Preventive antibiotic therapy in acute stroke

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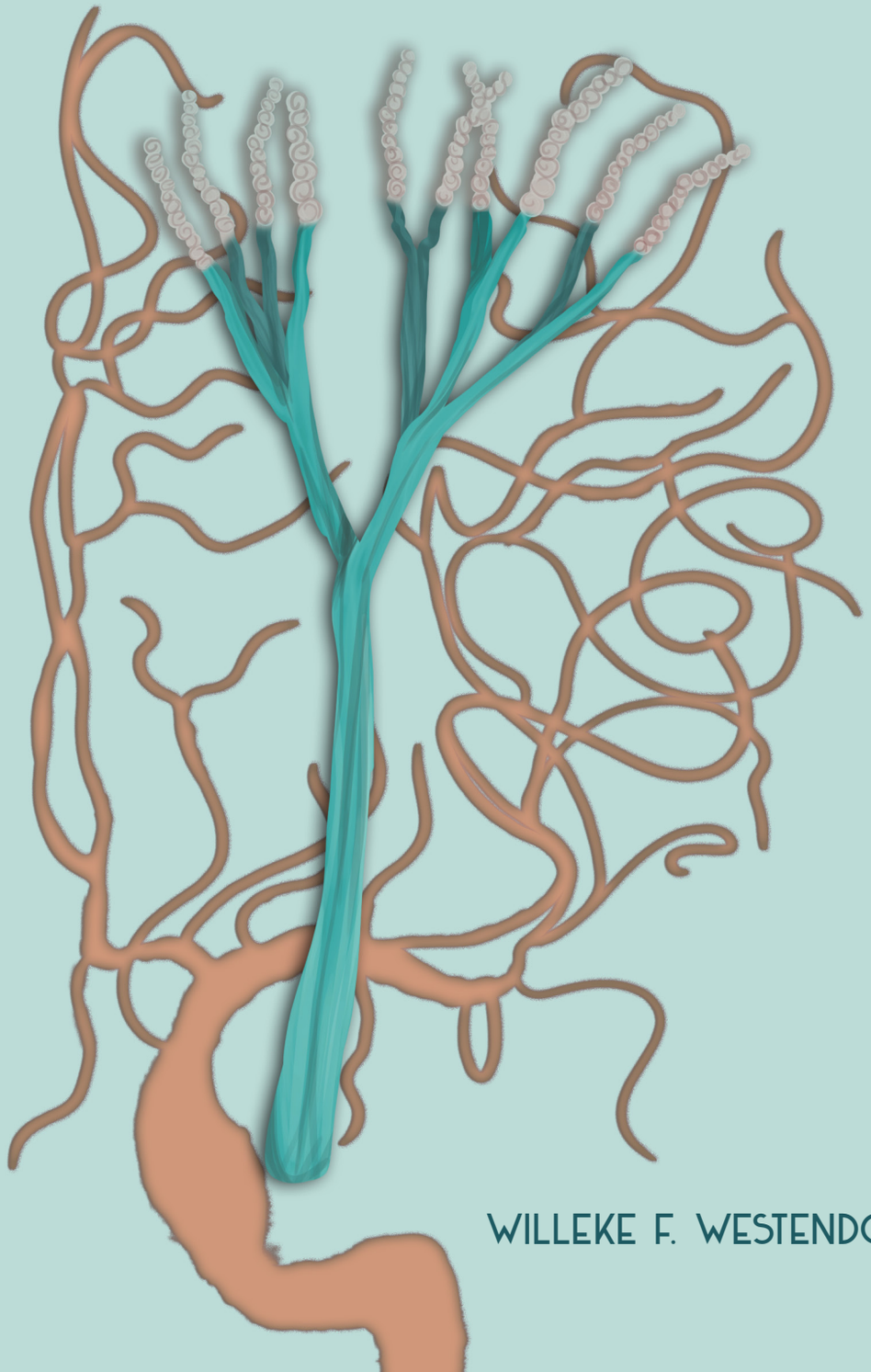

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Willeke F. Westendorp

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PREVENTIVE ANTIBIOTIC THERAPY IN ACUTE STROKE

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Faculteit der Geneeskunde

voor mijn ouders Jan en Riekie

Contents

CHAPTER 1

Introduction

Introduction

Stroke is the second most common cause of death worldwide, accounting for 11.8% of all deaths, and the third most common cause of disability.(1) The absolute number of patients affected by stroke is still increasing due to the aging and growing population. In the Netherlands, there were 41.300 new patients with stroke in 2015, and 53.800 with a TIA. The prevalence of patients who ever had a stroke was 437.100 in 2015.(2)

Ischaemic stroke is caused by an arterial thrombus or occlusion and is responsible for 80% of all strokes.(3) Haemorrhagic stroke accounts for the remaining 20% and is due to rupture of a blood vessel. Stroke causes acute focal deficits, such as speech disturbances (aphasia, dysarthria), weakness of arms and legs, sensory loss, neglect, ataxia, and visual field defects. These deficits can be scored on the National Institute of Health Stroke Scale (NIHSS) to assess stroke severity (Appendix). A few stroke patients have a lowered consciousness, for example patients with a basilar artery occlusion. Consciousness can be scored on the Glasgow Coma Scale (GCS) (Appendix). Both the NIHSS and the GCS are scales often used in stroke studies.

One month after the stroke, about 15% of patients have died, and 40% of the survivors are disabled. One year after ischaemic stroke 25% of patients have died, compared to 55% of patients with haemorrhagic stroke.(4) Prognosis of stroke is mostly expressed in mortality and in functional outcome on the modified Rankin Scale (mRS, Appendix). In general, 'dependency' and 'unfavourable outcome' are defined by a modified Rankin Scale score of 3-6.

Therapeutic strategies in stroke can be subdivided in acute therapies, preventive strategies and treatment or prevention of complications after stroke. In recent years, great progression has been made in the treatment of acute ischaemic stroke. Since 1995 treatment with intravenous thrombolysis was widely adapted by neurologists after the first trial with positive results.(5) A large meta-analysis of 27 thrombolysis studies showed that per 1000 patients, death or dependency was avoided in 41 patients treated with thrombolysis within 6 hours, and 95 patients treated with thrombolysis within 3 hours.(6) Next, treatment of stroke patients in stroke-units for early detection of post-stroke complications has also improved outcome.(7) And very recently, different randomised clinical trials proved the benefit of endovascular treatment of patients with ischaemic stroke (mechanical removal of the thrombus from a blood vessel by thrombus retrieval or disruption, direct intra-arterial thrombolysis, or a combination of both).(8) The number needed to treat for improvement of disability (of at least 1 mRS score) is 2.6 with this treatment. Unfortunately, not all stroke patients are eligible for these therapies. Recanalisation therapies can

only be used for ischaemic stroke within a limited time frame, endovascular therapies are only possible when an arterial occlusion is present. Therefore, there is an urgent need for further research to improve outcome in acute stroke patients.

Another area of stroke research focusses on the prevention of complications in acute stroke patients in order to improve outcome. Directly after the stroke many other risks are present, such as cerebral edema, hypertension, hyperglycemia, fever, and infections. Many of these complications have a strong association with poor functional outcome.

Studies from 1995 and 1996 showed that infections, especially pneumonia and urinary tract infection, are amongst the most common complications in patients with acute stroke.(9, 10) In a Dutch study of 521 acute stroke patients, 78 patients were diagnosed with an infection. Infections occurred more often in patients who were older and had more severe strokes.(11) Also use of urinary catheters or tracheal tubes increase the risk for infection. For pneumonia, a major risk factor is the presence of swallowing difficulties, e.g. dysphagia. In the same study, the patients with infections had poorer outcomes at discharge and at 1 year after stroke than patients without infections (OR 2.6). Pneumonia had the strongest association with poor outcome at 1 year (OR 10).(12) How infections contribute to unfavourable outcome is not certain, but theories include the following: infections could contribute to unfavourable outcome by induction of inflammation or autoimmunity, or by systemic effects like fever, hypotension and hypoxia and deterioration of the clinical condition of a patient. Preventive antibiotic therapy could theoretically reduce infections and possibly improve functional outcome in patients with stroke. In 2010, little evidence was available on the role of preventive antibiotics in stroke.(13)

Preventive antibiotics in acute stroke: aims and outline of this thesis

Aim of this thesis is to investigate whether preventive antibiotic therapy can be an effective strategy to improve functional outcome in acute stroke patients. Another aim of this thesis is to investigate frequency of infections after stroke, outcome after post-stroke infections, risk factors for these infections and possible other preventive strategies to prevent infections after stroke.

In **Chapter 2** we aim to investigate the frequency of infections after stroke. Studies reported varying frequencies of these infections, from 5 to 65%. Frequency seemed to depend on the setting of the studies (ICU vs. non-ICU studies), country, the year of the study and methods of detection of infection (prospective, retrospective studies). In chapter 1 we undertake a large systematic review and meta-analysis to assess the true incidence of infections after stroke and to assess which factors cause the varying frequencies reported. We also report the association with outcome after post-stroke infections.

Chapter 3 describes a systematic review and meta-analysis of all randomised controlled trials (RCTs) on preventive antibiotic therapy in acute stroke, performed until October 2010. Aim of this study was to compare infection rate and the risk of dependency and death at follow-up in patients treated with preventive antibiotic therapy in addition to standard care vs. patients receiving standard care.

We describe the study protocol for a new large randomised clinical trial on preventive antibiotic therapy in acute stroke, the 'Preventive Antibiotics in Stroke Study' in chapter 4. Also in this chapter, we present an update of the trial protocol in this chapter. This update was done to change the primary analysis of primary outcome during the course of the study. Finally, we describe the statistical analysis plan of the trial, which was published before data were unblinded.

In chapter 5 we describe the results of the 'Preventive Antibiotics in Stroke Study (PASS)'. In this study, 2538 stroke patients were randomised to preventive treatment with ceftriaxone 4 grams intravenously in addition to standard care or to standard care alone. We describe the effect of this preventive strategy on the primary outcome, functional outcome on the modified Rankin Scale, as well as secondary outcomes such as infection rate. In chapter 6 we report the cost-effectiveness analysis of the PASS.

In chapter 7 and 8, we focus on future strategies to prevent infection in acute stroke. In chapter 7 we describe an explanatory analysis of the PASS. Previous studies suggested a protective effect of beta-blockers against infection after stroke. In this chapter we aim to investigate whether pre-stroke use of beta-blockers lowers infection rate after stroke. For future studies, it is necessary to be able to select the patients at the highest risk for infection. In chapter 8 we present an internally validated prediction model for infection and pneumonia after stroke. After external validation, this can be used to select the patients at the highest risk, for example for inclusion in trials.

In chapter 10 we give an overview of the characteristics of infection after stroke, such as frequency, causative pathogens and diagnosis of infection. Next, we summarize and discuss the evidence on preventive antibiotic therapy in stroke up until so far, and will compare the results of the PASS with other large trials on preventive antibiotic therapy. We also discuss future directions for research in this area.

References

1. Feigin VL, Norrving B, Mensah GA. Global Burden of Stroke. Circulation research. 2017;120(3):439-48.
2. RIVM. volksgezondheidenzorg.info (2017): https://www.volksgezondheidenzorg.info/onderwerp/beroerte/cijfers-context/huidige-situatie#!node-prevalentie-en-nieuwe-gevallen-van-beroerte. Bilthoven2017.
3. van der Worp HB, van GJ. Clinical practice. Acute ischemic stroke. N Engl J Med. 2007;357(6):572-9.
4. Hankey GJ. Stroke. Lancet. 2017;389(10069):641-54.
5. Tissue plasminogen activator for acute ischemic stroke. The New England journal of medicine. 1995;333(24):1581-7.
6. Wardlaw JM, Murray V, Berge E, del Zoppo GJ. Thrombolysis for acute ischaemic stroke. The Cochrane database of systematic reviews. 2014(7):Cd000213.
7. Organised inpatient (stroke unit) care for stroke. The Cochrane database of systematic reviews. 2013(9):Cd000197.
8. Mokin M, Rojas H, Levy EI. Randomized trials of endovascular therapy for stroke--impact on stroke care. Nature reviews Neurology. 2016;12(2):86-94.
9. Davenport RJ, Dennis MS, Wellwood I, Warlow CP. Complications after acute stroke. Stroke. 1996;27(3):415-20.
10. Kalra L, Yu G, Wilson K, Roots P. Medical complications during stroke rehabilitation. Stroke. 1995;26(6):990-4.
11. Davenport RJ, Dennis MS, Wellwood I, Warlow CP. Complications After Acute Stroke. Stroke. 1996;27(3):415-20.
12. Vermeij FH, Scholte op Reimer WJ, de Man P, van Oostenbrugge RJ, Franke CL, de Jong G, et al. Stroke-associated infection is an independent risk factor for poor outcome after acute ischemic stroke: data from the Netherlands Stroke Survey. Cerebrovasc Dis. 2009;27(5):465-71.
13. van de Beek D, Wijdicks EF, Vermeij FH, de Haan RJ, Prins JM, Spanjaard L, et al. Preventive antibiotics for infections in acute stroke: a systematic review and meta-analysis. Arch Neurol. 2009;66(9):1076-81.

CHAPTER 2

Post-stroke infection: a systematic review and meta-analysis

Willeke F. Westendorp, Paul J. Nederkoorn, Jan-Dirk Vermeij,
Marcel G. Dijkgraaf, Diederik van de Beek

BMC Neurol. 2011;11:110.

Abstract

Background

Stroke is the main cause of disability in high-income countries, and ranks second as a cause of death worldwide. Patients with acute stroke are at risk for infections, but reported post-stroke infection rates vary considerably. We performed a systematic review and meta-analysis to estimate the pooled post-stroke infection rate and its effect on outcome.

Methods

MEDLINE and EMBASE were searched for studies on post-stroke infection. Cohort studies and randomised clinical trials were included when post-stroke infection rate was reported. Rates of infection were pooled after assessment of heterogeneity. Associations between population- and study characteristics and infection rates were quantified. Finally, we reviewed the association between infection and outcome.

Results

87 studies were included involving 137817 patients. 8 studies were restricted to patients admitted on the intensive care unit (ICU). There was significant heterogeneity between studies ($P<0.001$, $I^2=97\%$). The overall pooled infection rate was 30% (24-36%); rates of pneumonia and urinary tract infection were 10% (95% confidence interval [CI] 9-10%) and 10% (95%CI 9-12%). For ICU studies, these rates were substantially higher with 45% (95% CI 38-52%), 28% (95%CI 18-38%) and 20% (95%CI 0-40%). Rates of pneumonia were higher in studies that specifically evaluated infections and in consecutive studies. Studies including older patients or more females reported higher rates of urinary tract infection. Pneumonia was significantly associated with death (odds ratio 3.62 (95%CI 2.80-4.68).

Conclusions

Infection complicated acute stroke in 30% of patients. Rates of pneumonia and urinary tract infection after stroke were 10%. Pneumonia was associated with death. Our study stresses the need to prevent infections in patients with stroke.

Background

Infection is a common complication in the acute phase after stroke. Reported infection rates after stroke vary considerably, ranging 5-65%. Differences in patient populations, study design and definition of infection may account for these large variations in post stroke infection rates.(1) However, a reliable pooled estimate of the infection rate in patients with stroke is lacking. Pneumonia is the most common post-stroke infection and been associated with a relative risk of 3.0 for mortality in a study including 14293 patients with stroke.(2) Consequently, new treatment strategies, *i.e.* preventive antibiotics, are currently under investigation.(3) In this systematic review and meta-analysis we calculated the pooled post-stroke infection rates, identified study and population characteristics associated with infection, and estimated the impact of pneumonia on outcome after stroke.

Methods

Selection of studies

Cohort studies and randomised clinical trials (RCT) on ischaemic or haemorrhagic stroke with reported rates of infections in the acute phase were included. In- and exclusion criteria are listed in additional file table 1. A literature search in MEDLINE (1950 to present) and EMBASE (1980 to present) was performed without language restrictions (additional file table 2). Cross references were checked and experts in the field were consulted. Infection was defined according to the criteria used in included studies. Studies including a small subgroup of patients, *i.e.*, those restricted to patients with dysphagia, were excluded to minimize selection bias. If two publications described one similar patient group data was used only once; this occurred three times.(4-9) If studies reported data of different treatment arms separately, these were also included as separate groups in our analysis.(10, 11) Treatment arms in randomised controlled trials on preventive antibiotic therapy were excluded, since this is likely to influence infection rate and is not part of standard stroke care.(12)

Data extraction

Two independent observers (WFW and JDV) extracted data from selected articles according to predefined definitions. Disagreement was resolved by discussion. Overall infection rate and rate of pneumonia and urinary tract infection – the most common post-stroke infections - were extracted.(13) When percentages were given, these were converted into absolute numbers. We did not calculate an overall infection rate in each study by adding data on different infections, because of the possibility of two infections occurring in one patient.

We also extracted the study characteristics prospective design, consecutive enrollment, type of stroke, study aim on infection and observation period. Population characteristics extracted were income country, age, gender, ICU study, infarction or bleeding, stroke severity, lowered consciousness, dysphagia and urinary incontinence/retention. Definitions can be found in additional file table 3.

Analyses

For each study we calculated the proportion of overall infection, pneumonia and urinary tract infection. Next, these proportions were pooled using Review Manager to obtain one estimate for each infection. A fixed or random effects model was chosen after tests of heterogeneity (heterogeneity was defined with p-value < 0.05). Subgroup analysis was performed on Intensive Care Unit (ICU) *vs.* non-ICU studies. Subsequently, we performed univariate analyses investigating the association between population and study characteristics and the pooled proportion of infection, pneumonia and urinary tract infection. Pearson or Spearman correlation, Students T-Test, Mann-Whitney U Test or 1-way ANOVA were used when appropriate. Characteristics with a p-value < 0.10 were included in a multivariate regression analysis. To transform the proportion of pneumonia and urinary tract infection in a normally distributed variable we used arc sin-square root and square root transformations. Review Manager 5 and SPSS (version 16.0) were used for the statistical analyses.

Results

Included studies

Figure 1 summarizes the study selection process. 87 studies were included involving 137817 patients. 8 studies included patients admitted on the ICU and 79 studies included patients admitted on stroke-unit, medium care facility or ward (non-ICU studies). Reasons for exclusion are noted in additional file table 4. Not all characteristics could be extracted from all studies: age was reported in 77 studies; gender in 80; observation period in 73; stroke severity in 21; lowered consciousness in 25; urinary incontinence/retention in 6; and dysphagia in 26 studies. All extracted data is shown additional file table 5.

Pooled infection rates

Infection rates were evaluated in all 87 studies (Table 1). Pooled values were calculated in a random effects model because of significant heterogeneity between studies (P<0.001, I^2=97%). The overall pooled infection rate was 30% (95%CI 24-36%). The pooled pneumonia rate was 10% (95%CI 9-10%) and of urinary tract infection 10% (95%CI 9-12%). In ICU studies, overall infection rate was 45% (95%CI 38-52%) and rates of pneumonia

and urinary tract infection were 28% (95%CI 18-38%) and 20% (95%CI 0-40%). In non-ICU studies, overall infection rate was 28% (95%CI 22-34%) and rates of pneumonia and urinary tract infection were 9% (95%CI 9-10%) and 10% (95%CI 8-11%).

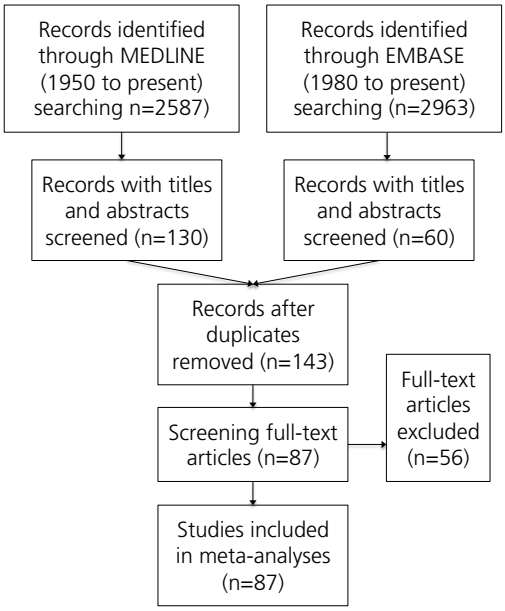

Figure 1. Flowchart of study selection

Table 1. Pooled infection rates in 137817 patients with stroke

	All studies	ICU studies	Non-ICU studies
No. of included studies	87	8	79
No. of evaluated patients	137817	871	136946
Infection rates	**% (95%CI)**	**% (95%CI)**	**% (95%CI)**
All infections	30 (24-36)	45% (38-52%)	28% (22-34%)
Pneumonia	10 (9-10)	28% (18-38%)	9% (9-10%)
Urinary tract infection	10 (9-12)	20% (0-40%)	10% (8-11%)

CI denotes confidence interval

Associations with population- and study characteristics

Associations between population and study characteristics with infection rates are noted in Table 2 and 3. In univariate analysis, infection rate was higher in ICU-studies (P = 0.05) and in studies with a longer observation period (P = 0.03); a trend towards significance was seen for lowered consciousness (P = 0.06). We did not perform multivariate analysis for infection, because few studies reported all 3 variables. In the subgroup of non-ICU studies, an even stronger association was seen for observation period (P = 0.003).

Table 2. Univariate analysis between study or population characteristics and reported infection rates

Study/population characteristic*	No. of studies	No. of evaluated patients	P-value
Study design			
Prospective design	18	9174	0.82
Consecutive enrollment	18	9174	0.42
Study aim on infection	18	9174	0.89
Observation period	17	8844	0.03
Population characteristics			
Income country	18	9174	0.94
Age	18	9174	0.35
Gender	18	9174	0.86
ICU population	18	9174	0.05
Infarction of bleeding	18	9174	0.15
Stroke severity (NIHSS)	5	1818	0.14
Lowered consciousness	6	2365	0.06
Dysphagia	6	395	0.97

*Definitions of characteristics can be found in additional file table 3

The rate of pneumonia was higher in ICU studies (P = 0.001), prospective studies (P = 0.02), studies that specifically evaluated infections (P = 0.004), studies with consecutive enrollment (P = 0.005), studies with a higher stroke severity (P = 0.01) and studies with higher proportions of patients with a lowered consciousness (P = 0.001).

