acute ischaemic or haemorrhagic stroke, aged 18 years or older. We included trials that did not differentiate between ischaemic and haemorrhagic stroke by computed tomography (CT) or magnetic resonance imaging (MRI) before inclusion in the trial, on the basis that 75% to 90% of strokes are ischaemic and occur in predominantly white populations.

Types of interventions

Preventive antibiotic therapy for systemic use (oral, intramuscular, or intravenous administration) at any dose or length of treatment, starting after stroke onset, versus placebo or open control.

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Types of outcome measures

At least, investigators had to record the incidence of infection or mortality for studies to be included.

Primary outcomes

Functional status of the patient. Since the aim of antibiotic treatment should be to prevent disability, we assessed two outcome parameters, both assessing dependency in activities of daily living, scored at the end of follow-up (either end of treatment, or one to three months after the initial event). These were preferably measured with the modified Rankin Scale (mRS), or otherwise the Barthel Index (BI). Our main outcome parameters were:

- case fatality (mRS 6); and
- 'poor functional outcome' (death or dependency; preferably mRS≥3)

Secondary outcomes

- occurrence of 'any infection' in the acute phase of stroke (throughout the manuscript called 'overall' infections to make a distinction with (sub)types of infection)
- occurrence of urinary tract infections in the acute phase of stroke
- occurrence of pneumonia in the acute phase of stroke
- occurrence of elevated body temperature (temperature ≥ 38°C) in the acute phase of stroke
- length of hospital stay
- occurrence of adverse events likely to be related to antibiotic therapy

Search methods for identification of studies

See the 'Specialized Register' section in the Cochrane Stroke Group module.[19] We searched for trials published in all languages and arranged translation of trial reports when required.

Electronic searches

We searched the Trials Register of the Cochrane Stroke Group and the Cochrane Central Register of Controlled Trials (CENTRAL; 2017, Issue 5) in the Cochrane Library (last searched 25 June 2017) (Appendix 1); MEDLINE Ovid (1950 to 11 May 2017) (Appendix 2); and Embase Ovid (1980 to 11 May 2017) (Appendix3).

We developed the MEDLINE Ovid and Embase Ovid search strategies with the help of the Cochrane Stroke Group's Information Specialist and adapted them for use with the other databases. In an effort to identify additional published, unpublished, and ongoing trials, we searched the following trials and research registers (24 August 2017) (Appendix 4). We conducted broad searches to ensure that no trials were missed.

- ClinicalTrials.gov (www.clinicaltrials.gov)
- ISRCTN Registry (www.isrctn.com)
- Stroke Trials Registry (www.strokecenter.org/trials)
- World Health Organization (WHO) Registry Platform (apps.who.int/trialsearch)

Searching other resources

We scanned the reference lists of relevant articles and contacted trial authors, colleagues, and researchers in the field.

Data collection and analysis

Selection of studies

Two review authors (JDV, WW) independently screened the titles and abstracts of studies identified through database searches, and excluded obviously irrelevant articles. We obtained the full text of remaining articles and independently selected studies meeting the inclusion criteria for this review. We resolved disagreements by discussion and by consultation with a third review author (PJN), if necessary.

Data extraction and management

Two review authors (JDV, WW) independently extracted and recorded trial data on specially designed forms, and subsequently cross-checked the data. We discussed and resolved discrepancies at a consensus meeting with a third review author (PJN). We collected the following data from identified studies: study design, inclusion and exclusion criteria, participant characteristics, intervention characteristics, and outcome and complication measures. Participant characteristics included age, sex, stroke type, stroke severity, and the number of dysphagic patients. Intervention characteristics included type, dosage, and duration of the intervention; co-treatment with antipyretic medication; time from symptom onset to intervention (with intended dichotomisation at 24 hours from onset); and the number of participants with incomplete treatment. Outcome measures included body temperature in the acute phase of stroke, occurrence of infections, types of infection, elapsed time from start of treatment to occurrence of infection, data on functional outcome, length of hospital stay, and death. Complication measures consisted of complications and adverse events noted during follow-up, including incidence of colonisation with antibiotic-resistant micro-organisms.

Assessment of risk of bias in included studies

One review author (DWJD) assessed the risk of bias for each study, except for 'Westendorp 2015'[18] owing to co-authorship, which was assessed by an external observer (BvdV). For each study, we evaluated methodological quality using the Cochrane 'Risk of bias' tool. We rated each criterion as having 'low' risk of bias, 'high' risk of bias, or 'unclear' risk of bias, indicating lack of information or uncertainty over the potential for bias.[20] We assessed:

- adequacy of sequence generation;
- allocation concealment;
- blinding of participants and personnel;
- blinding of outcome assessment;
- incomplete outcome data;
- selective reporting; and
- other sources of bias.

Measures of treatment effect

For dichotomous outcomes, we calculated a weighted estimate of treatment effects across trials using the risk ratio (RR). When using continuous scales of measurement to assess effects of treatment, we used the mean difference (MD).

Unit of analysis issues

We calculated a weighted estimate of the typical treatment effect across trials (RR) by using a fixed-effect model and Review Man-ager 5.3.[21] However, in case of heterogeneity of treatment effects, we used the random-effects model to assess the overall treatment effect. We did not expect to find any trials with a cross-over design. For cluster-randomised trials, we considered effect estimates (RR) with adjustment for a cluster effect.

Dealing with missing data

In case of missing data, for example when mRS or BI scores were not available, we contacted the corresponding publication author to request follow-up data that were as complete as possible for all randomised participants.

Assessment of heterogeneity

We used tests for heterogeneity between trial results along with the Cochrane Q statistic and the I^2 statistic (percentage of total variation across studies due to heterogeneity). We considered values exceeding 50% as representing substantial heterogeneity. We also assessed heterogeneity qualitatively by comparing the population and design of each study for the primary outcomes using a sensitivity analysis.

Assessment of reporting biases

We used funnel plots to assess reporting bias and assessed funnel plots qualitatively.

Data synthesis

GRADE and 'Summary of findings' table

We created a 'Summary of findings for the main comparison' using the following outcomes.

- Primary outcome measure: case fatality at the end of follow-up.
- Primary outcome measure: poor functional outcome at the end of follow-up.
- Secondary outcome measure: number of ('overall') infections at the end of follow-up.
- Secondary outcome measure: number of urinary tract infections at the end of follow-up.
- Secondary outcome measure: number of pneumonias at the end of follow-up.
- Secondary outcome measure: occurrence of elevated body temperature.
- Secondary outcome measure: number of serious adverse events.

We assessed the overall quality of evidence for main outcomes (the two primary outcomes and the secondary outcome 'any infection', which can be considered a mediator) by taking into account the five GRADE considerations: risk of bias, consistency of effect, imprecision, indirectness, and publication bias.[20] We also applied GRADE criteria for the 'Summary of findings for the main comparison'.

Subgroup analysis and investigation of heterogeneity

We aimed to conduct the following subgroup analyses.

- Stroke severity.
- Early (within 24 hours) versus late (after 24 hours) start of treatment after stroke.

Sensitivity analysis

When we found substantial heterogeneity on efficacy analysis, we explored heterogeneity by stratifying for risk of bias and including only studies with 'low' risk of bias. We therefore used the Cochrane 'Risk of bias' tool and assessed the risk of bias of each individual study. We considered studies at 'moderate' risk of bias if we found more than two items to be at 'high' risk or 'unclear' risk. We considered studies at 'low' risk of bias if we scored fewer than two items as having 'high' risk or 'unclear' risk in the 'Risk of bias' summary.

Regarding primary outcomes, we also performed a sensitivity analysis on study design: placebo and/or double-blinded versus open-label trial.

Results

Description of studies

Results of the search

Through electronic searching, we found 830 MEDLINE Ovid and 3792 Embase Ovid abstracts. We deliberately used broad search criteria for trials and research registers and found one extra study in CENTRAL. We identified three additional studies as initially applicable by searching other trials and research registers. We assessed 15 full-text articles for eligibility, of which we excluded eight before qualitative synthesis (one appeared to be a double hit, two were review articles, and five articles appeared to be unusable after we considered the paper in greater detail: one was a conference abstract of a long-term follow-up of an earlier study that had not been published, one was a conference abstract on ventilator-associated pneumonia, one included only people with indwelling catheters, and two did not apply the study design needed to be eligible). In addition to the remaining seven studies, we identified one extra trial that had just been completed. We received permission to include the data in this meta-analysis. After qualitative synthesis, we included all eight studies in the meta-analysis. We have presented a PRISMA flow chart of study selection in Figure 2.

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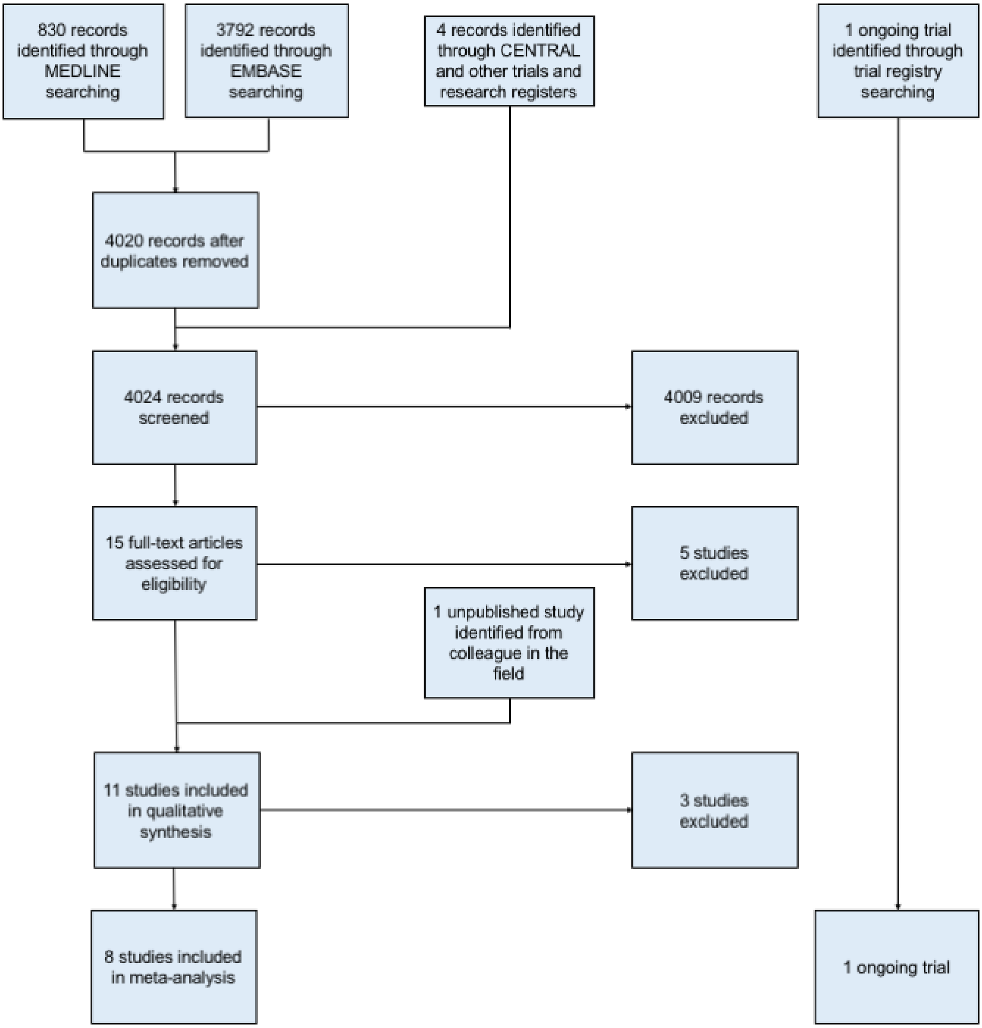
Figure 2. Study flow diagram.



Included studies

We found eight studies eligible for inclusion in the meta-analysis (Chamorro 2005 [22]; De Falco 1998 [23]; Harms 2008 [24]; Kalra 2015 [17]; Lampl 2007 [25]; Schwarz 2008 [26]; Ulm 2016 [27]; Westendorp 2015 [18]). These studies included 4488 participants in total: 2230 participants were randomised to preventive antibiotic therapy, and 2258 were randomised to control groups. In the control groups, 186 participants were randomised to placebo[22,24,25], and 2072 participants to conventional management.[17,18,23,26,27] Sample size calculation was performed in six studies[17,18,22,24,25,27] : One of these studies was terminated prematurely after the first 130 participants were analysed, because no effect was expected[22], and one study was terminated earlier than the initial sample size after an amendment was made to the primary outcome (from dichotomous to ordinal regression), mainly owing to insufficient funding.[18] All studies included adult participants; one study included participants of all ages.[23] Three studies included people with ischaemic and haemorrhagic stroke[17,18,22] ; all other studies only included people with ischaemic stroke.

The mean age in the preventive antibiotics group was 74.2 years, and in the control group 74.8 years. In both treatment groups, the percentage of men was 52%. Seven studies reported baseline median stroke severity scores on the National Institutes of Health Stroke Scale (NIHSS)[17,18,22,24-27] ; these scores ranged from 5 to 17 in the preventive antibiotics group, and from 5 to 15.5 in the control group. One study reported stroke severity on the Canadian Neurological Scale (CNS), with median CNS score of 4.5 (standard deviation (SD) 2.3) in the preventive antibiotics group versus 4.1 (SD 2.1) in the control group.[23] One study excluded people with swallowing difficulties at presentation[25], whereas another study used swallowing difficulties as an inclusion criterion.[17] One other study reported the number of people with dysphagia.[18]

Study intervention differed in all eight studies: fluoroquinolones in two studies - levofloxacin in the study by Chamorro *et al.*.[22] and moxifloxacin by Harms *et al.*[24]; a tetracycline (minocycline) by Lampl *et al.*[25]; a bèta-lactam antibiotic by Westendorp *et al.*[18]; a combination of a bèta-lactam antibiotic and a bèta-lactamase inhibitor by Schwarz *et al.*[26]; and penicillinin by De Falco *et al.*[23] In two studies, the choice of the (type of) antibiotic was made according to the local antibiotic policy of the participating centre (Kalra *et al.*[17]; Ulm *et al.*[27]). The aim of both of these studies was to treat (stroke-associated) pneumonia. Route of administration was intravenous in four studies[18,22,24,26] ; oral in one[25]; and intramuscular in one.[23] Investigators in two studies chose the antibiotic and route of administration according to local policy.[17,27] Amongst studies with predescribed antibiotics, one study did not report dosage.[23] Six studies required that participants had to be included within 24 hours of stroke onset[18,22-26] ; and two studies required enrolment within 48 hours.[17,27] The duration of (predescribed) antibiotic therapy ranged from three to five days, and one study did not report this information.[23] Two studies described mean elapsed time from start of symptoms to intervention for both treatment groups. In these

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Description of studies

Name – year Ref.	Methods	Participants
Chamorro 2005 [22]	Randomised, double-blind	People older than 18 years with non-septic ischaemic or haemorrhagic stroke enrolled within 24 hours from clinical onset
De Falco 1998 [23]	Randomised, unblinded	People with ischaemic stroke within 12 hours from clinical onset
Harms 2008 [24]	Randomised, double-blind	People older than 17 years with ischaemic stroke in MCA territory and NIHSS≥12within 9 to 36 hours after onset
Kalra 2015 [17]	Cluster-randomised, open-label, blinded endpoint	People aged older than 18 years, confirmed diagnosis of new stroke (ischaemic or haemorrhagic), onset of symptoms within 48 hours at recruitment, unsafe to swallow because of impaired consciousness, failed bedside swallow test, or presence of a nasogastric tube
Lampl 2007 [25]	Quasi-randomised (8th number of identity card), open-label, blinded outcome assessment	People older than 18 years with ischaemic stroke, NIHSS > 5, and onset of stroke 6 to24 hours before start of treatment
Schwarz 2008 [26]	Randomised, unblinded	People aged at least 18 years with ischaemic stroke and onset of symptoms less than 24 hours ago, bedridden (mRS > 3), with estimated premorbid mRS < 2 and stable deficits
Ulm 2016 [27]	Randomised, open-label with blinded outcome assessment	People aged 18 years or older, with severe ischaemic stroke (score > 9 on the NIHSS) and clinical diagnosis of a stroke in the MCA territory
Westendorp 2015 [18]	Randomised, open-label, blinded endpoint	People aged 18 years or older with clinical symptoms of stroke (ischaemic or haemorrhagic), onset of symptoms within less than 24 hours, score ≥ 1 on the NIHSS

Footnotes to table

BI: Barthel Index.

CDT: *Clostridium difficile* toxin.

CNS: Canadian Neurologic Scale.

CRP: C-reactive protein.

EuroQoL: measure of health-related quality of life.

MCA: middle cerebral artery.

mRS: modified Rankin Scale.

MRSA: methicillin-resistant *Staphylococcus aureus.*

NIHSS: National Institutes of Health Stroke Scale.

Interventions	Outcomes
Intravenous levofloxacin 500 mg/100 mL/d, for 3 days	Early infection (within the first 7 days after stroke), case fatality, favourable outcome atday 90 (mRS < 2, NIHSS < 2, BI 95 or 100)
Penicillin intramuscularly	Infectious complications, case fatality, functional outcome (BI, CNS)
Intravenous moxifloxacin 400 mg/d for 5 days	Infection rate within 11 days after stroke onset, bacterial spectrum, moxifloxacin resistance, daily maximum body temperature, CRP, survival and functional outcome (BI) at day 180 after stroke (BI was dichotomized ≥ 60 and < 60)
Antibiotic choice conformed to local antibiotic policy by dose and by route according tolocal guidelines. Amoxicillin or co-amoxiclav, together with clarithromycin for 7 days,was recommended if no restrictions were applied	Poststroke pneumonia determined by a statistician masked to allocation using a criteria-based hierarchical algorithm; pneumonia diagnosed by the local treating physician. NIHSS at 14 days; death at 14 and 90 days; functional outcome at 90 days on mRS; CDT-positive diarrhoea; MRSA colonisation; EuroQoL at 90 days; physician-diagnosed pneumonia at baseline and at 2, 7, 10, and 14 days; length of hospital stay; time to death
Orally minocycline 200 mg/d for 5 days	NIHSS on day 90; NIHSS, mRS, BI, and death on days 7, 30, and 90
Intravenous mezlocillin 2 g and sulbactam 1 g every 8 hours for 4 days (12 infusions in total)	mRS at day 90, infection, daily temperature. Infection was assessed by blinded observer; primary outcome (mRS at day 90) was assessed by telephone interview with unknown blinding procedure
Standard stroke care plus daily ultra-sensitive procalcitonin (PCTus)-guided antibiotic treatment. If PCTus concentration was higher than 0.05 ng/mL, a bacterial infection was considered likely and use of antibiotics was recommended. Type and duration of antibiotic treatment - focusing on stroke-associated pneumonia - were left to the treating physician	mRS at 7 days and 3 months, death at 7 and 30 days, infection rate, total antibiotic use, BI at 3 months
Intravenous ceftriaxone 2 g/d for 4 days	mRS at 3 months, death at discharge and 3 months after randomisation, infection rate, total antimicrobial use, length of hospital stay, volume of poststroke care, and quality-adjusted life-years and costs

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two studies, 141 participants were included in the preventive antibiotics group with a mean time to treatment of 13.3 hours; 146 participants were included in the control group with mean time to treatment of 12.2 hours.[22,25] One study described time to treatment for the total group of participants as 24 hours.[24] Completeness of treatment was described in four studies, with a total of 3970 participants; treatment was incomplete in 2.8% (56 out of 1976) of participants in the preventive antibiotic therapy group.[17,18,22,24] Ulm *et al.* provided ultrasensitive pro-calcitonin (PCTus)-guided antibiotic treatment and reported 65% adherence to PCT guidance.[27] Three studies did not provide information on completeness of treatment.[23,25,26] No studies provided data on co-treatment with antipyretic medication.

Case fatality was reported as a primary outcome in one study[23]; seven studies reported case fatality as a secondary out-come.[17,18,22,24-27] All eight studies presented data on functional outcome; however, outcome scales and duration of follow-up varied. Investigators used three different scales: mRS in six studies[17,18,22,25-27] ; BI in five studies[22-25,27] ; and CNS in one study.[23] Three studies did not report the number of dependent participants.[22,23,25] Attempts to collect additional information from the study authors failed in one case and succeeded in two.[22,25] Dependency was defined as BI <60[24] or mRS score ≥3.[17,18,22,26,27] Included studies assessed case fatality and functional outcomes during different follow-up periods; one study reported case fatality during hospital stay[23]; six studies reported both case fatality and functional outcomes at three months[17,18,22,25-27] ; and one study at six months.[24]

Infection rate was reported as a primary outcome in two studies[22,24] , with varying duration of follow-up (seven and 11 days). In five studies, infection rate was presented as a secondary outcome: two studies reported infection rate during hospital stay[18,23] , one study during seven days[27], one study during an observation period of 10 days[26], and one study during 90 days.[17] One study did not report infection rate.[25] Type of infection was specified in five of the seven studies that reported infection rate[17,18,24,26,27] , one study reported only the pneumonia rate.[23] One study did not specify the type of infection.[22] Definitions used for the diagnosis of infection differed substantially between studies; we have provided these in Appendix 5.

Two studies reported the occurrence of elevated body temperature.[24,26] One study reported data on length of hospital stay[17], none on the incidence of opportunistic infections in the two treatment groups. Five stud-ies reported the incidence of adverse events.[17,18,24,26,27] Three studies reported data on the occurrence of colonisation with antibiotic-resistant micro-organisms on day 11 after stroke.[17,18,24]

We identified one ongoing trial (ISRCTN82217627). This study aims to assess functional outcome and infection rate amongst participants allocated in a 2 × 2 × 2 factorial design to any combination of metoclopramide (10 mg three times daily), intravenous ceftriaxone (2000 mg once daily), or paracetamol (1000 mg fourtimes daily), or to usual care.

Excluded studies

We excluded no studies after qualitative synthesis of manuscript data.

Risk of bias in included studies

Figure 1 shows a summary of the risk of bias in all included studies. A 'Risk of bias' table for each study is provided below.

Allocation

A randomised sequence generation was a requirement for inclusion in the meta-analysis and therefore was present in all studies. However, one study randomised by using the eighth number on the participant's identity card. This can be considered a random number but might also be considered inferior, because treatment allocation was not concealed.[25] One study did not specify allocation concealment.[23] The other six studies sufficiently described allocation concealment.

Blinding

Two studies used a double-blind design[22,24] ; the other six studies used an open-label design. In three of these open-label studies, outcomes were assessed blindly by trial office researchers masked to allocation.[17,18,27] In one study, outcomes were reported to be assessed blindly, although the method of blinding was not specified.[25] One study described blinded assessment of infections but did not describe blinded assessment of secondary outcomes such as mRS.[26] One study did not describe blinding.[23]

Incomplete outcome data

In three studies, incomplete outcome data were completely addressed: One study had no losses to follow-up at all[26] , and two studies described losses to follow-up in detail for primary or secondary outcomes, or both.[17,18] Three studies provided incomplete insight on missing data: for one study, this can be explained by the fact that the data are not yet published and secondary outcomes currently are not known[27] ; in another study, researchers assessed the primary outcome for all participants but did not describe the number of participants according to treatment allocation for the secondary outcomes[22] ; in the third study, trial authors reported no further details on the (seven) participants lost to follow-up.[24] Two studies did not describe completeness of follow-up and outcome assessment at all.[23,25]

Selective reporting

Five studies performed an intention-to-treat (ITT) analysis.[17,18,22,25,26] Two studies performed both ITT and per-protocol analyses.[24,27] One study performed a per-protocol analysis alone.[23]

Other potential sources of bias

Data show no other potential sources of bias.