Stroke severity was closely related to ICU-study (Mann-Whitney U, Z -2,154, P = 0.02). In multivariate analysis pneumonia rate was associated with study characteristics: ICU studies (B 0.207, standard error (se) 0.042, P < 0.01), study aim on infection (B 0.062, se 0.026, P = 0.02), consecutive enrollment (B 0.058, se 0.024, P = 0.02). Stroke severity and lowered consciousness were excluded from this multivariate analysis since these characteristics were only reported in a relatively small number of studies. In non-ICU studies, rate of

pneumonia was also higher in studies that specifically evaluated infections (B 0.06, se 0.025, P = 0.02) and studies with consecutive enrollment (B 0.065, se 0.022, P = 0.005) in multivariate analysis. No associations were found between age (P = 0.18) or dysphagia (P = 0.16) and pneumonia rate.

Table 3. Univariate analysis between study or population characteristics and reported pneumonia rates

Study/population characteristic*	No. of studies	No. of evaluated patients	P-value
Study design			
Prospective design	87	137779	0.02
Consecutive enrollment	87	137779	0.005
Study aim on infection	87	137779	0.004
Observation period	73	102280	0.37
Population characteristics			
Income country	87	137779	0.23
Age	77	118755	0.30
Gender	80	132750	0.76
ICU population	87	137779	0.001
Infarction of bleeding	87	137779	0.13
Stroke severity (NIHSS)	20	31493	0.01
Lowered consciousness	25	52939	0.001
Dysphagia	26	17937	0.19

*Definitions of characteristics can be found in additional file table 3

The rate of urinary tract infections was higher in studies with a higher stroke severity (P = 0.01), a lower proportion of male patients (P = 0.005), prospective studies (P = 0.02) and in studies with a longer observation period (P = 0.10). A trend towards significance was seen for studies with a higher mean age (P = 0.08). In multivariate analysis, advanced age was independently associated with urinary tract infection (B 0.008, se 0.004, P = 0.04). Gender was associated with urinary tract infection, but only in a sub analysis of non-ICU studies (B -0,341, se 0.164, P = 0.04).

Outcome

To estimate the effect of post-stroke infection on outcome we pooled the mortality rates in patients with and without infection of the studies reporting these rates. Of all patients with an infection, 48% died vs. 18% of patients without infection (N = 1839; OR 2.08, 95% CI 1.63 - 2.67). Mortality rates were also higher in patients with pneumonia (26% vs. 5%, N = 16433; OR 5.58, 95% CI 4.76, 6.55) and a little higher in patients with urinary tract infection (12% vs. 10%, N = 2528; OR 1.12, 95% CI 0.76, 1.66). Since

this data was not corrected for potential confounders, we also estimated the effect of pneumonia on death by pooling corrected odds ratios (OR) in a random effects model. This meta-analysis included four studies (19971 patients) and resulted in an OR in-hospital mortality of 3.62 (95% CI 2.80-4.68) (Figure 2). Five studies reported corrected OR of pneumonia for unfavourable outcome (Table 4); meta-analysis was not performed because of differences in outcome measure and follow-up. These studies showed an increased risk of unfavourable outcome in post-stroke patients with pneumonia. Urinary tract infection had no association with death according to four studies reporting on this.(14-17) Three studies evaluated the effect on functional outcome for urinary tract infection, two studies showed an association with poor outcome in multivariate analysis.(14, 18, 19) OR's for unfavourable functional outcome were 2.72 (95% CI 4.73-11.84) and 3.1 (95% CI 1.6-5.9).

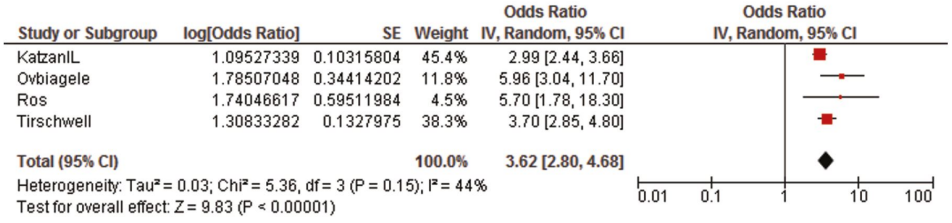

Figure 2. Pooled odds ratios: effect of pneumonia on in-hospital mortality

Table 4. Effect of pneumonia on functional outcome

Study	No. of patients	Outcome (modified Rankin Scale)	OR (95 % CI)	Correction for confounders
Vermeij et al(1)	521	> 2 at discharge > 2 at 1 year	9.5 (1.7-52) 10.95 (2.2-46)	Yes
Hong et al(18)	1254	3-6 at 3 months	4.44 (2.20-8.99)	Yes
Huang et al(20)	66	< 3 at 1 month	0.50 (0.38-0.64)	No
Ovbiagele(16)	663	0-3 at discharge	0.16 (0.09-0.29)	Yes
Aslanyan et al(14)	1455	≥ 2 at 3 months	3.4 (1.4-8.3)	Yes

Discussion

This meta-analysis shows that infections commonly complicate the acute phase after stroke. The pooled overall infection rate was 30% and pneumonia and urinary tract infections occurred each in 10% of patients. Previous studies showed a wide range of post-stroke infection rates, from 5%- 65% for infections, 1%-33% for pneumonia, and 2%-27% for urinary tract infection.(1, 21) This meta-analysis included a large number of patients and therefore provides a reliable estimate of infection after stroke.

We identified several study and population characteristics that were associated with infection rate. Given the prevalence of pneumonia and urinary tract infection in general wards in Dutch hospitals - 1.1% and 1.7% respectively - our findings confirm that infection rate in patients in the acute phase of stroke is high.(22) This increased vulnerability of patients in the acute phase of stroke for infections can be attributed to different factors.

First, infection was associated with study characteristics. Studies aimed on infection and those with consecutive enrolment were associated with higher infection rate. Possibly, these studies benefit of more rigorous detection of infection. Rate of infection was also higher in studies with a longer observation time. In a prospective observational study, most post stroke infections occurred within three days of hospital admission.(1) Nevertheless, 25% of infections occurred after these three days. Also, most urinary tract infections occur after 48 hours, i.e. most UTI's are hospital acquired, and therefore it seems logical that a longer observation time yielded a higher urinary tract infection rate in our study.(17) The absence of an association between observation time and pneumonia is not surprising. Pneumonia is mostly diagnosed within the first days following a stroke, both in studies performed on general wards as well as ICU's.(1, 13, 23-25)

Microbiologic data of patients with post-stroke pneumonia shows a pattern of mostly 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.* were commonly identified; also *Streptococcus species* are occasionally found. Gram-negative bacteria and *Staphylococcus aureus* are known to cause pneumonia by aspiration of endogenous material from the colonized oropharynx.(26, 27) These pathogens are often seen in nosocomial infections.(28) *Streptococcus species* is still the most detected pathogen in community acquired pneumonia.(29) In stroke patients, it could be a cause of 'community acquired aspiration pneumonia', with aspiration occurring at the stroke ictus.(30) Often, no causative organism is detected in post-stroke pneumonia. Yield of cultures is usually not very high in patients with pneumonia; collection of material could be even more difficult in

stroke patients due to neurologic deficit or lowered level of consciousness. Also, some cases of suspected pneumonia could in fact be a non-infectious aspiration pneumonitis, or the infection could be caused by anaerobic bacteria that require special culturing techniques. However, the role of anaerobic bacteria in the development of pneumonia is unclear.(30, 31)

Second, infection rate was associated with the patients' clinical condition. Studies including patients with a higher stroke severity or lower levels of consciousness showed higher infection rates, in particular for pneumonia. This effect corresponds with previous studies that often report both characteristics as risk factors for pneumonia.(14, 32, 33) Risk for aspiration is increased in these patients due to the absence of protective reflexes, and this risk is related to the degree of consciousness impairment.(34) Most stroke related pneumonias are believed to result from dysphagia and subsequent aspiration of oropharyngeal material or gastric content. A systematic review by Martino *et al* showed that dysphagia occurs in 37- 78% of stroke patients and increases the risk for pneumonia 3-fold and 11-fold in patients with confirmed aspiration.(35) However, up to half of patients with post stroke pneumonia do not aspirate, which implies that also other mechanisms are involved, *e.g.*, stroke-induced immunodepression which is discussed below.(2)

We also found higher infection rates in ICU studies. Patients admitted to an ICU generally suffer from more severe strokes and also the frequency of invasive procedures is higher.(15, 25, 33, 36) The use of invasive procedures – i.e. urinary catheterization or mechanical ventilation in ICU patients – increase infection risk by facilitating the entry of a pathogen.(17, 36)

We identified a higher age and female sex as risk factors for urinary tract infection. Both of these characteristics have previously been reported as risk factors for urinary tract infection.(16, 17) Studies frequently report urinary catheterization as an important risk factor; we could not investigate this association because this characteristic was mostly not reported.(17) Advanced age as risk factor for post-stroke infection has been reported previously.(16)

In addition to above mentioned characteristics, acute stroke may lead to stroke-induced immunodepression, a systemic anti-inflammatory response that is related to susceptibility to infection.(21, 37, 38) This anti-inflammatory response was found in different clinical studies in acute stroke patients, and includes an excessive counter-inflammatory cytokine responses and impairments in cell-mediated immunity.(39) Certain features of this response – *i.e.*, reduced lymphocyte count, delay in the recovery of T-lymphocyte loss - were more pronounced in patients developing post stroke infections.(38, 40) These results suggest that immunological changes could facilitate infection in acute stroke.

Outcome is affected by post-stroke infections, as shown in our review. Pneumonia and urinary tract infection both increase the risk for unfavourable outcome and pneumonia is associated with mortality with an OR of 3.62. Infections could affect outcome in several ways. Firstly, they lead to immobilization, general frailty and a delay in rehabilitation due to prolonged hospital stay.(1, 41) More importantly, immunological effects of infections could worsen outcome. Evidence from experimental studies suggests that infection promotes antigen presentation and autoimmunity against the brain.(42) However, the evidence on this topic is scarce and the exact pathofysiology remains to be investigated.

Our systematic review has limitations. First, results are limited by publication bias. Data was derived from randomised controlled trials, cohort studies and stroke registries; infection rate could differ in hospitals without stroke research or complication registries. Second, included studies were heterogeneous in definition of infection, which was based on clinical grounds and in some cases not described. A standardized definition for infection - as described by the Centers for Disease Control and Prevention (43) – is preferred since stricter criteria could permit identification of fewer infections. Next, not all relevant characteristics - for example use of antibiotics, differences in primary stroke care or use of a urinary catheter – could be evaluated, due to lack of data in included studies. Some of these characteristics have previously been described as a risk factor for infection and could have confounded our results. Thirdly, for some characteristics data was lacking in many studies. Surprisingly, no association was found between age or dysphagia and pneumonia rate, often described risk factors for pneumonia.(25, 32, 33, 36, 44, 45) Dysphagia was not reported in all studies, which was a limiting factor in the analysis. Lack of these significant associations could be due to few studies reporting adequate information on all these characteristics and therefore the analyses have low explanatory power. Finally, many of the characteristics act on patient level, but studies report aggregate data (*i.e.*, mean age of study population) also reducing explanatory power. Due to these limitations – most importantly the heterogeneity in definition of infections - results of this review need to be interpreted with caution.

Our review shows the potential of strategies aiming to prevent infections in patients with stroke. Some of these strategies – *i.e.,* prevention of aspiration and reduction of urinary catheterisation – are incorporated in stroke unit care, which reduces the risk of death after stroke through the prevention of infections in particular. (46) Infections can also be prevented by use of preventive antibiotic therapy, as shown in a recent meta-analysis. This meta-analysis did not establish a reduction in mortality, however, included studies were small and heterogeneous and functional outcome was not evaluated. (12) Also, limited data was reported on the effect on antibiotic resistance. Currently, the use of preventive antibiotics in stroke, and the effect of this therapy on antibiotic resistance, is investigated

in a large randomised controlled trial with functional endpoint.(3) This trial will be able to establish whether preventive antibiotic treatment is an effective strategy to prevent infection and its adverse effect on outcome in patients in the acute phase of stroke.

Conclusions

Results of this meta-analysis show an overall infection rate in the acute phase of stroke of 30%. Rates of pneumonia and urinary tract infection were both 10%. Infection rates are related with study characteristics and the patients' clinical condition, *e.g.*, age, gender, stroke severity, level of consciousness and whether a patient is admitted on ICU. Pneumonia is an independent risk factor for unfavourable outcome and death after stroke. Our data stress the need of interventions to prevent infections in patients with stroke.

Competing interests

None

Authors' contributions

WFW participated in the conceptualization and design of the review, performed the selection of studies, data-extraction and –analysis, and drafted the review. PJN and DvdB were involved in the conceptualization and design of the review, and the data analysis. JDV participated in the selection of studies and data-extraction. MGD carried out the statistical analysis and interpretation of data. All authors participated in revising the manuscript and the final approval of the manuscript.

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Reference List

1. Vermeij FH, Scholte op Reimer WJ, de Man P, van Oostenbrugge RJ, Franke CL, de Jong G, et al. Stroke-associated infection is an independent risk factor for poor outcome after acute ischemic stroke: data from the Netherlands Stroke Survey. Cerebrovasc Dis. 2009;27(5):465-71.
2. Katzan IL, Cebul RD, Husak SH, Dawson NV, Baker DW. The effect of pneumonia on mortality among patients hospitalized for acute stroke. Neurology. 2003;60(4):620-5.
3. Nederkoorn PJ, Westendorp WF, Hooijenga IJ, de Haan RJ, Dippel DW, Vermeij FH, et al. Preventive antibiotics in stroke study: rationale and protocol for a randomised trial. Int J Stroke. 2011;6(2):159-63.
4. Dziedzic T, Pera J, Klimkowicz A, Turaj W, Slowik A, Rog TM, et al. Serum albumin level and nosocomial pneumonia in stroke patients. Eur J Neurol. 2006;13(3):299-301.
5. Dziedzic T, Slowik A, Pera J, Szczudlik A. Beta-blockers reduce the risk of early death in ischemic stroke. J Neurol Sci. 2007;252(1):53-6.
6. Lees KR, Zivin JA, Ashwood T, Davalos A, Davis SM, Diener HC, et al. NXY-059 for acute ischemic stroke. The New England journal of medicine. 2006;354(6):588-600.
7. Schwab S, Georgiadis D, Berrouschot J, Schellinger PD, Graffagnino C, Mayer SA. Feasibility and safety of moderate hypothermia after massive hemispheric infarction. Stroke. 2001;32(9):2033-5.
8. Schwab S, Schwarz S, Spranger M, Keller E, Bertram M, Hacke W. Moderate hypothermia in the treatment of patients with severe middle cerebral artery infarction. Stroke. 1998;29(12):2461-6.
9. Shuaib A, Lees KR, Lyden P, Grotta J, Davalos A, Davis SM, et al. NXY-059 for the treatment of acute ischemic stroke. N Engl J Med. 2007;357(6):562-71.
10. Newell SD, Jr., Englert J, Box-Taylor A, Davis KM, Koch KE. Clinical efficiency tools improve stroke management in a rural southern health system. Stroke. 1998;29(6):1092-8.
11. Webb DJ, Fayad PB, Wilbur C, Thomas A, Brass LM. Effects of a specialized team on stroke care. The first two years of the Yale Stroke Program. Stroke. 1995;26(8):1353-7.
12. van de Beek D, Wijdicks EF, Vermeij FH, de Haan RJ, Prins JM, Spanjaard L, et al. Preventive antibiotics for infections in acute stroke: a systematic review and meta-analysis. Arch Neurol. 2009;66(9):1076-81.
13. Davenport RJ, Dennis MS, Wellwood I, Warlow CP. Complications After Acute Stroke. Stroke. 1996;27(3):415-20.
14. Aslanyan S, Weir CJ, Diener HC, Kaste M, Lees KR. Pneumonia and urinary tract infection after acute ischaemic stroke: a tertiary analysis of the GAIN International trial. Eur J Neurol. 2004;11(1):49-53.
15. Harms H, Prass K, Meisel C, Klehmet J, Rogge W, Drenckhahn C, et al. Preventive antibacterial therapy in acute ischemic stroke: a randomized controlled trial. PLoS One. 2008;3(5):e2158.
16. Ovbiagele B, Hills NK, Saver JL, Johnston SC. Frequency and determinants of pneumonia and urinary tract infection during stroke hospitalization. J Stroke Cerebrovasc Dis. 2006;15(5):209-13.
17. Stott DJ, Falconer A, Miller H, Tilston JC, Langhorne P. Urinary tract infection after stroke. QJM. 2009;102(4):243-9.
18. Hong KS, Kang DW, Koo JS, Yu KH, Han MK, Cho YJ, et al. Impact of neurological and medical complications on 3-month outcomes in acute ischaemic stroke. Eur J Neurol. 2008;15(12):1324-31.
19. Pinto AN, Melo TP, Lourenco ME, Leandro MJ, Brazio A, Carvalho L, et al. Can a clinical classification of stroke predict complications and treatments during hospitalization? Cerebrovasc Dis. 1998;8(4):204-9.
20. Huang WY, Weng WC, Chien YY, Wu CL, Peng TI, Chen KH. Predictive factors of outcome and stroke recurrence in patients with unilateral atherosclerosis-related internal carotid artery occlusion. Neurol India. 2008;56(2):173-8.
21. Emsley HC, Hopkins SJ. Acute ischaemic stroke and infection: recent and emerging concepts. Lancet Neurol. 2008;7(4):341-53.
22. van der Kooi TI, Mannien J, Wille JC, van Benthem BH. Prevalence of nosocomial infections in The Netherlands, 2007-2008: results of the first four national studies. The Journal of hospital infection. 2010;75(3):168-72.
23. Georgilis K, Plomaritoglou A, Dafni U, Bassiakos Y, Vemmos K. Aetiology of fever in patients with acute stroke. J Intern Med. 1999;246(2):203-9.
24. Hassan A, Khealani BA, Shafqat S, Aslam M, Salahuddin N, Syed NA, et al. Stroke-associated pneumonia: microbiological data and outcome. Singapore Med J. 2006;47(3):204-7.
25. Hilker R, Poetter C, Findeisen N, Sobesky J, Jacobs A, Neveling M, et al. Nosocomial pneumonia after acute stroke: implications for neurological intensive care medicine. Stroke. 2003;34(4):975-81.
26. Gosney M, Martin MV, Wright AE. The role of selective decontamination of the digestive tract in acute stroke. Age Ageing. 2006;35(1):42-7.

27. Millns B, Gosney M, Jack CI, Martin MV, Wright AE. Acute stroke predisposes to oral gram-negative bacilli -- a cause of aspiration pneumonia? Gerontology. 2003;49(3):173-6.
28. Jones RN. Microbial etiologies of hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2010;51 Suppl 1:S81-7.
29. Bartlett JG. Community-acquired pneumonia. International journal of clinical practice Supplement. 2000(115):18-22.
30. Marik PE. Aspiration pneumonitis and aspiration pneumonia. N Engl J Med. 2001;344(9):665-71.
31. Marik PE, Careau P. The role of anaerobes in patients with ventilator-associated pneumonia and aspiration pneumonia: a prospective study. Chest. 1999;115(1):178-83.
32. Hinchey JA, Shephard T, Furie K, Smith D, Wang D, Tonn S. Formal dysphagia screening protocols prevent pneumonia. Stroke. 2005;36(9):1972-6.
33. Kwon HM, Jeong SW, Lee SH, Yoon BW. The pneumonia score: a simple grading scale for prediction of pneumonia after acute stroke. Am J Infect Control. 2006;34(2):64-8.
34. Adnet F, Baud F. Relation between Glasgow Coma Scale and aspiration pneumonia. Lancet. 1996;348(9020):123-4.
35. Martino R, Foley N, Bhogal S, Diamant N, Speechley M, Teasell R. Dysphagia after stroke: incidence, diagnosis, and pulmonary complications. Stroke. 2005;36(12):2756-63.
36. Walter U, Knoblich R, Steinhagen V, Donat M, Benecke R, Kloth A. Predictors of pneumonia in acute stroke patients admitted to a neurological intensive care unit. J Neurol. 2007;254(10):1323-9.
37. Chamorro A, Amaro S, Vargas M, Obach V, Cervera A, Torres F, et al. Interleukin 10, monocytes and increased risk of early infection in ischaemic stroke. J Neurol Neurosurg Psychiatry. 2006;77(11):1279-81.
38. Haeusler KG, Schmidt WU, Fohring F, Meisel C, Helms T, Jungehulsing GJ, et al. Cellular immunodepression preceding infectious complications after acute ischemic stroke in humans. Cerebrovasc Dis. 2008;25(1-2):50-8.
39. Emsley HC, Smith CJ, Hopkins SJ. Infection and brain-induced immunodepression after acute ischemic stroke. Stroke. 2008;39(1):e7.
40. Vogelgesang A, Grunwald U, Langner S, Jack R, Broker BM, Kessler C, et al. Analysis of lymphocyte subsets in patients with stroke and their influence on infection after stroke. Stroke. 2008;39(1):237-41.
41. Spratt N, Wang Y, Levi C, Ng K, Evans M, Fisher J. A prospective study of predictors of prolonged hospital stay and disability after stroke. J Clin Neurosci. 2003;10(6):665-9.
42. Iadecola C, Anrather J. The immunology of stroke: from mechanisms to translation. Nature medicine. 2011;17(7):796-808.
43. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 2008;36(5):309-32.
44. Kammersgaard LP, Jorgensen HS, Reith J, Nakayama H, Houth JG, Weber UJ, et al. Early infection and prognosis after acute stroke: the Copenhagen Stroke Study. J Stroke Cerebrovasc Dis. 2001;10(5):217-21.
45. Yilmaz GR, Cevik MA, Erdinc FS, Ucler S, Tulek N. The risk factors for infections acquired by cerebral hemorrhage and cerebral infarct patients in a neurology intensive care unit in Turkey. Jpn J Infect Dis. 2007;60(2-3):87-91.
46. Govan L, Langhorne P, Weir CJ. Does the prevention of complications explain the survival benefit of organized inpatient (stroke unit) care?: further analysis of a systematic review. Stroke. 2007;38(9):2536-40.