Name – year Ref.	**Random sequence generation (selection bias)**	**Allocation concealment (selection bias)**	
Chamorro 2005 [22]	Low risk. Participants were randomised via a computer-generated number sheet	Low risk. Participants were randomly allocated to 1 of the 2 treatment groups; a pharmacist, nurse, or fellow opened a numbered sealed envelope. Study treatment was prepared at the central pharmacy of the institution and kept within its premises until allocation	
De Falco 1998 [23]	Unclear risk. The study was described as 'randomised'	Unclear risk. Allocation concealment was not mentioned	
Harms 2008 [24]	Low risk. A computer-generated allocation schedule was used	Low risk. Trial pharmacists at each site labelled trial drugs with sequential study numbers according to randomisation lists prepared by the trial statistician and dispensed drugs	
Kalra 2015 [17]	Low risk. Minimisation algorithm, stratifying centres for number of stroke admissions per year and proportion admitted directly to specialist care. Randomisation was computer generated and was done away from the trial office	Low risk. Participants, research staff obtaining data, and statisticians undertaking analyses of outcome data were unaware of stroke unit allocation. Randomisation was computer generated and was done away from the trial office	
Lampl 2007 [25]	High risk. The 8th number on the participant's identity card was used	High risk. As stated via email from the epidemiologist of the trail. The attending physician in the emergency room was blind to the treatment assignment. We do not know for sure wheter blinding was maintained on the ward of the hospital	
Schwarz 2008 [26]	Low risk. Randomisation was performed via a computer-generated number sheet and a numbered, sealed envelope	Low risk. Randomisation was performed via a computer-generated number sheet and a numbered, sealed envelope	
Ulm 2016 [27]	Low risk. Use of random number generator	Low risk. Numbered sealed, opaque envelopes	
Westendorp 2015 [18]	Low risk. Online tool, stratified according to study centre (university hospital, large non-university hospital, or small non-university hospital) and stroke severity (score on NIHSS of 1 to 9 vs score ≥ 10), with permuted blocks of varying block size (with maximum block size of 6)	Low risk. Online tool within 24 hours after symptom onset. Local investigators and participants were not masked, but research nurses who did the follow-up interviews were masked to treatment allocation	

Blinding (performance bias and detection bias) All outcomes	**Incomplete outcome data (attrition bias) All outcomes**	**Selective reporting (reporting bias)**	**Other bias**
Low risk. Double-blind design, placebo controlled. Outcome assessment (e.g. occurrence of infections) was assessed blindly because physicians were not aware of treatment allocation	Low risk. Table 2 indicates that all participants were seen at 90 days. Counts of participants with secondary outcomes by treatment were not provided	Low risk. All outcomes were reported (infections, case fatality, unfavourable functional outcome)	Low risk. No other sources of bias were found
High risk. This study appears to have an open-label design; blinding of outcome assessment was not described	High risk. Nothing was reported about completeness of follow-up and outcome assessment	High risk. Outcome assessment was performed at discharge instead of at a fixed time point	Low risk. No other sources of bias were found
Low risk. Study investigators and enrolling staff were masked to assignments	High risk. 7 participants were lost to follow-up; no details were mentioned	Low risk. All outcomes (infection, neurological outcome, adverse events, and case fatality) were reported at prespecified intervals	Low risk. No other sources of bias were found
Low risk. Participants, research staff obtaining data, and statisticians undertaking analyses of outcome data were unaware of stroke unit allocation. Detection bias for the primary outcome between groups was minimised by a criteria-based algorithm for diagnosis of poststroke pneumonia, applied blind to the whole dataset. Outcomes at 90 days were assessed by trial office researchers masked to allocation	Low risk. No participants were lost to follow-up at the primary endpoint; 2 participants in the antibiotic group withdrew consent, and 16 (3%) in the control group and 18 (3%) in the antibiotic group could not be contacted for the 90-day follow-up	Low risk. All outcomes (poststroke pneumonia, neurological outcome, functional outcome, mortality, CDT-positive diarrhoea, MRSA colonisation, EuroQoL scores, hospital stay) were reported at prespecified intervals	Low risk. No other sources of bias were found
Low risk. Blinded study; outcomes were assessed blindly (although the adequacy of blind was not described)	High risk. The number of participants lost to follow-up was not reported. Scores on NIHSS, BI, and mRS were presented as means	Low risk. All outcomes were reported	Low risk. No other sources of bias were found
High risk. This was an open-label design. Assessment of infections during the study period was done by a blinded observer, but assessment of secondary outcomes, such as NIHSS and mRS, was not done in a blinded fashion	Low risk. Data show no losses to follow-up	Low risk. Data show no losses to follow-up in this study, not even at 90 days	Low risk. No other sources of bias were found
Low risk. This was an open-label study. Outcome assessment of mRS at 3 months was adequately blinded: centrally assessed by structured telephone interviews conducted by trained staff members masked to allocation	Low risk. Primary outcome was centrally assessed by structured telephone interviews conducted by staff members at the study centre. All secondary outcomes were reported, except for length of stay and use of antibiotics, owing to methodological restrictions	Low risk. Figure 2 showing patient flow through the study was missing from the manuscript; cannot be sure if all participants were accounted for at follow-up	Low risk. No other sources of bias were found
Low risk. Local investigators and participants were not masked, but research nurses who did the follow-up interviews were masked to treatment allocation. Trained and masked research nurses based at the Academic Medical Center assessed functional outcomes at 3 months using a validated structured telephone interview	Low risk. 24 participants were lost to follow-up (11 in the ceftriaxone group and 13 in the control group); details are mentioned in the appendix of the manuscript	Low risk. All outcomes were reported	Low risk. No other sources of bias were found

Effects of interventions

See also the 'Summary of findings for the main comparison'

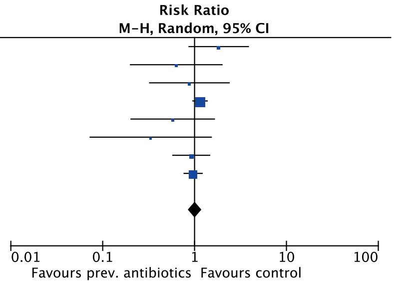
Primary outcomes

The overall number of participants who died was 373 of 2208 (17%) in the preventive antibiotics group versus 360 of 2214 (16%) in the control group (RR 1.03, 95% CI 0.87 to 1.21; high-quality evidence) (Figure 3; Analysis 1.1). Data show no substantial heterogeneity (P = 0.31; I^2 = 15%).

Figure 3. Analysis 1.1. Forest plot of comparison: Case fatality at the end of follow-up.

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
Chamorro 2005	16	67	9	69	4.8%	1.83 [0.87, 3.85]
De Falco 1998	4	38	7	42	2.1%	0.63 [0.20, 1.99]
Harms 2008	6	39	7	40	2.7%	0.88 [0.32, 2.38]
Kalra 2015	184	595	158	586	42.4%	1.15 [0.96, 1.37]
Lampl 2007	5	74	9	77	2.5%	0.58 [0.20, 1.64]
Schwarz 2008	2	30	6	30	1.2%	0.33 [0.07, 1.52]
Ulm 2016	25	97	28	100	11.4%	0.92 [0.58, 1.46]
Westendorp 2015	131	1268	136	1270	32.9%	0.96 [0.77, 1.21]
Total (95% CI)		**2208**		**2214**	**100.0%**	**1.03 [0.87, 1.21]**
Total events	373		360			

Heterogeneity: $Tau^2 = 0.01$; $Chi^2 = 8.28$, df = 7 (P = 0.31); $I^2 = 15\%$
Test for overall effect: Z = 0.30 (P = 0.76)



The number of participants with a poor functional outcome (death or dependency) was 1158 of 2168 (53%) in the preventive antibiotics group versus 1182 of 2164 (55%) in the control group (RR 0.99, 95% CI 0.89 to 1.10; moderate-quality evidence) (Figure 4; Analysis 1.2). Substantial heterogeneity was present (P < 0.0001; I^2 = 79%) and can be noted in the funnel plots (Figure 5). One study was not included in this analysis: De Falco *et al.* did not report the number of dependent participants at the end of follow-up. In this study, mean scores for outcome of both treatment groups were reported and showed a better functional outcome in the preventive antibiotics group, with a mean BI of 38.2 (SD 32.4) in the preventive antibiotics group versus 21.8 (SD 27.6) in the control group.

Figure 4. Analysis 1.2. Forest plot of comparison: Death or dependency at the end of follow-up.

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
Chamorro 2005	46	67	43	69	10.2%	1.10 [0.86, 1.41]
Harms 2008	14	39	18	40	3.1%	0.80 [0.46, 1.37]
Kalra 2015	486	595	465	586	23.0%	1.03 [0.97, 1.09]
Lampl 2007	7	72	36	69	1.8%	0.19 [0.09, 0.39]
Schwarz 2008	30	30	30	30	22.5%	1.00 [0.94, 1.07]
Ulm 2016	88	97	83	100	19.2%	1.09 [0.98, 1.22]
Westendorp 2015	487	1268	507	1270	20.2%	0.96 [0.87, 1.06]
Total (95% CI)		**2168**		**2164**	**100.0%**	**0.99 [0.89, 1.10]**
Total events	1158		1182			

Heterogeneity: $Tau^2 = 0.01$; $Chi^2 = 28.72$, df = 6 (P < 0.0001); $I^2 = 79\%$
Test for overall effect: Z = 0.21 (P = 0.84)

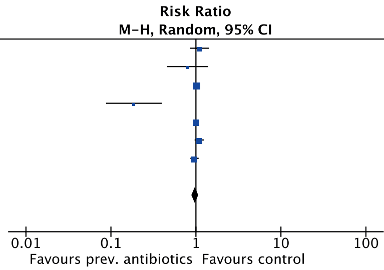
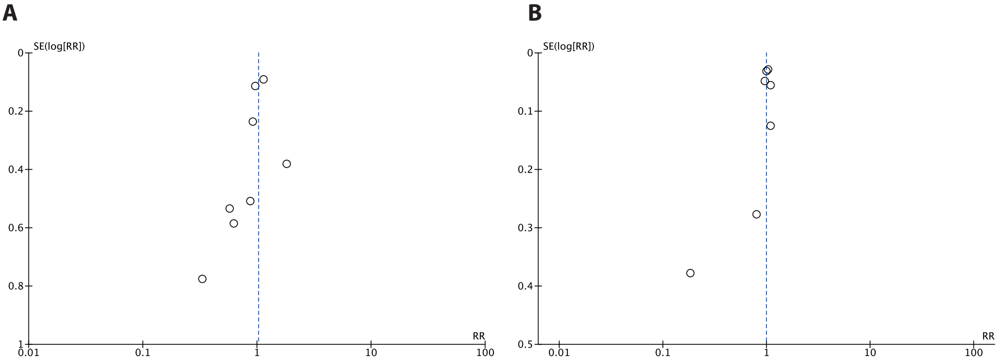


Figure 5. Funnel plots of comparison. (A) Case fatality at the end of follow-up. (B) Death or dependency at the end of follow-up.



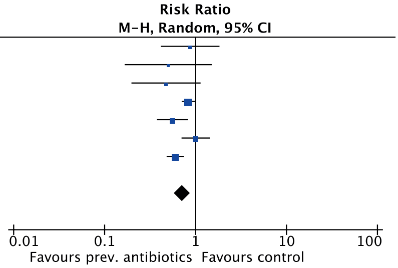
Secondary outcomes

The number of participants with any 'overall' infection at the end of follow-up was significantly reduced in the preventive antibiotics group: 408 of 2161 (19%) compared with 558 of 2156 (26%) in the control group (RR 0.71, 95% CI 0.58 to 0.88; high-quality evidence) (Figure 6; Analysis 2.1). Heterogeneity was substantial (P = 0.03; I^2 = 56%).

Figure 6. Analysis 2.1. Forest plot of comparison: Number of infections at the end of follow-up.

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
Chamorro 2005	11	67	13	69	6.4%	0.87 [0.42, 1.81]
De Falco 1998	4	30	8	30	3.2%	0.50 [0.17, 1.48]
Harms 2008	6	39	13	40	4.9%	0.47 [0.20, 1.12]
Kalra 2015	201	615	237	602	27.8%	0.83 [0.71, 0.97]
Schwarz 2008	15	30	27	30	15.6%	0.56 [0.38, 0.81]
Ulm 2016	41	112	42	115	17.2%	1.00 [0.71, 1.41]
Westendorp 2015	130	1268	218	1270	24.8%	0.60 [0.49, 0.73]
Total (95% CI)		**2161**		**2156**	**100.0%**	**0.71 [0.58, 0.88]**
Total events	408		558			

Heterogeneity: $Tau^2 = 0.03$; $Chi^2 = 13.56$, df = 6 (P = 0.03); $I^2 = 56\%$
Test for overall effect: Z = 3.22 (P = 0.001)



Regarding type of infection, urinary tract infections were highly significantly reduced: 81 of 2131 (4%) participants in the preventive antibiotics group versus 204 of 2126 (10%) in the control group (RR 0.40, 95% CI 0.32 to 0.51; high-quality evidence) (Figure 7; Analysis 2.2) (heterogeneity: P = 0.70; I^2 = 0%), whereas no significant difference for pneumonia was found: 222 of 2131 (10%) in the preventive antibiotics group versus 235 of 2126 (11%) in the control group (RR 0.95, 95% CI 0.80 to 1.13; high-quality evidence) (Figure 8; Analysis 2.3) (heterogeneity: P = 0.45; I^2 = 0%) (see funnel plots in Figure 8).

Figure 7. Analysis 2.2. Forest plot of comparison: Number of UTIs at the end of follow-up.

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Risk Ratio M–H, Random, 95% CI
Chamorro 2005	1	67	4	69	1.3%	0.26 [0.03, 2.24]
Harms 2008	3	39	5	40	3.3%	0.62 [0.16, 2.40]
Kalra 2015	15	615	39	602	17.7%	0.38 [0.21, 0.68]
Schwarz 2008	8	30	18	30	13.8%	0.44 [0.23, 0.86]
Ulm 2016	8	112	11	115	7.9%	0.75 [0.31, 1.79]
Westendorp 2015	46	1268	127	1270	56.1%	0.36 [0.26, 0.50]
Total (95% CI)		**2131**		**2126**	**100.0%**	**0.40 [0.32, 0.51]**
Total events	81		204			

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 3.00$, df = 5 (P = 0.70); $I^2 = 0\%$
Test for overall effect: Z = 7.25 (P < 0.00001)

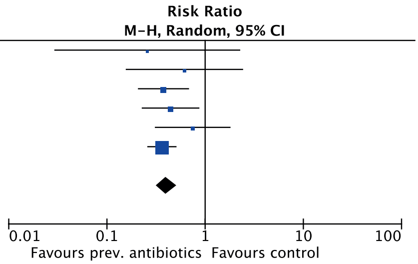
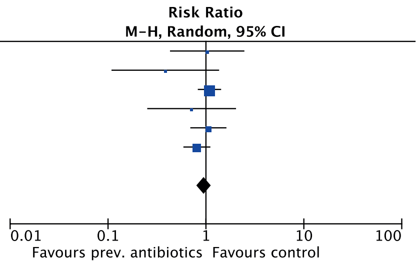


Figure 8. Analysis 2.3. Forest plot of comparison: Number of pneumonias at the end of follow-up.

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Risk Ratio M–H, Random, 95% CI
Chamorro 2005	9	67	9	69	3.9%	1.03 [0.44, 2.44]
Harms 2008	3	39	8	40	1.8%	0.38 [0.11, 1.34]
Kalra 2015	101	615	91	602	42.8%	1.09 [0.84, 1.41]
Schwarz 2008	5	30	7	30	2.7%	0.71 [0.25, 2.00]
Ulm 2016	33	112	32	115	17.2%	1.06 [0.70, 1.60]
Westendorp 2015	71	1268	88	1270	31.6%	0.81 [0.60, 1.09]
Total (95% CI)		**2131**		**2126**	**100.0%**	**0.95 [0.80, 1.13]**
Total events	222		235			

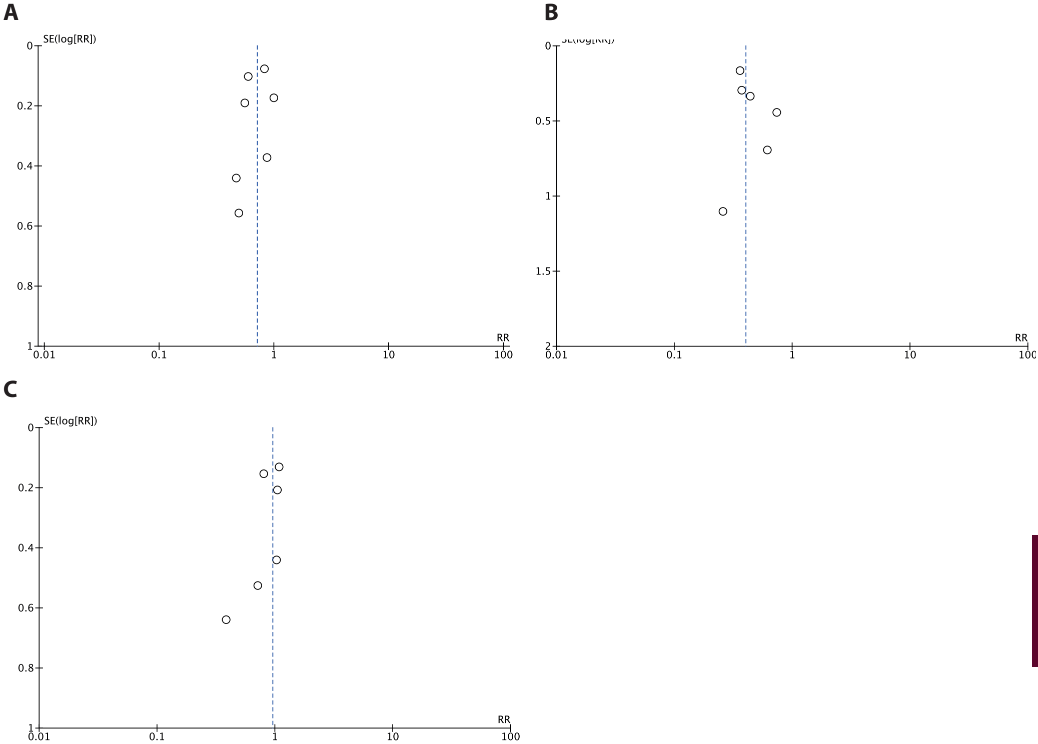
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 4.74$, df = 5 (P = 0.45); $I^2 = 0\%$
Test for overall effect: Z = 0.55 (P = 0.58)



The occurrence of elevated body temperature was reported in two studies.[24,26] Data were assessed qualitatively and could therefore not be pooled. Body temperature did not differ significantly between treatment groups according to one study, whereas temperatures were significantly higher in the conventional treatment group at days one, two, and three in the other study.

Only two studies reported data on length of hospital stay.[17,18] Data differed significantly, with a mean of six days in both treatment arms for Westendorp *et al.* versus 24 days in the antibiotics group and 26 days in the control group for Kalra *et al.*

Figure 9. Funnel plots of comparison. (A) Number of infections at the end of follow-up. (B) Number of UTIs at the end of follow-up. (C) Number of pneumonias at the end of follow-up.



Regarding adverse events, two studies reported none related to study medication.[24,27] In the other three studies reporting the occurrence of adverse events, the most frequently reported event was elevated liver or renal enzymes, or both (161 of 1887 participants; 9%).[17,18,26] Clinical consequences were not reported for any of these participants. It has to be taken into account that a similar rate of adverse events was also reported in 136 of 1872 (7%) of the control participants. Other reported adverse events included drug-induced exanthema in one participant[26], 'allergic reaction causing cessation of ceftriaxone' in seven of 1242 participants (1%)[18] , oliguria or raised plasma creatinine in 101of 1242 participants (8%) (compared with 112 of 1270 (9%) in control participants)[18] , phlebitis in 15 of 1242 participants (1%) (compared with nine of 1270 participants (1%) in the control group)[18] , and infection by a ceftriaxone-resistant micro-organism in six of 1242 participants (1%) (compared with five of 1270 (1%) participants in the control group).[18] The occurrence of colonisation with an antibiotic resistant micro-organism was also reported in another study: one infection with methicillin-resistant Staphylococcus aureus (MRSA); however, colonisation was present before start of study medication.[24]

Subgroup analyses

Data were insufficient for predefined subgroup analyses regarding stroke severity and time of initiation of treatment. Two studies reported effects of stroke severity[17,18] ; however, data do not overlap. Kalra *et al.* assessed the relation between baseline NIHSS and risk of pneumonia and found no relationship. Westendorp *et al.* also found no relationship between baseline NIHSS and functional outcomes. No study reported the time of the start of treatment.

Based on the finding that the number of participants with pneumonia was not significantly different for preventive antibiotics than for current best medical care, we performed a subanalysis of the studies in which any antibiotic could be used with the goal of preventing pneumonia.[17,27] Again, we found no beneficial effect of preventive antibiotics: 131 of 727 (18%) pneumonias in the antibiotics group versus 123 of 717 (17%) in the control group (RR 1.08, 95% CI 0,87 to 1.34; moderate-quality evidence). Also within this subgroup, we found no beneficial effect of preventive antibiotics on mortality: 209 out of 602 (35%) in the antibiotics group versus 186 out of 686 (28%) in the control group (RR 1.11; 95% CI 0.94 to 1.32; moderate-quality evidence). This was also the case for poor functional outcome: 574 of 692 participants (83%) in the preventive antibiotics group versus 548 of 686 participants (80%) in the control group (RR 1.04; 95% CI 0.99 to 1.10; moderate-quality evidence).

Sensitivity analyses

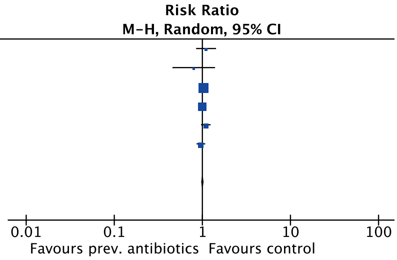
Two above-mentioned analyses showed significant heterogeneity, warranting stratification of the included studies for risk of bias. Using the Cochrane 'Risk of bias' tool, we considered two of the eight included studies to be at 'moderate' risk of bias[23,25]; we considered the other six to be at 'low' risk of bias (Figure 1).

For the primary outcome 'death or dependency' (I^2 = 79%), we excluded only Lampl *et al.* from the initial analysis when stratifying studies with 'low risk of bias', because De Falco *et al.* did not report functional outcomes on the mRS. When stratifying the six studies with 'low' risk of bias, again we noted no differences between both treatment arms: The number of participants with a poor functional outcome was 1151 of 2096 (55%) in the preventive antibiotics group versus 1146 of 2095 (55%) in the control group (RR 1.02, 95% CI 0.98 to 1.06; high-quality evidence) (heterogeneity: P = 0.39; I^2 = 4%) (Figure 9; Analysis 3.1).

Figure 9. Analysis 3.1. Forest plot of comparison: Functional outcome: low risk of bias studies.

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
Chamorro 2005	46	67	43	69	2.3%	1.10 [0.86, 1.41]
Harms 2008	14	39	18	40	0.5%	0.80 [0.46, 1.37]
Kalra 2015	486	595	465	586	39.7%	1.03 [0.97, 1.09]
Schwarz 2008	30	30	30	30	31.6%	1.00 [0.94, 1.07]
Ulm 2016	88	97	83	100	11.5%	1.09 [0.98, 1.22]
Westendorp 2015	487	1268	507	1270	14.4%	0.96 [0.87, 1.06]
Total (95% CI)		**2096**		**2095**	**100.0%**	**1.02 [0.98, 1.06]**
Total events	1151		1146			

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 5.21$, df = 5 (P = 0.39); $I^2 = 4\%$
Test for overall effect: Z = 0.90 (P = 0.37)

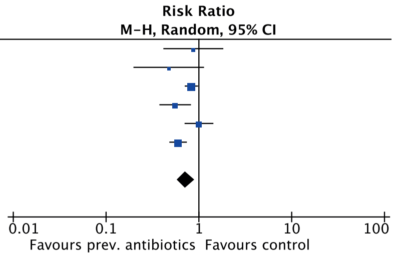


For the secondary outcome ’occurrence of overall infections’ ($I^2 = 56\%$), we excluded only De Falco *et al.* from the initial calculation, because Lampl *et al.* did not report the infection rate. Within the six studies with ’low’ risk of bias, the numbers of participants with any ’overall’ infection remained significantly lower: 404 of 2131 (19%) in the preventive antibiotics group versus 550 of 2126 (26%) in the control group (RR 0.72, 95% CI 0.58 to 0.89; high-quality evidence). Heterogeneity remained present as well (P = 0.02; $I^2 = 62\%$) (Figure 10; Analysis 3.2).