Additional file

Additional file table 1. In- and exclusion criteria

Inclusion criteria	Exclusion criteria
Adult patients with acute stroke	Rehabilitation setting
Cohort study or Randomised Controlled Trial	Reviews, case reports, studies with < 25 patients
Post stroke infection rate reported	Infection preceding stroke of after discharge
English, German, French, Spanish language Full text available	Study performed solely on subgroup of patients

Additional file table 2. Synonyms for MEDLINE and EMBASE search

Synonyms for MEDLINE Search
(cerebrovascular disorders/ or exp basal ganglia cerebrovascular disease/ or exp brain ischemia/ or stroke/ or exp brain infarction/ or hypoxia-ischemia, brain/ or exp intracranial arterial diseases/ or exp "Intracranial Embolism and Thrombosis"/ or exp intracranial hemorrhages/ or (stroke or poststroke or post-stroke or cerebrovasc$ or brain vasc$ or cerebral vasc$ or cva$ or apoplex$ or isch?emi$ attack$ or tia$1 or neurologic$ deficit$).tw. or ((brain$ or cerebr$ or cerebell$ or cortical or vertebrobasilar or hemispher$ or intracran$ or intracerebral or infratentorial or supratentorial or MCA or anterior circulation or posterior circulation or basal ganglia) adj5 (isch?emi$ or infarct$ or emboli$ or occlus$ or hypox$ or obstruction or vasculopathy)).tw. or ((lacunar or cortical) adj5 infarct$).tw. or ((brain$ or cerebr$ or cerebell$ or intracerebral or intracran$ or parenchymal or intraventricular or infratentorial or supratentorial or basal gangli$ or subarachnoid or putaminal or putamen or posterior fossa) adj5 (haemorrhage$ or hemorrhage$ or haematoma$ or hematoma$ or bleed$)).tw. or ((brain or intracranial or basal ganglia or lenticulostriate) adj5 (vascular adj5 (disease$ or disorder or event))).tw. or ((isch?emic or apoplectic) adj5 (event or events or insult or attack$)).tw. or ((intracranial or cerebral art$ or basilar art$ or vertebral art$ or vertebrobasilar or vertebral basilar) adj5 (stenosis or ischemia or insufficiency or arteriosclero$ or atherosclero$ or occlus$)).tw. or ((unilateral or visual or hemispatial or attentional or spatial) adj5 neglect).tw.) not (stroke adj volume).tw. not ((exp child/ or exp infant/) not ((exp child/ or exp infant/) and exp adult/))
AND
exp incidence/ or exp prevalence/ or exp epidemiology/ or occurrence.tw. or frequenc$.tw. or incidence.tw. or prevalence.tw. or exp Cross-Sectional Studies/ or exp Health Surveys/ or (health adj3 survey$).ti,ab. or (population adj3 based).ti,ab. or outcome.ti,ab. or prognos$.ti,ab or (follow adj 2 up adj2 stud$).ti,ab or mortality.ti,ab or predict$.ti,ab
AND
exp Urinary Tract Infections/ or uti.ti,ab or exp Cystitis/ or Cystitis.ti,ab. or exp Pneumonia/ or Pneumonia.ti,ab. or rti.ti,ab or fever.ti,ab or (stroke adj4 complication$).ti,ab or ((pulmonary or lung or airway or chest or respiratory or urinary) adj2 (inflammation$ or infection$)).ti,ab

Mesh terms for EMBASE search
exp stroke/ OR exp brain ischemia/ OR exp intracranial hemorrhage/
AND
exp pneumonia/ OR exp respiratory tract infection/ OR exp cystitis/ OR exp urinary tract infections/

Additional file table 3. Definitions of characteristics

Study/population characteristic	Definition
Study design	
Prospective design	Whether the study design was prospective
Consecutive enrollment	Whether patients were included consecutively. When not reported, this was regarded as not consecutive
Study aim on infection	Whether post stroke infection was the primary focus of the study or not
Observation period	The time in days in which occurrence of infection was scored (length of stay or a predetermined interval; when occurrence of infection was scored during different time periods, the shortest interval was used)
Population characteristics	
Income country	Economies are divided into high (= 0) and low (= 1) income, according to 2008 GNI per capital (low income: $975 or less and lower middle income, $976 - $3,855, high: upper middle income, $3,856 - $11,905 and high income, $11,906 or more) (worldbank.org)
Age	Mean age of included patients *
Gender	Number of male patients **
ICU study	Whether the study was performed on an ICU or not
Infarction or bleeding	Ischaemic stroke (IS), haemorrhagic stroke (HS), or both (S)
Stroke severity (NIHSS)	Mean NIHSS-score on admission (other scales were not used, neither were median values) *
Lowered consciousness	Number of patients with a reduced consciousness on admission
Dysphagia	Number of patients with dysphagia on admission (according to the definition used in the study)
Urinary incontinence/retention	Number of patients with urinary incontinence or urinary retention on admission (as defined in the study)
Outcome	
Infection	Number of patients with any infection, defined on criteria used in study** (only used when exact number was given and not calculated by adding numbers of different infections, because 2 infections could have occurred in 1 patient)
Pneumonia	Number of patients with pneumonia, defined on criteria used in study*
Urinary tract infection	Number of patients with urinary tract infection, defined on criteria used in study**

* when mean values for different groups were given, a general mean was calculated. ** when percentages were given, numbers were calculated

Additional file table 4. Reason for exclusion on full-text

Infection rate not reported	Bamford et al, 1990; Broessner G et al 2009; Christensen et al 2009; Czlonkowska et al, 2002; Dumas et al 1994; Elkind MS et al 2004/2007; Gray et al, 2007; Lodder et al 2006; MacWalter et al 1995; Poungvarin et al 2006; Reggiani M et al, 2009; Ryglewicz D; Silver et al 1984; Sweileh WM et al 2006-2007; Vernino et al 2003; Marti-Vilalta et al: Barcelona stroke registry, 1999.
Rehabilitationsetting after acute phase of stroke	Addington et al 1999; Arai et al 2004; Culebras et al, 2007; Doshi et al 2003; Dromerick et al 2003; Ersoz 2007;Falsetti et al 2009; Harada et al, 2006; Kalra et al, 1995; Langhorne et al, 2000; Lipson et al, 2005; Luk et al, 2006; Marciniak et al; Meng et al, 2000; Rocco A et al 2007; Roth EJ 2001; Werner et al 1998.
No fulltext available	(after request in medical library and attempts to contact the author by e-mail) Gonzales MJ et al 1995; Lee HC et al, 2008; Suwanwela et al, 2007; Morales-Ortiz et al, 2001; Werner et al 1998 (1572); Roth et al, 2007; Huang et al, 2005
Small study	Chang KC et al ; Knoll T et al 2002
Different domain (e.g. study on subgroup, or SAH included)	Commichau C et al 2003; Farooq et al, 2008; Kalra et al 2000 ; Kim H et al 2000; Ali M et al, 2009; Ding R et al, 2000
Language	Aslan et al, 2007
Similar patient population	Dziedzic et al, 2009; Lees et al, 2006; Schwab S et al,1999

Additional file table 5. Extracted data

Name, Year	Number of Patients	Age	Infection rate	Pneumonia rate	UTI rate	High (0) /low (1) income country	Study Design (prospective =0, retrospective=1)
Minnerup J	591	67,7	237	72	65	0	0
Pilar Grajales Cuesy, 2010 [1]	255	73,3	-	44	-	0	0
Eriksson M, 2009[2]	24633	76	-	988	-	0	1
Den Hertog, 2009 [3]	1400	69,9	-	33	10	0	0
Langdon, 2009[4]	330	71,1	115	51	52	0	0
Levy DE, 2009[5]	500	69,9	170	23	86	0	0
Saposnik, 2008[6]	3631	72	-	240	-	0	0
Sorbello D., 2009 [7]	71	74,7	-	11	14	0	0
Stott DJ, 2009 [8] Sellars C, 2007 [9]	412	67,9	-	78	65	0	0
Vermeij, 2009 [10]	521	70,5	78	39	23	0	0
Gargano, 2008 [11]	2566	68,4	-	146	204	0	1
Harms, 2008 [12]	40	72,7	13	8	5	0	0
Hong, 2008 [13]	1254	66,5	-	151	86	0	0
Horner, 2008 [14]	13831	70,5	-	763	680	0	1
Huang, 2008 [15]	66	67,6	-	6	2	1	0
Indredavik, 2008 [16]	489	77,2	-	55	78	0	0
Naidech, 2008 [17]	56	63,9	-	9	-	0	0
Navarro JC, 2008 [18]	1153	62	-	95	50	1	0
Reid JM, 2008[19]	2725		-	141	353	0	1
Schwarz, 2008 [20]	30	73	27	7	18	0	0
Sposato, 2008 [21]	1991	69,4	-	285	-	0	0
Yan, 2008 [22]	137	71	67	63	19	1	1
Lee M, 2007 [23]	947	69,6	-	104	119	1	0
Ros, 2007 [24]	258	74,9	102	24	38	0	0
Roth, 2007	2457	63,4	-	379	498	0	1
Shuaib, 2007 [25]	3195	68,9	-	193	325	0	0
Sundar U, 2007 [26]	184	-	-	29	8	1	0
Walter U, 2007 [27]	236	69,8	-	51	-	0	0
Yilmaz, 2007 [28]	171	66	71	44	70	0	0
Chiu, 2006 [29]	46	79	-	7	18	1	0

	Consecutive study (=1)	Domain	Gender	ICU study(=1)	Time of observation	Stroke severity	Dysphagia	Study aim (1 = study on infection)	Lowered consciousness	Disorders of urinary tract
	1	S	325	0	14	7	-	1	105	-
	0	IS	102	0	-	-	43	1	143	-
	0	S	12350	0	17	-	-	0	4419	-
	0	S	784	0	-	6	0	0	-	-
	0	IS	170	0	30	-	74	1	-	127
	0	S	246	0	90	11	-	0	-	-
	1	IS	1895	0	30	-	418	0	483	-
	0	S	38	0	90	-	-	0	-	-
	1	S	205	0	90	-	-	1	-	-
	1	IS	281	0	7	-	-	1	58	-
	0	S	1185	0	-	-	-	0	-	-
	0	IS	29	0	11	-	27	1	-	-
	1	IS	703	0	28	4	-	0	-	-
	0	IS	7161	0	90	6	-	0	-	-
	1	IS	51	0	30	-	-	0		-
	1	S	233	0	7	-	-	0	104	-
	1	HS	29	1	8	-	-	0	43	-
	1	S	666	0	14	-	-	0		49
	1	S	1414	0	-	-	-	0	-	-
	0	IS	30	0	10	15	-	1	-	-
	1	IS	1100	0	8	-	-	0	-	-
	1	S	84	1	5	-	62	0	80	-
	1	IS	502	0	18	-	-	0	129	-
	1	S	126	0	11	13	114	1	-	-
	1	S	1214	0	17	9	-	0	-	-
	0	IS	1768	0	90	13	NR	0	-	-
	1	IS	-	0	14	-	43	0	-	-
	0	IS	124	1	5	-	69	1	65	-
	0	S	90	1	11	-	-	1	-	-
	1	IS	14	0	17	19	-	0	-	-

Additional file table 5. Extracted data

Name, Year	Number of Patients	Age	Infection rate	Pneumonia rate	UTI rate	High (0) /low (1) income country	Study Design (prospective =0, retrospective=1)
Dziedzic, 2006 [30]	833	69,0	-	89	-	0	1
Dziedzic, 2006 [31]	705	69,7	-	74	-	0	1
Gosney, 2006 [32]	100	-	-	7	-	0	0
Hassan A, 2006 [33]	443	58	-	102	-	1	1
Kwan J, 2007 [34]	439	74	73	45	30	0	0
Kwon HM, 2006 [35]	286	62,8	-	47	-	0	0
Langdon PC, 2006 [36]	88	74,6	25	16	10	0	0
Maramattom, 2006	144	71,3	-	28	-	0	0
Matz, 2006[39]	238	71,8	-	33	17	0	0
Ovbiagele B, 2006 [37]	663		-	66	84	0	1
Vargas, 2006 [38]	229	72,6	60	33	13	0	0
Secades, 2006 [39]	38	70,8	-	-	1	0	0
Garbusinski, 2005[40]	148	-	-	27	-	1	0
Hanchaiphiboolkul	332	62	22	12	13	1	1
Hinchey,2005 [41]	2532	70,5	-	114	-	0	0
Misra, 2005 [42]	141	57,2	-	12	-	1	0
Heuschman, 2004 [43]	10800	70	-	648	-	0	1
Field, 2004 [44]	11642	-	-	541	-	0	1
Kwan, 2004 [45]	351	74,5	-	37	25	0	-
Lees, 2004 [46]	2386	70,3	-	19	-	0	0
Steger, 2004[47]	992	76,2	-	135	142	0	0
Upadya A, 2004 [48]	55	69,6	-	26	-	0	1
Aslanyan S, 2003 [49]	1455	70	-	159	146	0	0
Broadley, 2003 [50]	149	70	-	7	-	0	0
FOOD-collaboration, 2003 [51]	3012	73,3	-	367	-	0	0
Hamidon BB, 2003 [52]	163	62,2	26	20	6	0	0
Hilker, 2003 [53]	124	63,8	-	26	-	0	0
Katzan IL, 2003 [54]	11286	76,8	-	635	-	0	1
Lang, 2003 [55]	2030		-	73	40	0	1
Martinsson, 2003 [56]	45	67,3	-	6	3	0	0
Pittock SJ, 2003 [57]	117	69,9	-	11	4	0	0

	Consecutive study (=1)	Domain	Gender	ICU study(=1)	Time of observation	Stroke severity	Dysphagia	Study aim (1 = study on infection)	Lowered consciousness	Disorders of urinary tract
	1	IS	389	0	30	-	-	0	-	-
	1	IS	320	0	12	-	-	1	-	-
	0	S	106	0	21	-	33	1	-	-
	0	S	-	0	4	-	-	1	-	-
	1	S	215	0	5	0	149	1	118	183
	1	S	192	0	30	13	96	1	-	-
	0	IS	43	0	30	-	58	0	36	-
	1	HS	89	0	6	-	-	0	69	-
	1	S	109	0	10	5	-	0	-	-
	0	IS	297	0	-	-	-	1	-	-
	1	S	117	0	7	-	-	1	-	-
	0	HS	19	0	90	12	-	0	-	-
	1	S	73	0		16	90	0	68	-
	0	IS	209	0	3	-	-	0	31	-
	0	IS	1262	0	6	-		1	-	-
	0	HS	101	0	30	-	-	0	-	-
	1	IS	5751	0	11	-	-	0	-	-
	?0	S	5856	0	-	-	-	0	-	-
	1	S	173	0	5	-	149	0	-	-
	0	IS	1277	0	2	-	-	0	-	-
	1	S	425	0	-	-	-	0	-	-
	0	S	31	1	16	17	-	1	-	-
	0	IS	815	0	7	13	-	1	-	-
	1	S	88	0	-	-	74	0	18	44
	0	S	1520	0	40	-	732	0	-	-
	0	IS	105	0	3	-	-	1	-	-
	1	S	82	1	8	-	36	1	-	-
	0	S	7888	0		-	-	1	1696	-
	0	IS	-	0	5	-	-	0	-	-
	0	IS	26	0	90	-	-	0	7	-
	1	IS	68	0	14	-	33	0	-	29

Additional file table 5. Extracted data

Name, Year	Number of Patients	Age	Infection rate	Pneumonia rate	UTI rate	High (0) /low (1) income country	Study Design (prospective =0, retrospective=1)
Spratt, 2002 [58]	257	73	51	26	33	0	0
Weimar, 2002 [59]	3866	66,6	-	286	244	0	0
Evans, 2001 [60]	304	76	-	58	-	0	0
Kammersgaard LP, 2001 [61]	1156	74,2	225	82	143	0	0
Koennecke, 2001 [62]	42	70,3	-	2	2	0	0
Enlimomab Stroke Trial, 2001 [63]	625	68,9	-	12	-	0	0
Schwab, 2001 [64]	50	57	-	24	-	0	0
Georgilis K, 1999 [65]	330	72,8	75	33	38	0	1
Grau AJ, 1999 [66]	119	61	17	10	2	0	0
Tirschwell DL, 1999[67]	4757	75	-	338	442	0	1
Hacke, 1998 [68]	37	68,5	-	3	-	0	0
Hinds, 1998 [69]	115	74,9	-	27	-	0	0
Johnston, 1998 [70]	279	69	-	27	30	0	0
Newell, 1995 [71]	356	-	-	23	-	0	1
Newell, 1997	399	-	-	11	-	0	1
Nilsson, 1998 [72]	100	75,4	-	5	-	0	0
Pinto, 1998 [73]	213	59,2	-	21	37	0	0
Sala, 1998	187	73,3	-	13	-	0	0
Schneider, 1998[75]	32	63	-	10	-	0	0
Grotta J, 1997 [76]	721	70,5	-	59	95	0	0
Davenport, 1996 [77]	607	73	-	70	98	0	1
Kidd, 1995 [78]	60	72	-	19	-	0	0
Webb, 1995 [79]	383	-	-	12	64	0	1
Webb	303	-	-	5	35	0	1
Oddersson, [80]	211	74,1	-	11	24	0	0
Spitzer, 1988 [81]	63	50,1	-	20	12	0	1
Gordon, 1987 [82]	91	-	-	11	-	0	0
Przelomski, 1986 [83]	104	71,8	-	13	2	0	0

	Consecutive study (=1)	Domain	Gender	ICU study(=1)	Time of observation	Stroke severity	Dysphagia	Study aim (1 = study on infection)	Lowered consciousness	Disorders of urinary tract
	1	IS	127	0	21	-	-	0	43	-
	?0	IS	2241	0	7	8	-	0	766	-
	0	S	155	0	90	-	-	0	-	-
	1	S	531	0	3	-	-	1	-	-
	1	IS	22	1	10	-	-	0	-	-
	0	IS	344	0	5	15	-	0	-	-
	1	IS	35	1	-	25	-	0	-	-
	0	S	184	0	-	-	-	0	-	-
	1	IS	79	0	2	-	-	1	-	-
	0	IS	2041	0	8	-	266	0	-	-
	0	IS	21	0	28	-	-	0	-	-
	1	S	51	0	13	-	62	0	22	-
	0	IS	159	0	90	-	15	0	-	14
	0	IS	-	0	10	-	-	0	-	-
	0	IS	-	0	7	-	-	0	-	-
	1	S	36	0	-	-	14	0	-	-
	1	S	113	0	10	-	-	0	14	-
	1	S	95	0	10	-	135	0	55	-
	0	S	14	0		-	-	0	-	-
	0	IS	-	0	7	15	-	0	-	-
	1	S	279	0	37	-	-	0	-	-
	1	S	25	0	14	-	25	1	-	-
	0	S	-	0	10	-	-	0	-	-
	0	S	-	0	8	-	-	0	-	-
	0	IS	-	0	8	-	-	0	-	-
	1	HS	31	0	-	-	-	0	-	-
	1	S	38	0		-	41	0	55	-
	1	S	55	0	5	-	-	0	-	-

Reference List additional file

1. Cuesy PG, Sotomayor PL, Pina JO. Reduction in the incidence of poststroke nosocomial pneumonia by using the "turn-mob" program. J Stroke Cerebrovasc Dis 2010;19(1):23-28.
2. Eriksson M, Glader EL, Norrving B, Terent A, Stegmayr B. Sex differences in stroke care and outcome in the Swedish national quality register for stroke care. Stroke 2009;40(3):909-914.
3. den Hertog HM, van der Worp HB, van Gemert HM et al. The Paracetamol (Acetaminophen) In Stroke (PAIS) trial: a multicentre, randomised, placebo-controlled, phase III trial. Lancet Neurol 2009;8(5):434-440.
4. Langdon PC, Lee AH, Binns CW. High incidence of respiratory infections in 'nil by mouth' tube-fed acute ischemic stroke patients. Neuroepidemiology 2009;32(2):107-113.
5. Levy DE, del Zoppo GJ, Demaerschalk BM et al. Ancrod in acute ischemic stroke: results of 500 subjects beginning treatment within 6 hours of stroke onset in the ancrod stroke program. Stroke 2009;40(12):3796-3803.
6. Saposnik G, Hill MD, O'Donnell M, Fang J, Hachinski V, Kapral MK. Variables associated with 7-day, 30-day, and 1-year fatality after ischemic stroke. Stroke 2008;39(8):2318-2324.
7. Sorbello D, Dewey HM, Churilov L et al. Very early mobilisation and complications in the first 3 months after stroke: further results from phase II of A Very Early Rehabilitation Trial (AVERT). Cerebrovasc Dis 2009;28(4):378-383.
8. Stott DJ, Falconer A, Miller H, Tilston JC, Langhorne P. Urinary tract infection after stroke. QJM 2009;102(4):243-249.
9. Sellars C, Bowie L, Bagg J et al. Risk factors for chest infection in acute stroke: a prospective cohort study. Stroke 2007;38(8):2284-2291.
10. Vermeij FH, Scholte op Reimer WJ, de MP et al. Stroke-associated infection is an independent risk factor for poor outcome after acute ischemic stroke: data from the Netherlands Stroke Survey. Cerebrovasc Dis 2009;27(5):465-471.
11. Gargano JW, Wehner S, Reeves M. Sex differences in acute stroke care in a statewide stroke registry. Stroke 2008;39(1):24-29.
12. Harms H, Prass K, Meisel C et al. Preventive antibacterial therapy in acute ischemic stroke: a randomized controlled trial. PLoS One 2008;3(5):e2158.
13. Hong KS, Kang DW, Koo JS et al. Impact of neurological and medical complications on 3-month outcomes in acute ischaemic stroke. Eur J Neurol 2008;15(12):1324-1331.
14. Horner S, Niederkorn K, Schnabl S, Fazekas F. [Gender aspects of Ischemic stroke. An analysis of the Austrian Stroke-Unit Registry]. Wien Med Wochenschr 2008;158(15-16):446-452.
15. Huang WY, Weng WC, Chien YY, Wu CL, Peng TI, Chen KH. Predictive factors of outcome and stroke recurrence in patients with unilateral atherosclerosis-related internal carotid artery occlusion. Neurol India 2008;56(2):173-178.
16. Indredavik B, Rohweder G, Naalsund E, Lydersen S. Medical complications in a comprehensive stroke unit and an early supported discharge service. Stroke 2008;39(2):414-420.
17. Naidech AM, Bendok BR, Tamul P et al. Medical complications drive length of stay after brain hemorrhage: a cohort study. Neurocrit Care 2009;10(1):11-19.
18. Jose C Navarro, Ester Bitanga, Nijasri Suwanwela et al. Complication of acute stroke: A study in ten Asian countries. Neurology Asia [13: 33-39]. 2010.
19. Reid JM, Dai D, Gubitz GJ, Kapral MK, Christian C, Phillips SJ. Gender differences in stroke examined in a 10-year cohort of patients admitted to a Canadian teaching hospital. Stroke 2008;39(4):1090-1095.
20. Schwarz S, Al-Shajlawi F, Sick C, Meairs S, Hennerici MG. Effects of prophylactic antibiotic therapy with mezlocillin plus sulbactam on the incidence and height of fever after severe acute ischemic stroke: the Mannheim infection in stroke study (MISS). Stroke 2008;39(4):1220-1227.
21. Sposato LA, Esnaola MM, Zamora R, Zurru MC, Fustinoni O, Saposnik G. Quality of ischemic stroke care in emerging countries: the Argentinian National Stroke Registry (ReNACer). Stroke 2008;39(11):3036-3041.
22. Yan F, Zhang D, Xu H, Guo H. Risk factors for fever in critically ill patients with acute new-onset stroke. Neurol Res 2008;30(4):394-399.
23. Lee M, Huang WY, Weng HH, Lee JD, Lee TH. First-ever ischemic stroke in very old Asians: clinical features, stroke subtypes, risk factors and outcome. Eur Neurol 2007;58(1):44-48.
24. Ros L, Garcia M, Prat J et al. [Predictors of nosocomial infection in acute stroke. Relation with morbimortality and outcome]. Med Clin (Barc) 2007;128(12):441-447.
25. Shuaib A, Lees KR, Lyden P et al. NXY-059 for the treatment of acute ischemic stroke. N Engl J Med 2007;357(6):562-571.
26. Sundar U, Mehetre R. Etiopathogenesis and predictors of in-hospital morbidity and mortality in posterior circulation strokes--a 2 year registry with concordant comparison with anterior circulation strokes. J Assoc Physicians India 2007;55:846-850.

27. Walter U, Knoblich R, Steinhagen V, Donat M, Benecke R, Kloth A. Predictors of pneumonia in acute stroke patients admitted to a neurological intensive care unit. J Neurol 2007;254(10):1323-1329.
28. Yilmaz GR, Cevik MA, Erdinc FS, Ucler S, Tulek N. The risk factors for infections acquired by cerebral hemorrhage and cerebral infarct patients in a neurology intensive care unit in Turkey. Jpn J Infect Dis 2007;60(2-3):87-91.
29. Chiu EH, Liu CS, Tan TY, Chang KC. Venturi mask adjuvant oxygen therapy in severe acute ischemic stroke. Arch Neurol 2006;63(5):741-744.
30. Dziedzic T, Slowik A, Pera J, Szczudlik A. Beta-blockers reduce the risk of early death in ischemic stroke. J Neurol Sci 2007;252(1):53-56.
31. Dziedzic T, Pera J, Klimkowicz A et al. Serum albumin level and nosocomial pneumonia in stroke patients. Eur J Neurol 2006;13(3):299-301.
32. Gosney M, Martin MV, Wright AE. The role of selective decontamination of the digestive tract in acute stroke. Age Ageing 2006;35(1):42-47.
33. Hassan A, Khealani BA, Shafqat S et al. Stroke-associated pneumonia: microbiological data and outcome. Singapore Med J 2006;47(3):204-207.
34. Kwan J, Hand P. Infection after acute stroke is associated with poor short-term outcome. Acta Neurol Scand 2007;115(5):331-338.
35. Kwon HM, Jeong SW, Lee SH, Yoon BW. The pneumonia score: a simple grading scale for prediction of pneumonia after acute stroke. Am J Infect Control 2006;34(2):64-68.
36. Langdon PC, Lee AH, Binns CW. Dysphagia in acute ischaemic stroke: severity, recovery and relationship to stroke subtype. J Clin Neurosci 2007;14(7):630-634.
37. Ovbiagele B, Hills NK, Saver JL, Johnston SC. Frequency and determinants of pneumonia and urinary tract infection during stroke hospitalization. J Stroke Cerebrovasc Dis 2006;15(5):209-213.
38. Vargas M, Horcajada JP, Obach V et al. Clinical consequences of infection in patients with acute stroke: is it prime time for further antibiotic trials? Stroke 2006;37(2):461-465.
39. Secades JJ, Alvarez-Sabin J, Rubio F, Lozano R, Davalos A, Castillo J. Citicoline in intracerebral haemorrhage: a double-blind, randomized, placebo-controlled, multi-centre pilot study. Cerebrovasc Dis 2006;21(5-6):380-385.
40. Garbusinski JM, van der Sande MA, Bartholome EJ et al. Stroke presentation and outcome in developing countries: a prospective study in the Gambia. Stroke 2005;36(7):1388-1393.
41. Hinchey JA, Shephard T, Furie K, Smith D, Wang D, Tonn S. Formal dysphagia screening protocols prevent pneumonia. Stroke 2005;36(9):1972-1976.
42. Misra UK, Kalita J, Pandey S, Mandal SK, Srivastava M. A randomized placebo controlled trial of ranitidine versus sucralfate in patients with spontaneous intracerebral hemorrhage for prevention of gastric hemorrhage. J Neurol Sci 2005;239(1):5-10.
43. Heuschmann PU, Kolominsky-Rabas PL, Misselwitz B et al. Predictors of in-hospital mortality and attributable risks of death after ischemic stroke: the German Stroke Registers Study Group. Arch Intern Med 2004;164(16):1761-1768.
44. Field TS, Green TL, Roy K, Pedersen J, Hill MD. Trends in hospital admission for stroke in Calgary. Can J Neurol Sci 2004;31(3):387-393.
45. Kwan J, Hand P, Dennis M, Sandercock P. Effects of introducing an integrated care pathway in an acute stroke unit. Age Ageing 2004;33(4):362-367.
46. Muir KW, Lees KR, Ford I, Davis S. Magnesium for acute stroke (Intravenous Magnesium Efficacy in Stroke trial): randomised controlled trial. Lancet 2004;363(9407):439-445.
47. Steger C, Pratter A, Martinek-Bregel M et al. Stroke patients with atrial fibrillation have a worse prognosis than patients without: data from the Austrian Stroke registry. Eur Heart J 2004;25(19):1734-1740.
48. Upadya A, Thorevska N, Sena KN, Manthous C, Amoateng-Adjepong Y. Predictors and consequences of pneumonia in critically ill patients with stroke. J Crit Care 2004;19(1):16-22.
49. Aslanyan S, Weir CJ, Diener HC, Kaste M, Lees KR. Pneumonia and urinary tract infection after acute ischaemic stroke: a tertiary analysis of the GAIN International trial. Eur J Neurol 2004;11(1):49-53.
50. Broadley S, Croser D, Cottrell J et al. Predictors of prolonged dysphagia following acute stroke. J Clin Neurosci 2003;10(3):300-305.
51. Poor nutritional status on admission predicts poor outcomes after stroke: observational data from the FOOD trial. Stroke 2003;34(6):1450-1456.
52. Hamidon BB, Raymond AA, Norlinah MI, Jefferelli SB. The predictors of early infection after an acute ischaemic stroke. Singapore Med J 2003;44(7):344-346.
53. Hilker R, Poetter C, Findeisen N et al. Nosocomial pneumonia after acute stroke: implications for neurological intensive care medicine. Stroke 2003;34(4):975-981.

54. Katzan IL, Cebul RD, Husak SH, Dawson NV, Baker DW. The effect of pneumonia on mortality among patients hospitalized for acute stroke. Neurology 2003;60(4):620-625.
55. Lang W, Lalouschek W. [Acute therapy of ischemic stroke]. Wien Med Wochenschr 2003;153(1-2):21-24.
56. Martinsson L, Wahlgren NG. Safety of dexamphetamine in acute ischemic stroke: a randomized, double-blind, controlled dose-escalation trial. Stroke 2003;34(2):475-481.
57. Pittock SJ, Meldrum D, Hardiman O, Thornton J, Brennan P, Moroney JT. The Oxfordshire Community Stroke Project classification: correlation with imaging, associated complications, and prediction of outcome in acute ischemic stroke. J Stroke Cerebrovasc Dis 2003;12(1):1-7.
58. Spratt N, Wang Y, Levi C, Ng K, Evans M, Fisher J. A prospective study of predictors of prolonged hospital stay and disability after stroke. J Clin Neurosci 2003;10(6):665-669.
59. Weimar C, Roth MP, Zillessen G et al. Complications following acute ischemic stroke. Eur Neurol 2002;48(3):133-140.
60. Evans A, Perez I, Harraf F et al. Can differences in management processes explain different outcomes between stroke unit and stroke-team care? Lancet 2001;358(9293):1586-1592.
61. Kammersgaard LP, Jorgensen HS, Reith J et al. Early infection and prognosis after acute stroke: the Copenhagen Stroke Study. J Stroke Cerebrovasc Dis 2001;10(5):217-221.
62. Koennecke HC, Leistner S. Prophylactic antipyretic treatment with acetaminophen in acute ischemic stroke: a pilot study. Neurology 2001;57(12):2301-2303.
63. Use of anti-ICAM-1 therapy in ischemic stroke: results of the Enlimomab Acute Stroke Trial. Neurology 2001;57(8):1428-1434.
64. Schwab S, Georgiadis D, Berrouschot J, Schellinger PD, Graffagnino C, Mayer SA. Feasibility and safety of moderate hypothermia after massive hemispheric infarction. Stroke 2001;32(9):2033-2035.
65. Georgilis K, Plomaritoglou A, Dafni U, Bassiakos Y, Vemmos K. Aetiology of fever in patients with acute stroke. J Intern Med 1999;246(2):203-209.
66. Grau AJ, Buggle F, Schnitzler P, Spiel M, Lichy C, Hacke W. Fever and infection early after ischemic stroke. J Neurol Sci 1999;171(2):115-120.
67. Tirschwell DL, Kukull WA, Longstreth WT, Jr. Medical complications of ischemic stroke and length of hospital stay: experience in Seattle, Washington. J Stroke Cerebrovasc Dis 1999;8(5):336-343.
68. Hacke W, Donnan G, Fieschi C et al. Association of outcome with early stroke treatment: pooled analysis of ATLANTIS, ECASS, and NINDS rt-PA stroke trials. Lancet 2004;363(9411):768-774.
69. Hinds NP, Wiles CM. Assessment of swallowing and referral to speech and language therapists in acute stroke. QJM 1998;91(12):829-835.
70. Johnston KC, Li JY, Lyden PD et al. Medical and neurological complications of ischemic stroke: experience from the RANTTAS trial. RANTTAS Investigators. Stroke 1998;29(2):447-453.
71. Newell SD, Jr., Englert J, Box-Taylor A, Davis KM, Koch KE. Clinical efficiency tools improve stroke management in a rural southern health system. Stroke 1998;29(6):1092-1098.
72. Nilsson H, Ekberg O, Olsson R, Hindfelt B. Dysphagia in stroke: a prospective study of quantitative aspects of swallowing in dysphagic patients. Dysphagia 1998;13(1):32-38.
73. Pinto AN, Melo TP, Lourenco ME et al. Can a clinical classification of stroke predict complications and treatments during hospitalization? Cerebrovasc Dis 1998;8(4):204-209.
74. Schwab S, Schwarz S, Spranger M, Keller E, Bertram M, Hacke W. Moderate hypothermia in the treatment of patients with severe middle cerebral artery infarction. Stroke 1998;29(12):2461-2466.
75. Schneider D, Berrouschot J, Brandt T et al. Safety, pharmacokinetics and biological activity of enlimomab (anti-ICAM-1 antibody): an open-label, dose escalation study in patients hospitalized for acute stroke. Eur Neurol 1998;40(2):78-83.
76. Grotta J. Lubeluzole treatment of acute ischemic stroke. The US and Canadian Lubeluzole Ischemic Stroke Study Group. Stroke 1997;28(12):2338-2346.
77. Davenport RJ, Dennis MS, Wellwood I, Warlow CP. Complications After Acute Stroke. Stroke 1996;27(3):415-420.
78. Kidd D, Lawson J, Nesbitt R, MacMahon J. The natural history and clinical consequences of aspiration in acute stroke. QJM 1995;88(6):409-413.
79. Webb DJ, Fayad PB, Wilbur C, Thomas A, Brass LM. Effects of a specialized team on stroke care. The first two years of the Yale Stroke Program. Stroke 1995;26(8):1353-1357.
80. Odderson IR, McKenna BS. A model for management of patients with stroke during the acute phase. Outcome and economic implications. Stroke 1993;24(12):1823-1827.
81. Spitzer K, Thie A, Kunze K. Spontaneous subarachnoid haemorrhage: expert system for appraisal of the prognosis and computer-supported decision for therapy. J Neurol 1988;235(6):335-342.
82. Gordon C, Hewer RL, Wade DT. Dysphagia in acute stroke. Br Med J (Clin Res Ed) 1987;295(6595):411-414.
83. Przelomski MM, Roth RM, Gleckman RA, Marcus EM. Fever in the wake of a stroke. Neurology 1986;36(3):427-429.

CHAPTER 3

Antibiotic therapy for preventing infections in patients with acute stroke

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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 infections and improve outcome.

Objectives

1. To assess whether preventive antibiotic therapy in patients with acute stroke reduces the risk of dependency and death at follow-up.
2. To assess whether preventive antibiotic therapy in patients with acute stroke reduces infection rate.

Search methods

We searched the Cochrane Stroke Group's Trials Register (October 2010); The Cochrane Central Register of Controlled Trials (CENTRAL) (*The Cochrane Library* 2010, Issue 3); MEDLINE (1950 to October 2010) and EMBASE (1980 to October 2010). In an effort to identify further published, unpublished and ongoing trials we searched trials and research registers, scanned reference lists and contacted authors, colleagues and researchers in the field.

Selection criteria

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

Data collection and analysis

Two authors independently selected articles and performed data extraction; we discussed and resolved discrepancies in a consensus meeting with a third observer. We contacted the study authors to obtain missing data when required. An independent observer assessed methodological quality. We calculated relative risks (RRs) for dichotomous outcomes, assessed heterogeneity amongst included studies and performed subgroup analyses on study quality.

Main results

We included five studies involving 506 patients. Study population, study design, type of antibiotic and definition of infection differed considerably. The number of patients who died in the preventive antibiotic group was non-significantly reduced (33/248 (13%) versus 38/258 (15%), RR 0.85, 95% confidence interval (CI) 0.47 to 1.51); the number of dependent patients in the preventive antibiotic therapy group was also non-significantly reduced (97/208 (47%) versus 127/208 (61%), RR 0.67, 95% CI 0.32 to 1.43). Preventive antibiotic therapy did reduce the incidence of infections in patients with acute stroke from 36% to 22% (36/166 (22%) versus 61/169 (36%), RR 0.58, 95% CI 0.43 to 0.79). No major side-effects of preventive antibiotic therapy were reported.

Authors' conclusions

In this meta-analysis, preventive antibiotic therapy seemed to reduce the risk of infection, but did not reduce the number of dependent or deceased patients. However, the included studies were small and heterogeneous. Large randomised trials are urgently needed.

Plain language summary

Antibiotic therapy for preventing infections in patients with acute stroke

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 frequently. Infections may adversely affect outcome after stroke. Preventive antibiotic therapy may reduce the number of infections, thereby improving the outcome of patients with acute stroke. This review of five studies on preventive antibiotic therapy in 506 stroke patients shows that preventive antibiotic treatment reduces the number of infections after stroke. A substantial effect on dependency and case fatality was not provided but could not be excluded, since included studies were small and heterogeneous. Further studies are warranted to investigate the effect of preventive antibiotic therapy on dependency and case fatality.

Background

Stroke is a main cause of disability and death worldwide, affecting both high income and developing countries. It is often followed by complications, especially infections, which occur frequently. Approximately 30% of all patients in the acute phase of stroke are diagnosed with an infection, in particular pneumonia and urinary tract infections are common.(1-6) The increased risk of infection in patients in the acute phase of stroke can be attributed to different factors. Firstly, infections are associated with a patient's clinical condition. Older patients with more severe strokes experience infections more frequently. Also, patients with swallowing disturbances with subsequent aspiration are at increased risk of pneumonia.(7-10) Secondly, the use of invasive procedures, such as urinary catheterisation or mechanical ventilation, is associated with the occurrence of infections.(11, 12) In addition, acute stroke may lead to stroke-induced immunodepression, a systemic anti-inflammatory response that is thought to increase the vulnerability for infection in patients in the acute phase of stroke.(13-15) Several studies investigated the association between post-stroke infections and morbidity and case fatality. Most studies show that infections are associated with poor short- and long-term functional outcome.(1, 3, 6, 16, 17) However, one study could not confirm this association.(5) Several interventions have been proposed and evaluated to prevent infection after stroke, such as trained nurses using a protocol for managing patients with dysphagia (difficulty with swallowing) and avoidance of urinary catheters, but these are at most only partially effective.(18) An additional approach to reduce the incidence of infections after acute stroke may be preventive antibiotic treatment. Current guidelines on the management of stroke do not advocate the use of preventive antibiotic therapy because it has not been proven effective.(19, 20) For critically ill patients in intensive care

units (ICUs), treatment with antibiotics (selective decontamination) to prevent infections has been proven to be effective in reducing the incidence of infections and case fatality.(21-23) In a previous review and meta-analysis on preventive antibiotic therapy in stroke patients, preventive antibiotics reduced the risk of infection but did not reduce case fatality.(24) This review aims to assess the current knowledge on the effect of preventive antibiotic therapy on functional outcome after stroke, the incidence of infections and the length of hospital stay. It will address the number of adverse events occurring in antibiotic therapy as well.

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 patients without infection at presentation.

How the intervention might work

Infections occurring within seven days after stroke are associated with poor outcome, independent of stroke severity and other prognostic factors.(6) Preventive antibiotic therapy may prevent infections in patients with acute stroke. Hence, prevention of infections may result in lower case fatality rates and better functional outcome. Preventive antibiotic treatment may also have adverse effects like 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). Additionally, it 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

Several studies have suggested an association between the occurrence of infections after stroke and poor outcome. Preventive use of antibiotic therapy could potentially prevent infections in stroke patients, thereby improving outcome.

Objectives

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

1. reduces the risk of dependency and death at follow up;
2. reduces the occurrence of infections in the acute phase of stroke;
3. reduces the occurrence of elevated body temperature (temperature of 38°C or higher) in the acute phase of stroke;

4. reduces the length of hospital stay; or
5. 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

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

Types of interventions

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

Types of outcome measures

At least incidence of infection or case fatality had to be recorded for studies to be included.

Primary outcomes

Death or dependency (poor functional outcome) at the end of follow-up (end of treatment or one to three months), measured with the modified Rankin Scale (mRS), Barthel Index (BI) or another method assessing dependency in activities of daily living. Poor functional outcome is the most important measure of outcome since the aim of treatment should not only be to prevent death but also to prevent disability and dependency in survivors.

Secondary outcomes

1. The occurrence of infections in the acute phase of stroke.
2. The occurrence of elevated body temperature (temperature 38o C or higher) in the acute phase of stroke.

3. The length of hospital stay.
4. The occurrence of adverse events likely to be related to antibiotic therapy.

Search methods for identification of studies

Electronic searches

We searched the Cochrane Stroke Group's Trials Register (last searched October 2010); the Cochrane Central Register of controlled Trials (CENTRAL) (*The Cochrane Library* 2010, Issue 3); MEDLINE (1950 toOctober 2010) (**Appendix 1**); and EMBASE (1980 to October 2010) (**Appendix 2**). We developed the MEDLINE search strategy with the help of the Cochrane Stroke Group's Trials Search Coordinator and adapted it for the other databases.

Searching other resources

In an effort to identify further published, unpublished and ongoing trials, we searched the following trials and research registers (October 2010):

- ClinicalTrials.gov (www.clinicaltrials.gov);
- Current Controlled Trials (www.controlled-trials.com); and
- Stroke Trials Registry (www.strokecenter.org/trials).

We scanned reference lists of relevant articles and contacted authors, colleagues and researchers in the field. We searched for trials in all languages and arranged translation of trial reports published in languages other than English.

Data collection and analysis

Selection of studies

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

Data extraction and management

Two review authors (WW, JDV) independently extracted and recorded the data on specially designed forms, and subsequently cross-checked the data. We discussed and resolved discrepancies in a consensus meeting with a third observer (PJN).We collected the following data from the studies: study design, inclusion and exclusion criteria, patient's characteristics, intervention characteristics, and outcome and complication measures. Patient characteristics included age, sex, stroke type, stroke severity and the number of dysphagic patients. Intervention characteristics included type, dosage and duration of intervention,

co-treatment with antipyretic medication, time from symptom onset to intervention (with an intended dichotomisation at 24 hours from onset) and number of patients with incomplete treatment. The outcome measures included body temperature in the acute phase of stroke, occurrence of infections, type of infection, elapsed time from start of treatment to the occurrence of infection, data on functional outcome, length of hospital stay and death. Complication measures were complications and adverse events during follow-up, which included incidence of colonisation with antibiotic resistant microorganisms.

Assessment of risk of bias in included studies

For each study, an independent observer (DWJD) assessed the risk of bias for the following items:

- 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.

We rated each criterion in accordance with the Cochrane Collaboration's tool for assessing risk of bias as either "low risk of bias", "high risk of bias" or "unclear risk of bias" (indicating either lack of information or uncertainty over the potential for bias.(25)

Measures of treatment effect

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

Unit of analysis issues

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 cases of missing data, for example when mRS or BI scores were not available, we contacted the corresponding publication author to get as complete follow-up data as possible on all randomised patients.

Assessment of heterogeneity

We used tests for heterogeneity between trial results with the Cochrane Q statistic and 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.

Assessment of reporting biases

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

Data synthesis

We calculated a weighted estimate of the typical treatment effect across trials (RR) by means of a fixed-effect model using RevMan 5.1.(26) However, in case of heterogeneity of treatment effects, we used the random-effects model to assess the overall treatment effect.

Subgroup analysis and investigation of heterogeneity

Where possible, we performed subgroup analyses for differences in study design and population, such as time since onset of symptoms and stroke severity. When substantial heterogeneity was found on efficacy analysis, we explored heterogeneity by stratifying for trial quality (A: low risk of bias versus B: unclear risk of bias or C: high risk of bias).

Sensitivity analysis

When we found evidence of heterogeneity that could not be explained by study quality we conducted the following subgroup analysis and sensitivity analysis:

- placebo versus open controlled trials; and
- early (within 24 hours) versus late (after 24 hours) start of treatment after stroke.