Figure 10. Analysis 3.2. Forest plot of comparison: Number of infections: low risk of bias studies.

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
Chamorro 2005	11	67	13	69	6.9%	0.87 [0.42, 1.81]
Harms 2008	6	39	13	40	5.3%	0.47 [0.20, 1.12]
Kalra 2015	201	615	237	602	28.2%	0.83 [0.71, 0.97]
Schwarz 2008	15	30	27	30	16.3%	0.56 [0.38, 0.81]
Ulm 2016	41	112	42	115	17.9%	1.00 [0.71, 1.41]
Westendorp 2015	130	1268	218	1270	25.4%	0.60 [0.49, 0.73]
Total (95% CI)		**2131**		**2126**	**100.0%**	**0.72 [0.58, 0.89]**
Total events	404		550			

Heterogeneity: $Tau^2 = 0.04$; $Chi^2 = 13.06$, df = 5 (P = 0.02); $I^2 = 62\%$
Test for overall effect: Z = 2.98 (P = 0.003)



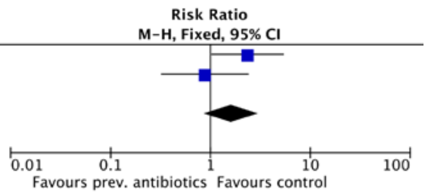
Regarding the predefined sensitivity analysis on study design, the primary outcome ’case fatality’ remained unchanged: subanalysis of the two double-blinded randomised trials showed no significant effect on mortality, with a broad confidence interval: 22 of 106 (21%) participants died in the preventive antibiotics group versus 14 of 109 (13%) in the control group (RR 1.62, 95% CI 0.87 to 3.00; moderate-quality evidence), with substantial heterogeneity (P = 0.13; $I^2 = 55\%$) (Figure 11; Analysis 3.3). Subanalysis of the open-label trials showed a narrower confidence interval, again with no significant differences between treatment arms: 347 of 2064 (17%) participants in the preventive antibiotics group died versus 337of 2063 (16%) in the control group (RR 1.03, 95% CI 0.90 to 1.17; high-quality evidence), with no substantial heterogeneity (P = 0.26; $I^2 = 24\%$) (Figure 10; Analysis 3.4).

Figure 11. Analysis 3.3 and 3.4. Forest plot of comparison: Case fatality: (A) double-blind design and (B) open-label design.

A

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Chamorro 2005	16	67	7	69	49.9%	2.35 [1.03, 5.36]
Harms 2008	6	39	7	40	50.1%	0.88 [0.32, 2.38]
Total (95% CI)		**106**		**109**	**100.0%**	**1.62 [0.87, 3.00]**
Total events	22		14			

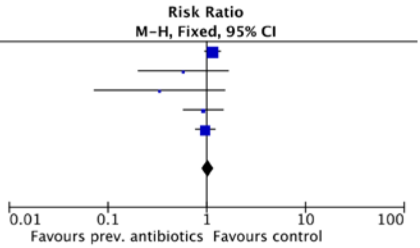
Heterogeneity: Chi² = 2.24, df = 1 (P = 0.13); I² = 55%
Test for overall effect: Z = 1.52 (P = 0.13)



B

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Kalra 2015	184	595	158	586	47.2%	1.15 [0.96, 1.37]
Lampl 2007	5	74	9	77	2.6%	0.58 [0.20, 1.64]
Schwarz 2008	2	30	6	30	1.8%	0.33 [0.07, 1.52]
Ulm 2016	25	97	28	100	8.2%	0.92 [0.58, 1.46]
Westendorp 2015	131	1268	136	1270	40.3%	0.96 [0.77, 1.21]
Total (95% CI)		**2064**		**2063**	**100.0%**	**1.03 [0.90, 1.17]**
Total events	347		337			

Heterogeneity: Chi² = 5.24, df = 4 (P = 0.26); I² = 24%
Test for overall effect: Z = 0.37 (P = 0.71)



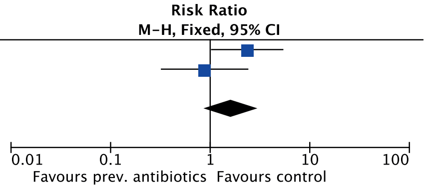
For the predefined sensitivity analysis on study design for the primary outcome 'functional outcome', we also found no significant differences in an analysis of the two double-blinded randomised trials: The number of participants with a poor functional outcome at the end of follow-up was 60 of 106 (57%) in the preventive antibiotics group versus 61 of 109 (56%) in the control group (RR1.02, 95% CI 0.78 to 1.35; moderate-quality evidence), with no substantial heterogeneity ($P = 0.27$; $I^2 = 17\%$) (Figure 12; Analysis 3.5). In the subanalysis of the five open-label trials, 1198 of 2062 (58%) participants in the preventive antibiotics group had a poor functional outcome versus 1121 of 2055 (55%) in the control group (RR 0.98, 95% CI 0.93 to 1.03; high-quality evidence), with substantial heterogeneity ($P < 0.0001$; $I^2 = 86\%$) (Figure 12; Analysis 3.6).

Figure 12. Analysis 3.5 and 3.6. Forest plot of comparison: Functional outcome: (A) double-blind design and (B) open-label design.

A

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Chamorro 2005	16	67	7	69	49.9%	2.35 [1.03, 5.36]
Harms 2008	6	39	7	40	50.1%	0.88 [0.32, 2.38]
Total (95% CI)		**106**		**109**	**100.0%**	**1.62 [0.87, 3.00]**
Total events	22		14			

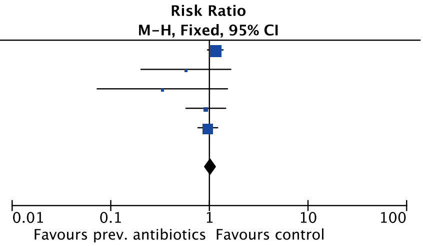
Heterogeneity: $Chi^2 = 2.24$, df = 1 (P = 0.13); $I^2 = 55\%$
Test for overall effect: Z = 1.52 (P = 0.13)



B

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Kalra 2015	184	595	158	586	47.2%	1.15 [0.96, 1.37]
Lampl 2007	5	74	9	77	2.6%	0.58 [0.20, 1.64]
Schwarz 2008	2	30	6	30	1.8%	0.33 [0.07, 1.52]
Ulm 2016	25	97	28	100	8.2%	0.92 [0.58, 1.46]
Westendorp 2015	131	1268	136	1270	40.3%	0.96 [0.77, 1.21]
Total (95% CI)		**2064**		**2063**	**100.0%**	**1.03 [0.90, 1.17]**
Total events	347		337			

Heterogeneity: $Chi^2 = 5.24$, df = 4 (P = 0.26); $I^2 = 24\%$
Test for overall effect: Z = 0.37 (P = 0.71)



We have summarised the main results and have provided an explanation for determination of the quality of evidence in the Summary of findings for the main comparison.

Discussion

Summary of the main results

This meta-analysis shows that preventive antibiotic therapy in people with ischaemic or haemorrhagic stroke did not reduce the risk of dependency and/or death. However, preventive antibiotic therapy did significantly reduce the occurrence of 'overall' infections from 26% to 19%. Regarding type of infection, this was highly significant for urinary tract infections (4% vs 10%), whereas no effect on pneumonia was found (10% vs 11%). No major side effects of preventive antibiotic therapy were reported.

Overall completeness and applicability of evidence

The former version of this Cochrane review included a total of 506 participants from five studies with considerable heterogeneity in study population, study design, type of antibiotic, and definition of infection.[16] Owing to the large confidence intervals, it was not possible to draw conclusions on the effect of 'any' preventive antibiotic on mortality or stroke outcome (mortality: risk ratio (RR) 0.85, 95% confidence interval (CI) 0.47 to 1.51; dependency: RR 0.67, 95% CI 0.32 to 1.43). Besides, study sizes were too small to specify the effect of preventive antibiotics on specific types of infection. It was concluded that the meta-analysis did not allow a very robust conclusion on the use of preventive antibiotic therapy in acute stroke.

With the completion of three large randomised controlled trials (RCTs) since 2012, the increase in study population to 4488 participants has now made it possible to evaluate the effect of preventive antibiotics on functional outcome and mortality. In general, the much larger study population has led to neutralisation of the former heterogeneity between studies. The current review shows that preventive antibiotic therapy did not reduce the risk of dependency or death or both in participants with acute stroke, whereas this therapy did significantly reduce the occurrence of 'overall' infections. The latter can now be specified, showing that this reduction is highly significant for urinary tract infections whereas no effect on pneumonia was found.

As was the case in the previous version of this meta-analysis, available studies have not adequately addressed several issues. Type of antibiotic therapy, dosage and duration varied between all eight studies, making it impossible to draw 'overall' conclusions regarding specific types of antibiotic regimens. Five studies used preventive antibiotic therapy that covered the common causative organisms in poststroke infections. Participants in two studies could use any antibiotic to prevent poststroke pneumonia. One study used minocycline to investigate a possible neuroprotective effect. This preventive antibiotic therapy did not effectively cover the antimicrobial spectrum of poststroke infections, and trial authors did not report infection rates.[25]

Regarding the primary outcomes of this meta-analysis, only data on case fatality was reported in all studies. Dependency was reported as a mean score in one study[23] , whereas the absolute number of dependent patients was necessary for a pooled analysis. This study showed a favourable effect of preventive antibiotic therapy on functional outcome on the Barthel Index (BI) and the Canadian Neurologic Scale (CNS). The pooled analysis does not include data from this study and was based on the seven studies that did report the number of dependent patients, along with mortality rates.

The number of infections was not reported in one study[25] ; therefore, we based the pooled estimate on seven studies. These seven studies used different definitions for the diagnosis of infection. Less strict definitions might lead to an overestimation of the number of infections, which could be a particular problem in studies with an open-label design. In a systematic meta-analysis on the incidence of poststroke infection, researchers found the overall pooled infection rate to be 30%, and the rate of pneumonia and of urinary tract infection to be 10%.[1] These data appear to be in line with the observed overall infection rate in the current control group of 26%, as well as the incidence of urinary tract infection (10%) and of pneumonia (10%).

No conclusions can be drawn on the effects of preventive antibiotic therapy on occurrence of elevated body temperature, length of hospital stay, and occurrence of opportunistic infections owing to lack of data. Limited data was also available on adverse events likely to be related to antibiotic therapy.

To account for heterogeneity in study design (double-blind vs open-label studies), types of antibiotic therapy (adequately covering all pathogens in poststroke infections vs those mostly chosen for neuroprotective properties), and definitions of infection, we chose a random-effects model for the pooled analyses. In a fixed-effect model, it is assumed that differences between studies are due to chance, not to differences in study design. It is likely that results of this meta-analysis varied owing to the obvious heterogeneity between studies - not only owing to chance. We therefore preferred to use a random-effects model.

Quality of evidence

Despite the fact that data have become more robust as compared with the previous version of this meta-analysis, and conclusions can now better be drawn, the decision of whether to use preventive antibiotic therapy in acute stroke should still be made with care owing to various factors. First, study design was heterogeneous, and, although this is better than in the previous version of this meta-analysis, the total of eight studies is limited. However, with a total of 4488 participants, it has now become possible to draw first ’overall’ conclusions on the net effect of preventive antibiotic therapy in stroke. Second, as shown in the ’Risk of bias’ table for each study, only four studies scored an overall ’low’ risk of bias, and several biases may have influenced the results of included studies. Selection bias could have confounded our results. Case fatality rates were low in all included studies, ranging from 9% to 29%, with an average of 17%. Usually, case fatality rates in acute stroke range from 15% to 25%.[28] Three studies excluded people with a life expectancy of less than 90days.[18,26,27] Selection of less severely affected people may lead to an overestimation of the effects of preventive antibiotic therapy, because less effect could be expected in people with a high a priori case fatality risk. On the other hand, severely affected people might benefit the most from preventive antibiotic therapy. Stroke severity has previously been reported as a risk factor for poststroke infection, and the incidence of infection is higher amongst people with more severe stroke.[1,2,29,30]

According to blinding, six studies used an open-label design; in three of these studies, blinding of outcome parameters was incomplete or was not adequately described.[23,25,26] Knowledge of the intervention in a trial can affect the outcomes when provided care differs between treatment groups. Conduct of a study on preventive antibiotic therapy might have increased use of antibiotic therapy in the control group, which could lead to underestimation of a possible effect. Included studies did not specify prescription of antibiotic therapy in the control group; therefore these data could not be compared.

Detection bias might influence results when outcome is not assessed blindly. Two studies used a double-blind design in which all outcomes were assessed blindly.[22,24] Three studies used an open-label design in which outcomes were assessed blindly by researchers masked to allocation.[17,18,27] In one study, infection was assessed blindly, but other outcomes were not[26] ; and two studies did not assess outcomes blindly or did not describe outcome assessment.[23,25] Despite the open-label design and/or the incomplete/unclear method of blinding in six of the

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eight included studies, it is unlikely that one of the primary endpoints in current meta-analysis (case fatality) was influenced because this is a hard endpoint. Less objective end-points, such as score on the modified Rankin Scale (mRS), could have been influenced, but this effect might be overcome by blinding of the endpoint. The most vulnerable outcome measurement, however, is infection rate. Because treating physicians were probably aware of participation in a study on (preventive) antibiotics, (over)alertness about the occurrence of infection in study participants is likely. With an open-label design, this might even be more applicable to the group not given preventive antibiotic treatment. On the other hand, infections might be less easily diagnosed in participants allocated to the experimental group, who are receiving preventive antibiotics. (Over)alertness to infection in the control group, as compared with possible underdiagnosis of infection in the experimental group, might lead to overestimation of a possible effect.

At least the overdiagnosis of infections in the control group was not found in current meta-analysis. The overall incidence of any infection in the acute phase after stroke is approximately 30%[1] ; in current meta-analysis, infections occurred in 19% in the preventive antibiotic group versus 26% in the control group in studies with an open-label design; in comparison with 16% in the preventive antibiotic group versus 24% in the placebo group in studies with a double-blind design.

A recent review described considerable variation in terminology and in the diagnostic approach to pneumonia.[31] Recently, consensus operational criteria for the terminology and diagnosis of stroke-associated pneumonia have been proposed based on criteria of the Centers for Disease Control and Prevention (CDC).[32] For future studies, standardised definitions such as those provided by the CDC are preferable, especially in open-label trials. Four of eight studies applied criteria derived from the CDC, mainly as secondary analyses.[17,18,22,27] The incidence of infection based on these criteria appears to be relatively lower.

Attrition bias can occur when trial participants are withdrawn after randomisation. For example, side effects of medication (nausea, diarrhoea, exanthema, etc) might lead to exclusion of participants owing to their inability to complete the course, introducing bias in favour of one study arm. In this current meta-analysis, only one study reported no loss to follow-up at all.[26] In seven of the eight included studies, attrition bias might have occurred. Kalra *et al.* reported no loss to follow-up for the primary endpoint (assessed after 14 days), but did describe loss to follow-up for the secondary endpoints (assessed after 90 days).[17] Chamorro *et al.* also reported all participants in the primary analysis but did not provide counts of participants for secondary outcomes; loss to follow-up might have occurred.[22] Westendorp *et al.* described in detail the numbers of participants lost to follow-up; these were equally distributed over both study groups.[18] Harms *et al.* (seven participants) and Ulm *et al.* (30 participants) reported numbers of participants lost to follow-up, without mentioning further details.[24,27] De Falco *et al.* and Lampl *et al.* did not report at all the numbers of participants lost to follow-up.[23,25]

Potential biases in the review process

Using multiple overlapping searches of various databases, we aimed to include all relevant publications in this review. However, we cannot totally exclude the possibility that small randomised clinical trials, published in journals with a lower impact factor, might have been missed. We aimed to minimise this risk by developing a new search strategy with the help of the Cochrane Stroke Group's Information Specialist, instead of using the strategy of the former version of this meta-analysis, as well as completely reperforming the selection of studies, instead of only updating the selection from the date of the former search.

Another strategy that we used to minimise the risk of missing small studies was to search trials registries. In the trial registries, we identified only one study that was still ongoing. The funnel plots do show heterogeneity (Figures 5 and 8), but they have improved in comparison with the previous version of this meta-analysis. Furthermore, a high prevalence of incomplete outcome reporting exists[33], which also could have affected our meta-analysis: in only three of the eight included studies were incomplete outcome data adequately addressed. By contacting trial authors to request additional information for inclusion in our prespecified analyses, we tried to minimise this influence.

Agreements and disagreements with other studies or reviews

The previous version of this Cochrane review - Westendorp *et al.* dating from 2012[16] - was an update of a systematic review and meta-analysis performed in 2009 by Van de Beek *et al.*[34] Results of previous meta-analyses are consistent with the findings of current version.

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Authors' Conclusions

Implications for practice

Currently, use of preventive antibiotic therapy is not included in standard care for people with acute stroke.[35] Results of this meta-analysis do not provide evidence that current practice should be changed. Despite the large number of participants, the included studies were heterogeneous, and further research is warranted to translate current conclusions of 'overall' preventive antibiotics to specific antibiotic regimens and/or stroke subtypes.

Implications for research

Compared with the previous version of this meta-analysis, the more robust results in the current analyses, leading to smaller confidence intervals and the shift of effect sizes on functional outcomes and mortality towards no effect, have now made it possible to draw first conclusions on the effects of 'overall' preventive antibiotics in stroke. However, studies remain heterogeneous and it is not possible to draw conclusions on specific types of antibiotics. Besides, it could be suggested that people with different subtypes of stroke do benefit from preventive antibiotics, as was noted for thrombolysed patients in a recent post hoc analysis.[36] A pooled analysis of current large trials is being planned to further evaluate effects of preventive antibiotics on this, and other, potential subgroups.

In addition to research on the potential beneficial effects of preventive antibiotics, an interesting finding in the current meta-analysis is the fact that the incidence of 'overall' infections and urinary tract infections can be significantly reduced, whereas pneumonia – which of all infections is clearly most often associated with poor outcome[1] – cannot be prevented. Additional studies on this finding are necessary, since this might explain the absence of beneficial effects of preventive antibiotics on dependency and mortality. A possible explanation for the observed effect is that pneumonia is not simply a bacterial infection, but may result from (poststroke) chemical and immunological alterations resulting in a 'pneumonitis' in which the role of cultured bacteria is very limited. In case of this pneumonitis prevention by antibiotics is impossible.

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Contribution of authors

J-D Vermeij (JDV): performed the search, extracted data, analysed and interpreted data and drafted the review. WF Westendorp (WW): performed the search, extracted data and analysed and interpreted data. DWJ Dippel (DWJD): conceived and designed the review, assessed methodological quality, and provided final approval of the review for publication. PJ Nederkoorn (PJN): performed analysis and interpretation of the data and commented on the review for its intellectual content. D van de Beek (DVDB): commented on the review for intellectual content and provided final approval of the review for publication.

Declarations of interest

All authors or this review are member of the study group of the Preventive Antibiotics in Stroke Study (ISRCTN66140176). D van de Beek (DVDB) and PJ Nederkoorn (PJN) are the principal investigators.

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Index Terms

Medical Subject Headings (MeSH)

Anti-Bacterial Agents [*therapeutic use]; Antibiotic Prophylaxis [methods]; Bacterial Infections [mortality; *prevention & control]; Brain Ischemia [complications]; Randomised Controlled Trials as Topic; Stroke [*complications; mortality]

MeSH check words

Humans

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Appendix 1.

CENTRAL search strategy

We used the following search strategy for the Cochrane Stroke Group's Trials Register and the Cochrane Central Register of Controlled Trials (CENTRAL).

- Stroke AND antibiotics.
- Haemorrhage AND antibiotics.
- Stroke AND prophylaxis.
- Haemorrhage AND prophylaxis.
- Stroke AND infection.
- Haemorrhage AND infection.

Appendix 2.

MEDLINE Ovid search strategy

We used the following search strategy for MEDLINE Ovid.

- cerebrovascular disorders/ or exp basal ganglia cerebrovascular disease/ or exp brain ischemia/ or exp carotid artery diseases/ or exp cerebrovascular trauma/ or exp intracranial arterial diseases/ or exp intracranial arteriovenous malformations/ or exp "intracranial embolism and thrombosis"/ or exp intracranial hemorrhages/ or stroke/ or exp brain infarction/ or vasospasm, intracranial/ or vertebral artery dissection/
- (stroke$ or poststroke$ or cva$ or cerebrovascular$ or cerebral vascular).tw.
- ((cerebral or cerebellar or brain$ or vertebrobasilar) adj5 (infarct$ or isch?emi$ or thrombo$ or apoplexy or emboli$)).tw.
- ((cerebral or intracerebral or intracranial or brain or cerebellar or subarachnoid) adj5 (haemorrhage or hemorrhage or haematoma or hematoma or bleeding or aneurysm)).tw.
- 1 or 2 or 3 or 4
- Antibiotic Prophylaxis/
- exp Anti-Bacterial Agents/
- (antibiotic$ or anti-bacterial or anti bacterial or antibacterial or bacteriocid$ or anti-mycobacterial or anti mycobacterial or antimycobacterial or anti-infect$ or anti infect$).tw.
- (amoxicillin or amphotericin b or ampicillin or calcimycin or cephalosporin$ or cephalothin or cephamycin$ or chloramphenicol or dactinomycin or doxycycline or erythromycin or fluoroquinolone$ or gentamicin$ or kanamycin or minocycline or neomycin or oxytetracycline or penicillin or streptomycin or tetracycline or vancomycin).tw.
- 7 or 8 or 9

- exp infection/ or exp bacterial infections/ or exp infection control/ or exp fever/ or exp inflammation/
- (infection$ or sepsis or septicaemia or septicemia or pneumonia or bacteremia or bacteraemia or inflammation or fever or blood poisoning).tw.
- 11 or 12
- (prophyla$ or prevent$ or premedicat$ or incidence or occurrence).tw.
- prevention control.fs.
- 15 or 14
- 10 and 13 and 16
- 6 or 17
- 5 and 18

Appendix 3.

Embase Ovid search strategy

We used the following search strategy for Embase Ovid.

- cerebrovascular disease/ or basal ganglion hemorrhage/ or exp brain hematoma/ or exp brain hemorrhage/ or exp brain infarction/ or exp brain ischemia/ or exp carotid artery disease/ or exp cerebral artery disease/ or cerebrovascular accident/ or exp cerebrovascular malformation/ or exp intracranial aneurysm/ or exp occlusive cerebrovascular disease/ or stroke/
- stroke unit/ or stroke patient/
- (stroke$ or poststroke$ or cva$ or cerebrovascular$ or cerebral vascular).tw.
- ((cerebral or cerebellar or brain$ or vertebrobasilar) adj5 (infarct$ or isch?emi$ or thrombo$ or apoplexy or emboli$)).tw.
- ((cerebral or intracerebral or intracranial or brain or cerebellar or subarachnoid) adj5 (haemorrhage or hemorrhage or haematoma or hematoma or bleeding or aneurysm)).tw.
- 1 or 2 or 3 or 4 or 5
- antibiotic prophylaxis/
- exp antibiotic agent/
- (antibiotic$ or anti-bacterial or anti bacterial or antibacterial or bacteriocid$ or anti-mycobacterial or anti mycobacterial or antimycobacterial or anti-infect$ or anti infect$).tw.
- (amoxicillin or amphotericin b or ampicillin or calcimycin or cephalosporin$ or cephalothin or cephamycin$ or chloramphenicol or dactinomycin or doxycycline or erythromycin or fluoroquinolone$ or gentamicin$ or kanamycin or minocycline or neomycin or oxytetracycline or penicillin or streptomycin or tetracycline or vancomycin).tw.