Table 1. Characteristics of included studies

Name, year	Methods	Participants	Interventions	Outcomes
Chamorro, 2005	Randomised, double-blind	Patients older than 18 years with non-septic ischaemic or haemorrhagic stroke enrolled within 24 hours from clinical onset	Intravenous levofloxacin 500 mg/100 mL/d, for 3 days	Early infection (within the first 7 days after stroke), case fatality, favourable outcome at day 90 (mRS < 2, NIHSS < 2, BI 95 or 100)
De Falco, 1998	Randomised, unblinded	Patients with ischaemic stroke within 12 hours from clinical onset	Penicillin intramuscularly	Infectious complications, case fatality, functional outcome (BI, CNS)
Harms, 2008	Randomised, double-blind	Patients older than 17 years with ischaemic stroke in MCA territory and NIHSS ≥ 12 within 9 to 36 hours after onset	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 dichotomised ≥ 60 and < 60)
Lampl, 2007	Quasi-randomised (8th number of identity card), open-label, blinded outcome assessment	Patients older than 18 years with ischaemic stroke, NIHSS > 5 and onset of stroke 6 to 24 hours prior to beginning treatment	Orally minocycline 200 mg/d for 5 days	NIHSS on day 90; NIHSS, mRS, BI, death on day 7, 30, 90
Schwarz, 2008	Randomised, unblinded	Patients aged at least 18 years with ischaemic stroke with onset of symptoms less than 24 hours ago, bedridden (mRS > 3), an estimated premorbid mRS < 2 and stable deficits	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 assessed by blinded observer, primary outcome (mRS at day 90) assessed by telephone interview with unknown blinding procedure

Results

Description of studies

See Table 1 'Characteristics of included studies', Table 2 'Characteristics of excluded studies' and Table 3 'Author's judgement and support for judgement for risk of bias in included studies'.

Results of the search

From the electronic searching we found 477 MEDLINE and 1743 EMBASE abstracts, and 21 CENTRAL studies. We assessed 14 full-text articles for eligibility; we included six studies in the qualitative synthesis. From there, we identified four ongoing trials (ISRCTN37118456; ISRCTN66140176; NCT00836355; NCT00930020). After qualitative synthesis, we included five studies in the meta-analysis. A PRISMA-flowchart of study selection is shown in Figure 1.

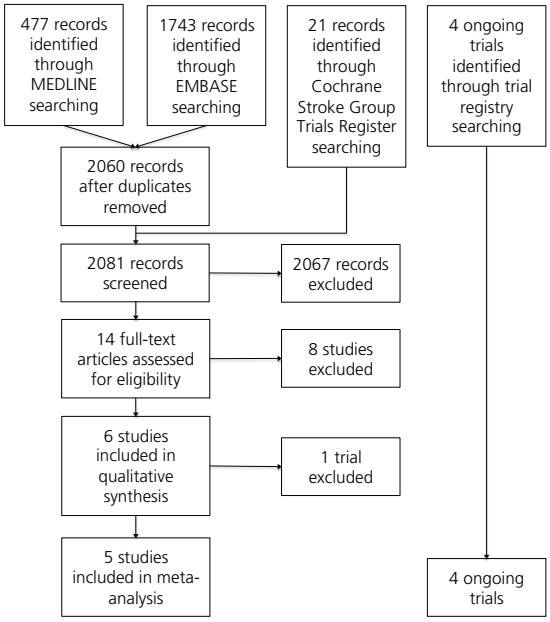

Figure 1. PRISMA flowchart of study selection

Included studies

We found five studies eligible for inclusion in the meta-analysis.(27-31) These studies included 506 patients in total; 248 patients were randomised to preventive antibiotic therapy; 258 patients were randomised to the control groups. In the control groups, 228

patients were randomised to placebo and 30 patients did not receive additional therapy in an open-label study design.(31) Sample size calculation was performed in three studies. (27-29) One of these studies was terminated prematurely after analysis of the first 130 patients because no effect was to be expected.(27) All but one of the studies included adult participants; in one study patients of all ages were included.(30). Patients with both ischaemic and haemorrhagic stroke were included in one study(27); all other studies included solely patients with ischaemic stroke. The mean age in the preventive antibiotics group was 71.7 years versus 70.8 years in the control group. In both treatment groups, the percentage of male patients was 52%. Four studies reported baseline median stroke severity scores on the National Institutes of Health Stroke Scale (NIHSS)(27-29, 31); these scores ranged from 7.5 to 17 in the preventive antibiotics group versus 7.6 to 15 in the control group. One study reported the 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. (30) Patients with swallowing difficulties were excluded in one study;(29) all other studies did not report the number of dysphagic patients.

Table 2. Characteristics of excluded studies

Study	Reason for exclusion
Acquarolo 2005	Study population: also non-stroke patients included
Gavriliuc 2010	Study population: only febrile stroke patients included
Gosney 2006	Intervention: preventive antibiotic therapy was not systemically administered
Kalra 2010	Conference abstracts describing the ongoing trial of ISRCTN37118456
Maijkowski 1982	No randomisation
Mountokalakis 1985	Study population: only patients with indwelling catheters included
Nyren 1981	Study population: only patients with indwelling catheter s included
Sirvent 1997	Study population: also non-stroke patients included
Smithard 2009	Conference abstracts describing the ongoing trial of ISRCTN3711845

Study intervention differed in all five studies; this consisted of fluoroquinolones in two studies: levofloxacin in the study performed by *Chamorro et al(27)* and moxifloxacin in the study performed by *Harms et al(28)*. Minocycline (a tetracycline) was used in one study(29), a combination of a bèta-lactam antibiotic with bèta-lactamase inhibitor in one study (31) and penicillin in one study.(30) Route of administration was intravenous in three studies(27, 28, 31), oral in one study(29) and intramuscular in one study.(30) Dosage was not reported in one study.(30) Therapy had to be started within 24 hours of stroke onset in all studies. The duration of antibiotic therapy varied between three to five days and was not reported in one study.(30) Two studies described the mean elapsed time from

start of symptoms to intervention for both treatment groups. In these two studies, 141 patients were included in the preventive antibiotics group and time to treatment in this group was 13.3 hours; 146 patients were included in the control group and mean time to treatment was 12.2 hours.(27, 29) One study described the time to treatment for the total group of patients as 24 hours.(28) Completeness of treatment was described in two studies with 215 patients; treatment was incomplete in 13% (14 out of 106) of patients in the preventive antibiotic therapy group versus 12% (13 out of 109) in the placebo group. (28, 32) Three studies did not provide information on completeness of treatment.(29-31) Data on co-treatment with antipyretic medication was not reported in any of the studies.

Case fatality was reported as a primary outcome in one study(30); four studies reported case fatality as a secondary outcome.(27-31) All five studies presented data on functional outcome; however, outcome scales and duration of follow-up varied. Three different scales were used: mRS was used in three studies(27, 29-31), the BI in four studies(27-30) and the CNS in one study.(30) Three studies did not report the number of dependent patients.(27, 29, 30) Attempts to collect additional information from the authors failed in one case and succeeded in two.(27, 29) Dependency was defined as a BI < 60 (28) or a score on the mRS > 2.(27, 31) Case fatality and functional outcome were assessed during different follow-up periods in included studies: one study reported case fatality during hospital stay (De Falco 1998), three studies reported both case fatality and functional outcome at three months(27, 29, 31); one study at six months.(28) Infection rate was reported as a secondary outcome in two studies; one of these two studies reported infection rate during hospital stay(30), the other study during an observation period of 10 days.(31) One study did not report infection rate.(29) Out of the four studies that reported infection rate, two studies specified the type of infection.(28, 31), one study reported only pneumonia rate(30) and one study did not specify the type of infection. (27) Definitions used for the diagnosis of infection differed substantially between studies and are described in **Appendix 3**.

The occurrence of elevated body temperature was reported by two studies.(28, 31) Infection rate was reported as a primary outcome in two studies(27, 28), with varying duration of follow-up (7 and 11 days). None of the studies reported data on the length of hospital stay or the incidence of opportunistic infections in the two treatment groups. The incidence of adverse events likely to be related to antibiotic therapy was reported in two studies.(28, 31) One study reported data on the occurrence of colonisation with antibiotic resistant micro-organisms on day 11 after stroke.(28) We identified four ongoing trials (ISRCTN37118456; ISRCTN66140176; NCT00836355; NCT00930020). One study examining the effect of enoxaparin or minocycline, or both, was prematurely terminated because too few acute stroke patients were available to meet enrolment requirements

(NCT00836355). The NeuMast trial is currently recruiting participants, aiming to include a total of 330 patients (NCT00930020). The trial performed by *Kalra et al* investigates the preventive use of antibiotic therapy in stroke patients with swallowing problems and has a target number of participants of 800 (ISRCTN37118456). The effect of preventive antibiotic therapy in patients in the acute phase of stroke is investigated in the PASS trial; this trial aims to include 3200 patients (ISRCTN66140176).

Excluded studies

We excluded eight studies after full-text assessment of eligibility. We excluded two studies because not solely stroke patients were included(33, 34) and one study because only febrile stroke patients were included.(35) Two studies were performed solely in patients with indwelling catheters.(36, 37) We excluded one study because preventive antibiotic therapy was not systemically administered, but topically.(38) Two references were conference abstracts describing the ongoing trial of ISRCTN37118456.(39, 40). During qualitative synthesis we excluded one study because randomisation procedure was unclear.(41)

Risk of bias in included studies

Figure 2 shows a summary of the risk of bias in all included studies. A risk of bias table for each study is provided in Table 2.

Allocation

A randomised sequence generation was a requirement for inclusion in the meta-analysis and therefore 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 could also be considered inferior, since treatment allocation was not concealed.(29) In one study, allocation concealment was not specified.(30) In the other three studies allocation concealment was sufficient.

Blinding

Two studies used a double-blind design(27, 28) and two studies used an open-label design. In one of these open-label studies, outcomes were assessed blindly, although the adequacy of blinding was not specified.(29) One study described blinded assessment of infection but did not describe blinded assessment of secondary outcomes, such as mRS.(31) Blinding was not described in one study.(30)

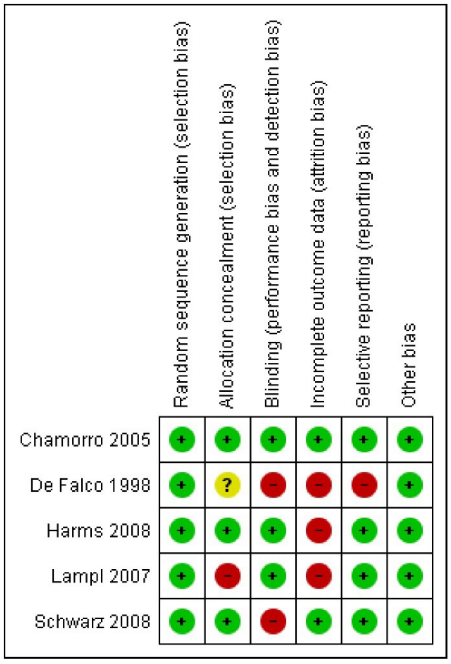

Figure 2. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

Incomplete outcome data

In one study incomplete outcome data were addressed.(31) In one study the primary outcome was assessed for all patients however, counts of patients with secondary outcomes by treatment were not described.(27) Seven patients were lost to follow-up in one study, no further details are mentioned.(28) Two studies did not describe completeness of follow-up and outcome assessment at all.(29, 30)

Selective reporting

Intention-to-treat (ITT) analysis was performed in three studies.(29, 31) Both ITT and per-protocol analysis were performed in one study.(28) Per-protocol analysis alone was performed in one study.(30)

Other potential sources of bias

One study presented a very limited amount of baseline characteristics, thereby limiting a baseline comparison of both arms.(30)

Table 3. Author's judgement and support for judgement for risk of bias in included studies

	Random sequence generation (selection bias	Allocation concealment (selection bias)	Blinding (performance bias and detection bias) All outcomes
Chamorro, 2005	Low risk. Patients were randomised by using a computer-generated number sheet	Low risk. Patients 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	Low risk. Double-blind design, placebo controlled. Outcome assessment (e.g. the occurrence of infections) was assessed blindly because physicians were not aware of treatment allocation
De Falco, 1998	Low risk. The study is described as 'randomised'	Unclear risk. Allocation concealment is not mentioned	High risk. This study appears to have an open label design; blinding of outcome assessment is not described
Harms, 2008	Low risk. A computer generated allocation schedule was used	Low risk. Trial pharmacists in each site labelled the trial drugs with sequential study numbers according to randomisation lists prepared by the trial statistician and dispensed the drugs	Low risk. Study investigators and enrolling staff were masked to the assignment
Lampl, 2007	Low risk. The 8th number on the participant's identity card was used	High risk. * see below for explanation.	Low risk. Blinded study, outcomes were assessed blindly (although the adequacy of blind was not described)
Schwarz, 2008	Low risk. Randomi-sation was performed using a computer-generated number sheet and by opening a numbered, sealed envelope	Low risk. Randomisation was performed using a computer-generated number sheet and by opening a numbered, sealed envelope	High risk. This was an open label design. The assessment of infections during the study period was done by a blinded observer, but the assessment of secondary outcomes, such as NIHSS and mRS, was not done in a blinded fashion

* After contacting the author by e-mail we received an e-mail from the epidemiologist of this trial. The allocation concealment was described as follows "A patient arrived at the emergency room with signs of stroke. Emergency room personnel were aware that the study was recruiting participants and identified patients who met study inclusion criteria. Once this identification was made, the attending physician in the emergency room phoned me regardless of the time, day or night. In the emergency room were sealed, numbered packages containing medication. The attending physician read me the eighth digit of the patient's National Identity number. I referred to a randomisation list which had been computer-generated prior to study onset, and based on whether the eighth digit was odd or even, the

	Incomplete outcome data (attrition bias) All outcomes	Selective reporting (reporting bias)	Other bias
	Low risk. Table 2 indicates that all patients were seen at 90 days. Counts of patients with secondary outcomes by treatment however, are not provided	Low risk. All outcomes are reported (infections, case fatality, unfavourable functional outcome)	Low risk. No other sources of bias were found
	High risk. Nothing is reported about completeness of follow-up and outcome assessment	High risk. Outcome assessment is performed at discharge instead of a fixed time point	Low risk. No other sources of bias were found
	High risk. 7 patients were lost to follow-up, no details are mentioned	Low risk. All outcomes (infection, neurological outcome, adverse events and case fatality) are reported in prespecified intervals	Low risk. No other sources of bias were found
	High risk. The number of patients lost to follow-up is not mentioned. Scores on NIHSS, BI and mRS are presented as means	Low risk. All outcomes are reported	Low risk. No other sources of bias were found
	Low risk. There were no losses to follow-up	Low risk. There were no losses to follow-up in this study, not even at 90 days	Low risk. No other sources of bias were found

randomisation list assigned the patient to a numbered package. The attending physician then provided the medication inside the appropriately numbered package to the patient. Thus, the attending physician in the emergency room was blind to the treatment assignment. I was not blind to the treatment assignment, however, and was aware of the patient's treatment assignment. I, therefore, consider this trial open label." In conclusion, we do not know for sure whether blinding was maintained on the ward of the hospital. It could be possible that physicians were aware of the treatment because they knew that patients with even/odd NID numbers would get a certain treatment

Effects of interventions

Primary outcome

The overall number of patients who died was 33 out of 248 (13%) in the preventive antibiotics group versus 38 out of 258 (15%) in the placebo group (RR 0.85, 95%CI 0.47 to 1.51) (Figure 3 'Analysis 1.1'). The number needed to treat to prevent death is 50; however, this is not significant. In the preventive antibiotics group, the number of dependent patients was 97 out of 208 (47%) versus 127 out of 208 (61%) in the placebo group (RR 0.67, 95%CI 0.32 to 1.43) (Figure 4 'Analysis 1.2). Substantial heterogeneity was present in the analysis ($P < 0.00001$; $I^2 = 97\%$). In the predefined subanalysis of the two double-blinded randomised trials, the number of dependent patients at the end of follow-up was 60 out of 106 (57%) in the preventive antibiotics group versus 61 out of 109 (56%) in the placebo group (RR 1.02, 95% CI 0.78 to 1.35) and no substantial heterogeneity was found ($P = 0.27$; $I^2 = 17\%$). In contrast, in a subanalysis of the two open-label trials, 37 out of 102 (36%) patients in the preventive antibiotic group were dependent at the end of follow-up versus 66 out of 109 (67%) in the control group (RR 0.43, 95% CI 0.00 to 387.09), and substantial heterogeneity was present ($P < 0.0001$; $I^2 = 100\%$). All analyses were performed using a random-effects model. In a second analysis that excluded one study with pseudo-randomisation(29) no substantial heterogeneity was found ($P = 0.53$; $I^2 = 0\%$), this study showed a strong effect. One study was not included in the pooled analysis of functional outcome: *De Falco et al* did not report the number of dependent patients at the end of follow-up, but mean scores for both treatment groups.(30) This study showed a better mean functional outcome in the preventive antibiotics group, with a mean BI of 38.2 (SD32.4) in the preventive antibiotics group versus 21.8 (SD 27.6). Both case fatality and dependency at the end of treatment were not reported in any of the included studies.

Secondary outcomes

The number of infections at the end of follow-up was significantly reduced in the preventive antibiotics group compared to the placebo group: 36 out of 166 (22%) versus 61 out of 169 (36%) (RR 0.58, 95% CI 0.43 to 0.79) (Figure 5 'Analysis 2.1'). The occurrence of elevated body temperature was reported in two studies.(28, 31) This was assessed qualitatively and, therefore, data could not be pooled. Body temperature per day did not differ significantly between both 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. In one study, no adverse events related to study medication were present.(28) In the other study reporting on the occurrence of adverse events likely to be related to antibiotic therapy, two patients in the treatment group developed minor adverse events: drug-induced exanthema and asymptomatic, mildly elevated liver enzymes, possibly linked to the study drug. In both patients, various comedications and other conditions could also have caused

these symptoms.(31) In the only study that reported the occurrence of colonisation with antibiotic resistant micro-organisms, one infection with methicillin-resistant *Staphylococcus aureus* (MRSA) occurred in the preventive antibiotics group; however, colonisation was present before start of study medication.(28)

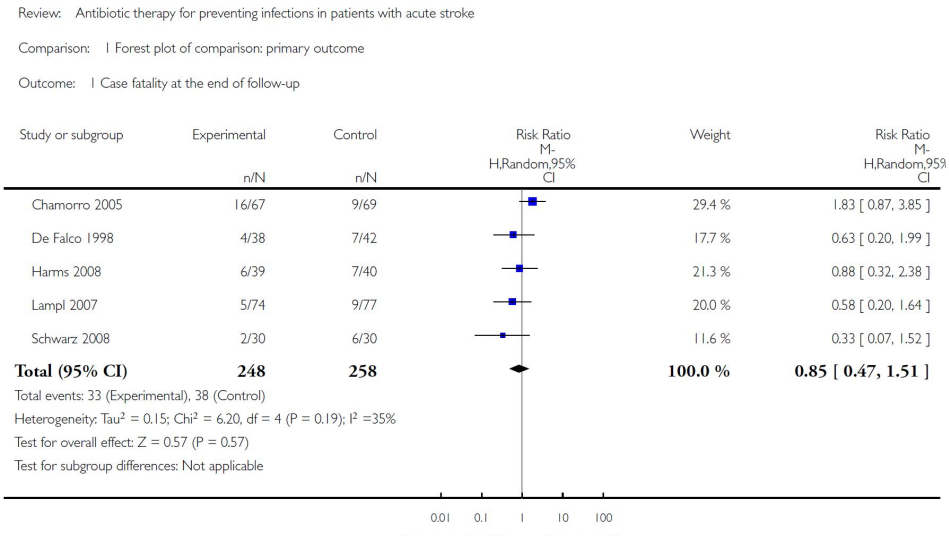

Figure 3. Analysis 1.1. Forest plot of comparison: primary outcome, case fatality at the end of follow-up

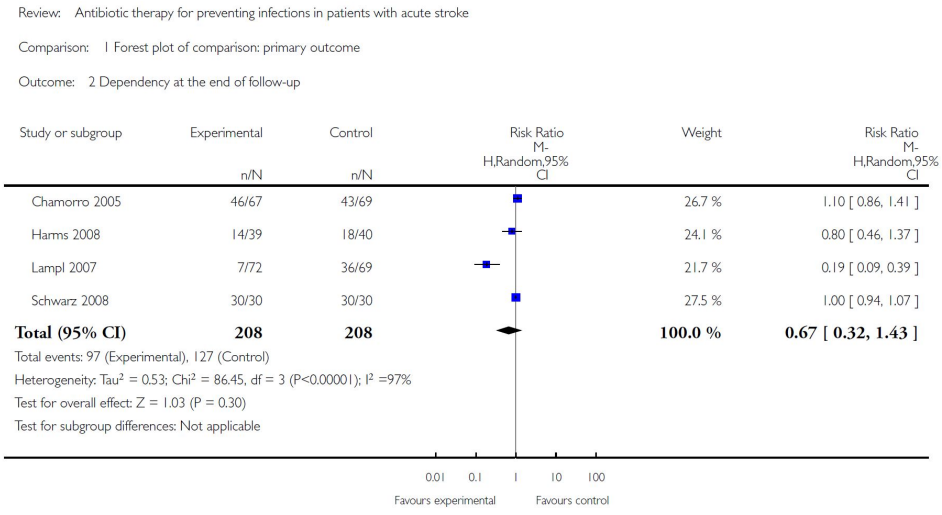

Figure 4. Analysis 1.2. Forest plot of comparison: primary outcome, dependency at the end of follow-up

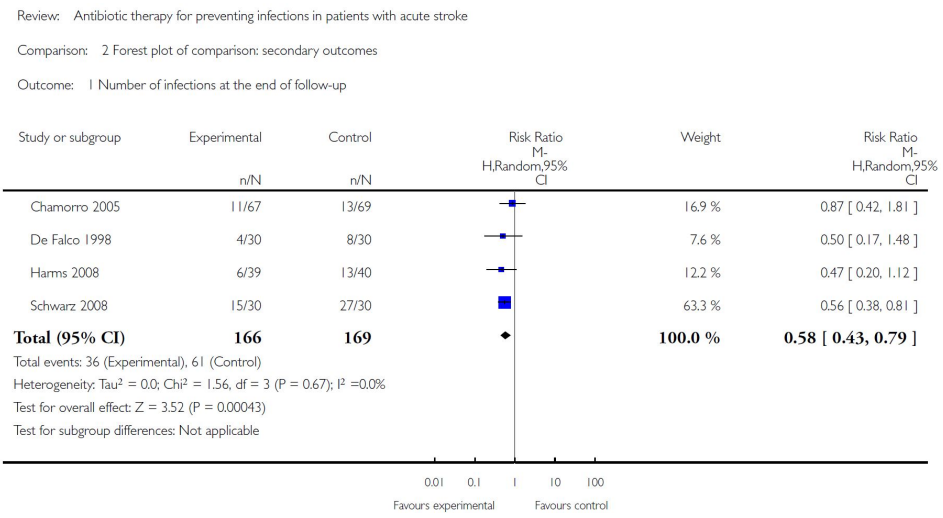

Figure 5. Analysis 2.1. Forest plot of comparison: number of infections at the end of follow-up

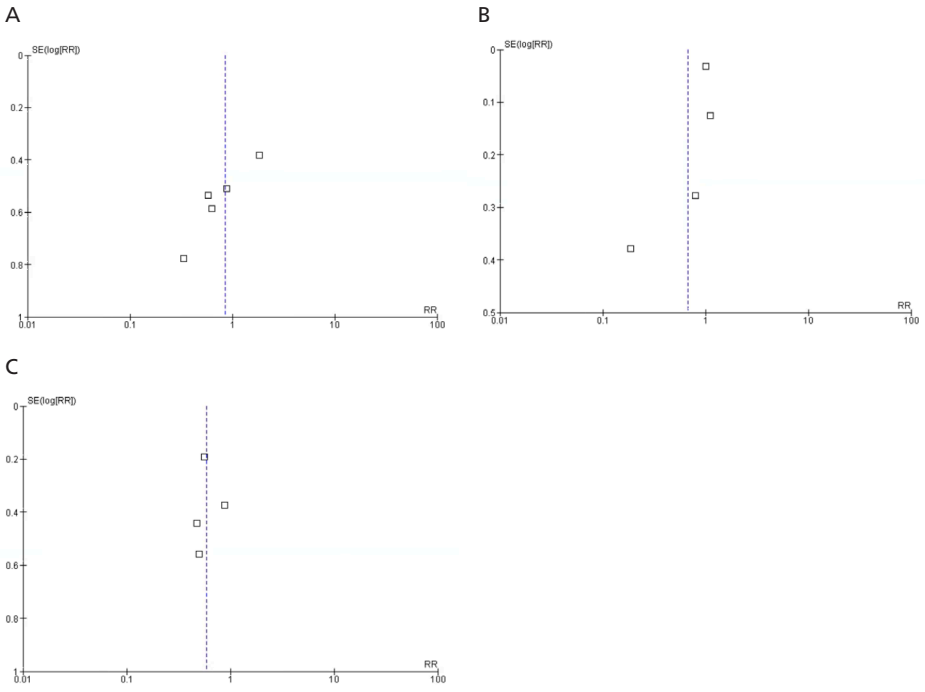

Figure 6. Funnel plots of comparison. A. Case fatality at the end of follow-up. B. Dependency at the end of follow-up. C. Number of infections at the end of follow-up.

Discussion

Summary of main results

This meta-analysis shows a non-significant reduction in the number of deceased or dependent acute stroke patients treated with preventive antibiotic therapy. Preventive antibiotic therapy did significantly reduce the occurrence of infections in patients with acute stroke from 36% to 22%. However, the included studies were small and heterogeneous.No major side-effects of preventive antibiotic therapy have been reported.

Overall completeness and applicability of evidence

Several issues are not adequately addressed in the available studies. Firstly, type of antibiotic therapy, dosage and duration varied between all five studies. Four studies used preventive antibiotic therapy that covered the common causative organisms in post-stroke infections. In contrast, in one study minocycline was used in order to investigate a possible neuroprotective effect. This preventive antibiotic therapy did not effectively cover the antimicrobial spectrum of post-stroke infections.(29) This study also did not report infection rate. Second, all studies included patients with ischaemic stroke only, except for one study that included 26 patients with haemorrhagic stroke. Therefore, no conclusions can be drawn on the effect of preventive antibiotic therapy in patients with haemorrhagic stroke. Regarding the primary outcomes of this meta-analysis, only data on case fatality were reported in all studies. Dependency was reported as a mean score in one study, 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 BI and CNS.The pooled analysis does not include data from this study and is based on the four studies that did report the number of dependent patients. The number of infections was not reported in one study, the pooled estimate is, therefore, based on four studies. All these four studies used different definitions for the diagnosis of infection. Less strict definitions might overestimate the number of infections, which could be a particular problem in studies with an open-label design.

No conclusions can be drawn on the effect of preventive antibiotic therapy on the occurrence of elevated body temperature, the length of hospital stay and the occurrence of opportunistic infections, due to lack of data. Limited data were also available on adverse events likely to be related to antibiotic therapy; this was reported in two studies. To account for the heterogeneity in study design (double-blind versus open-label studies), type of antibiotic therapy (adequately covering all pathogens in post-stroke infections versus mostly chosen for neuroprotective properties) and definitions of infection (**Appendix 3**), 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 design

of the studies. It is likely that results of this meta-analysis varied due to the obvious heterogeneity between the studies, and not only due to chance. Therefore, we preferred using a random-effects model.

Quality of the evidence

The evidence included in this meta-analysis does not allow a very robust conclusion on the use of preventive antibiotic therapy in acute stroke yet. This is due to different factors. First, the total number of studies and participants is limited: five studies were included with a total number of 506 participants. Second, as shown in the risk of bias table for each study, only one study scored a "low risk of bias" overall, and several biases may have influenced the results of the included studies. Selection bias could have confounded the results. Case fatality rates were very low in all included studies, ranging from 0% to 14%. Usually, case fatality rates in acute stroke range between 15% to 25%.(42) Also, one study excluded patients with a life expectancy of fewer than 90 days.(31) Selection of less severely affected patients might overestimate the effect of preventive antibiotic therapy, since 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. Stroke severity has previously been reported as a risk factor for post-stroke infection and incidence of infection is higher in these patients.(7, 43, 44) Foreknowledge of forthcoming allocations might have influenced selection in the open-label study that used quasi-randomisation. In this study, baseline characteristics were mostly similar in both arms, however, a larger proportion of the minocycline group received treatment with angiotensin-converting enzyme (ACE) inhibitors and sulphonylurea and a smaller proportion had a history of peptic ulcer disease. A possible effect of this baseline imbalance is not easily predicted. Two studies used an open-label design and one study did not specify blinding. Knowledge of the intervention in a trial can affect outcome when provided care differs in the two 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 an underestimation of a possible effect. Prescription of antibiotic therapy in the control group was not specified in included studies and can therefore not be compared. A double-blind design was used by two studies and outcomes were assessed blindly.(27, 28) In one study infection was assessed blindly, but other outcomes were not.(31) Outcome was not assessed blindly or outcome assessment was not described in two studies.(29, 30) Detection bias might influence results when outcome is not assessed blindly. However, it is not very likely that this influenced our primary endpoint case fatality, since this a hard endpoint. It could have affected the score on the mRS, since this is a less objective endpoint. The assessment of infection rate, however, might have been influenced more by an unblinded assessment, especially since criteria for the diagnosis of infection were not clearly specified. Physicians could detect infections more easily in the group without preventive antibiotic treatment, or less easily in the group treated with

preventive antibiotic therapy. In this analysis, infections occurred overall more frequently in studies with open-label design: 32% in the preventive antibiotic group versus 58% in the control group, in comparison to double-blind studies with 16%in the preventive antibiotic group versus 24% in the placebo group. For future studies, standardised definitions such as made by the Centers for Disease Control and Prevention (CDC) are preferable, especially in open-label trials. One study in this analysis used criteria derived from these CDC criteria, and this double-blind study showed a relatively low number of infections.(27) Attrition bias can occur when patients are withdrawn after randomisation. For example, excluding participants because of inability to complete the course of antibiotics owing to minor side effects (exanthema, diarrhoea) clearly introduces bias in favour of the studymedication. In four of the five included studies, attrition bias might have occurred. *Chamorro et al* did not describe counts of patients with secondary outcomes for each treatment group. *Harms et al* reported that seven patients were lost to follow-up without mentioning further details, this study had a positive effect in the per-protocol analysis only. The number of patients lost to follow-up was not mentioned at all by *De Falco et al* and *Lampl et al.* One study reported no losses to follow-up at all.(31)

Potential biases in the review process

Using multiple overlapping searches of a number of databases we aimed to include all relevant publications in this review. However, the possibility of missing small randomised clinical trials, published in journals with a lower impact, cannot be totally excluded. By searching trial registries and performing funnel plots (Figure 6) we aimed to analyze this influence. In the trial registries, only studies were identified that were either still ongoing or published. The funnel plots of studies included in this meta-analysis do not allow strong conclusions, since the total number of included studies and the number of included patients are small. Furthermore, in these studies a high prevalence of incomplete outcome reporting exists(45), which could also have affected our meta-analysis. By contacting the authors with requests for additional information (in order to get all the information for our prespecified analyses) we tried to minimize this influence. However, we did not obtain additional information from one study.(30) We excluded one trial from the analyses because the randomisation procedure was unclear; this trial showed no reduction of infectious complications in the preventive antibiotics group.(41)

Agreements and disagreements with other studies or reviews

One systematic review and meta-analysis on preventive antibiotic therapy in stroke was performed in 2009.(24) This Cochrane review is an upgrade of this review with the addition of one study.(30) The results of this review agree with the results of the previous meta-analysis.

Author's conclusions

Implications for practice

Currently, the use of preventive antibiotic therapy is not included in standard care for patients with acute stroke.(20) Results of this meta-analysis do not yet provide evidence that current practice has to be changed. However, the results do warrant further research on this topic since included studies were small and heterogeneous.

Implications for research

The observed effect in this meta-analysis warrants evaluation of preventive antibiotics in new stroke trials. These trials should use functional clinical outcomes and standardised definitions of infection and the type of antibiotic therapy should adequately cover all relevant pathogens in post-stroke infection. Also, use of antibiotic therapy in the control group should be monitored. To establish with certainty whether preventive antibiotic therapy has a place in the treatment of patients with acute stroke, an RCT enrolling a very large number of patients would need to be undertaken, aiming to detect even a small effect. This study should also evaluate the occurrence of antibiotic-resistant micro-organisms. Currently, different trials of preventive antibiotic therapy are enrolling patients. With the results of these trials, it is very likely that the necessary answers to our research questions will be provided.

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Contributions of authors

WF Westendorp (WW): performed the search, data extraction, analysis and interpretation of data and drafted the review. J-D Vermeij (JDV): performed the search, data extraction, analysis and interpretation of data. FH Vermeij (FV): conceived and designed the review, assessed methodological quality and drafted the protocol. HM den Hertog (HMDH): conceived and designed the review, commented on the review for intellectual content 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. DWJ Dippel (DWJD): conceived and designed the review, commented on it critically for intellectual content, assessed methodological quality and provided final approval of the review for publication. D van de Beek (DVDB): commented on the review for intellectual content and provided final approval of the review for publication.

Declarations of interest

Diederik van de Beek (DVDB) and Paul JNederkoorn (PJN) are the principal investigators of an ongoing RCT of preventive antibiotics in stroke, the Preventive Antibiotics in Stroke Study (ISRCTN66140176). All authors of this review and meta-analysis are members of the study group of this trial.

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Differences between protocol and review

The number of objectives has been shortened in the review in comparison with the protocol. In the protocol, seven objectives were described. In the review, we combined objectives five, six and seven (e.g. increases the incidence of opportunistic infections, leads to colonisation with antibiotic resistant micro-organisms and leads to an increased rate of serious adverse events) of the protocol into one objective (objective 5: leads to an increased rate of serious adverse events, such as anaphylactic shock, skin rash or colonisation with antibiotic resistant micro-organisms) in the review. This was done to shorten the text and increase the readability of the review.

Indexterms

Medical Subject Headings (MeSH)

Anti-Bacterial Agents [_therapeutic use]; Antibiotic Prophylaxis [methods]; Bacterial Infections [mortality; _prevention & control]; Brain Ischemia [complications]; Randomized Controlled Trials as Topic; Stroke [_complications; mortality]

MeSH check words

Humans. Antibiotic.

References

1. Aslanyan S, Weir CJ, Diener HC, Kaste M, Lees KR. Pneumonia and urinary tract infection after acute ischaemic stroke: a tertiary analysis of the GAIN International trial. Eur J Neurol. 2004;11(1):49-53.
2. Davenport RJ, Dennis MS, Wellwood I, Warlow CP. Complications After Acute Stroke. Stroke. 1996;27(3):415-20.
3. Kwan J, Hand P. Infection after acute stroke is associated with poor short-term outcome. Acta Neurol Scand. 2007;115(5):331-8.
4. Langhorne P, Stott DJ, Robertson L, MacDonald J, Jones L, McAlpine C, et al. Medical complications after stroke: a multicenter study. Stroke. 2000;31(6):1223-9.
5. Vargas M, Horcajada JP, Obach V, Revilla M, Cervera A, Torres F, et al. Clinical consequences of infection in patients with acute stroke: is it prime time for further antibiotic trials? Stroke. 2006;37(2):461-5.
6. Vermeij FH, Scholte op Reimer WJ, de Man P, van Oostenbrugge RJ, Franke CL, de Jong G, et al. Stroke-associated infection is an independent risk factor for poor outcome after acute ischemic stroke: data from the Netherlands Stroke Survey. Cerebrovasc Dis. 2009;27(5):465-71.
7. Kammersgaard LP, Jorgensen HS, Reith J, Nakayama H, Houth JG, Weber UJ, et al. Early infection and prognosis after acute stroke: the Copenhagen Stroke Study. J Stroke Cerebrovasc Dis. 2001;10(5):217-21.
8. Lee M, Huang WY, Weng HH, Lee JD, Lee TH. First-ever ischemic stroke in very old Asians: clinical features, stroke subtypes, risk factors and outcome. Eur Neurol. 2007;58(1):44-8.
9. Martino R, Foley N, Bhogal S, Diamant N, Speechley M, Teasell R. Dysphagia after stroke: incidence, diagnosis, and pulmonary complications. Stroke. 2005;36(12):2756-63.
10. Yilmaz GR, Cevik MA, Erdinc FS, Ucler S, Tulek N. The risk factors for infections acquired by cerebral hemorrhage and cerebral infarct patients in a neurology intensive care unit in Turkey. Jpn J Infect Dis. 2007;60(2-3):87-91.
11. Stott DJ, Falconer A, Miller H, Tilston JC, Langhorne P. Urinary tract infection after stroke. QJM. 2009;102(4): 243-9.
12. Walter U, Knoblich R, Steinhagen V, Donat M, Benecke R, Kloth A. Predictors of pneumonia in acute stroke patients admitted to a neurological intensive care unit. J Neurol. 2007;254(10):1323-9.
13. Chamorro A, Amaro S, Vargas M, Obach V, Cervera A, Torres F, et al. Interleukin 10, monocytes and increased risk of early infection in ischaemic stroke. J Neurol Neurosurg Psychiatry. 2006;77(11):1279-81.
14. Emsley HC, Smith CJ, Hopkins SJ. Infection and brain-induced immunodepression after acute ischemic stroke. Stroke. 2008;39(1):e7.
15. Haeusler KG, Schmidt WU, Fohring F, Meisel C, Helms T, Jungehulsing GJ, et al. Cellular immunodepression preceding infectious complications after acute ischemic stroke in humans. Cerebrovasc Dis. 2008;25(1-2):50-8.
16. Hilker R, Poetter C, Findeisen N, Sobesky J, Jacobs A, Neveling M, et al. Nosocomial pneumonia after acute stroke: implications for neurological intensive care medicine. Stroke. 2003;34(4):975-81.
17. Katzan IL, Cebul RD, Husak SH, Dawson NV, Baker DW. The effect of pneumonia on mortality among patients hospitalized for acute stroke. Neurology. 2003;60(4):620-5.
18. Carnaby G, Hankey GJ, Pizzi J. Behavioural intervention for dysphagia in acute stroke: a randomised controlled trial. Lancet Neurol. 2006;5(1):31-7.
19. Guidelines for management of ischaemic stroke and transient ischaemic attack 2008. Cerebrovascular diseases (Basel, Switzerland). 2008;25(5):457-507.
20. Adams HP, Jr., del ZG, Alberts MJ, Bhatt DL, Brass L, Furlan A, et al. Guidelines for the early management of adults with ischemic stroke: a guideline from the American Heart Association/American Stroke Association Stroke Council, Clinical Cardiology Council, Cardiovascular Radiology and Intervention Council, and the Atherosclerotic Peripheral Vascular Disease and Quality of Care Outcomes in Research Interdisciplinary Working Groups: The American Academy of Neurology affirms the value of this guideline as an educational tool for neurologists. Circulation. 2007;115(20):e478-e534.
21. de Smet AM, Kluytmans JA, Cooper BS, Mascini EM, Benus RF, van der Werf TS, et al. Decontamination of the digestive tract and oropharynx in ICU patients. N Engl J Med. 2009;360(1):20-31.
22. Falagas ME, Siempos, II, Bliziotis IA, Michalopoulos A. Administration of antibiotics via the respiratory tract for the prevention of ICU-acquired pneumonia: a meta-analysis of comparative trials. Critical care (London, England). 2006;10(4):R123.
23. Liberati A, D'Amico R, Pifferi, Torri V, Brazzi L. Antibiotic prophylaxis to reduce respiratory tract infections and mortality in adults receiving intensive care. The Cochrane database of systematic reviews. 2004(1):Cd000022.
24. van de Beek D, Wijdicks EF, Vermeij FH, de Haan RJ, Prins JM, Spanjaard L, et al. Preventive antibiotics for infections in acute stroke: a systematic review and meta-analysis. Arch Neurol. 2009;66(9):1076-81.

25. (editors) HJGS. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. 2011.
26. Review Manager (RevMan). 2011.
27. Chamorro A, Horcajada JP, Obach V, Vargas M, Revilla M, Torres F, et al. The Early Systemic Prophylaxis of Infection After Stroke study: a randomized clinical trial. Stroke. 2005;36(7):1495-500.
28. Harms H, Prass K, Meisel C, Klehmet J, Rogge W, Drenckhahn C, et al. Preventive antibacterial therapy in acute ischemic stroke: a randomized controlled trial. PLoS One. 2008;3(5):e2158.
29. Lampl Y, Boaz M, Gilad R, Lorberboym M, Dabby R, Rapoport A, et al. Minocycline treatment in acute stroke: an open-label, evaluator-blinded study. Neurology. 2007;69(14):1404-10.
30. P. DFFSRMLA. Antimicrobial prophylaxis in the management of ischemic stroke. Rivista di Neurobiologia 1998;44(1):63–7.1998.
31. Schwarz S, Al-Shajlawi F, Sick C, Meairs S, Hennerici MG. Effects of prophylactic antibiotic therapy with mezlocillin plus sulbactam on the incidence and height of fever after severe acute ischemic stroke: the Mannheim infection in stroke study (MISS). Stroke. 2008;39(4):1220-7.
32. Chamorro A, Urra X, Planas AM. Infection after acute ischemic stroke: a manifestation of brain-induced immunodepression. Stroke. 2007;38(3):1097-103.
33. Acquarolo A, Urli T, Perone G, Giannotti C, Candiani A, Latronico N. Antibiotic prophylaxis of early onset pneumonia in critically ill comatose patients. A randomized study. Intensive care medicine. 2005;31(4):510-6.
34. Sirvent JM, Torres A, El-Ebiary M, Castro P, de Batlle J, Bonet A. Protective effect of intravenously administered cefuroxime against nosocomial pneumonia in patients with structural coma. American journal of respiratory and critical care medicine. 1997;155(5):1729-34.
35. Gavriliuc M UA, Manole E, Cosciug L. Antimicrobial therapy in febrile patients with acute stroke. . European Journal of Neurology 2005;12 Suppl 2:53.2005.
36. Mountokalakis T, Skounakis M, Tselentis J. Short-term versus prolonged systemic antibiotic prophylaxis in patients treated with indwelling catheters. The Journal of urology. 1985;134(3):506-8.
37. Nyren P, Runeberg L, Kostiala AI, Renkonen OV, Roine R. Prophylactic methenamine hippurate or nitrofurantoin in patients with an indwelling urinary catheter. Annals of clinical research. 1981;13(1):16-21.
38. Gosney M, Martin MV, Wright AE. The role of selective decontamination of the digestive tract in acute stroke. Age Ageing. 2006;35(1):42-7.
39. Kalra L CR, Da vis A, Gulliford M, Patel A, Rudd A, et al. . 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. Proceedings of the 4th UK Stroke Forum 2009.2009.
40. Smithard D KL, Wolfe C, Patel A, Rudd A, Gulliford M. A cluster randomised trial of different strategies of antibiotic use to reduce the incidence and consequence of chest infection in acute stroke patients with dysphagia (Stroke-Inf). . Dysphagia 2009;24:4612009.
41. Majkowski J, Kunicka J, Szabelska K, Cendrowski W. [Prophylactic use of penicillin G and ampicillin in stroke. I. Clinical observations]. Neurologia i neurochirurgia polska. 1982;16(4):261-7.
42. van der Worp HB, van GJ. Clinical practice. Acute ischemic stroke. N Engl J Med. 2007;357(6):572-9.
43. Hamidon BB, Raymond AA. Risk factors and complications of acute ischaemic stroke patients at Hospital Universiti Kebangsaan Malaysia (HUKM). Med J Malaysia. 2003;58(4):499-505.
44. Kwon HM, Jeong SW, Lee SH, Yoon BW. The pneumonia score: a simple grading scale for prediction of pneumonia after acute stroke. Am J Infect Control. 2006;34(2):64-8.
45. Smyth RM, Kirkham JJ, Jacoby A, Altman DG, Gamble C, Williamson PR. Frequency and reasons for outcome reporting bias in clinical trials: interviews with trialists. Bmj. 2011;342:c7153.

Appendix 1. MEDLINE (Ovid)

We used the following search strategy for MEDLINE and adapted it for CENTRAL.