- 8 or 9 or 10
- exp infection/ or infection control/ or infection risk/ or fever/ or exp inflammation/
- (infection$ or sepsis or septicaemia or septicemia or pneumonia or bacteremia or bacteraemia or inflammation or fever or blood poisoning).tw.
- 12 or 13
- (prophyla$ or prevent$ or premedicat$ or incidence or occurrence).tw.
- prophylaxis/
- 16 or 15
- 11 and 14 and 17
- infection prevention/ or exp infection/pc
- 11 and 19
- 7 or 18 or 20
- 6 and 21

Appendix 4.

Clinical trials and research registers search strategy

We used the following search strategy for ClinicalTrials.gov (www.clinicaltrials.gov).

- Stroke AND antibiotics.
- Stroke AND infection.

We used the following search strategy for the ISRCTN Registry (www.isrctn.com).

- Stroke AND antibiotics.
- Stroke AND infection.

We used the following search strategy for the Stroke Trials Registry (www.strokecenter.org/trials).

- Antibiotics.
- Infection.

We used the following search strategy for the WHO Registry Platform (apps.who.int/trialsearch).

- Stroke AND antibiotics.
- Stroke AND infection.

Appendix 5.

Definitions used for infection

Name – year	Definition
Chamorro 2005	Temperature > 37.5°C in 2 determinations; or > 37.8°C in a single determination in participants with suggestive symptoms; white blood cell count > 11,000/mL or < 4000/mL; pulmonary infiltrate on chest x-rays, or cultures positive for a pathogen. Early infection: within 7 days, late: 8 to 90 days
De Falco 1998	Infectious complications: bronchopulmonary, urinary, or hyperthermia of unspecified origin. No definitions specified
Harms 2008	Pneumonia: > 1 of the following: abnormal respiratory examination, or pulmonary infiltrates in chest x-rays; productive cough with purulent sputum; microbiological cultures from lower respiratory tract or blood cultures; leukocytosis and elevation of C-reactive protein (CRP). Urinary tract infection (UTI): > 1 of the following: fever (temperature > 38.0°C), urine sample positive for nitrite, leukocyturia, and significant bacteriuria
Kalra 2015	The algorithm for pneumonia was derived from criteria for pneumonia from the Centers for Disease Control and Prevention that assess 8 clinical or laboratory findings at 6 time points (baseline, 2, 7, 10, and 14 days) for patient's temperature ≥ 37.5°C on 2 consecutive measurements, or 1 measurement ≥ 38.0°C and a respiratory rate ≥ 20 breaths per minute, or cough and breathlessness, or purulent sputum, and a white blood cell count > 11.0×10^9/L, or chest infiltrates on radiograph, or positive sputum culture or microbiology, or positive blood culture
Lampl 2007	Not evaluated
Schwarz 2008	Pneumonia: new infiltrate on chest x-ray compatible with the diagnosis of infection plus ≥ 1 of the following: fever (temperature > 38°C), leukocytosis > 12,000/µL or leukopenia < 3000/µL, purulent tracheal secretions Tracheobronchitis: purulent tracheal secretions or sputum plus ≥ 1 of the following: fever (temperature > 38°C), leukocytosis > 12,000/µL, or leukopenia < 3000/µL UTI: > 25 leukocytes/µL in the urine if not explained by other findings. Bacteraemia: bacteria in blood cultures Sepsis: clinical evidence of an infection with ≥ 2 of the following: temperatures > 38°C or < 35°C, tachycardia > 90/min, tachypnoea > 20/min, leukocytosis > 12,000/µL, or leukopenia < 3000/µL Infection of unclear origin or other infections: clinical evidence of an infection of unknown origin or any other systemic infection
Ulm 2016	Infection in the control group is diagnosed by the clinician. In the intervention group, presence of an infection is guided by ultrasensitive procalcitonin (PCTus) monitoring. In patients with a PCT concentration > 0.05 ng/mL, a bacterial infection is considered likely, and the use of antibiotics is recommended. The responsible physician will then decide on appropriate treatment based on the suspected type of infection according to CDC criteria.
Westendorp 2015	Infections were categorised as diagnosed by the clinician, and as judged by an independent adjudication committee (masked to treatment allocation) according to modified Centers for Disease Control and Prevention criteria. The scoring algorithms for infections used by this committee have been described previously.

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Post-stroke infections and preventive antibiotics in stroke: update of clinical evidence

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Abstract

This review provides an update of evidence on post-stroke infections and the use of preventive antibiotics in stroke. Infection is a common complication after stroke, affecting between 15% and 30% of the patients. The predictors for post-stroke infection can be divided into three categories: clinical factors, anatomical (stroke related) factors and immunological factors. The relation between the occurrence of a post-stroke infection and functional outcome remained subject of debate, but it seems likely that the occurrence of these infections has a causal relation with poor functional outcome and mortality.

In the first meta-analysis on preventive antibiotic therapy, almost a decade ago, its beneficial effect on post-stroke infection rate was clear, however, the effect on functional outcome remained uncertain because included studies were small and heterogeneous. Afterwards, three large phase-3 RCTs were published and a Cochrane meta-analysis was performed.

It has now become clear that, despite the finding that overall infections are reduced, preventive antibiotic therapy in the acute phase of stroke does neither improve functional outcome, nor decrease mortality rates.

This does not yet mean that further research on preventive antibiotics in stroke is useless: the pathophysiology and etiology of post-stroke infections are unclear and the use of preventive antibiotics in specific subgroups of stroke patients could still be very effective. This is currently being studied. Besides, preventive antibiotic therapy might be cost-effective by increasing quality-adjusted life years. Thirdly, research for the upcoming years might put more emphasis on the effect of stroke on immunological alterations.

Background

Stroke is a main cause of disability and death worldwide, with an estimated 42 million prevalent cases and over 6 million deaths in 2015.[1] Before this same year, the only proven effective therapy for acute ischemic stroke was intravenously administered recombinant tissue-type plasminogen activator (rt-PA).[2] Treatment options were extended in 2015, after multiple large multicenter randomized clinical trials showed beneficial effect of intra-arterial thrombectomy (IAT) when an occlusion occurs in the distal internal carotid or the proximal middle cerebral artery.[3] With the introduction of this second proven treatment option for acute ischemic stroke, it is very likely that in the upcoming years outcome will improve and that mortality rates of stroke will further decrease.

With the aim to further improve outcome, different other therapeutic strategies for acute stroke have been proposed. While the first studies date from 1998, in 2008 the first review suggested that there might be a relation between the occurrence of post-stroke infections and outcome, and that the preventive use of antibiotics could be beneficial.[4] In the subsequent years, a possible negative association between post-stroke infections and clinical outcome was reported in different studies.[5-7] The relation between preventive antibiotics and outcome, however, remained unclear. In a Cochrane meta-analysis in 2012, it was found that preventive antibiotics did decrease the post-stroke infection rate, but its effect on functional outcome remained uncertain because the included studies were rather small and heterogeneous[8]. Large phase-3 RCTs were needed and since treatment has not yet been proven effective, guidelines on acute stroke management currently state that preventive administration of antibiotics is not indicated.[9]

Since the publication of this meta-analysis, three large phase-3 RCTs on the effect of preventive antibiotics in acute stroke have been completed.[10-12] As a result, general insights on this subject have markedly changed, as may have recommendations for guidelines and the implications for future research. This review aims to give an update on current knowledge on post-stroke infections and preventive antibiotics.

Post-stroke infections: prevalence, risk factors and their impact on clinical outcome

There is debate about the actual prevalence of post-stroke infections. Most literature mainly report the broad variety in infection rates, ranging between 5 and 65%, because of large heterogeneity of study populations, and definitions for post-stroke infection. Current most complete approximation on the actual post-stroke infection rate is based on a large meta-analysis, including 137,817 patients from 87 different studies with a maximum observation period of 90 days, that showed an overall pooled infection rate of 30% (95% confidence interval [CI] 24–36%).[13] Regarding type of infection, the pneumonia rate was 10% (95%CI 9–10%) as was the urinary tract infection rate (95%CI 9–12%).

One could argue, however, that these numbers are overestimated since data are based on selected populations that were prone to detection bias: numbers were derived from randomized controlled trials, cohort studies and stroke registries and detection rate might differ between hospitals with and without stroke research. In a large unselected consecutive cohort study including patients with suspected ischemic stroke, the incidence of a post-stroke infection within seven days was much lower: 15% of the patients had developed 'any' overall post-stroke infection. The prevalence for pneumonia was 7.5% and for urinary tract infection 4.4%.[7]

Microbiologic data on post-stroke infection mainly shows an early onset nosocomial pneumonia or a community acquired aspiration syndrome. *Staphylococcus aureus* and Gram-negative bacteria such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* or *Enterobacter spp.* are commonly identified, as are *Streptococcus species*. Pneumonia by aspiration are frequently caused by *Staphylococcus aureus*, community acquired pneumonia is often caused by *Streptococcus spp.* Often, no causative organism is detected in post-stroke pneumonia.[13]

Predictors for the occurrence of an infection can be divided into three categories: clinical factors, anatomical (stroke related) factors and immunological factors (see also Appendix 1).

Different clinical factors that appear to influence post-stroke infection rate have been described. These can be subdivided into either patient characteristics or factors related to hospital admission. There are general risk factors, like: older age, sex for different types of infection, stroke severity, bedridden state or diabetes mellitus; and factors that directly increase the risk of a certain infection, like: invasive maneuvers such as feeding tube placement or urinary catheterization, mechanical ventilation, presence of chronic obstructive pulmonary disease, dysphagia, or decreased consciousness and reduced bulbar reflexes resulting in aspiration of nasopharyngeal secretions.[13,14]

Regarding anatomical (stroke related) factors, to date only little is known. An association has been suggested between lesion size and the occurrence of infections: patients with larger stroke volume might be at higher risk for pneumonia (Odds ratio [OR] 3.5, $p < 0.001$), compared with patients with smaller stroke volume, and patients with a small lesion have a lower risk for urinary tract infections (OR 0.4, $p < 0.01$) compared with those with a larger lesion.[15] Regarding location or even stroke lateralization, no clear association has been found. To our knowledge, only little additional studies have been performed to repeat these findings: two studies also found a small association between stroke volume and the risk of infection[16,17] , whereas another study did not find an independent association between stroke volume and post-stroke infection, but did find a relation with location: an infarction in the anterior middle cerebral artery cortex was significantly associated with the occurrence of infections (OR 5.68; 95%CI 1.00–32.29).[18]

The association between intracerebral hemorrhage volume, localization and the risk of post-stroke infections remains uncertain as well: one prospective cohort study found an association with a hematoma volume larger than 30cc (OR 2.0; 95%CI 1.1–3.5) as well as a deep location of the hemorrhage (reference lobar: OR 1.90; 95%CI 1.28–2.88)[19] , whereas in two other cohort studies these associations did not remain significant after logistic regression.[20,21]

Thirdly, brain injury itself may influence the risk of a post-stroke infection. It is believed that the central nervous system and the immune system are closely linked and continuously interact via long-distance feedback loops between the central nervous system and peripheral immune organs using the hypothalamo-pituitary-adrenal axis and the sympathetic nervous system. As a result of central nervous system injury, disturbance of these normally well-balanced brain-immune interactions leads to a state called stroke-induced immunodepression.[22-24] The background of this complex interaction is beyond the scope of this review and is yet not fully understood, but in general brain injury leads to an overactivation of the autonomic nervous system and an increased release of stress hormones resulting in changes in the innate and adaptive immune system. Described features include lymphocyte apoptosis, lymphopenia, altered cytokine production and atrophy of lymphoid organs.[17]

The effect of the occurrence of a post-stroke infection on functional outcome has been discussed in different reviews.[4,14] The majority of studies show an independent association between the occurrence of an infection within the first days after stroke and poor functional outcome and/or mortality.[5,7,25-33] This effect appears to be the particularly present for pneumonia. An overview of the main and most cited studies on this subject has been summarized in Appendix 2.

In general, the occurrence of 'any' overall post-stroke infection is associated with an odds ratio for a poor functional outcome that varies between 0.9 and 4.4. The odds ratio for mortality varies between 1.5 and 6.0 (with one outlier with a broad confidence interval[25]). Despite the fact that different outcome parameters have been used, six of the seven major studies show a significant negative effect of infections on stroke outcome.

Regarding pneumonia, the evidence for functional outcome is limited and odds ratio's vary with broad confidence intervals of 1.7 to 52. The odds ratio for mortality, however, ranges between 2.1 and 3.0 with small variation between studies. All four major studies showed a significant association.

The effect of post-stroke urinary tract infections on clinical outcome remains subject of debate: of the four major studies looking at this effect, three showed no significant effect.

While the association between infection and a worse functional outcome seems to be proven, the potential mechanisms that could link both parameters remains subject of debate. One could argue that different confounding factors that are more frequent in patients with infections are also predictors of poor outcome, e.g. comorbidity, impaired consciousness and medical interventions. On the other hand, infections could also directly affect outcome via different ways. Firstly, immobility and frailty as a result of a post-stroke infection might lead to a prolonged hospital stay and a delay in rehabilitation. Secondly, infections might induce immunological effects that could worsen outcome.[13] Fever as a result of the infection, for example, affects the penumbra, increases the metabolic demands of the cell, exaggerates free oxygen radical production, increases breakdown of the blood-brain barrier and accelerates their transformation into ischemic tissue.[6,34]

Preventive antibiotics in stroke: "the early days"

The hypothesis that post-stroke infection might be prevented by antibiotic prophylaxis dates from the 1980's: two studies that were published in 1981 and 1985 respectively, investigated the effect of antibiotic prophylaxis for preventing urinary tract infections in stroke patients in need of an indwelling catheter.[35,36] The first publication on the general prevention of 'any' overall post-stroke infection dates from 1982, and investigated the effect of ampicillin and penicillin.[37] The latter study showed no significant effect and after the publication of the aforementioned three manuscripts, it would take years before this subject gained attention again.

The effect of preventive antibiotics on stroke outcome, besides infection rate, gained attention over 20 years later. Starting from 2004, different animal models showed the beneficial effect of e.g. moxifloxacin, minocycline and ceftriaxone administered within the first hours after stroke on neurological outcome, mortality and even infarct size.[4] The first large clinical trial, including over 100 patients, was published in 2005 and investigated the effect of intravenous levofloxacin on infection rate, neurological outcome and mortality.[38] This study also did not show a beneficial effect above optimal care.

Within the next three years, four studies on the effect of preventive antibiotics on infection rate and stroke outcome were published. Although study intervention and duration of therapy varied, these results were compared in a first systematic review and meta-analysis on this subject, published in 2009.[39] There were many major remarks and in general, the study mainly provided a plead for the upcoming large randomized controlled trials on this subject that were to be published within the upcoming years.

A systematic meta-analysis on preventive antibiotic therapy in the acute phase of stroke was published in the Cochrane Library in 2012.[8] By that time, five studies involving 506 patients were identified. It was concluded that by the use of prophylactic antibiotic therapy, the incidence of post-stroke infections was significantly reduced (RR 0.58; 95%CI 0.43–0.79), whereas this did not affect stroke outcome, both in mortality (RR 0.85; 95%CI 0.47–1.51) and dependency (RR 0.67; 95%CI 0.32–1.43). The evidence, however, did still not allow a robust and definitive conclusion on the use of preventive antibiotic therapy in acute stroke: the total number of studies and participants was again too limited and there was a broad variety in study design, type of antibiotic therapy (adequately covering all pathogens in post-stroke infections versus mostly chosen for neuroprotective properties) and definitions of infection.

By the end of 2012, after the publication of the first Cochrane meta-analysis, it was concluded that there is not yet evidence that the use of preventive antibiotic therapy should be included in standard care for patients with acute stroke, nor that its potentially beneficial effect should now be rejected. Since included studies were small and heterogeneous, the results warranted evaluation in new stroke trials that needed to enroll large numbers of patients aiming to detect even a small effect, to establish with certainty whether preventive antibiotic therapy has a place in the treatment of patients with acute stroke.

The "new era" of preventive antibiotics in stroke trials

The plead for large clinical trials on the effect of preventive antibiotics on stroke outcome was heard: after the publication of the first Cochrane meta-analysis, three large studies were completed that, in two times, included over the total amount of participants of the former meta-analysis.[10-12]

The first large trial was the 'Preventive Antibiotics in Stroke Study' (PASS), that was published in The Lancet in 2015.[10] In this study with a prospective, randomised, open-label, blinded endpoint (PROBE) design, a total of 2,538 patients were assigned to receive either intravenous ceftriaxone at a dose of 2g, given every 24h intravenously for 4 days, or standard care.

In PASS, the number of infections was significantly reduced (OR 0.55; 95%CI 0.44–0.70), which was mainly driven by a reduction in urinary tract infections (OR 0.34; 95%CI 0.24–0.48) whereas the pneumonia rate did not differ (OR 0.80; 95%CI 0.58–1.10). This study showed no beneficial effect of ceftriaxone on stroke outcome: the distribution of scores on the modified Rankin Scale (mRS) was similar to the control group (adjusted common OR 0.94; 95%CI 0.82–1.09), as were the percentage of patients with an unfavorable outcome (mRS 3-6) when the mRS was dichotomized (OR 0.94; 95%CI 0.80–1.11). Mortality rate at 3 months follow-up did not differ as well (OR 0.96; 95%CI 0.74–1.24).

The second large trial was the 'Cluster randomised trial of different strategies of antibiotic use to reduce the incidence and consequences of chest infection in acute stroke patients with swallowing problems' (STROKE-INF), that was published a few months later in The Lancet as well.[11] In this study with a PROBE design 1,217 stroke patients with swallowing difficulties were enrolled. 48 stroke units in the UK were cluster randomized to preventive antibiotic therapy, in which the choice of the antibiotic was conformed to the local guidelines of participating centers aiming to reduce the incidence of post-stroke pneumonia, or standard care. The incidence of pneumonia, however, was not significantly reduced (OR 1.01; 95%CI 0.61-1.68), nor was the percentage of patients with a good

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functional outcome increased (mRS 0-2) (OR 0.87; 95%CI 0.6–1.24) or the mortality rate at 90 days reduced (aOR 1.22; 95%CI 0.9–1.64).

The third most recent trial was the 'Stroke Adverse Outcome is Associated With Nosocomial Infections: PCTus-Guided Antibacterial Therapy in Severe Ischemic Stroke Patients' (STRAWINSKI) study, that was published in 2017.[12] In this RCT 227 patients were randomized to receive either standard stroke care plus Procalcitonin ultrasensitive (PCTus)-guided antibiotic treatment or to standard stroke care alone. Procalcitonin is an early marker of severe bacterial infections and was assessed daily in the intervention group. At a concentration >0.05 ng/ml, a bacterial infection was considered likely and patients were treated with prophylactic antibiotics. Type and duration of antibiotic treatment – aiming to prevent stroke-associated pneumonia – was left to the local guidelines of the participating center. As in the former two studies, the intervention group did not have a better functional outcome on the mRS at 90 days (OR 0.79; 95%CI 0.45–1.39), nor have a lower mortality rate (OR 1.20; 95%CI 0.65–2.24). The overall number of infections, as well as the pneumonia and urinary tract infection rate within the first seven days did not differ as well.

Current insights in the use of preventive antibiotics in the acute phase of stroke

The completion of three recent large clinical trials warranted an update of the Cochrane meta-analysis on this subject. The recent large clinical trials have led to an almost nine-fold increase in study population: from the former five studies involving 506 patients, to eight studies involving 4,488 patients. This increase in participants has led to a neutralization of the heterogeneity, making it for the first time possible to draw conclusions on the effect of the 'overall' use of preventive antibiotics on functional outcome. The Cochrane meta-analysis has been completely reperformed and was published in January 2018[40] , an overview of the characteristics of the included clinical trials has been provided in Appendix 3.

Preventive antibiotics lead to a significant reduction in the general incidence of 'any' overall post-stroke infection: from 26% in the control group to 19% (RR 0.71, 95%CI 0.58–0.88). Regarding type of infection, this effect is highly significant for urinary tract infections which were reduced from 10% to 4% of the antibiotic-treated patients (RR 0.40; 95%CI 0.32–0.51), whereas they seem to be unable to prevent pneumonia, which occur in 11% in the control group versus 10% in the antibiotic group (RR 0.99; 95%CI 0.80–1.13).

In a subanalysis of the studies with the aim of preventing pneumonia by leaving the choice of the antibiotic to local guidelines, there was still no significant difference in the occurrence of pneumonia: 18% in the antibiotics group versus 17% in the control group (RR 1.08; 95%CI 0.87–1.34). As expected, functional outcome did not differ as well.

Regarding functional outcome, prophylactic antibiotics do not have a beneficial effect: the number of patients with a poor functional outcome (dependency or death) do not significantly differ: 55% in the preventive antibiotics group versus 53% in the control group (RR 0.99; 95%CI 0.89–1.10). Mortality is even increased, although not significantly: 17% of the preventive antibiotics group versus 16% in the control group (RR 1.03; 95%CI 0.87–1.21).

No major adverse events are reported. The most reported adverse event was elevated liver and/or renal enzymes, which occurred in 9% of the patients treated with preventive antibiotics. This was also reported in 7% of the patients in the control groups and in none of the patients clinical consequences were reported. Antibiotic resistance, a fearful side effect of the increased use of antibiotics, does currently not seem to be an issue. Infection by a resistant bacterium was reported in 1% of the patients in one study, but happened in the same percentage in the control group. Another study reported the presence of colonisation with an antibiotic resistant micro-organism in one patient as well; however, this was already present before the start of the study.

Implications for daily practice

With the completion of the recent large clinical trials, the meta-analysis has gained enough power to state that, despite the heterogeneity, preventive antibiotics do not affect functional outcome after acute stroke and should therefore not be applied in standard stroke care of all patients. The risk of 'any' overall post-stroke infection, however, is significantly reduced. This reduction is highly significant for urinary tract infections, whereas no effect on pneumonia is found.

It is considered unlikely that with the completion of new trials this advice will change: in comparison with the former meta-analysis from 2012, point estimates are similar, all confidence-intervals have become smaller and effect sizes on functional outcome and mortality have shifted towards no effect.

Future directions: "Can we conclude that no further research on preventive antibiotics in stroke is necessary?"

Although functional outcome may not be influenced by the use of preventive antibiotics in all stroke patients, it may be false to jump to the conclusion that the research on preventive antibiotics in stroke should now be considered finished. There are different aspects that should be taken into consideration.

First, current data considers the 'overall' effect on functional outcome, using 'any' type of antibiotic. Although even the large trials and different antibiotic regimens show that functional outcome and mortality are not influenced – even the trials in which the choice of antibiotic was left to local guidelines – data is too limited to draw conclusions on specific antibiotic regimens. It might for instance be possible that the observed beneficial effect of certain antibiotic regimens in animal models are indeed based on neuroprotection instead of the prevention of infections. These results may be neutralized in current meta-analysis.

Second, current data considers the overall effect of antibiotics on ‘any' patient with stroke. It might be possible that different subgroups of stroke patients do benefit from prophylaxis with antibiotics. In a post-hoc analysis, for instance, a beneficial effect was found in thrombolysed patients.[41] These findings, however, are yet based on a single observation and could still be a finding by chance. Data is currently too limited to make a distinction between ischemic and hemorrhagic stroke, let alone subgroups of stroke patients. Within the next years, data will increase since one international multicenter trial is still ongoing: in the “PREvention of Complications to Improve OUtcome in elderly patients with acute Stroke” (PRECIOUS) study, a targeted 3,800 patients will be randomly allocated in a 2x2x2 factorial design to ceftriaxone, paracetamol, metoclopramide, any combination of these, or to ‘standard' treatment (ISRCTN82217627).

The next indispensable step within this research field is to identify the patient groups that are most likely to benefit from preventive antibiotic therapy. Based on the hypothesis that the beneficial effect can be explained by the prevention of infections, a prediction rule for post-stroke pneumonia and all post-stroke infections is currently being developed to identify patients at high risk of such infections.[42] Data from PASS is used to develop and internally validate these models and, after external validation, these prediction rules could be an important step for the selection of patients for future trials.

Another possibility to further identify subgroups of patients that might benefit from preventive antibiotic therapy is via a pooled analysis of individual patient data. This is currently being performed for the most recent large trials on this subject. Results are expected soon.

The conclusion that there is no role for the use of preventive antibiotics with the aim of improving functional outcome does not exclude that this therapy might still be implied in general practice for its added value in the prevention of post-stroke infections. A recent cost-effectiveness study of PASS showed that three months after stroke, the number of quality-adjusted life years (QALYs) was significantly higher in the ceftriaxone treated patients, compared with the control group ($p = 0.006$) and that preventive ceftriaxone in adults with acute stroke is indeed cost-effective.[43] Whether costs justify the increasing use of antibiotics, however, remains subject of debate.

One argument against the more widespread use of antibiotics, especially for a financial reason, might be the occurrence of bacterial resistance. In none of the participants resistance due to the study intervention has been proven for current studies. The frequent prophylactic administration of antibiotics has already been applied in the intensive care unit by means of selective digestive decontamination (SDD) and selective oropharyngeal decontamination (SOD). One of the aims of this therapy is even to reduce the incidence of ventilator-associated pneumonia. The extensive use of preventive antibiotics in the ICU do not show an increased incidence of colonisation or infection with antimicrobial resistant pathogens.[44] Indeed, for all pathogens other than meticillin-resistant *Staphylococcus aureus* (MRSA), even a lower level of antibiotic-resistance was found. One explanation might be that "the use of prophylactic selective decontamination antimicrobials could lead to reductions in the need for therapeutic antimicrobials. If the overall net use of antimicrobials is unchanged (or even decreased) with selective decontamination, then there would be no increased antimicrobial selection pressure."

Fourth, current studies have all been performed in Western Europe. It is unknown if current data also applies to other parts of the world, like Asia, where different pathogens are seen and aspects of acute stroke management and strategies for stroke prevention significantly vary.[45]

Besides arguments that plead for the continuation of the research on preventive antibiotics in stroke, current insights have also led to different new questions that provide implications for future research.

As shown in Appendix 2, pneumonia appears to have the strongest association with poor outcome and mortality of all types of infection. The finding that preventive antibiotics do not influence the post-stroke pneumonia rate, even in the trials in which the choice of antibiotic was left to the local guidelines, might therefore be a plausible explanation for the non-beneficial effect of antibiotics on functional outcome. Indeed, urinary-tract infections can be significantly reduced; however, its effect on stroke outcome is uncertain.

Additional studies to explain this finding are necessary. One suggestion might be that stroke-associated pneumonia is not a bacterial infection, but the result of chemical and immunological alterations that result in a 'pneumonitis' that cannot be prevented by antibiotics.[10] Currently, there is no prove for this finding. However, it has been suggested before that post-stroke infections are only a marker of stroke severity without an independent outcome effect.[33]

While research within this field for the last decade mainly drew attention to the effect of preventive antibiotics on infection rate and consequently functional outcome, this might lead to a shift of the main focus for the upcoming years. Instead of infections, research might put more emphasis on the effect of stroke on immunological alterations, e.g. the physiology of stroke-induced immunodepression. If proven that functional outcome may not be influenced by any antibiotic regimen and in any subgroup of stroke patients, the next therapeutic opportunity might become the field of immunological alteration.

Conclusion

Infection is a common complication after stroke. There are different predictors for the occurrence of an infection that can be divided into: clinical factors, anatomical (stroke related) factors and immunological factors. The majority of reviews show an independent association between the occurrence of a post-stroke infection and poor functional outcome and/or higher mortality. This mainly accounts for pneumonia; the relation between urinary tract infections and stroke outcome is doubtful.

After the completion of three large RCTs that were added to a recent Cochrane meta-analysis, it has now become possible to state that, despite that overall infections are significantly reduced, preventive antibiotic therapy does not improve functional outcome or decrease mortality rates.

This does not yet mean that research on preventive antibiotics in stroke should now be considered finished: a beneficial effect might still be applicable for certain antibiotic regimens and/or subgroups of patients. This is currently being studied. Besides, preventive antibiotic therapy might be cost-effective by increasing the QALYs.

If proven that functional outcome may not be influenced by any antibiotic regimen, in any subgroup of patients, research for the upcoming years might put more emphasis on the effect of stroke on immunological alterations. Consequently, the next therapeutic opportunity to improve stroke outcome might be within immunological alteration.

Contribution of authors

Jan-Dirk Vermeij provided the first draft of the manuscript. Willeke F. Westendorp reviewed the manuscript. Diederik van de Beek reviewed the manuscript. Paul J. Nederkoorn reviewed and finished the final version of the manuscript.

Declarations of interest

All authors were member of the study group of the Preventive Antibiotics in Stroke Study (ISRCTN66140176), which has been included in this review.

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6

Appendix 1.

Predictors for the occurrence of a post-stroke infection

Clinical factors
General risk factors
Age
Sex
Diabetes mellitus
Stroke severity
Bedridden state
Risk factors that increase the risk of certain infections
Feeding tube placement
Urinary catheterization
Mechanical ventilation
Chronic obstructive pulmonary disease
Dysphagia
Decreased consciousness
Reduced bulbar reflexes
Anatomical (stroke related) factors
Stroke lesion size
Stroke lesion location (?)
Hematoma volume (?)
Hematoma location (?)
Immunological factors
Stroke-induced immunodepression

Appendix 2.

Overview of the main and most cited** studies on the association between post-stroke infection and functional outcome

Name – year [Ref]	Objective	Study design	No. of patients	
Kammersgaard 2001 [1]	To establish the frequency of early post-stroke infections (within 3 days) and its relation to functional outcome.	Prospective consecutive cohort study.	1,156	
Katzan 2003 [2]	To assess the effect of pneumonia on 30-day mortality.	Retrospective cohort study.	14,293	
Hamidon 2003 [3]	To assess predictors of early infection and their relation functional outcome.	Prospective.	163	
Hilker 2003 [4]	To assess the incidence of stroke-associated pneumonia (SAP) and its effect on mortality in ICU treated stroke patients.	Prospective consecutive cohort study.	124	
Vargas 2006 [5]	To assess the effect of stroke associated infection on functional outcome.	Prospective consecutive cohort study.	229	
Ovbiagele 2006 [6]	To identify factors associated with an increased risk for pneumonia or urinary tract infections and determining their effect on functional outcome.	Retrospective cohort study.	663	
Kwan 2007 [7]	To assess the frequency, risk factors and clinical consequences of post-stroke infections.	Prospective consecutive cohort study.	439	
Hong 2008 [8]	To assess the effect of neurological and medical complications on 3-month outcome.	Prospective consecutive cohort study.	1,254	
Vermeij 2009 [9]	To assess the effect of infection on mortality and functional outcome at discharge and 1 year	Prospective consecutive cohort study.	521	
Finlayson 2011 [10]	To assess the impact of pneumonia on 30-day and 1 year mortality. Secondary outcome: functional outcome at discharge.	Retrospective cohort study.	8,251	
Rohweder 2015 [11]	To assess the association of medical complications with functional outcome at 90 days.	Prospective consecutive cohort study.	244	

aRR = adjusted Relative Risk, aOR = adjusted Odds Ratio, OR = Odds Ratio, RR = Risk Ratio, ICU = Intensive Care Unit, * = not statistically sign

** 'Main and most cited' indicates all studies that were referred to in the limited number of reviews on the effect of post-stroke infections c

	Main results		
	Any infection	**Pneumonia**	**Other infections**
	After multiple logistic regression, the length of hospital stay is prolonged by a mean of 9.3 days (p<0.0001). There is no significant effect on death during hospital stay (p=.78) stroke severity at discharge (p=.32) or rate of discharge to nursing home (p-.17).*		
		aRR for 30-day mortality 2.99 (95% CI 2.44–3.66)	
	OR for early infection on mortality 14.83 (95% CI 4.31–51.07).		
		SAP patients had a significantly worse clinical outcome (p<0.05). RR for mortality in SAP patients 2.5 (95% CI 1.0–5.9).	
	OR for poor outcome 0.9 (95% CI 0.9–1.0).*		
	aOR for good functional outcome 0.16 (95% CI 0.01–0.29). aOR for mortality during hospitalization 5.96 (95% CI 3.02–11.7).		Urinary tract infection: aOR for good functional outcome 0.65 (95% CI 0.32–1.58). aOR for mortality during hospitalization 0.83 (95% CI 0.43–1.57).*
	aOR for discharge home 0.47 (95% CI 0.26–0.83). aOR for mortality within the first 5 days 2.67 (95% CI 1.11–6.38). aOR for mortality during hospitalization 2.50 (95% CI 1.27–4.90).		
	aOR for poor outcome at 90 days 4.44 (95% CI 2.20–8.99).		Urinary tract infection: aOR for poor outcome at 90 days 2.72 (95% CI 1.32–5.60).
	aOR for poor outcome at discharge 2.6 (95% CI 1.0–6.7) and at 1 year 3.8 (95% CI 1.8–8.9). aOR for mortality 1.5 (95% CI 1.0–2.4).	aOR for poor outcome at discharge 9.5 (95% CI 1.7–52) and at 1 year 10 (95% CI 2.2–46). aOR for mortality 2.1 (95% CI 1.2–3.7).	Urinary tract infection: aOR for poor outcome at discharge 1.7 (95% CI 0.3–8.5) and at 1 year 2.7 (95% CI 0.6–12).* aOR for mortality 1.2 (95% CI 0.7–2.3).*
		OR for good functional outcome at discharge 0.2 (95% CI 0.14–0.29). aOR for mortality at 30 days 2.2 (95% CI 1.8–2.7) and at 1 year 3.0 (95% CI 2.5–3.7).	
			Chest infection: OR for bad functional outcome 2.52 (95% CI 1.14–8.53). Urinary tract infection: OR for bad functional outcome 1.33 (95% CI 0.91–1.93).* Infections other than chest or urinary tract infection: OR for bad functional outcome 2.09 (95% CI 1.15–3.80).

ificant
n functional outcome (4, 14) as well as the references of these studies.

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Appendix 3.

Characteristics of the clinical trials on preventive antibiotics in acute stroke, as included in the most recent Cochrane meta-analysis

Name – year [Ref]	Design	No. of patients	Intervention	
De Falco 1998 [1]	Randomised, unblinded	80	Penicillin i.m.	
Chamorro 2005 [2]	Randomised, double-blind	136 (sample size 240, early termination after interim analysis)	Levofloxacin 500mg for 3 days	
Lampl 2007 [3]	Quasi-randomised, open-label, blinded outcome assessment	151	Minocycline 200mg for 5 days	
Harms 2008 [4]	Randomised, double-blind	79	Moxifloxacin 400mg for 5 days	
Schwarz 2008 [5]	Randomised, unblinded	60	Mezlocillin 2g and sulbactam 1g every 8 hours for 4 days	
Westendorp 2015 [6]	Randomised, open-label, blinded endpoint	2,538	Ceftriaxone 2g for 4 days	
Kalra 2015 [7]	Cluster randomised, open-label, blinded endpoint	1,217	Antibiotic choice conformed to local policy	
Ulm 2017 [8]	Randomised, open-label, blinded endpoint	227	PCTus-based antibiotic therapy guidance in addition to stroke unit care.	

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Primary outcome	Main results
Unclear. Scored outcomes were: infectious complications, case fatality and functional outcome.	Treatment with penicillin i.m. was associated with lower rates of infections and better clinical outcome.
Early infection (< 7 days)	Prophylactic administration of levofloxacin is not better than optimal care for the prevention of infections. Using logistic regression, favorable outcome at day 90 was inversely associated with allocation to levofloxacin.
Changes from baseline to day 90 in functional outcome (NIHSS*)	Patients with acute stroke had significantly better outcome with minocycline treatment compared with placebo.
Infection rate within 11 days.	Preventive administration of moxifloxacin is superior in reducing infections after severe non-lacunar ischemic stroke compared to placebo. Neurological outcome and survival were not significantly influenced.
Incidence and height of fever	In patients with acute severe stroke, prophylactic administration of mezlocillin plus sulbactam over 4 days decreases body temperature, lowers the rate of infection, and may be associated with a better clinical outcome.
Functional outcome at 3 months, defined according to the modified Rankin Scale	Preventive ceftriaxone does not improve functional outcome at 3 months in adults with acute stroke.
Pneumonia rate	Antibiotic prophylaxis cannot be recommended for prevention of post-stroke pneumonia in patients with dysphagia after stroke managed in stroke units.
Functional outcome at 3 months, defined according to the modified Rankin Scale	PCTus-guided antibiotic therapy did not improve functional outcome at 3 months after severe ischemic stroke.

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7

Summary and general discussion

Summary and general discussion

This thesis describes the effect of preventive antibiotic therapy on functional outcome after stroke. Current discussion starts with an overview regarding insights on the incidence of post-stroke infections and their effect on stroke outcome – which was the initial reason for the hypothesis that patients might benefit from preventive antibiotic therapy in the acute phase after stroke. Next, the study design and the main results of the 'Preventive Antibiotics in Stroke Study' (PASS) are summarised and discussed, followed by the results of a subgroup analysis of patients in which the effect of prophylactic ceftriaxone on functional outcome differed from other subgroups in PASS. Afterwards, we put current insights in a broader perspective, having performed a systematic meta-analysis including all current studies on preventive antibiotic therapy in stroke. We end the chapter with implications for daily practice as well as a discussion regarding future directions.

Post-stroke infections: prevalence, risk factors and the effect on outcome

Infection is a common complication after stroke, but literature on the exact prevalence varies broadly, ranging between 5 to 65% due to study differences in length of follow-up, baseline characteristics, patient-risk as well as clinical definitions of infection.[1] Probably the most accurate estimate on the prevalence of post-stroke infections originates from a meta-analysis including 87 studies with a total of 137,817 stroke patients: despite significant heterogeneity between studies, the overall pooled infection rate was 30% (95%CI: 24 – 36%). If patients were admitted to the ICU, this number increased to 45% (95%CI: 38 – 52%).[2]

The most common types of infection are pneumonia and urinary tract infections, which both occur in 10% of the patients. In ICU studies, the prevalence of pneumonia increases to 28% and of urinary-tract infections increases to 20%.[2]

Based on microbiologic analyses, post-stroke pneumonia does not seem to be caused by the bacteria one would initially expect. The nasopharynx and oropharynx in healthy people are normally colonised by facultative Gram-positive aerobic bacteria such as alpha-haemolytic *Streptococcus pneumoniae*, as well as the Gram-negative anearobic *Haemophilus influenzae*, both of which are therefore overall the most common causes of community-acquired pneumonia.[3,4] Studies on post-stroke pneumonia, however, show that aerobic Gram-negative bacteria are the most frequently isolated micro-organisms: *Escherichia coli, Enterobacter spp., Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.[4,5] Methicillin-resistant *Staphylococcus aureus* is becoming more common, but also other Staphylococcus species are prevalent.[6] It is not known why patients acquire especially these bacteria after stroke; a combination of physical change in the cleansing of the oral cavity, altered epithelial surfaces and changes in saliva flow and distribution have been postulated, as well as the presence of dentures in the mouth.[5] Often, no causative organism is detected in post-stroke pneumonia. The diagnosis of pneumonia may be mistaken for an ‘aspiration pneumonitis’ in which large volumes of gastric contents, including food, are aspirated. This results in a chemical burn of the tracheobronchial tree and pulmonary parenchyma, causing an intense local parenchymal inflammatory reaction.[3]

Risk factors for the occurrence of any post-stroke infection can be divided into three main categories: clinical factors, anatomical factors and immunological factors.

Clinical factors include patient related (individual) factors (e.g. prior history), and factors related to hospital admission (e.g. invasive manoeuvres).[2,7]

Regarding anatomical factors, different studies found an association between stroke volume and/or location and the chance of a post-stroke infection, both for ischaemic and haemorrhagic stroke. Currently, sample sizes are too small to provide meaningfull conclusions. Details are described in Chapter 6; however, more research needs to be done before providing solid statements.

Immunological factors comprise the interaction between the central nervous system and the systemic immune system. This is a two-way communication. On one hand, the immune system influences the brain following stroke. Leukocytes head towards the lesion within the brain and induce local inflammation. Invasion of pathogens results in tissue destruction that causes brain damage, however, key of the process is clearing of already damaged tissue and induction of remodelling processes like angiogenesis and neuroregeneration.[8] On the other hand, stroke-induced injury to the brain is able to negatively influence the systemic immune system. This modulation can be achieved via both humoral and neuronal pathways, and include the hypothalamic-pituitary-adrenal (HPA) axis, the vagus nerve and the sympathetic nervous system.[9] The background of this complex interaction is not yet fully understood. Summarized, the humoral pathway is run via the HPA axis: cytokines released by stroke-induced inflammation may activate neurons in the hypothalamus – as can be achieved by blood-borne cytokines in ‘healthy individuals’. Activation of the hypothalamus results in a cascade of events that eventually induces the secretion of glucocorticoids in the adrenal gland. As a result, these glucocorticoids suppress the systemic production of pro-inflammatory cytokines and promote the release of anti-inflammatory cytokines, limiting the inflammatory response. The neuronal pathway is run through the vagus nerve and the sympathetic nervous system. Activation results in peripheral immune alteration: the vagus nerve mainly induces a reduction in the peripheral release of cytokines through alteration of the macrophages; the sympathetic nerve fibers induce the release of norepinephrine in different peripheral tissues, including bone marrow, thymus, spleen and lymph nodes; resulting in inhibition of T helper pro-inflammatory activities and predominance of T helper anti-inflammatory activities. Finally, the sympathetic nervous system can also induce innervation of the adrenal gland, resulting in the release of catecholamines in the bloodstream where they act as hormones in the same way as glucocorticoids do. The humoral pathway and the neuronal pathway are believed to interact via nerves connecting the hypothalamus with the nuclei of the vagal and sympathetic nerves. Neuroendocrine (humoral) responses can thereby also induce cholinergic (neuronal) effects.[9,10] Whether induced via the humoral pathway, or via the neuronal pathway, the brain is able to induce a pronounced systemic anti-inflammatory phenotype that can consequently (also) facilitate the emergence of infections such as pneumonia and urinary tract infections.[10]

The association between post-stroke infection and clinical outcome has been investigated in several studies, which were summarised in Chapter 6. Most studies find a clear negative association, in which post-stroke infections lead to higher levels of disability on the modified Rankin Scale (mRS) or the Barthel Index.[11] In general, the odds ratio for a poor functional outcome after 'any' overall post-stroke infection ranges between 0.9 and 4.4 amongst different studies; regarding pneumonia the amount of studies are limited but odds ratios vary between 1.7 to 52. In the meta-analysis regarding the prevalence of post-stroke infections, 48% of patients with an infection died, compared with 18% of patients without an infection (OR 2.08; 95%CI 1.63 – 2.67). After pneumonia, this difference is even higher: 26% vs 5% (OR 5.58; 95%CI 4.76 – 6.55).[2]

Not all studies conclude that there is a direct relation between post-stroke infection rate and functional outcome. Some studies suggest that the occurrence of an infection is more like a marker of stroke severity without an independent effect on outcome.[12] It might be possible that either factors inherent in the patient, or the effects of stroke predispose to infectious complications, instead of the opposite possibility in which infectious complications independently affect the functional outcome of the patient. Different confounding factors can be opposed that have not always been taken into account in studies regarding this subject, including: stroke severity, infarct size, age, impaired pulmonary function and malnutrition.[11,12]

The amount of studies that did not find an independent outcome effect, however, are in the minority and several arguments can be opposed as to why infections do affect outcome. First, the occurrence of an infection leads to immobilisation ('prolongation of the bedridden state'), general frailty and prolongation of hospital stay resulting in a delay in rehabilitation. Second, the occurrence of an infection causes immunological effects that negatively influence nerve survival: electrolytic unbalance, fever, hypoxia, increased metabolic demands in already damaged brain tissue, the presentation of inflammatory cytokines and the production of harmful free oxygen radicals – all accelerating ischaemic tissue formation.[2,12]

The Preventive Antibiotics in Stroke Study

In **Chapter 2**, we present an update on the study design of the 'Preventive Antibiotics in Stroke Study' (PASS). The original design was published in 2011[13], however, primary outcome was changed.

PASS is a clinical phase three multicentre, prospective, randomised, open-label, blindend end-point (PROBE) trial, investigating whether the preventive use of the antibiotic ceftriaxone – by preventing infections – improves functional outcome in acute stroke. Adult patients with stroke (both ischaemic and haemorrhagic) were included, provided that they had a score ≥ 1 on the National Institutes of Health Stroke Scale (NIHSS) and stroke onset was within the last 24 hours before inclusion. Patients were excluded in case of an infection at admission, use of antibiotics within 24 hours before admission, previous hypersensitivity or anaphylaxis to cephalosporins or penicillin, subarachnoid haemorrhage, pregnancy or when death seemed imminent.

Patients in the intervention group received ceftriaxone at a dose of 2 grams, given every 24 hours intravenously, for 4 days, in addition to regular stroke unit care. Ceftriaxone was discontinued if patients were discharged or when active treatment was withdrawn. Patients in the control group received regular stroke unit care without preventive antibiotic therapy. The treating clinician could decide whether or not to treat a patient with a suspected infection with (additional) antimicrobials.

Primary outcome was functional outcome on the modified Ranking Scale (mRS) at 3 months follow-up, as assessed by a structured telephone interview by a trained assessor. The PROBE design implies that an open-label was used: patient and treating physician were aware of treatment allocation; however, the blinded endpoint implies that the outcome assessor was unaware of treatment allocation.

Secondary outcomes included death at discharge and at 3 months follow-up, infection rate during hospital admission, and the use of antibiotics during hospital admission.

In the initial trial protocol, a binary logistic regression analysis on the dichotomised mRS (favourable outcome: mRS 0 to 2 vs unfavourable outcome: mRS 3 to 6) was presented as the primary outcome. We explain in **Chapter 2.1** the reason for a change to an ordinal regression analysis – without prior knowledge of any of the outcome data and without breaking the randomisation code.

Chapter 3 provides the study results of PASS. In total, 2,550 adult patients from 30 Dutch sites were enrolled. 12 patients withdrew consent, leaving 2,538 patients available for the intention-to-treat analysis: 1,268 patients in the ceftriaxone group and 1,270 patients in the control group.

Baseline demographic and clinical characteristics of the two groups were similar. 87% of patients had a cerebral infarction or transient ischaemic attack, 11% had cerebral haemorrhage and 2% had an alternative diagnosis.

Preventive ceftriaxone did not improve functional outcome: distribution of scores on the mRS were not significantly shifted: adjusted common OR 0.94 (95%CI 0.82 – 1.09; $p = 0.41$). In the dichotomised analysis of the mRS, also no significant difference in the percentage of patients with an unfavourable outcome was found: 38% in the ceftriaxone group vs 40% in the control group; OR 0.94 (95%CI 0.80 – 1.11; $p = 0.49$). In none of the predefined subgroups, a beneficial effect of ceftriaxone was found; neither was an association found between time to start of prophylactic treatment and outcome.