1. cerebrovascular disorders/ or exp basal ganglia cerebrovascular disease/ or exp brain ischemia/ or exp carotid arte ry 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/
2. (stroke$ or poststroke$ or cva$ or cerebrovascular$ or cerebral vascular).tw.
3. ((cerebral or cerebellar or brain$ or vertebrobasilar) adj5 (infarct$ or isch?emi$ or thrombo$ or apoplexy or emboli$)).tw.
4. ((cerebral or intracerebral or intracranial or brain or cerebellar or subarachnoid) adj5 (haemorrh age or he morrh age or haematoma or hematoma or bleeding or aneurysm)).tw.
5. 1 or 2 or 3 or 4
6. Antibiotic Prophylaxis/
7. exp Anti-Bacterial Agents/
8. (antibiotic$ or anti-bacterial or anti bacterial or antibacterial or bacteriocid$ or anti-mycobacterial or anti mycobacterial or antimycobacterial or anti-infect$ or anti infect$).tw.
9. (amoxicillin or amphotericin b or ampicillin or calcimycin or cephalosporin$ 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.
10. 7 or 8 or 9
11. exp infection/ or exp bacterial infections/ or exp infection control/ or exp fever/ or exp inflammation/
12. (infection$ or sepsis or septicaemia or septicemia or pneumonia or bacteremia or bacteraemia or inflammation or fever or blood poisoning).tw.
13. 11 or 12
14. (prophyla$ or prevent$ or premedicat$ or incidence or occurrence).tw.
15. prevention control.fs.
16. 15 or 14
17. 10 and 13 and 16
18. 6 or 17
19. 5 and 18

Appendix 2. EMBASE (Ovid)

We used the following search strategy for EMBASE.

1. 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 ce rebrovascular accident/ or exp cerebrovascular malformation/ or exp intracranial aneurysm/ or exp occlusive cerebrovascular disease/ or stroke/
2. stroke unit/ or stroke patient/
3. (stroke$ or poststroke$ or cva$ or cerebrovascular$ or cerebral vascular).tw.
4. ((cerebral or cerebellar or brain$ or vertebrobasilar) adj5 (infarct$ or isch?emi$ or thrombo$ or apoplexy or emboli$)).tw.
5. ((cerebral or intracerebral or intracranial or brain or cerebellar or subarachnoid) adj5 (haemorrh age or he morrh age or haematoma or hematoma or bleeding or aneurysm)).tw.
6. 1 or 2 or 3 or 4 or 5
7. antibiotic prophylaxis/
8. exp antibiotic agent/
9. (antibiotic$ or anti-bacterial or anti bacterial or antibacterial or bacteriocid$ or anti-mycobacterial or anti mycobacterial or antimycobacterial or anti-infect$ or anti infect$).tw.
10. (amoxicillin or amphotericin b or ampicillin or calcimycin or cephalosporin$ 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.
11. 8 or 9 or 10
12. exp infection/ or infection control/ or infection risk/ or fever/ or exp inflammation/
13. (infection$ or sepsis or septicaemia or septicemia or pneumonia or bacteremia or bacteraemia or inflammation or fever or blood poisoning).tw.
14. 12 or 13
15. (prophyla$ or prevent$ or premedicat$ or incidence or occurrence).tw.
16. prophylaxis/
17. 16 or 15
18. 11 and 14 and 17
19. infection prevention/ or exp infection/pc
20. 11 and 19
21. 7 or 18 or 20
22. 6 and 21

Appendix 3. Definitions used for infection

Source	Definition
Chamorro 2005	Temperature > 37.5°C in two determinations; or > 37.8 in a single determination in patients 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: 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 CRP. UTI: > 1 of the following: fever, temperature > 38.0°C), urine sample positive for nitrite, leucocyturia, and significant bacteriuria
Lampl 2007	Not evaluated
Schwarz 2008	Pneumonia: new infiltrate on chest x-ray compatible with the diagnosis of infection plus at least one 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 at least 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. Bacteremia: bacteria in blood cultures Sepsis: clinical evidence of an infection with at least two of the following: temperatures > 38°C or < 35°C, tachycardia > 90/minute, tachypnoea > 20/minute, 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

CHAPTER 4.1

Preventive antibiotics in stroke study: rationale and protocol for a randomised trial

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Abstract

Rationale

Stroke is a leading cause of death worldwide. Fever after stroke is a strong predictor of a poor outcome. Infections occur in up to 40% of patients with stroke and have also been associated with poor outcomes. Preventive antibiotic therapy lowers the infection rates in patients after stroke, as shown in a recent meta-analysis of randomised studies. Phase III trials evaluating the effect of antibiotic prophylaxis on clinical outcomes in sufficient numbers of patients with stroke have, however, not been performed to date. Ceftriaxone, an off-patent medicine, is an antibiotic with a broad defence against the bacteria that cause the most common infections after stroke. Preventive antibiotic therapy with ceftriaxone may potentially reduce poor outcome after acute stroke and, therefore, a randomised clinical trial is warranted.

Aim

The aim of the present study is to investigate whether the preventive use of the antibiotic ceftriaxone improves functional outcome in patients with stroke.

Design

We will conduct a multicentre prospective, randomised, open-label, blinded end point trial of standard care with preventive ceftriaxone treatment and compare it with standard care without preventive ceftriaxone.

Study

Adult patients with stroke (both ischaemic and haemorrhagic) and a score≥1 on the National Institutes of Health Stroke Scale will be included. The 3200 patients will be randomly assigned to two groups of 1600 patients. One group will receive standard care and ceftriaxone at a dose of 2 g, given every 24 h intravenously for four-days, and the other group will receive stroke-unit care without preventive antibiotic treatment.

Outcomes

The primary end point will be functional outcome at a three-month follow-up on the modified Rankin Scale, dichotomised as a favourable outcome (0–2) or an unfavourable outcome (3–6). Secondary outcome measures will be death rate at discharge and three-months, infection rate during hospital admission, length of hospital admission, volume of poststroke care, use of antibiotics during the three-month follow-up, functional outcome using the full ordinal scoring range of the modified Rankin Scale, quality adjusted life years and costs.

Key words

antibiotics, infection, pneumonia, prevention, stroke

Introduction

Stroke is a leading cause of death worldwide. The incidence of stroke is rapidly increasing because of the ageing population.(1) The only proven effective therapy for patients with ischaemic acute stroke is intravenous tissue plasminogen activator.(2) The 30-day case fatality rate for stroke varies between 15% and 25% and the rate of patients with a poor outcome remains unacceptably high, at 50%.(1, 2) The annual costs of stroke are high. In high-income countries, stroke ranks second after ischaemic heart disease from the perspective of costs for society.(1) The mean lifetime costs after stroke have been estimated to be between €38 000 and €133 000 per person. Half of these costs concern (nursing) care. New cost-effective acute stroke therapies are warranted.

A promising area to see benefits for acute stroke patients (with both ischaemic and haemorrhagic stroke) is the prevention of infection.(3) Infection has been associated with an unfavourable outcome after stroke.(3) This association is also present in patients admitted on a stroke-unit with frequent monitoring and an early start to treatment if infection is suspected. Pneumonia is the most common infection complicating acute stroke. Patients with an acute neurological deficit and swallowing disturbances are at a high risk of developing pneumonia in the first days after the onset of symptoms. Urinary tract infection is the second most common infection after pneumonia and may lead to severe systemic illness. There is also increasing evidence for 'stroke-induced immunodepression', an impaired cellular immunity that occurs in patients after stroke.(3) Ceftriaxone is an off-patent antibiotic with a broad action against the causative bacteria of infection after acute stroke. Recent studies suggested that ceftriaxone also has neuroprotective properties. In a rat model of ischaemic stroke, ceftriaxone reduced mortality and neurological deficits.(4) Neuronal survival was improved within the penumbra and ceftriaxone led to an upregulation of neurotrophins in the peri-infarct zone.(4) The combination of an effective antibiotic and neuroprotective agent makes ceftriaxone a highly interesting drug for the proposed clinical trial. Infections can be prevented by the use of preventive antibiotics in acute stroke. We performed a meta-analysis of four trials on preventive antibiotics in acute stroke, which included 426 patients.(5) The proportion of patients with infection was significantly smaller in the antibiotic group than in the placebo/control group [32/136 (23.5%) vs. 53/139 (38.1%)]. The pooled odds ratio on infection was 0.44 (95% confidence interval, 0.23–0.86). The use of preventive antibiotics was not associated with a significant reduction in death. We have found no major harm or toxicity. Current international guidelines do not recommend routine preventive antibiotic treatment in stroke patients.(6) The observed effect in our meta-analysis warrants evaluation, using functional clinical outcomes, of preventive antibiotics in a new stroke trial. The aim of the proposed study is to investigate whether the preventive use of the antibiotic ceftriaxone

improves functional outcome in patients with stroke by preventing infection. This will be done in a large multicentre randomised-controlled trial. Within this trial, we will also assess the cost-effectiveness of this preventive treatment.

Methods

Design

We will conduct a multicentre prospective, randomised, open label, blinded end point (PROBE) trial of standard care plus preventive treatment with ceftriaxone, as compared with standard care without ceftriaxone.

Patient population

All adult patients with stroke (both ischaemic and haemorrhagic) with a score ≥1 on the National Institutes of Health Stroke Scale (NIHSS) are eligible for the study. Patients with a stroke and NIHSS score of ≥1 (i.e., those with dysarthria or swallowing disturbance) have a substantial risk of developing stroke-associated infections. The NIHSS is a validated scale with good interobserver variability, and has been widely used in stroke research (2).We will include patients within the first 24 h after the onset of stroke symptoms. Exclusion criteria are kept simple (Table 1). When all the selection criteria are fulfilled, the patient will be asked for written informed consent. When the patient has diminished decision-making capacity as a result of the stroke, e.g. due to aphasia or cognitive impairment, informed consent will be obtained from the patient's representative. Exclusion of these noncommunicative stroke patients would lead to a selective patient sample.

Randomisation, blinding and treatment allocation

By using the PROBE design, by definition, blinding is lost, but only as to treatment. The randomisation procedure will be available online using permuted blocks, and stratified by study centre and stroke severity. Only after registration in the database can treatment allocation be performed and from this moment it will not be possible to remove a patient from the database. Information regarding the treatment allocation will be kept separate from the study database. The patient and the treating physician will be aware of the treatment assignment; in contrast, the assessors of outcome will be blinded for the treatment allocation. A trial statistician will report unblended data to the Data Safety and Monitoring Board (DSMB) for evaluation and interim analysis. The steering committee will be kept unaware of these results unless necessary (as judged by the DSMB) and the code will not be broken until the last patients have completed three-months of follow-up.

Table 1. Inclusion and exclusion criteria

Inclusion criteria
Age > 18 years
Stroke (ischaemic and haemorrhagic)
Any measurable neurological deficit defined as NIHSS ≥ 1
Stroke onset < 24 h
Admission
Exclusion criteria
Clinical signs of infection on admission requiring antibiotic therapy
Use of antibiotics < 24 h before admission
Pregnancy
Hypersensitivity for cephalosporin
Anaphylaxis for penicillin derivates
Subarachnoidal haemorrhage
Death seems imminent

NIHSS, National Institutes of Health Stroke Scale.

Intervention

The study medication will be ceftriaxone 2 g, intravenously, once daily for four-days. Ceftriaxone is started within 24 h after stroke onset. If patients are discharged before day 4 after admission, the study medication will be stopped. The study medication may also be stopped if the treating physician decides to withdraw active treatment in a patient with a poor or an infaust prognosis. The treating physician will decide whether or not to treat a patient with suspected infection with (additional) antibiotics. Recommendations will be made for the treatment of infections according to the Dutch Working Party on Antibiotic Policy [Stichting Werkgroep Antibiotica Beleid (SWAB)] guidelines.(7)

Study procedures

At baseline, patient history will be recorded, and physical, as well as additional examinations, will be performed according to standard care in acute stroke patients.(6, 8) Patients with an infection on admission will be excluded from the study. The diagnosis of infection on admission will be made by the treating physician and is pragmatically defined as signs or symptoms of infection requiring antibiotic therapy. At discharge, we will record data on infection as the secondary end point two ways: first, according to the treating physician, who will register whether pneumonia, a urinary tract infection or another infection was diagnosed in the clinical setting. Second, according to the judgement of two experienced infectious disease specialists, who will be blinded for treatment allocation, using the modified criteria of the United States Centers for Disease Control and Prevention.(8) For this purpose, we will collect data on the diagnostic procedures in patients with 'clinical

infection' during admission. Recommendations for these procedures are a chest X-ray, two blood cultures, urine analysis and urine culture, a sputum culture, leucocyte count and C-reactive protein from a venous blood sample. This recommendation is based on standard procedures for identifying a focus of infection in poststroke care and therefore with no additional burden to the patient. In patients with diarrhoea, faeces will be tested for *Clostridium difficile* toxin. After three months, a structured interview by telephone will be performed in order to assess the primary outcome, expressed in a modified Rankin Scale (mRS). A short questionnaire will be sent regarding the amount of poststroke care, and patients will be asked to return this to the study centre.

Primary outcome

The primary efficacy end point will be functional outcome at the three-month follow-up, as assessed by the mRS dichotomized as a favourable outcome (mRS 0–2) or as an unfavourable outcome (mRS 3–6).(9) The proportional odds model will be used in a secondary analysis of the primary end point (see 'Discussion').

Secondary outcomes

Secondary outcomes will be the death rate at discharge and three-months, the infection rate during hospital admission, the length of hospital admission, volume of poststroke care, the use of antibiotics during the three-month follow-up, functional outcome using the full ordinal scoring range of the mRS, quality-adjusted life years (QALYs) and costs.

Data safety and monitoring board

The DSMB is an independent committee comprised of three trial experts in neurology, microbiology and statistics. It will monitor the safety of the trial and perform an interim analysis. Based on this information, they will advise the steering committee on prespecified grounds, as formulated by the DSMB.

Sample size

It is expected that 50% of the stroke patients included will have an unfavourable health outcome (mRS scores 3–6, including death). Calculation of the required sample size is carried out based on the assumption that ceftriaxone will reduce the proportion of patients with an unfavourable outcome from 50% to 45%. A two-group chi^2-test with a 0.05 two-sided significance level will have 80% power to detect the difference between a standard care proportion of 0.50 and a treatment group proportion of 0/45 (odds ratio of 0.818) when the sample size in each group is 1565. Although we expect that all (or at least a very high proportion) of the patients included will be available for evaluation at the end of the study, we intend to enrol 1600 patients per treatment arm: 3200 patients in total.

Statistical analyses

Statistical analysis will be based on the intention-to-treat principle. Baseline assessments and outcome parameters will be summarised using simple descriptive statistics. The main analysis will focus on a comparison between the trial treatment groups of the primary outcome and a dichotomised mRS score expressed in a relative risk (RR) estimate. In addition, the proportional odds model will be used to analyse the ordinal outcome data on the mRS. The secondary outcome parameters (death rate, infection rate, length of hospital stay, volume of poststroke care, volume of antibiotics and functional outcome using the full ordinal scoring range of the mRS) will be analysed using the w2-test (including RR estimates), the two-group t-test or the Mann–Whitney test, when appropriate. Finally, we will perform predefined subgroup analyses for stroke type (infarction or haemorrhage), severe strokes (NIHSS score >9) time to treatment (0-6 h, 6-12 h, 12-24 h) and the presence of swallowing disorder. In all analyses, statistical uncertainties will be quantified via corresponding 95% confidence intervals.

Economic evaluation

The economic evaluation of preventive antibiotic therapy with ceftriaxone against standard care without ceftriaxone after stroke will be performed from a societal perspective as a costeffectiveness analysis (CEA), as well as cost-utility analysis. The costs per patient with favourable outcome and the costs per QALY as the primary outcome will be measured. All relevant direct and indirect medical and nonmedical costs will be assessed. The net health benefits of preventive antibiotic therapy will be calculated (including 95% confidence intervals) for several willingness-to-pay values per extra patient with a favourable outcome and per extra QALY.

Discussion

The current trial will have a PROBE design. A double-blind, placebo-controlled designed trial might have been superior, but this design would be associated with considerably higher costs as compared with the PROBE design. In the current design, blinding will be maintained regarding the assessment of the primary outcome. A potential advantage of the PROBE design is that the effect of ceftriaxone found in our trial will resemble the effect in clinical practice after implementation. Physicians will be aware of treatment allocation and an antibiotic intervention can easily be adjusted in the case of a clinical suspicion on infection. We will include the entire spectrum of patients with stroke and also those admitted with a relatively low NIHSS score (≥1). Patients in this subgroup could also be at risk for infection, because of the hypothesis of immunodepression. Hopefully, this will enhance the inclusion rate because 3200 patients are needed. Dysphagia occurs

in many patients with acute stroke, and is a strong predictor of pneumonia independent of NIHSS scores.(10-12) Aspiration is expected in patients with a large hemispheric lesion but also with a lower brainstem lesion. Acute stroke, ischaemic stroke in particular, may also lead to stroke-induced immunodepression, a functional decrease in cellular immune response that is related to susceptibility to infection.(3) Finally, sympathetic activation is increased in patients with acute stroke, resulting in gastrointestinal dysmotility, which also poses a risk for aspiration pneumonia. Pneumonia is a well-recognised predictor of a poor outcome and mortality in patients after acute stroke. The primary outcome will be a dichotomised outcome on the mRS. This is in line with other large stroke trials.(9) A secondary analysis based on the concept of 'proportional odds' will also be performed. The proportional odds model provides additional information from ordinal outcome data, as it takes into account improvements at any point on the mRS.(13) Therefore, we will use this method in a secondary analysis of the primary end point. Beneficial effects on this outcome should be accompanied by effects on the classic end points in the same direction to be considered convincing. Rationale for choosing the antibiotic ceftriaxone is as follows: first, to prevent infections, the antimicrobial spectrum should cover most common causative bacteria of pneumonia and urinary tract infections. *Streptococcus pneumoniae, Haemophilus influenzae, Staphylococcus aureus* and *Enterobacteriaceae* predominate in patients with aspiration pneumonia that occurs within four-days after admission (community acquired aspiration syndrome).(14) The most common causative bacteria of urinary tract infections are *Escherichia coli* and other *Enterobacteriaceae*.(15) Ceftriaxone, a third generation cephalosporin and b-lactam antibiotic, is an offpatent antibiotic with a broad action against causative bacteria of infection after acute stroke. Second, antibiotics after acute stroke may also offer neuro protection. Ceftriaxone has such a neuroprotective action.(4, 16, 17) In a rat model of ischaemic stroke, administration of ceftriaxone resulted in reduced mortality and neurological deficits.(4) Neuronal survival was improved within the penumbra, and ceftriaxone led to an upregulation of neurotrophins in the peri-infarct zone. Finally, ceftriaxone has a favourable safety profile. Treatment with ceftriaxone has shown to be safe in numerous trials and patient series and most side effects are minor, without clinical consequences.(5) Nevertheless, serious adverse events can occur and will be reported to the Preventive Antibiotics in Stroke Study (PASS) trial office. Serious adverse events of ceftriaxone that will be recorded are *C. difficile* infection, severe allergic reaction or pancreatitis (in patients with risk factors for formation of biliary sludge or stasis). Also, minor side effects of ceftriaxone will be scored. The final aspect to address is increasing antibiotic resistance due to an increase of antibiotic usage. In a previous phase II trial evaluating antibiotic prophylaxis in patients with acute stroke, no difference was found in the antibiotic resistance patterns between treatment and placebo groups.(18) Nevertheless, the increasing use of antibiotics will lead to increasing resistance rates.(19) Regarding the use of ceftriaxone in this study, the

most important issue to address is the increase of bacteria (mostly *Enterobacteriaceae*) capable of forming extended-spectrum-b-lactamase (ESBL). The ESBL is an enzyme that hydrolyses b-lactam antibiotics by which they become ineffective. In the Netherlands, the rates of ESBL-producing bacteria are increasing and estimates of the prevalence of E. coli resistant to third-generation cephalosporins in the general population are reported from 1% to 5%, and *Klebsiella pneumoniae* 5–10%.(20) In our study, we want to monitor a possible change in prevalence to 10% for *E. coli* and to 15% for *K. pneumoniae*. We will therefore perform analyses in a subgroup of 556 patients on stool cultures, obtained on day 0, day 7 and after three-months. Patients will be consecutively drawn from selected centres and diagnostic procedures will be performed centrally. This problem will also be evaluated model-wise in the CEA. The results of this subanalysis will be interpreted in the light of the growing burden of antimicrobial resistance and carefully weighted with regard to the potential benefit for individual patients. In conclusion, with regard to the points discussed, we think that PASS will provide a reliable estimate of the clinical effect of preventive antibiotic therapy.

Summary

Infections (i.e., pneumonia and urinary tract infections) occur in up to 40% of patients with stroke and have been associated with a poor outcome. Recent randomised studies have shown that preventive antibiotic therapy lowers the infection rate in patients after stroke. Phase III trials evaluating the effect of preventive antibiotic therapy on the clinical outcome in a sufficient number of patients with stroke have not been performed to date. In the PASS, we will investigate whether the preventive use of the antibiotic ceftriaxone improves the functional outcome in patients with stroke by preventing infection. The design is a multicentre PROBE trial. Adult patients with stroke (both ischaemic and haemorrhagic) and a score ≥1 on the NIHSS will be included. Patients will be randomly assigned to two groups of 1600 patients: one to receive standard care plus ceftriaxone at a dose of 2 g, given every 24 h intravenously for four-days, and the other given standard care without ceftriaxone. The primary end point will be the functional outcome at the three-month follow-up, as assessed using the mRS, dichotomised as a favourable outcome (0–2) or as an unfavourable outcome (3–6). In addition, a CEA will be performed.