During hospital stay, 14% of all patients were diagnosed with an infection by the treating physician. According to an expert panel using the algorithms of to the modified Centers for Disease Control and Prevention criteria, 5% of the patients were diagnosed with an infection.

The occurrence of an infection did influence stroke outcome. In a multiple regression analysis, the adjusted OR for an unfavourable outcome after the clinical diagnosis of an infection was 3.48 (95%CI 2.53 – 4.77; $p < 0.0001$). This was 4.37 (95%CI 2.51 – 7.59; $p < 0.0001$) when the diagnosis was based on the criteria by the expert panel. For pneumonia the adjusted OR for unfavourable outcome was 9.64 (95%CI 5.06 – 18.42; $p < 0.0001$) and for urinary tract infection the adjusted OR for unfavourable outcome was 1.86 (95%CI 1.24 – 2.79; $p = 0.003$).

Preventive administration of ceftriaxone did prevent infections: for the diagnosis by the treating physician, the OR was 0.55 (95%CI 0.44 – 0.70; $p < 0.0001$); for the diagnosis by the expert panel, the OR was 0.44 (95%CI 0.30 – 0.65; $p < 0.0001$). It should be noted that this effect was mainly driven by the reduction in urinary tract infections. Rates of pneumonia did not differ between both treatment groups. Total antimicrobial use during hospital stay was higher in the ceftriaxone group than in the control group: 4,979 defined daily doses vs 2,2120 defined daily doses. However, only 904 defined daily doses within the ceftriaxone group were 'rescue medication'; the other 4,075 were study medication.

The use of preventive ceftriaxone did not induce an increased occurrence of adverse events.

We conclude in **Chapter 3** that preventive antimicrobial therapy with ceftriaxone does not improve functional outcome, nor does it reduce mortality or shorten the length of hospital stay. The number of infections, however, was significantly and safely reduced.

The number of infections in PASS was quite low, according to the currently known prevalence of about 30%. This could be explained by the study population and the definitions used for infection: patients with mild strokes (low NIHSS) were also included and the diagnosis of infection could be based upon two definitions; a pragmatic diagnosis by the treating physician or a strict approach by the criteria of the expert panel.

Infections in PASS were associated with a higher odds for poor functional outcome, ceftriaxone was found to decrease the total number of infections; however, ceftriaxone did not affect functional outcome. Several explanations could be opposed. Fist, infections could be a marker or bystander of functional outcome in which antibiotic use might not change the course of the disease. Second, preventive ceftriaxone might not be superior to optimum stroke unit care – if an infection is suspected, treatment might already be initiated early enough. Third, it was found that pneumonia is most strongly associated with outcome and this type of infection was not prevented. It could be possible that ceftriaxone did not optimally prevent pneumonia (e.g. in case of a *Staphylococcal* infection), or that post-stroke pneumonia might not be a bacterial infection but an inflammatory syndrome.

PASS has several limitations. First, the open-label design might have led to detection bias. Physicians were aware of treatment allocation, which could potentially have affected their decisions about diagnostics and non-scheduled treatment regarding suspected infections. This was tried to be solved by reconsidering the diagnosis of infection by the expert panel, however, also in that case only patients were assessed in which previously an infection was suspected by the treating physician. Second, a low infection rate was observed which might have led to an underestimation of the effect. A sub-analysis in patients with severe stroke, however, also did not show a beneficial effect.

In conclusion, PASS does not support the use of preventive ceftriaxone in adults with acute stroke. Even in the predefined subgroups (including age, stroke type and stroke severity), no clues were found for patient populations that might benefit from this treatment… or were there?!

Do patients treated with intravenous thrombolysis benefit from preventive ceftriaxone?

Although derived from a post-hoc analysis, one subgroup was found that indeed did benefit from preventive ceftriaxone: patients who received intravenous thrombolysis (IVT) with alteplase. In **Chapter 4** we look at this group in more detail. Since the finding originates from an unplanned post-hoc analysis, interpretation should be taken with extreme care. Aim of the chapter was to further investigate this finding, to conclude if the observed effect is a finding by chance, or if a subgroup has been found that could potentially become the study population in future trials.

Different analyses were performed to critically interpret the observed beneficial treatment effect. First, the interaction analysis was reperformed (‘Is IVT an effect modificator?’). Next, we searched for possible confounding factors regarding baseline characteristics and outcome parameters that could have led to a false positive result. We both compared ‘IVT treated patients’ with ‘non-IVT treated patients’ as well as patients within the IVT-treated group according to treatment allocation. Finally, individual confounders were compared that could influence functional outcome.

Regarding the interaction analysis, a dichotomous analysis in PASS shows that – in patients who received IVT with alteplase – unfavourable outcomes occurred significantly less often in patients who received ceftriaxone (33%) as compared with patients from the control group (40%), with an adjusted common OR of 0.77 (95%CI 0.61 – 0.99; $p = 0.04$). Including all patients, the p value for the interaction between IVT with alteplase and preventive ceftriaxone on functional outcome was 0.03.

When comparing IVT treated patients (n = 836) with non-IVT treated patients (n = 1,433), no explanatory differences were found regarding baseline characteristics or outcome parameters. The same accounts for a comparison regarding treatment allocation within the IVT treated patients: ceftriaxone-treated patients (n = 437) vs patients from the control group (n = 399).

Preventive ceftriaxone was associated with a significant beneficial distribution of scores on the mRS: unadjusted common OR 0.75 (95%CI 0.59 – 0.96; p = 0.02), which remained after predefined adjustments, as well as after extra adjustments for small differences in baseline characteristics: OR 0.74 (95%CI 0.55 – 0.97; p = 0.02). Mortality did not significantly decrease after discharge: OR 0.83 (95%CI 0.45 – 1.52), or after 3 months: OR 0.75 (95%CI 0.48 – 1.18).

Among the IVT treated patients, patients in the ceftriaxone group were significantly less often diagnosed with an infection as compared with the control group (8% vs 17%; OR 0.43; 95%CI 0.28 – 0.66). Again, this was mainly driven by a reduction in urinary tract infections (3% vs 7%; OR 0.34; 95%CI 0.17 – 0.70) whereas pneumonia was not significantly reduced (4% vs 7%; OR 0.63; 95%CI 0.34 – 1.15). Results for infections according to the expert panel were similar.

Symptomatic intracerebral haemorrhage (sICH), a feared complication after IVT, developed in 15 of 836 IVT patients (1.8%) in PASS. Although non-significantly, preventive ceftriaxone was associated with the development of post-IVT-sICH: 2.3% vs 0.8% in the control group: OR 3.09 (95%CI 0.85 – 11.31).

Median time from inclusion until death was 5 days for both the ceftriaxone and the control group. Patients in the ceftriaxone group tended to die significantly more often of post-IVT-sICH (33% vs 9%; p = 0.06), whereas patients in the control group died more often of infection (39% vs 0%; p = 0.002). Other causes of death were equally distributed.

IVT patients received ceftriaxone earlier as compared with non-IVT patients (median 8 vs 12 hours). Timing of administration of ceftriaxone in IVT vs non-IVT patients did not influence stroke outcome after 3 months: aOR 1.00 (95%CI 0.98 – 1.03; p = 0.85).

We conclude **Chapter 4** with the suggestion that preventive antimicrobial therapy with ceftriaxone in IVT treated patients indeed might improve functional outcome, despite a trend towards an increased rate of sICH.

It should be taken into account that current patient population slightly differs from the original population in PASS: patients suffering from cerebral haemorrhage were excluded. Slight differences in baseline characteristics between the original population and current subgroup are possible, since risk factors between intracerebral infarction and haemorrhage differ.

Several possible explanations are available for the observed beneficial effect.

First, the effect could be due to chance since data is derived from a post-hoc observation. However, current treatment effect remains present after adjustment for multiple possible confounders and might therefore be genuinely caused by the treatment effect.

Second, as compared with the total population in PASS, the absolute risk reduction regarding the occurrence of infections in current subpopulation is slightly higher. This accounts for the overall number of infections, but is even more pronounced for pneumonia. It seems unlikely, however, that only the increased absolute risk reductions would explain the observed treatment effect; in that case, more subgroup analyses within PASS would have shown beneficial effect of the intervention.

Third, a possible interaction between ceftriaxone and alteplase might enlarge the effect of both drugs. The observed trend towards an increased post-IVT-sICH rate might therefore be an indication.

Fourth, ceftriaxone has been shown to have neuroprotective properties. When ceftriaxone and alteplase show an interaction regarding neuroprotection, this might explain the beneficial effect within IVT-treated patients.

In summary, this post-hoc analysis might have indicated a subgroup of patients that benefits from preventive ceftriaxone. Setting and study design, however, are too limited to draw valid and reliable conclusions. This chapter provides a hypothesis for future research, starting with a similar subgroup analysis in other trials or a novel meta-analysis regarding this subgroup.

Preventive antibiotics in a broader perspective: a Cochrane meta-analysis

After the completion of PASS and the subsequent publication of two other large clinical trials, we performed a Cochrane meta-analysis with the aim to, for the first time, draw conclusions regarding the effect of preventive antibiotic therapy on functional outcome in addition to their effect on the incidence of post-stroke infections. Results were presented in **Chapter 5**.

We included patients with either ischaemic or haemorrhagic stroke. The intervention was preventive antibiotic treatment, at any dose or length of treatment, starting after stroke onset.

Primary outcome was the functional status of the patient at the end of follow-up. Two outcome parameters were used: case fatality (mRS 6) and 'poor functional outcome', defined as death or dependency (preferably mRS $\geq$ 3). Secondary outcomes included the occurrence of 'any' infection (called 'overall' infections to make a distinction with (sub)types of infection), the occurrence of urinary tract infections, the occurrence of pneumonia, and the occurrence of adverse events likely to be related to antibiotic therapy.

We searched in MEDLINE, Embase, CENTRAL and different trials and research registers. Selection of studies and data extraction were performed by two review authors independently. Discrepancies were discussed and resolved in a consensus meeting with a third review author. Methodological quality and risk of bias of included studies were assessed by another independent author.

After screening 4,024 records, 8 studies were found to be eligible for quantitative synthesis. Together, these studies included 4,488 patients: 2,230 patients were randomised to preventive antibiotic therapy and 2,258 patients were randomised to control groups. Three studies included both patients with ischaemic or haemorrhagic stroke, the other five studies included only patients with ischaemic stroke.

Substantial heterogeneity was found between the studies: study intervention differed, as did route of administration, timing of inclusion, duration of antibiotic therapy and reporting of outcome parameters.

Preventive antibiotic therapy did not reduce mortality rate: 17% in the preventive antibiotics group vs 16% in the control group, with a relative risk (RR) of 1.03 (95%CI 0.87 – 1.21).

The number of patients with a poor functional outcome (death or dependency) was also not significantly reduced: 53% in the preventive antibiotics group vs 55% in the control group; RR 0.99 (95%CI 0.89 – 1.10).

The number of patients with any 'overall' infection at the end of follow-up was significantly reduced by preventive antibiotics: 19% vs 26% in the control group; RR 0.71 (95%CI 0.58 – 0.88). Regarding type of infection, urinary tract infections were highly significantly reduced (4% in the antibiotics group vs 10% in the control group; RR 0.40 (95%CI 0.32 – 0.51)), whereas the occurrence of pneumonia did not differ between both treatment groups (10% in the antibiotics group vs 11% in the control group; RR 0.96 (95%CI 0.80 – 1.13)).

Data regarding the occurrence of elevated body temperature and length of hospital stay were too limited to be pooled. No major side effects of preventive antibiotic therapy were reported.

A subgroup analysis of the studies in which antibiotic treatment was left to local guidelines, aiming to prevent pneumonia, also did not show a beneficial effect of antibiotic therapy: neither for the prevention of pneumonia, nor for lowering mortality or improving functional outcome.

We conclude in **Chapter 5** that preventive antibiotic therapy in patients with ischaemic or haemorrhagic stroke does not reduce the risk of dependency and/or death. However, the intervention does significantly reduce the occurrence of 'overall' infections from 26% to 19%. Regarding type of infection, this is highly significant for urinary tract infections (4% vs 10%), whereas no effect on pneumonia is found (10% vs 11%). No major side effects are reported.

The almost nine-fold increase in study population, as compared to a former version of the meta-analysis, results in a neutralisation of the former heterogeneity between studies, making it possible to draw first 'overall' conclusions on the effect of preventive antibiotics on functional outcome and mortality. It remains impossible to draw conclusions regarding specific types of antibiotic regimens.

The decision of whether or not to use preventive antibiotic therapy in acute stroke should still be made with care owing to various factors.

First, study design was heterogeneous and the total of eight studies is still limited.

Second, only four of the eight studies showed an overall 'low' risk of bias. Several biases may have influenced the results. (1) Selection bias may have resulted in an overestimation of the effects of preventive antibiotic therapy: case fatality rates were relatively low in all included studies. Less effect could be expected in patients with a high a priori case fatality risk. On the other hand, severely affected patients might benefit the most from preventive antibiotic therapy, since stroke severity has been reported as a risk factor for post-stroke infection. (2) Detection bias might influence results when outcome is not assessed blindly. Conduct of a study on preventive antibiotic therapy might have increased the use of antibiotic therapy in the control group, which could lead to an overestimation of a possible effect: even more patients in the control group are diagnosed with an infection, whereas the infection rate remains the same in the intervention group. Other outcome parameters are less vulnerable to detection bias: mortality is hard endpoint, functional outcome on the mRS might be overcome by blinding of the endpoint. (3) Attrition bias can occur when trial participants are withdrawn after randomisation. Increased side effects in one study arm, for example, may lead to the exclusion of participants owing to their inability to complete the course, introducing bias in favour of one study arm. (4) We tried to prevent potential biases in the review process by using multiple overlapping searches of various databases, as well as completely reperforming the selection of studies instead of updating the selection from a former version of the meta-analysis.

After current meta-analysis, the former suspicion that preventive antibiotic therapy might not affect functional outcome or mortality may be considered proven: as a result of the increased number of participants, power of the study has increased, confidence intervals have all become smaller and shifted towards no effect. Is the end of research on preventive antibiotics in stroke? This conclusion may yet be drawn too fast.

Implications for daily practice and future perspectives

In **Chapter 6**, we first provide an overview of current insights on post-stroke infections. The key points are also listed at the beginning of current chapter. The second half of the chapter starts with a historic overview of research on preventive antibiotic therapy in stroke. We end the chapter with implications for daily practice, as well as future directions regarding research on preventive antibiotic therapy in stroke. Its content will be used as a base for the implications and future directions mentioned below.

Implications for daily practice

After the completion of PASS, and especially after having performed a large meta-analysis, we can now conclude that – despite the heterogeneity between studies – overall, preventive antibiotic therapy in the acute phase of stroke does not affect functional outcome or mortality and should therefore not be applied in standard stroke care. The risk of 'any' post-stroke infection is significantly reduced and its use appears to be safe, however, this does currently not warrant its use.

We consider it unlikely that possible future trials regarding this subject will change the advice: over time, confidence-intervals on this subject have all become smaller and effect sizes on functional outcome and mortality have shifted towards no effect.

Future directions

The conclusion that 'overall' preventive antibiotic therapy should not be applied in standard care, does not mean that research regarding this subject should now be considered finished. In the course of this thesis, different findings warrant further research. This accounts for insights on preventive antibiotic therapy, as well as the initiation of potential new research fields.

Preventive antibiotic therapy in stroke

Current implications for daily practice are based upon the use of 'any' type of antibiotic, in 'any' patient with stroke. Data is still too limited to consider specific antibiotic regimens or subgroups of patients.

Antibiotic therapy in most studies focussed on the prevention of post-stroke infections. Indeed, two studies even left the choice of the antibiotic to the treating physician, aiming to use the best type of antibiotic for that specific geographical region, covering the most familiar pathogens. Still, functional outcome was not significantly improved. It is considered unlikely that this strategy will prove to be effective in the future.

Some studies have evaluated the neuroprotective properties of specific antibiotics. However, there is currently only one clinical trial that fully aims on the neuroprotective effect and that does emphatically not try to adequately cover the most common organisms in post-stroke infection. In this study, the neuroprotective property of minocycline was investigated.[14] Its hypothesis was based on the finding that in animal stroke models, minocycline increased neuronal survival from 10.5 to 77%, and in another study the infarct area was reduced between 63 and 76%; probably by a reduction of microglia activation, inhibition of pro-inflammatory cytokines and inhibition of apoptotic cell death. In current trial, 152 patients were randomised to receive either minocycline 200mg/day or placebo, initiated within 6 to 24h after the onset of the symptoms. Neurologic deficits on the NIHSS and functional outcome on the mRS after 90 days appeared to be significantly lower in minocycline-treated patients. This trial has also been included in the meta-analysis of Chapter 5. However, potential beneficial effects due to neuroprotection (of any type of antibiotic) may have been neutralised by the other studies aiming to prevent infection.

Apart from minocycline, a recent retrospective pooled analysis has also suggested a potential beneficial effect on functional outcome of macrolide antibiotics, however, data is still limited: macrolides were only used in a minority of post-stroke infections and were mainly used as combination therapy with other drugs.[15]

For other groups of antibiotics, a potential beneficial neuroprotective effect has only been shown in animal models. For beta-lactam antibiotics, including ceftriaxone, a neuroprotective effect was considered via increase in glutamate transporter function – which was clearly not reproduced in PASS.[16] Other antibiotics that were considered to have a potential neuroprotective action in animal models are: azithromycin, erythromycin, rapamycin, tetracycline and doxycycline.[17-21]

Future research for different classes of antibiotics are necessary. For minocycline, trials with larger patient populations might show if the beneficial effect following stroke is reproducible; data from animal models keep showing its neuroprotective properties as well as its reductive effect on infarct size. Data regarding macrolides look promising, but can currently only be considered a first indication warranting new clinical trials. For the other antibiotics with a potential neuroprotective effect, data is still limited and reproducible results are necessary before translation to human research.

We state that 'Preventive antibiotic therapy following stroke has no beneficial effect and should therefore not be applied in standard care'; however, this does not yet exclude that there might be specific subgroups of patients that do benefit from this therapy.

As shown in Chapter 4, in an unplanned post-hoc analysis of PASS we found that preventive ceftriaxone following IVT indeed might diminish the number of infections and improve functional outcome. However, data is based on a single observation, could still be a finding by chance and study design is not suitable for definitive conclusions. We were able to exclude potential confounding factors for the observation, indicating that the results might be 'real'; on the other hand, the observation could not be reproduced in another study with a similar design.[22] In this study, however, the choice of antibiotic was left to the treating physician; ceftriaxone was therefore not the standard intervention.

In the largest recent trials, PASS and STROKE-INF, no other (predefined) subgroups were found that benefitted from preventive antibiotic therapy. These analyses included age, stroke severity and stroke type. Numbers of patients are, however, still limited; even to make a distinction between ischaemic and haemorrhagic stroke.

Within the next years, data will increase since one international multicentre trial is still ongoing: the "PREvention of Complications to Improve OUtcome in elderly patients with acute Stroke” (PRECIOUS) study. Here, a targeted 3,800 patients will be randomly allocated in a 2 x 2 x 2 factorial design to ceftriaxone, paracetamol, metoclopramide or any combination of these, or to ‘standard’ treatment. Due to the large study population, power in the meta-analyses will increase for any subgroup analysis, especially for ceftriaxone-treated patients.

The next indispensable step within the ‘preventive antibiotics in stroke’-research field will be to identify the subgroups of patients that are most likely to benefit from preventive antibiotic therapy. Different strategies can be used besides increasing patient numbers (‘the power’) via new trials. Via a prediction rule for post-stroke pneumonia or other post-stroke infections, patients can be identified that are most likely to benefit from preventive antibiotic therapy – given the hypothesis that the prevention of infection improves functional outcome.[23] These rules might be used as an inclusion criterium in future trials. Besides, pooling of individual patient data might identify classes of antibiotics or subgroups of patients that improve stroke outcome in a better way than a meta-analysis does.[15]

In the implications for daily practice, we also state that the use of preventive antibiotics ‘appears to be safe, however, this does currently not warrant its use’. This opinion might change in the future. In a cost-effectiveness study of PASS, three months after stroke, the number of quality-adjusted life years was significantly higher in the group of ceftriaxone-treated patients, as compared with the control group. Preventive ceftriaxone indeed proved to be cost-effective.[24] Whether this finding can either only be attributed to ceftriaxone or can be translated to the ‘overall’ use of antibiotics, and whether costs justify the increasing use of antibiotics remains to be investigated. Regarding cost-effectiveness in future trials, safety should gain a lot of attention; especially if functional outcome and mortality are not improved.

The main concern regarding ‘safety’, probably in any antibiotics trial, is the occurrence of bacterial resistance. In none of the participants included in the meta-analysis of Chapter 5, resistance due to the study intervention has been proven. Prophylactic administration of antibiotics is widely applied in the Intensive Care Unit, by means of selective digestive decontamination (SDD) and selective oropharyngeal decontamination (SOD). One of the aims is even to reduce the incidence of ventilator-associated pneumonia, however, also in this field no increased incidence of colonisation or infection with antimicrobial-resistant pathogens was found. Indeed, for all pathogens other than MRSA, even a lower level of antibiotic-resistance was observed.[25] One explanation might be a reduction in the need for therapeutic antimicrobials. With an unchanged (or even decreased) overall use of (therapeutic) antimicrobials due to prophylaxis, there would be no increased antimicrobial selection pressure. Another opposed explanation is that by decreasing the total burden of colonisation with Gram-positive cocci and Gram-negative bacilli, selective decontamination might reduce the total denominator of pathogen, so that even if the proportion of resistant pathogens increases, the incidence of antimicrobials resistant pathogens per patients might still decrease. A third explanation could be that selective decontamination might decrease the burden of bacterial colonisation, and thereby leading to less transmission of pathogens (including antimicrobial-resistant pathogens) in patients. One last, and unwanted, explanation might be that resistance is indeed increased but either due to an insufficient follow-up period, these were not yet detected in the studies, or due to selective reporting in studies (e.g. reporting only a subset of antimicrobial-resistant organisms) these data were not published.[25]

Potential new fields for future research

Apart from insights regarding the use of preventive antibiotics in stroke, findings in this thesis also lead to different new questions that provide implications for future research.

A possible field for further research is post-stroke pneumonia. As shown in Chapter 5, as well as in different other publications[2,26], pneumonia appears to have the strongest association with poor outcome and mortality. The finding that preventive antibiotics are unable to prevent this type of infection, even in the trials in which the choice of antibiotic was left to the local guidelines, might be the key to the explanation for the non-beneficial effect of antibiotics on functional outcome.

The search for an explanation for this finding warrants insight in the background of post-stroke pneumonia. Regarding possible clues, there are two interesting findings.

First, as mentioned in the beginning of this chapter, the causative bacteria in post-stroke pneumonia appear to be different from the bacteria that normally colonise the nasopharynx and oropharynx and that most commonly cause community-acquired pneumonia. A ‘simple’ exacerbation of these pathogens following a combination of swallowing deficits, intubation and an impaired defence mechanism due to post-stroke immunodepression seems therefore unlikely. Different studies found that acute stroke patients have an increased rate of oral colonisation of aerobic Gram-negative bacilli (AGNB), which are not normally part of the oral flora of healthy people.[4,5] They are carried on admission to hospital by a proportion of stroke patients, whilst other acquire them nosocomially.[4] Why is it that stroke patients carry these potentially pathogenic micro-organisms?

A recent meta-analysis regarding bacterial causes of post-stroke pneumonia tried to provide some first hypotheses.[27] After analysis of 15 studies, including 7,986 patients, the meta-analysis confirmed AGNB (*Klebsiella pneumoniae, Escherichia coli,* and *Pseudomonas aeruginosa*) and Gram-positive cocci (*Staphylococcus aureus* and *Streptococcus pneumoniae*) were associated with the majority of pneumonias complicating stroke. The spectrum of identified organisms appeared to be more closely related to hospital-acquired pneumonia than to ventilator-acquired pneumonia or hospitalised community-acquired pneumonia. It might be possible that the variation in identified organisms and their relative contributions to pneumonia is not a clue after all, but merely caused by a broad heterogeneity between the included studies: setting, patient selection, geographical location and timing from stroke onset to sampling.

Second, despite the ‘uncommon’ causative micro-organisms (that should still be adequately treated with broad-spectrum antibiotics), the finding that antibiotics were unable to prevent pneumonia might indicate that stroke-associated pneumonia is not a bacterial infection. In that case, we should speak of a ‘post-stroke pneumonitis’, that could be the result of chemical and immunological alterations. Currently, there is no direct prove for this finding, but there are some indications that support this hypothesis. First, many patients experience swallowing difficulties, reduced consciousness and abnormal cough in the acute phase after stroke. The combination with bed ridden state and potential intubation, makes them susceptible to an ‘aspiration pneumonitis’ in which large volumes of gastric contents can be aspirated, resulting in a chemical burn of the tracheobronchial tree and pulmonary parenchyma, causing a local intense inflammatory reaction.[3] On the other hand, use of acid-suppressive medication (including proton pump inhibitors and histamine-2 receptor antagonists) in the acute phase after stroke appears to increase the odds for pneumonia.[28,29] Second, in an earlier mentioned retrospective pooled analysis macrolides appeared to have a beneficial effect on functional outcome, also in a secondary analysis regarding patients with pneumonia; it seems unlikely that its beneficial effect would be explained by a reduction in infections, since this type of antibiotic is mainly active against Gram-positive organism which appear to be less common in post-stroke pneumonia.[15] Animal models have shown an immune-modulatory effect that might either facilitate host immune responses to bacterial challenge, or limit excessive inflammation including in the lung.[15] The beneficial effect of macrolides might therefore indicate the ‘pneumonitis’-theory.

A possible second new field for future research is immunological alteration: if the occurrence of a post-stroke pneumonitis appears to be the cause of a poor outcome and infections are only a marker of stroke severity (instead of a factor influencing outcome-effect), immune modulation might be the target to improve outcome, e.g. by limiting excessive inflammation in the lung. Within the next few years, we might need to shift our attention from the effect of preventive antibiotics on infection rate, towards the effect of stroke on the immune system. With the aim to find potential treatment new targets, a first step within this research field would be to gain more insight in stroke-induced immunodepression. Direct (total systemic) suppression of the immune system with the aim to improve functional outcome seems impossible due to the increased susceptibility to infections following stroke. Indeed, in a double-blind randomised trial regarding the efficacy and safety of the murine monoclonal antibody ‘enlimomab’, with the aim to reduce inflammation, the number of adverse events, including infections and fever, significantly increased and functional outcome was significantly worse. Pneumonia within the first 5 days occurred almost five times more frequently.[30] Although the next therapeutic opportunity might become immunological alteration, current thesis proves that this field should at least be approached with care…

Conclusion

Infections are a common complication after stroke, especially pneumonia and urinary tract infections. Their effect on functional outcome remains uncertain: most literature report a negative outcome effect; a minority considers them a marker of stroke severity. Compared with placebo or standard care, preventive antibiotic therapy neither has a beneficial effect on functional outcome, nor on mortality. The 'overall' number of infections, as well as the amount of urinary tract infections are reduced; however, no preventive effect for pneumonia is found. Its use appears to be safe.

Future research should either study potential subclasses of antibiotics that might have a beneficial outcome effect, or subtypes of patients that might benefit from this therapy. Besides, new fields of research have arisen, including the background of post-stroke pneumonia or pneumonitis, as well as the background of stroke-induced immunodepression and the potential subsequent beneficial effect of immune modulation.

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Appendix

Nederlandse samenvatting

Hoofdstuk 1 betreft de inleiding. Beroertes komen wereldwijd veel voor. In 2017 waren er in Nederland 477.800 personen die ooit een beroerte hebben doorgemaakt; bij 38.000 personen was dit in het afgelopen jaar. Bijna 3% van de Nederlandse populatie ouder dan 12 jaar heeft ooit een beroerte doorgemaakt.

Het begrip 'beroerte' betreft een verzamelterm. Ongeveer 85% van de beroertes wordt veroorzaakt door een afsluiting van een slagader. Men spreekt dan van een herseninfarct of, wanneer de klachten korter dan 24 uur duren en geheel herstellen, een 'transient ischaemic attack' (TIA). Ongeveer 10% van de beroertes wordt veroorzaakt door een bloedvatscheur. In dat geval spreekt men van een hersenbloeding. De laatste 5% omvat een restgroep van alternatieve oorzaken, waarvan een bloeding vanuit een aneurysma (de subarachnoïdale bloeding) en een afsluiting van een ader (de sinustrombose) het meest voorkomen.

Ongeacht de oorzaak, is het gevolg van een beroerte dat een gedeelte van de hersenen onvoldoende- of geen zuurstof krijgt. Dit resulteert in zenuwuitval en is aan een patiënt te herkennen doordat er acuut klachten ontstaan. Kenmerkend zijn 'negatieve symptomen', ofwel het verlies van hersenfuncties. Dit kan onder andere plaatsvinden op het gebied van de kracht, het gevoel, de spraak of het zicht. De ernst van de uitvalsverschijnselen kan worden gescoord op de 'National Institutes of Health Stroke Scale' (NIHSS, zie Appendix) of, in geval van bewustzijnsverlies, op de 'Glasgow Coma Scale' (GCS, zie Appendix).

Van alle patiënten met een doorgemaakt herseninfarct is na één maand ongeveer 15% overleden; na een jaar betreft dit 25% van de patiënten. Van de patiënten met een hersenbloeding is na een jaar ongeveer 55% overleden. Circa 40% van de patiënten die een beroerte overleven zijn tussen 1 maand en 5 jaar na het voorval (tenminste deels) zorgafhankelijk. Prognose na een beroerte wordt in studieverband vaak uitgedrukt in het sterftecijfer (de mortaliteit) of in de functionele uitkomst, gescoord op de 'modified Rankin Scale' (mRS, zie Appendix). Deze schaal loopt van 0 ('geen symptomen') tot en met 6 ('overleden'). Vaak wordt de score gesplitst in een 'gunstige uitkomst' (mRS tussen 0 en 2 – patiënten hebben slechts lichte uitval en zijn niet afhankelijk van hulp van anderen) of een 'ongunstige uitkomst' (mRS tussen 3 en 6 – patiënten zijn tenminste deels zorgafhankelijk of overleden).

De behandelmogelijkheden na een beroerte kunnen worden onderverdeeld in acute behandeling, preventie van complicaties en verlaging van de recidiefkans. Acute behandelmogelijkheden bestaan vooralsnog enkel voor het herseninfarct. Dit omvat ofwel het via een ader toedienen van een hele sterke bloedverdunner om te pogen de bloedprop die de bloedvatafsluiting veroorzaakt op te lossen (trombolyse), ofwel het actief verwijderen van de bloedprop met een katheter die vanuit de lies via de bloedvaten wordt opgevoerd naar het afgesloten bloedvat in de hersenen (intra-arteriële trombectomie). Hoewel voor beide therapieën geldt dat na een geslaagde ingreep de uitkomst van een herseninfarct duidelijk beter is, komt slechts een zeer klein percentage voor deze behandelingen in aanmerking: 12-15% van de patiënten met een herseninfarct komt in aanmerking voor trombolyse en zelfs maar 3-5% van deze patiënten komt in aanmerking voor intra-arteriële trombectomie (11-18% van alle patiënten, indien ze binnen 6 uur op de spoedeisende hulp presenteren). Voor hersenbloedingen zijn er geen acute behandelmogelijkheden, enkel ingrepen om overlijden te voorkomen.

Om te pogen het ziektebeloop en de functionele uitkomst van alle patiënten met een beroerte te verbeteren (zowel na een herseninfarct als na een hersenbloeding) zijn andere strategieën noodzakelijk en zal de aandacht gericht moeten worden op interventies tijdens- en na de ziekenhuisopname. Complicaties tijdens de opname kunnen de opnameduur, de kans op revalidatie en de functionele uitkomst sterk negatief beïnvloeden. Beïnvloedbare factoren op dit gebied zijn onder andere: hyperglykemie, hyperthermie, blaasretenties, diepe veneuze trombose, hersenoedeem en epileptische aanvallen. Daarnaast hebben patiënten baat bij 'secundaire preventie': het verlagen van de kans op een recidief in de toekomst. Dit kan worden bewerkstelligd door het regelmatig controleren (en zo nodig tijdig behandelen) van vasculaire risicofactoren, zoals: hypertensie, hypercholesterolemie en diabetes mellitus.

Het optreden van een infectie is een complicatie die de eerste dagen na een beroerte bij circa 30% van de patiënten optreedt. Dit betreffen met name longontstekingen (de pneumonie) en blaasontstekingen (de urineweginfectie). Beiden ontwikkelen bij ongeveer 10% van de patiënten. Wanneer patiënten op de intensive care worden opgenomen ontwikkelt zelfs 45% een infectie. Verschillende studies onderzochten het effect van het optreden van een infectie op de prognose van een beroerte. De meerderheid concludeert dat het optreden van deze complicatie geassocieerd is met een slechtere functionele uitkomst en een hoger sterftecijfer. Het lijkt daarom plausibel dat de uitkomst na een beroerte zal verbeteren wanneer deze veelvoorkomende complicatie kan worden vermeden.

Voorkoming van infecties kan worden bewerkstelligd door het preventief toedienen van een antibioticum. Hiervoor is een antibioticum noodzakelijk dat de frequentst optredende bacteriële verwekkers van een infectie na een beroerte dekt. Daarnaast is van enkele antibiotica beschreven dat ze, ongeacht de werking tegen bacteriën, ook een beschermend effect hebben op de zenuwen, bijvoorbeeld door het bevorderen van hun herstellend vermogen waardoor het definitief beschadigde hersengebied na een beroerte afneemt (een neuroprotectieve werking). Een van de antibiotica die zowel adequaat lijkt te werken tegen de meest voorkomende bacteriële verwekkers na een beroerte, als die potentieel een neuroprotectief effect zou hebben, is het b-lactam antibioticum ceftriaxon. In potentie zou dit antibioticum daarom een gunstig effect hebben op de prognose van alle patiënten met een doorgemaakte beroerte – niet enkel het kleine percentage dat in aanmerking komt voor de acute therapie. Aan de andere kant kan toenemend antibioticagebruik leiden tot toenemende resistentievorming. Voor- en nadelen moeten goed tegen elkaar worden afgewogen.

Doel van deze thesis is om te bepalen of preventief gebruik van antibiotica bij patiënten met een beroerte een effectieve en veilige methode is om de functionele uitkomst te verbeteren.

Hoofdstuk 2 beschrijft het protocol van de 'Preventive Antibiotics in Stroke Study' (PASS). Dit fase III onderzoek met een PROBE-design (prospectief, gerandomiseerd, open-label met een geblindeerd eindpunt) onderzocht of het preventief toedienen van ceftriaxon leidt tot een betere functionele uitkomst na een beroerte. In deze studie werden zowel patiënten met een herseninfarct als met een hersenbloeding geïncludeerd, mits ze bij presentatie nog neurologische uitval hadden (NIHSS ≥ 1) en behandeling binnen 24 uur na het ontstaan van de klachten kon worden gestart. De interventie betrof toediening van ceftriaxon eenmaal per dag 2 gram intraveneus gedurende 4 dagen bovenop de reguliere 'standaard zorg'. De controlegroep kreeg 'standaard zorg'. In beide groepen mocht de behandelend arts besluiten tot (additionele) behandeling met antibiotica wanneer een infectie werd vermoed. Primaire uitkomstmaat was de functionele uitkomst op de mRS. Secundaire uitkomstmaten waren onder andere overlijden bij ontslag en na 3 maanden, incidentie van infecties en het gebruik van antibiotica tijdens opname.

In het oorspronkelijke protocol werd als primaire uitkomstmaat onderscheid gemaakt tussen een 'gunstige uitkomst' (mRS 0 tot 2) en een 'ongunstige uitkomst' (mRS 3 tot 6). In **hoofdstuk 2.1** beschrijven we een aanpassing van het studieprotocol: deze dichotome uitkomstmaat werd een secundaire analyse; als nieuwe primaire uitkomstmaat werd een ordinale analyse van de mRS gebruikt. Reden hiertoe is een tegenvallende inclusiesnelheid en het steeds gebruikelijker worden van deze laatste analyse: voor dezelfde ondezoeksvraag zijn minder patiënten nodig. In **hoofdstuk 2.2** beschrijven we het statisch analyseplan; we benoemen onder andere de manier van het diagnosticeren van infecties en staan stil bij de vooraf geplande subgroep analyses.

Hoofdstuk 3 presenteert de resultaten van de PASS. In totaal zijn er 2.550 patiënten geïncludeerd, waarvan er 12 op later termijn hun deelname introkken. Voor de analyses zijn 1.268 patiënten gerandomiseerd in de ceftriaxongroep en 1.270 patiënten in de controlegroep. Er waren geen grote verschillen in patiëntkenmerken tussen beide behandelarmen. Preventieve toediening van ceftriaxon had geen effect op de functionele uitkomst na drie maanden. Ook in de vooraf bepaalde subgroepen werd geen populatie gevonden die profiteerde van de interventie. Wel leidde het preventief toedienen van ceftriaxon tot significant minder infecties: zowel wanneer deze gediagnosticeerd werden door de behandelend arts, als wanneer een gestandaardiseerd diagnosemodel gehanteerd werd door een expert panel. Deze afname bleek vooral urineweginfecties te betreffen; er was geen verschil in het optreden van pneumonieën. Het preventief gebruik van ceftriaxon leidde niet tot meer bijwerkingen. Overigens werd opnieuw geconstateerd dat het optreden van infecties geassocieerd was met een slechtere functionele uitkomst.

Er zijn verschillende verklaringen mogelijk voor de neutrale bevindingen van PASS.

Allereerst zou het optreden van infecties slechts een marker kunnen zijn van ziekte-ernst zonder dat ze daadwerkelijk het ziektebeloop beïnvloedt. Daarnaast werden in PASS veel relatief lichte beroertes geïncludeerd, waardoor de frequentie van infecties laag was en het potentiële effect van de interventie op de uikomst daardoor kleiner is. Ook is het denkbaar dat preventieve toediening van ceftriaxon geen meerwaarde heeft boven de optimale beroertezorg in Nederland: wanneer een infectie wordt vermoed, wordt deze snel genoeg onderkend en behandeld. Tot slot is het zo dat in PASS de pneumonieën, die het duidelijkst negatief geassocieerd zijn met functionele uitkomst, niet voorkomen werden. Wellicht werden met ceftriaxon niet de juiste pathogenen gedekt.

Hoofdstuk 4 onderzoekt een subgroep van patiënten die in een post-hoc analyse van de PASS wel een gunstig effect had op preventieve ceftriaxon-toediening: patiënten met een herseninfarct die werden getrombolyseerd met alteplase. Gezien dit een post-hoc observatie betrof, dient de interpretatie echter met grote voorzichtigheid te zijn; doel van deze studie was vooral om te bepalen of de observatie reëel kon zijn, danwel een toevalsbevinding betrof.

Patiënten met een herseninfarct die werden getrombolyseerd en die preventief ceftriaxon ontvingen bleken significant minder vaak een 'ongunstige uitkomst' op de mRS te hebben in vergelijking met getrombolyseerde patiënten die geen preventieve ceftriaxon ontvingen. Het aantal overleden patiënten verschilde niet. De observatie bleek niet te worden verklaard door verschillen in patiëntkarakteristieken: zowel niet wanneer getrombolyseerde patienten werden vergeleken met niet-getrombolyseerde patiënten, als wanneer getrombolyseerde patiënten werden vergeleken naar studiearm binnen de PASS. Net als in de totale PASS-populatie was binnen de groep van getrombolyseerde patiënten het aantal infecties en het aantal urineweginfecties lager in de ceftriaxongroep dan in de controlegroep. Het aantal pneumonieën verschilde niet. Getrombolyseerde patiënten die preventief ceftriaxon ontvingen bleken vaker een hersenbloeding te ontwikkelen dan patiënten uit de controlegroep; zij overleden hier ook vaker aan. Patiënten uit de controlegroep overleden vaker aan een infectie. Andere doodsoorzaken waren min of meer gelijk verdeeld. Getrombolyseerde patiënten ontvingen de preventieve ceftriaxon eerder dan niet-getrombolyseerde patiënten; deze timing bleek echter niet van invloed op de functionele uitkomst na 3 maanden.

We concluderen dat vooralsnog niet verworpen kan worden dat getrombolyseerde patiënten inderdaad een gunstig effect hebben op preventieve toediening van ceftriaxon. De interpretatie dient echter met grote voorzichtigheid te zijn: een post-hoc analyse kan nog altijd puur gebaseerd zijn op toeval. Allereerst dienen de bevindingen bevestigd te worden in soortgelijke studies. Indien de bevinding reëel blijkt, zou ze wellicht verklaard kunnen worden door een interactie tussen ceftriaxon en alteplase waardoor één of beide middelen elkaar versterken. Daarnaast zou de neuroprotectieve werking van ceftriaxon de ongunstige bijwerkingen van alteplase kunnen onderdrukken. Tot slot is de absolute infectie-reductie in de huidge analyse groter ten opzichte van de totale populatie in PASS. Het is echter onwaarschijnlijk dat met dit minimale verschil de huidige observatie verklaard kan worden.

Hoofdstuk 5 beschrijft een systematische review en meta-analyse naar het effect van preventief antibioticagebruik op de functionele uitkomst na een beroerte, evenals op het optreden van infecties. Ten tijde van het schrijven waren er 8 gerandomiseerde studies met in totaal 4.488 geïncludeerde patiënten: 2.230 patiënten ontvingen preventieve antibiotica, 2.258 patiënten zaten in een controlegroep. De studies verschilden van elkaar in type antibiotica, de wijze van toediening, tijdsbestek waarbinnen behandeling gestart diende te worden, de duur van de behandeling en de gerapporteerde uitkomstmaten. In een gepoolde analyse blijkt preventief antibioticagebruik geen effect te hebben op de mortaliteit en ook niet op de kans op een slechte functionele uitkomst. Wel leidt preventief antibioticagebruik tot een afname van het totale aantal infecties. Opnieuw is het aantal urineweginfecties sterk gereduceerd, terwijl het aantal pneumonieën niet verschilt tussen de interventie- en de controlegroep. In geen van de studies werden ernstige bijwerkingen van de antibiotica gerapporteerd. Enkele studies lieten de behandelend arts vrij in de keus van het antibioticum – doel was voorkoming van pneumonieën. Een subanalyse van deze studies toonde opnieuw geen verschil in mortaliteit, functionele uitkomst of het optreden van pneumonieën.

Deze meta-analyse biedt een eerste indruk over het algemene effect van preventieve antibiotica op de functionele uitkomst na een beroerte. Het totale aantal studies omtrent dit onderwerp is echter nog beperkt en de heterogeniteit tussen de verschillende studies is groot. De resultaten wijzen er echter op dat 'overall' preventieve antibiotica geen effect lijken te hebben op de uitkomst na een beroerte.

Hoofdstuk 6 betreft een review over de huidige inzichten omtrent het optreden van infecties na een beroerte enerzijds en het effect van preventieve antibiotica anderzijds.

Er is een grote spreiding tussen verschillende studies rond het percentage van de patiënten dat na een beroerte een infectie ontwikkelt. De beste schatting ligt rond de 30%. Circa 10% van de patiënten ontwikkelt een urineweginfectie, hetzelfde percentage ontwikkelt een pneumonie. Met betrekking tot deze laatste infectie zijn verschillende bacteriële verwekkers gekweekt. Risicofactoren voor het ontwikkelen van een infectie kunnen worden opgedeeld in 'klinische factoren' (patiëntkenmerken en/of factoren gerelateerd aan een ziekenhuisopname), 'anatomische factoren' (locatie en/of grootte van de hersenbeschadiging) en 'immunologische factoren' (een interactie tussen de hersenen en het immuunsysteem van de patiënt). Inzichten in het effect van een infectie op de uitkomst van een beroerte wisselen – een tabel met een overzicht van de conclusies uit verschillende studies is weergegeven als appendix bij het hoofdstuk. De meeste studies concluderen een negatieve relatie tussen het optreden van een infectie en de functionele uitkomst na een beroerte. Dit geldt vooral voor de pneumonie; het effect van een urineweginfectie op het herstel na een beroerte is dubieus. Een minderheid concludeert dat het optreden van een infectie een uiting is van de ernst van de (klinische toestand na een) beroerte, zonder dat ze direct invloed heeft op de functionele uitkomst.

Het tweede deel van het hoofdstuk richt zich op de studies rondom preventieve antibiotica na een beroerte. Allereerst wordt een historisch overzicht gegeven. Aansluitend wordt stilgestaan bij de adviezen voor de dagelijkse praktijk en de implicaties voor toekomstig onderzoek. De inhoud is gebruikt als opzet voor het volgende hoofdstuk van het proefschrift.

Hoofdstuk 7 betreft de aanbevelingen voor de dagelijkse praktijk en de discussie naar aanleiding van de bevindingen in deze thesis. Dit wordt voorafgegaan door een samenvatting van de huidige inzichten omtrent de prevalantie-, de verwekkers-, de risicofactoren- en het effect van infecties op de functionele uitkomst na een beroerte; gevolgd door een samenvatting van de hoofdstukken uit dit proefschrift zoals ook hierboven beschreven.

Preventief gebruik van antibiotica heeft geen bewezen effect op de functionele uitkomst of op de mortaliteit na een beroerte. Wel wordt het risico op infecties verlaagd en lijkt de toepassing veilig. Dit rechtvaardigt het gebruik vooralsnog echter niet. Preventieve antibiotica worden momenteel niet geadviseerd als standaard behandeling in de beroertezorg.

Dit proefschrift biedt aanleiding tot toekomstig onderzoek op het gebied van preventief gebruik van antibiotica na een beroerte, maar biedt daarnaast ook nieuwe onderzoeksvragen.

De huidige adviezen omtrent preventief antibioticagebruik zijn gebaseerd op basis van elk type antibioticum en bij elke patiënt na een doorgemaakte beroerte; de inzichten zouden nog altijd kunnen verschillen voor specifieke subgroepen. Met betrekking tot het type antibioticum richten vrijwel alle studies zich uitsluitend op de indicatie van het voorkomen van infecties. Vooralsnog is er slechts één studie in de meta-analyse geïncludeerd die zich puur richt op het neuroprotectieve aspect van een antibioticum; in dat geval minocycline. Patiënten die dit antibioticum ontvingen hadden een betere functionele uitkomst ondanks dat dit antibioticum niet de juiste verwekkers dekt; de indicatie van neuroprotectie kan in de meta-analyse over preventieve antibiotica echter zijn geneutraliseerd door de andere studies die zich allen richtten op preventie. Ook van enkele andere antibioticatypen wordt een potentieel neuroprotectief effect gesuggereerd. Dit betreffen echter vooralsnog vooral proefdierstudies en zeker geen klinische trials. De gegevens voor wat betreft subgroepen van patiënten die potentieel een betere functionele uitkomst hebben door preventieve antibiotica zijn vooralsnog weinig hoopgevend. Alle vooraf gedefinieerde subgroep-analyses uit de recente grote studies blijken negatief; de post-hoc bevinding met betrekking tot de ‘getrombolyseerde patiënten’ in de PASS bleek niet reproduceerbaar in een andere studie.

Ook kosten-effectiviteit is een gebied dat in de toekomst aandacht verdient. De bevinding dat preventieve toediening van antibiotica leidt tot minder infecties zou op zichzelf al een indicatie voor deze therapie kunnen betekenen - ondanks dat er wellicht geen effect is op de functionele uikomst. Zeker met oog op deze indicatie is alertheid geboden op de veiligheid en eventuele resistentievorming.

Nieuwe onderzoeksvragen naar aanleiding van deze thesis omvatten zowel vragen rond de pneumonie, als vragen omtrent alternatieve behandelmethoden. Hoewel het optreden van een pneumonie het sterkst geassocieerd lijkt met een ongunstige uitkomst, bleek het niet mogelijk om dit type infectie met preventieve antibiotica te voorkomen. Dit zou enerzijds verklaard kunnen worden doordat de daadwerkelijke verwekker niet door het betreffende antibioticum wordt gedekt – in dat geval is meer inzicht noodzakelijk omtrent de bacteriële verwekkers van dit type pneumonie. Anderzijds is het denkbaar dat ten onrechte wordt geconcludeerd dat deze pneumonie een bacteriële infectie betreft. Wellicht is er eerder sprake van een inflammatoire reactie die niet met antibiotica kan worden verholpen. Dit laatste gegeven roept enerzijds nieuwe vragen op rondom de pathofysiologie van de (vermeende) pneumonie na een beroerte, anderzijds is het wellicht een indicatie dat de veranderingen in het immuunsysteem als gevolg van de beroerte wellicht de prognose bepaalt. Deze laatste hypothese impliceert potentiële nieuwe behandelmethoden: het zou denkbaar kunnen zijn dat de focus van toekomstig onderzoek niet meer moet liggen bij antibiotica, maar bij immuunmodulerende therapie.

Conclusie

Infecties zijn een veel voorkomende complicatie na een beroerte; met name de pneumonie en de urineweginfectie. Het effect van een infectie op de functionele uitkomst na een beroerte blijft onduidelijk: de meeste studies rapporteren een negatief effect, enkele studies beschouwen het optreden van een infectie als een uiting van de ernst van de (klinische toestand na een) beroerte zonder dat ze zelf de prognose beïnvloedt. Preventieve toediening van antibiotica na een beroerte heeft geen effect op de functionele uitkomst of het sterftecijfer. Het totale aantal infecties evenals het aantal urineweginfecties wordt er wel door verlaagd. Dit geldt niet voor de pneumonie. Het gebruik blijkt veilig.

Toekomstig onderzoek dient zich zowel te richten op potentiële subklassen van antibiotica die een potentieel gunstig effect op de functionele uitkomst hebben, als op subgroepen van patiënten die wellicht profiteren van deze behandeling. Daarnaast biedt dit proefschrift aanleiding voor nieuwe onderzoeksvragen, waaronder de achtergrond van de pneumonie na een beroerte, evenals de achtergrond van het effect van een beroerte op het immuunsysteem. Dit laatste zou potentiële alternatieve behandelmethoden kunnen impliceren: immuunmodulerende therapie in plaats van preventieve antibiotica.

Appendix

NIHSS, GCS and mRS

National Institutes of Health Stroke Scale (NIHSS)

Category		Score/Description
1a.	Level of Consciousness (LOC)	0 = **Alert;** keenly responsive 1 = **Not alert**; but arousable by minor stimulation to obey, answer or respond 2 = **Not alert**; requires repeated stimulation to attend, or is obtuned and requires strong or painful stimultation to make movements (not stereotyped) 3 = **No response**, only with reflex motor or autonomic effects or totally unresponsive, flaccid, and areflexic
1b.	LOC Questions	0 = **Answers** both questions correctly 1 = **Answers** one question correctly 2 = **Answers** neither question correctly
1c.	LOC Commands	0 = **Performs** both tasks correctly 1 = **Performs** one task correctly 2 = **Performs** neither task correctly
2.	Best Gaze	0 = **Normal** 1 = **Partial gaze palsy**; gaze is abnormal in one or both eyes, but forced deviation or total gaze paresis is not present 2= **Forced deviation**, or total gaze paresis not overcome by the oculocephalic maneuver
3.	Visual	0 = **No visual loss** 1 = **Partial hemianopia** 2 = **Complete hemianopia** 3 = **Bilateral hemianopia** (blind including cortical blindness)
4.	Facial Palsy	0 = **Normal** symmetrical movements 1 = **Minor paralysis** (flattened nasolabial fold, asymmetry on smiling) 2 = **Partial paralysis** (total or near-total paralysis of lower face) 3 = **Complete paralysis** of one or both sides (absence of facial movement in the upper and lower face)
5a. 5b.	Motor Arm Left Motor Arm Right	0 = **No drift**; limb hols 90 (or 45) degrees for full 10 seconds 1 = **Drift**; limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed or other support. 2 = **Some effort against gravity**; limb cannot get to or maintained (if cued) 90 (or 45) degrees, drifts down to bed, but has some effort against gravity. 3 = **No effort against gravity**; limb falls 4 = **No movement** UN = **Amputation** or joint fusion, explain …
6a. 6b.	Motor Leg Left Motor Leg Right	0 = **No drift**; leg hols 30-degree position for full 5 seconds 1 = **Drift**; leg falls by the end of the 5-second period but does not hit bed 2 = **Some effort against gravity**; leg falls to bed by 5 seconds, but has some effort against gravity 3 = **No effort against gravity**; leg falls to bed immediately 4 = **No movement** UN = **Amputation** or joint fusion, explain…
7.	Limb Ataxia	0 = **Absent** 1 = **Present in one limb** 2 = **Present in two limbs** UN = **Amputation** or joint fusion, explain…

8.	Sensory	0 = **Normal**; no sensory loss 1 = **Mild-to-moderate sensory loss**; patient feels pinprick is less sharp or dull on the affected side; or there is a loss of superficial pain with pinprick, but patient is aware of being touched 2 = **Severe to total sensory loss**; patient is not aware of being touched in the face, arm, and leg
9.	Best Language	0 = **No apahisa**; normal 1 = **Milt-to-moderate aphasia**; some obvious loss of fluency or facility of comprehension, without significant limitation on ideas expressed or form of expression. Reduction of speech and/or comprehension, however, makes conversation about provided materials difficult or impossible. 2 = **Severe aphasia**; all communication is through fragmentary expression; great need for inference, questioning, and guessing by the listener. Range of information that can be exchanged is limited; listener carries burden of communication. 3 = **Mute, global apahsia**; no usable speech or auditory comprehension
10.	Dysarthria	0 = **Normal** 1 = **Mild-to-moderate dysarthria**; patient slurs at least some words and, at worst, can be understood with some difficulty. 2 = **Severe dysarthria**; patient's speech is so slurred as to be unintelligible in the absence of or out proportion to any dysphasia, or is mute/anarthric. UN = **Intubated** or other physical barrier, explain…
11.	Extinction and Inattention	0 = **No abnormality** 1 = **Visual, tactile, auditory, spatial, or personal inattention** or extinction to bilateral simultaneous stimulation in one of the sensory modalities 2 = **Profound hemi-inattention or extinction to more than one modality**; does not recognize own hand or orients to only one side of space

Glasgow Coma Scale (GCS)

Eye opening		
4.	Spontaneous	Open before stimulus
3.	To sound	After spoken or shouted request
2.	To pressure	After finger tip stimulus
1.	None	No opening at any time, no interfering factor
NT.	Non testable	Closed by local factor
Best motor response		
6.	Obeys commands	Obey 2-part request
5.	Localising	Brings hand above clavicle to stimulus
4.	Normal flexion	Bends arm at elbow rapidly
3.	Abnormal flexion	Bends arm at elbow, features abnormal
2.	Extension	Extends arm at elbow
1.	None	No movement in arms/legs, no interfering
NT	Non testable	Paralysed or other limiting factor
Verbal response		
5.	Orientated	Correctly gives name, place and date
4.	Confused	Not orientated, but communication coherently
3.	Words	Intelligible single words
2.	Sounds	Only moans / groans
1.	None	No audible response, no interfering factor
NT.	Non testable	Factor interferring with communication

modified Rankin Scale (mRS)

Score	Description
0.	No symptoms at all
1.	No significant disability despite symptoms; able to carry out all usual duties and activities
2.	Slight disability; unable to carry out all previous activities, but able to look after own affairs without assistance
3.	Moderate disability; requiring some help, but able to walk without assistance
4.	Moderatly severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance
5.	Severe disability; bedridden, incontinent and requiring constant nursing care and attention
6.	Dead

List of abbreviations

ACE	antiotensin-converting enzyme
AMC	Academic Medical Centre
BI	Barthell Index
CBF	cerebral blood flow
CDC	Centers for Disease Control and Prevention
CDT	*Clostridium difficile* toxin
CI	confidence interval
CNS	Canadian Neurologic Scale
CONSORT	Consolidated Standards or Reporting Trials
CRF	case record form
CRP	C-reactive protein
CT	computed tomography
DALYs	disability adjusted life years
DDD	defined daily dose
ESBL	extended-spectrum-β-lactamase
GCS	Glasgow Coma Scale
GLT-1	glutamate transporter 1
HPA-axis	hypothalamic-pituitary-adrenal axis
IAT	antra-arterial thrombectomy
IQR	interquartile range
ITT	intention to treat
IVT	intravenous thrombolysis
LOCF	last observation carried forward
MCA	middle cerebral artery
MD	mean difference
MMPs	matrix metalloproteinases
MRI	magnetic resonance imaging
mRS	modified Rankin Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
NA	not applicable
NIHSS	National Institutes of Health Stroke Scale
OR	odds ratio
PASS	Preventive Antibiotics in Stroke Study
PCTus	procalcitonin ultrasensitive
PP	per protocol
PROBE	Prospective, Randomised, Open-label, Blinded End point
QALYs	quality adjusted life years
RCTs	randomised controlled trials

RR	relative risk
rt-PA	recombinant tissue plasminogen activator
SAE	serious adverse event
SAP	statistical analysis plan
SDD	selective digestive decontamination
sICH	symptomatic intracrebral haemorrhage
SOD	selective oropharyngeal decontamination
SUSAR	suspected unexpected serious adverse reactions
TIA	transient ischaemic attack
VAP	ventilator-associated pneumonia

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Research Portfolio

PhD portfolio

Name PhD student: Jan-Dirk Vermeij
PhD period: 2010 – 2019
Name PhD Supervisors: Diederik van de Beek, Paul J. Nederkoorn

	Year	Workload ECTS
Courses		
Advanced Immunology	2012	2.9
Basic Course in legislation and organization for clinical researchers (BROK)	2012	1.0
Basic course hospital management	2016	1.0
Update of the Basic Course in legislation and organization for clinical researchers (BROK)	2017	1.0
Advanced hospital management / Entrepreneurship with a 'medical specialist company' ('medisch specialistisch bedrijf')	2018	1.0
Specific courses		
Exchange to learn the "Transiënt Intraluminal middle cerebral artery occlusion" (MCAO) model in rats and mice. Academic Hospital of Barcelona, August Pi i Sunyer Biomedical Research, Barcelona, Spain. Supervisors: dr. AM. Planas, Prof.dr. A. Chamorro	2011	2.0
Seminars, workshops and master classes		
Weekly meetings with the Center of Infection and Immunity of the Academic Medical Center ('jelly belly')	2010 - 2012	4.0
Weekly journal club or research meeting	2010 - 2018	8.0
Weekly seminars from the neurology department	2010 - 2018	8.0
Weekly meetings with the cerebrovascular working-/research group ('vaatclub'), as well as with the neurologic infectious diseases group	2010 - 2018	8.0

Presentations

Oral Presentation. “Preventive Antibiotics in Stroke Study”. Dutch Neurovascular Working Group. Utrecht, the Netherlands	2011	1.0
Oral Presentation. “Preventive Antibiotics in Stroke Study”. Dutch Neurovascular Working Group. Utrecht, the Netherlands	2012	1.0
Oral Presentation. Yearly update at the “Investigators Meeting of the Preventive Antibiotics in Stroke Study”. Amsterdam, the Netherlands.	2010 - 2014	1.0
Oral Presentation. “Preventief ceftriaxon zou zowel het risico op infectie als functionele uitkomst kunnen verbeteren bij gethrombolyseerde patiënten”. Dutch Neurovascular Working Group. Utrecht, the Netherlands	2015	1.0
Oral Presentation. “Preventive ceftriaxone may improve both infection rate and functional outcome in IVT-treated patients”. 1st European Stroke Organisation Conference. Glasgow, United Kingdom.	2015	1.0
Oral Presentation. “Preventief antibioticagebruik na een beroerte: een meta-analyse.” Scientific Meeting of the Dutch Association of Neurology. Nunspeet, the Netherlands.	2016	1.0
Oral Presentation. “Antibiotic therapy for preventing infections in patients with acute stroke: a meta-analysis”. 2nd European Stroke Organisation Conference. Barcelona, Spain	2016	1.0
Poster Presentation. ‘Preventive Antibiotics in Stroke Study (PASS)’. Scientific Meeting Dutch Association of Neurology. Garderen, the Netherlands.	2011	0.5
Poster Presentation. “Preventive Antibiotics in Stroke Study – study progress”. 20th European Stroke Conference. Hamburg, Germany.	2011	0.5
Poster Presentation. “Preventive Antibiotics in Stroke Study (PASS)”. Scientific Meeting of the Dutch Association of Neurology. Nunspeet, the Netherlands.	2012	0.5

Poster Presentation. "Preventive Antibiotics in Stroke Study – study progress". 21st European Stroke Conference. Lisbon, Portugal.	2012	0.5
Poster Presentation. "Preventive Antibiotics in Stroke Study – study progress". 22nd European Stroke Conference. London, United Kingdom.	2013	0.5
Poster Presentation. "Preventive Antibiotics in Stroke: effect on functional outcome and infection rate: a Cochrane meta-analysis". Amsterdam Neuroscience annual meeting. Amsterdam, the Netherlands	2017	0.5

(International) conferences and meetings

20th European Stroke Conference, Hamburg, Germany	2011	1.0
21st European Stroke Conference, Lisbon, Portugal	2012	1.0
1st European Stroke Organisation Conference, Glasgow, UK	2015	1.0
2nd European Stroke Organisation Conference, Barcelona, Spain	2016	1.0

Teaching

Bachelor and Master study Medicine

Neuroanatomy course. Department of Anatomy, University of Amsterdam.

Course "the Neurological Physical Examination" both for Bachelor and Master students. University of Amsterdam.

Examination of the "Skillslab test" at the end of the Bachelor course. University of Amsterdam.

Nurse training

Training to become a "regular nurse". Education on different neurological diseases. Amstel Academy, Amsterdam.		
Training to become a "specialised nurse in neurology". Education on different neurological diseases and additional topics. University of Amsterdam.		

Student coaching and mentoring

Bachelor thesis (Medicine)	2011 - 2012	1.0
Author of various guidelines. Department of Neurology of the Academic Medical Center, Amsterdam.	2013 - 2018	1.0

Parameters of esteem

"Neurovascular Science Award 2016" by the Dutch Neurovascular Working Group.	2016

Professional memberships and other activities

Member of the Dutch Neurovascular Working Group	2011 - 2019
Member of the Dutch Association of Neurology	2013 - 2019
Member of the European Stroke Organisation	2015 - 2019
Designer and principal investigator of the "Evaluation of Secondary Prevention of Stroke Study in the Outpatient Clinic" (ESPRESSO study)	2012 - 2014

Publications by the author

International Publications – First Author

Vermeij JD, Winogrodzka A, Trip J, Weber WE. Parkinson's disease, levodopa-use and the risk of melanoma. Parkinsonism & related disorders 2009; 15(8): 551-3.

Vermeij JD, Nederkoorn PJ, Roos YB. Intravenous thrombolytic therapy for acute ischemic stroke. The New England journal of medicine 2011; 365(10): 965-6; author reply 6-7.

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Westendorp WF*, **Vermeij JD***, Zock E, Hooijenga IJ, Kruyt ND, Bosboom HJ, Kwa VI, Weisfelt M, Remmers MJ, ten Houten R, Schreuder AH, Vermeer SE, van Dijk EJ, Dippel DW, Dijkgraaf MG, Spanjaard L, Vermeulen M, van der Poll T, Prins JM, Vermeij FH, Roos YB, Kleyweg RP, Kerkhoff H, Brouwer MC, Zwinderman AH, van de Beek D, Nederkoorn PJ; PASS investigators. The Preventive Antibiotics in Stroke Study (PASS): a pragmatic randomised open-label masked endpoint clinical trial. Lancet 2015; 385(9977): 1519-26.

Vermeij JD, Westendorp WF, Roos YB, Brouwer MC, van de Beek D, Nederkoorn PJ. Preventive Ceftriaxone in Patients with Stroke Treated with Intravenous Thrombolysis: Post Hoc Analysis of the Preventive Antibiotics in Stroke Study. Cerebrovascular diseases 2016; 42(5-6): 361-9.

Vermeij JD, Westendorp WF, Dippel DW, van de Beek D, Nederkoorn PJ. Antibiotic therapy for preventing infections in people with acute stroke. The Cochrane database of systematic reviews 2018; 1: CD008530.

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International Publications – Co-Author

Westendorp WF, Nederkoorn PJ, **Vermeij JD**, Dijkgraaf MG, van de Beek D. Post-stroke infection: a systematic review and meta-analysis. BMC neurology 2011; 11: 110.

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Vermeij JD, Winogrodzka A, Trip J, Weber WEJ. Parkinson, levodopa en het risico op een melanoom. Tijdschrift voor Neurologie en Neurochirurgie. 2008; 5:214-220.

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Book chapter

van der Steen WE, **Vermeij JD**, Marquering HA, van den Berg R, Majoie CB, Nederkoorn PJ. Intracranial Carotid Artery Stenosis Diagnosed with CTA in a Western Population: Predictor for Poor Outcome. Carotid Artery Disease: Risk Factors, Prognosis and Management. Hauppauge NY: Nova Science Publishers Inc.; 2014, pp. 33-48

About the author

Jan-Dirk Vermeij was born on July 30th 1986 in Rotterdam. He grew up in Nuenen, Noord-Brabant.

In 2004, he finished secondary school at the 'Lorentz Casimir Lyceum' in Eindhoven. In the same year he started medical school at Maastricht University. At the end of his first academic year he joined the 'Honours Programme' and started research on 'deep brain stimulation' at the Department of Neurosurgery and Neurosciences (prof.dr. V. Visser-Vandewalle, prof.dr. Y. Temel) and later at the Department of Neurology (dr. A. Winogrodzka, dr. W.E.J.M. Weber) in which he focussed on the clinical aspects of Parkinson's Disease.

After having finished his medical school in 2010, he started a PhD on the use of preventive antibiotics in stroke at the Department of Neurology of the Academic Medical Center in Amsterdam (prof.dr. D. van de Beek, dr. P.J. Nederkoorn).

In 2013 he started his residency Neurology in the same hospital (prof.dr. J. Stam, prof. dr. M. Vermeulen, prof.dr. Y.B. Roos, prof.dr. I.N. van Schaik, dr. J.H.T.M. Koelman). In January 2016, his publication containing the main results of the 'Preventive Antibiotics in Stroke Study' was rewarded the scientific award of the Dutch Neurovascular Working Group. In 2018, he finished his residency at the 'Noordwest Ziekenhuisgroep' in Alkmaar (dr. B.M. van Geel) and continued working there as a neurologist with the main focus on 'acute neurology'.

In March 2019, he moved to Heusden-Zolder, Belgium, where he currently works in the Sint-Franciscusziekenhuis as well as in the 'Centrum voor Neurologie' in Hasselt. Jan-Dirk lives in Bolderberg, Belgium, together with his wife Zsuzsa and his son Philip.

Dankwoord

Dankwoord

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Het complete PASS-team: Imke Hooijenga, onze trial manager; Willeke Westendorp, mede PhD en inmiddels doctor; Annemiek de Jong en Irma Stijnman–Moerman, onze trial verpleegkundigen; en alle studenten die hebben geholpen. Wellicht is het een cliché, maar je beseft je pas hoe bijzonder een samenwerking is geweest op het moment dat deze er niet meer is. De vele uren die wij tussen 2010 en 2015 met elkaar hebben doorgebracht, de verzending van de wijn, alle reizen met onze OV-jaarkaart (‘PASS on Tour’) en de verschillende uitjes – etentjes, wijnproeverijen, ‘PASS in De Kas’, symposia, (inter) nationale congressen enz. – waren uniek. Ik kijk met een verschrikkelijk goed gevoel, een grote glimlach, maar ook met enig gevoel van weemoed terug op de tijd die we samen hebben beleefd. Bij aanvang realiseerden wij ons niet wat een werk wij ons op de hals hadden gehaald, maar ieder had zijn eigen specialiteit en samen waren we in de volle overtuiging dat wij dit enorme project aan konden… en het is ons gelukt!

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Beste Willeke, jij had samen met Diederik, Paul en Imke de eerste stappen gezet toen ik mij in augustus 2010 bij het PASS-team aansloot. Ik kon mij geen betere collega wensen. Volledig terecht was jij het gezicht van de PASS: je zat altijd uitstekend in de materie (zowel wetenschappelijk als organisatorisch) en hebt zowel onderzoekstechnisch als in de afronding van het promotietraject meermaals het voorwerk gedaan, waardoor ik de geijkte paden kon bewandelen. Zonder jouw hulp weet ik niet of dit proefschrift ooit zou zijn afgerond. Ik wil je ongelofelijk bedanken en hoop dat we nog vele jaren, ook met Matthijs en Abel, het goede contact kunnen onderhouden. Zet je wetenschappelijke potentie vooral door; in de discussie staan nog wat suggesties voor nieuwe onderzoeksvragen…

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Alle medewerkers van de deelnemende centra aan de PASS: lokale hoofdonderzoekers, neurologen, arts-assistenten, (onderzoeks-) verpleegkundigen, secretaresses en het ondersteunende personeel. Zonder jullie inzet was het nooit mogelijk geweest de studie – en daarmee dit proefschrift – succesvol te volbrengen. Wij hebben destijds bij onze 'introductiepresentatie' wellicht luchtig gedaan over de hoeveelheid administratie die deelname aan de PASS met zich meebracht; echter, zeker toen ik zelf startte met mijn opleiding tot neuroloog heb ik geleerd dat het niet vanzelfsprekend is om van jullie te verlangen dat jullie naast alle dagelijkse verplichtingen ook patiënten includeren en CRF's nauwkeurig en compleet invullen. Tel daar vele extra's bij op: lokale toetsing bij de METC, overleg met de apotheek rondom medicatieverstrekking, onderhandelen met de radiologie omtrent levering van de beeldvorming, mails vanuit ons trialbureau over missende gegevens etc. – en iedereen die dit dankwoord leest zal begrijpen dat jullie inzet niet vanzelfsprekend is geweest. Veel dank daarvoor!

Leden van de study group en advisory board van de PASS; prof.dr. D.W.J. Dippel, prof. M.G.W. Dijkgraaf, prof. J.M. Prins, dr. L. Spanjaard, prof.dr. T. Van der Poll, dr. F.H. Vermeij, prof.dr. M. Vermeulen en dr. R. De Haan, dank voor jullie inbreng en adviezen bij het tot stand komen van de PASS en de protocol updates.

Members of the Data Safety Monitoring Board; dear prof. G.J. Hankey, prof.dr. A. Algra and prof.dr. M.J.M. Bonten, thank you for participating in this board, the detailed reports of the interim analyses and for maintaining the safety of the PASS.

De leden van de promotiecommissie: prof.dr. Y.B. Roos, prof.dr. W.J. Wiersinga, dr. H.B. van der Worp, prof.dr. M. Vermeulen, prof.dr. C.M.J.E. Vandenbroucke–Grauls en prof. dr. R.M. Van den Berg–Vos. Bedankt voor het zitting nemen in de commissie en het beoordelen van dit manuscript op zijn wetenschappelijke waarde.

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