References

1. van der Worp HB, van GJ. Clinical practice. Acute ischemic stroke. N Engl J Med. 2007;357(6):572-9.
2. Hacke W, Donnan G, Fieschi C, Kaste M, von KR, Broderick JP, et al. Association of outcome with early stroke treatment: pooled analysis of ATLANTIS, ECASS, and NINDS rt-PA stroke trials. Lancet. 2004;363(9411):768-74.
3. Emsley HC, Hopkins SJ. Acute ischaemic stroke and infection: recent and emerging concepts. Lancet Neurol. 2008;7(4):341-53.
4. Thone-Reineke C, Neumann C, Namsolleck P, Schmerbach K, Krikov M, Schefe JH, et al. The beta-lactam antibiotic, ceftriaxone, dramatically improves survival, increases glutamate uptake and induces neurotrophins in stroke. J Hypertens. 2008;26(12):2426-35.
5. van de Beek D, Wijdicks EF, Vermeij FH, de Haan RJ, Prins JM, Spanjaard L, et al. Preventive antibiotics for infections in acute stroke: a systematic review and meta-analysis. Arch Neurol. 2009;66(9):1076-81.
6. Adams HP, Jr., del ZG, Alberts MJ, Bhatt DL, Brass L, Furlan A, et al. Guidelines for the early management of adults with ischemic stroke: a guideline from the American Heart Association/American Stroke Association Stroke Council, Clinical Cardiology Council, Cardiovascular Radiology and Intervention Council, and the Atherosclerotic Peripheral Vascular Disease and Quality of Care Outcomes in Research Interdisciplinary Working Groups: The American Academy of Neurology affirms the value of this guideline as an educational tool for neurologists. Circulation. 2007;115(20):e478-e534.
7. Stichting Werkgroep Antibiotica Beleid (SWAB) guidelines. . In: (SWAB) SWAB, editor. Available at http://www.swab.nl (last accessed 22 November 2010).
8. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 2008;36(5):309-32.
9. Banks JL, Marotta CA. Outcomes validity and reliability of the modified Rankin scale: implications for stroke clinical trials: a literature review and synthesis. Stroke. 2007;38(3):1091-6.
10. Hamidon BB, Nabil I, Raymond AA. Risk factors and outcome of dysphagia after an acute ischaemic stroke. Med J Malaysia. 2006;61(5):553-7.
11. Martino R, Foley N, Bhogal S, Diamant N, Speechley M, Teasell R. Dysphagia after stroke: incidence, diagnosis, and pulmonary complications. Stroke. 2005;36(12):2756-63.
12. Sharma JC, Fletcher S, Vassallo M, Ross I. What influences outcome of stroke--pyrexia or dysphagia? Int J Clin Pract. 2001;55(1):17-20.
13. McHugh GS, Butcher I, Steyerberg EW, Lu J, Mushkudiani N, Marmarou A, et al. Statistical approaches to the univariate prognostic analysis of the IMPACT database on traumatic brain injury. J Neurotrauma. 2007;24(2):251-8.
14. Marik PE. Aspiration pneumonitis and aspiration pneumonia. N Engl J Med. 2001;344(9):665-71.
15. Stamm WE, Hooton TM. Management of urinary tract infections in adults. N Engl J Med. 1993;329(18):1328-34.
16. Lee SG, Su ZZ, Emdad L, Gupta P, Sarkar D, Borjabad A, et al. Mechanism of ceftriaxone induction of excitatory amino acid transporter-2 expression and glutamate uptake in primary human astrocytes. J Biol Chem. 2008;283(19):13116-23.
17. Lipski J, Wan CK, Bai JZ, Pi R, Li D, Donnelly D. Neuroprotective potential of ceftriaxone in in vitro models of stroke. Neuroscience. 2007;146(2):617-29.
18. Harms H, Prass K, Meisel C, Klehmet J, Rogge W, Drenckhahn C, et al. Preventive antibacterial therapy in acute ischemic stroke: a randomized controlled trial. PLoS One. 2008;3(5):e2158.
19. Hawkey PM. The growing burden of antimicrobial resistance. J Antimicrob Chemother. 2008;62 Suppl 1:i1-i9.
20. EARSS. European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2015. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm: ECDC; 2017.

CHAPTER 4.2

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

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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.

Conclusion

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; www.controlled-trials.com; ISRCTN: 66140176. Date of registration: 6 April 2010.

Keywords

Stroke, Infection, Antibiotics

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.

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 hospital stay, 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.

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.

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.

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; www.controlled-trials.com; ISRCTN: 66140176
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 sponsor(s)	-
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 prospective randomised 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 amendments
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).

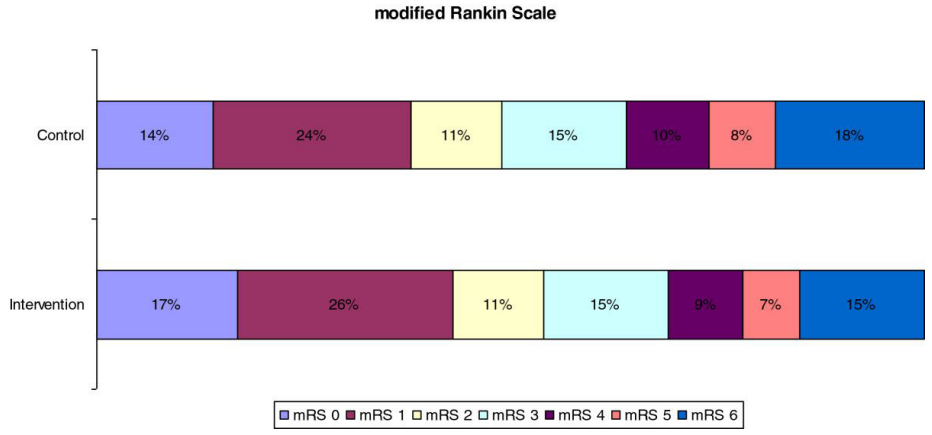

Figure 1. Expected distribution of the two treatment arms. The assumption for the distribution on mRS in the control-arm is based on the control-arm in the the Paracetamol (Acetaminophen) In Stroke (PAIS) trial, which had almost similar inclusion criteria as PASS.(13)

Recruitment target

By 12 February 2014, 2,470 patients were included in the PASS. Up-to-date statistics can be found at 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.

Abbreviations

AMC, Academic Medical Centre; ESBL, extended-spectrum-β-lactamase, mRS, modified Rankin Scale; 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.

Competing interests

All authors declare not to have any competing interests.

Authors' contributions

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.

Acknowledgements

The PASS study was funded by the Academic Medical Centre (AMC), by the Netherlands Organisation for Health Research and Development (ZonMW; 171002302) and the Netherlands Heart Foundation (Hartstichting; 2009B095). Principal investigators of the PASS are Dr. PJ Nederkoorn and Professor D van de Beek. DvdB is supported by grants from the European Research Council (ERC Starting Grant (Proposal/Contract number 281156)), Netherlands Organization for Health Research and Development (ZonMw;NWO-Vidi grant 2010 (Proposal/Contract number 016.116.358)). The study group comprises Professor DWJ Dippel; Dr. MGW Dijkgraaf; Professor JM Prins; Dr. L Spanjaard; Professor T van der Poll; Dr. FH Vermeij. Two PhD students working on the PASS are Dr. WF Westendorp and Dr. J-D Vermeij. Trial manager and nurses are Drs. IJ Hooijenga, AG de Jong and I Stijnman– Moerman**.** 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.

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

Centre	Local investigator
Academic Medical Centre, Amsterdam	PJ Nederkoorn; D van de Beek
Albert Schweitzer Hospital, Dordrecht	H Kerkhoff
Amphia Hospital, Breda	MJM Remmers
Amstelland Hospital, Amstelveen	DSM Molenaar
Atrium Medical Centre, Heerlen	T Schreuder
Boven-IJ Hospital, Amsterdam	M Janmaat
Bronovo Hospital, The Hague	SM Manschot
Catharina Hospital, Eindhoven	K Keizer
Erasmus Medical Centre, Rotterdam	DWJ Dippel
Groene Hart Hospital, Gouda	K de Gans
HAGA Hospital, The Hague	SF de Bruijn
Kennemer Gasthuis, Haarlem	M Weisfelt
Laurentius Hospital, Roermond	ML van Goor
Martini Hospital, Groningen	ES Schut
Medical Centre Haaglanden, The Hague	K Jellema
Medical Centre Alkmaar	R ten Houten
Onze Lieve Vrouwe Gasthuis Amsterdam	JLM Bosboom
Orbis Medical Centre, Sittard	N van Orshoven
Rijnstate Hospital, Arnhem	SE Vermeer
Reinier de Graaf Hospital, Delft	LAM Aerden
Slotervaart Hospital, Amsterdam	ND Kruyt
Spaarne Hospital, Hoofddorp	ISJ Merkies
St. Franciscus Gasthuis, Rotterdam	FH Vermeij
University Medical Centre Radboud, Nijmegen	E van Dijk
University Medical Centre, Utrecht	HB van der Worp
VU Medical Centre, Amsterdam	MC Visser
Westfries Gasthuis Hoorn	TC van der Ree
Ijsselland Hospital, Capelle aan den IJssel	AD Wijnhoud
Zaans Medical Centre, Zaandam	RM van den Berg - Vos
ZGT, Almelo	LJA Reichman

References

1. Vermeij FH, Scholte op Reimer WJ, de Man P, van Oostenbrugge RJ, Franke CL, de Jong G, et al. Stroke-associated infection is an independent risk factor for poor outcome after acute ischemic stroke: data from the Netherlands Stroke Survey. Cerebrovasc Dis. 2009;27(5):465-71.
2. Westendorp WF, Nederkoorn PJ, Vermeij JD, Dijkgraaf MG, van de Beek D. Post-stroke infection: A systematic review and meta-analysis. BMC Neurol. 2011;11:110.
3. van de Beek D, Wijdicks EF, Vermeij FH, de Haan RJ, Prins JM, Spanjaard L, et al. Preventive antibiotics for infections in acute stroke: a systematic review and meta-analysis. Arch Neurol. 2009;66(9):1076-81.
4. Westendorp WF, Vermeij JD, Vermeij F, den Hertog HM, Dippel DW, van de Beek D, et al. Antibiotic therapy for preventing infections in patients with acute stroke. Cochrane Database Syst Rev. 2012;1:CD008530.
5. Nederkoorn PJ, Westendorp WF, Hooijenga IJ, de Haan RJ, Dippel DW, Vermeij FH, et al. Preventive antibiotics in stroke study: rationale and protocol for a randomised trial. Int J Stroke. 2011;6(2):159-63.
6. Banks JL, Marotta CA. Outcomes validity and reliability of the modified Rankin scale: implications for stroke clinical trials: a literature review and synthesis. Stroke. 2007;38(3):1091-6.
7. Bath PM, Gray LJ, Collier T, Pocock S, Carpenter J. Can we improve the statistical analysis of stroke trials? Statistical reanalysis of functional outcomes in stroke trials. Stroke. 2007;38(6):1911-5.
8. Anderson CS, Heeley E, Huang Y, Wang J, Stapf C, Delcourt C, et al. Rapid blood-pressure lowering in patients with acute intracerebral hemorrhage. N Engl J Med. 2013;368(25):2355-65.
9. den Hertog HM, van der Worp HB, van Gemert HM, Algra A, Kappelle LJ, van GJ, et al. Correction: PAIS: paracetamol (acetaminophen) in stroke; protocol for a randomized, double blind clinical trial. [ISCRTN74418480]. BMC Cardiovasc Disord. 2008;8:29.
10. Miller SW, Palesch YY. Comments regarding the recent OAST article. Stroke. 2008;39(1):e14.
11. Hofmeijer J, Kappelle LJ, Algra A, Amelink GJ, van GJ, van der Worp HB. Surgical decompression for space-occupying cerebral infarction (the Hemicraniectomy After Middle Cerebral Artery infarction with Life-threatening Edema Trial [HAMLET]): a multicentre, open, randomised trial. Lancet Neurol. 2009;8(4):326-33.
12. Ananth CV, Kleinbaum DG. Regression models for ordinal responses: a review of methods and applications. Int J Epidemiol. 1997;26(6):1323-33.
13. den Hertog HM, van der Worp HB, van Gemert HM, Algra A, Kappelle LJ, van GJ, et al. The Paracetamol (Acetaminophen) In Stroke (PAIS) trial: a multicentre, randomised, placebo-controlled, phase III trial. Lancet Neurol. 2009;8(5):434-40.
14. Whitehead J. Sample size calculations for ordered categorical data. Stat Med. 1993;12(24):2257-71.
15. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 2008;36(5):309-32.
16. Katzan IL, Cebul RD, Husak SH, Dawson NV, Baker DW. The effect of pneumonia on mortality among patients hospitalized for acute stroke. Neurology. 2003;60(4):620-5.
17. Kwan J, Hand P. Infection after acute stroke is associated with poor short-term outcome. Acta Neurol Scand. 2007;115(5):331-8.
18. McHugh GS, Butcher I, Steyerberg EW, Marmarou A, Lu J, Lingsma HF, et al. A simulation study evaluating approaches to the analysis of ordinal outcome data in randomized controlled trials in traumatic brain injury: results from the IMPACT Project. Clin Trials. 2010;7(1):44-57.
19. Savitz SI, Lew R, Bluhmki E, Hacke W, Fisher M. Shift analysis versus dichotomization of the modified Rankin scale outcome scores in the NINDS and ECASS-II trials. Stroke. 2007;38(12):3205-12.

CHAPTER 4.3

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, randomised, open-label, blinded endpoint (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.

Keywords

Stroke, infection, antibiotics, randomised clinical trial, statistical analysis plan

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 randomised 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 randomisation code will not be broken before acceptance of the current paper for publication.

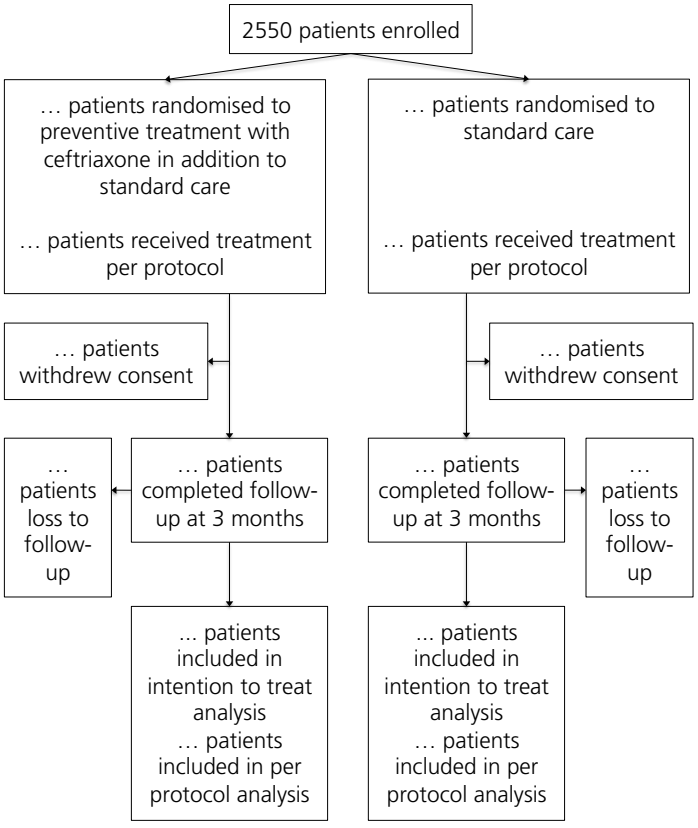

Figure 1. Flow-chart of patients

Summary study protocol

PASS is a multicenter prospective, randomised, 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 ischaemic and haemorrhagic), 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 haemorrhage, 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. Randomisation was performed through ALEA (online software for randomised trials; https://nl.tenalea.net/amc/ALEA/Login.aspx) and is based on a uniform distribution; weight of the arms is equal (1:1). Randomisation is stratified according to study center (academic hospital, large nonacademic 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 (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)

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		
No neurological symptoms (NIHSS = 0)		
Onset of stroke >24 hours ago		
Infection at admission		
Use of antibiotics <24 hours before admission		
Pregnancy		
Known hypersensitivity to cephalosporins		
Previous anaphylaxis for penicillin derivates		
Subarachnoidal haemorrhage		
Death is imminent		
Total number of protocol violations in eligibility		

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.

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 randomised more than once, the first randomisation outcome was used. Patients who withdrew consent directly after randomisation (that is, before treatment was initiated in those randomised for ceftriaxone in addition to standard care, or within 6 hours after randomisation in those randomised 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).

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). 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.

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

Patient number	Explanation	Treatment allocation

Table 3. Baseline characteristics

Baseline characteristics	Ceftriaxone + standard care	Standard care
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 inhibitor		
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 denotes National Institutes of Health Stroke Scale.

Protocol deviations in eligibility, consent procedure, treatment

When a patient was randomised 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 randomisation 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 randomised 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 the 'Outline of figures and tables' section (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.

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).

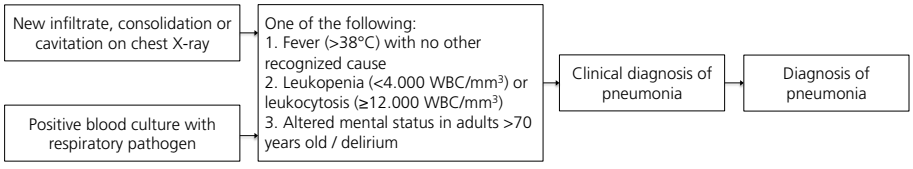

Figure 2. Diagnosis of pneumonia

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.

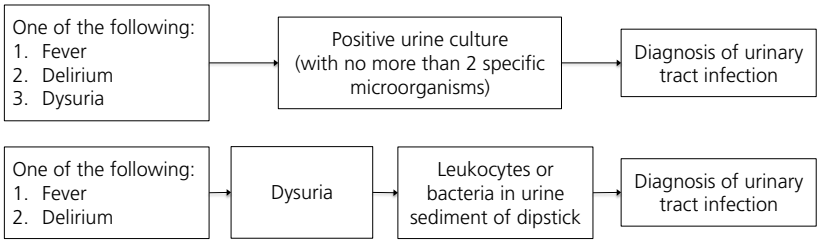

Figure 3. Diagnosis of urinary tract infection

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 haemorrhage, 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 randomisation 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.

Analysis of secondary outcomes

The number of patients with one or more in-hospital poststroke 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.

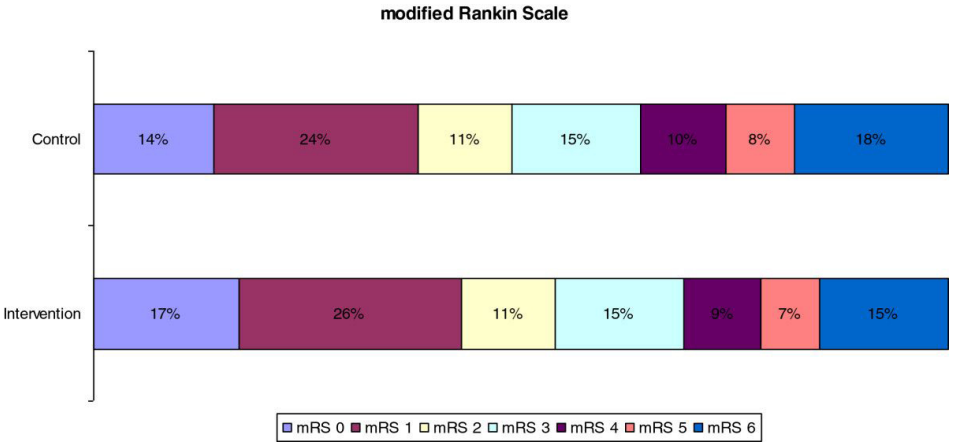

Figure 4. Graphic display of primary outcome

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).

Subgroup analysis of primary and secondary outcomes

We will perform the following sub-group analyses for the primary outcome: stroke type (infarction or haemorrhage), 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 4. Secondary outcomes

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

Authorships

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.

Table 5. Number and type of Serious Adverse Events

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 SAE's			

*SAE: serious adverse event

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 %.			

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.

Table 7. Subgroup analysis of primary outcome

	Ceftriaxone + standard care (n =…)	Standard care (n =…)	P	OR 95%
Favorable outcome (mRS* 0 to 2) - % n/N				
• Ischaemic stroke				
• Haemorrhagic stroke				
• Transient ischaemic attack (TIA)				
• Other				
Favorable outcome (mRS 0 to 2) - % n/N				
• NIHSS** 1 to 9				
• 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: modified Rankin Scale. **NIHSS: National Institutes of Health Stroke Scale.

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 randomised 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.

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 ischaemic attack; VAP: ventilator-associated pneumonia.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

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.

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sor DWJ Dippel; Dr. MGW Dijkgraaf; Professor JM Prins; Dr. L Spanjaard; Professor T van der Poll; Dr. FH Vermeij. Two PhD students working on the PASS are WF Westendorp and J-D Vermeij. Trial manager and nurses are IJ Hooijenga, AG de Jong and I Stijnman- Moerman. Thirty Dutch hospitals participate in the PASS; all centers with local investigators are shown in Table. 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.

References

1. Feigin VL, Forouzanfar MH, Krishnamurthi R, Mensah GA, Connor M, Bennett DA, et al. Global and regional burden of stroke during 1990-2010: findings from the Global Burden of Disease Study 2010. Lancet. 2014;383(9913):245-54.
2. Vermeij FH, Scholte op Reimer WJ, de Man P, van Oostenbrugge RJ, Franke CL, de Jong G, et al. Stroke-associated infection is an independent risk factor for poor outcome after acute ischemic stroke: data from the Netherlands Stroke Survey. Cerebrovasc Dis. 2009;27(5):465-71.
3. Westendorp WF, Nederkoorn PJ, Vermeij JD, Dijkgraaf MG, van de Beek D. Post-stroke infection: A systematic review and meta-analysis. BMC Neurol. 2011;11:110.
4. van de Beek D, Wijdicks EF, Vermeij FH, de Haan RJ, Prins JM, Spanjaard L, et al. Preventive antibiotics for infections in acute stroke: a systematic review and meta-analysis. Arch Neurol. 2009;66(9):1076-81.
5. Westendorp WF, Vermeij JD, Vermeij F, den Hertog HM, Dippel DW, van de Beek D, et al. Antibiotic therapy for preventing infections in patients with acute stroke. Cochrane Database Syst Rev. 2012;1:CD008530.
6. Nederkoorn PJ, Westendorp WF, Hooijenga IJ, de Haan RJ, Dippel DW, Vermeij FH, et al. Preventive antibiotics in stroke study: rationale and protocol for a randomised trial. Int J Stroke. 2011;6(2):159-63.
7. Westendorp WF, Vermeij JD, van GN, Dippel DW, Dijkgraaf MG, van der Poll T, et al. Update on the Preventive Antibiotics in Stroke Study (PASS): a randomised controlled phase 3 clinical trial. Trials. 2014;15:133.
8. National Institute of Health, National Institute of Neurological Disorders and Stroke. Stroke Scale. 2014.
9. ALEA Software for randomisation in clinical trials. In ALEA Version - Release: 2.2 build: 2070.
10. Rizopoulos D, Lesaffre E. Introduction to the special issue on joint modelling techniques. Stat Methods Med Res. 2014;23(1):3-10.
11. Janssen PM, Visser NA, Dorhout Mees SM, Klijn CJ, Algra A, Rinkel GJ. Comparison of telephone and face-to-face assessment of the modified Rankin Scale. Cerebrovasc Dis. 2010;29(2):137-9.
12. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 2008;36(5):309-32.
13. World Health Organization: ATC/DDD Index. 2014. http://www.whocc.no/atc_ddd_index/, last updated 2013-12-19.
14. Connolly SJ, Ezekowitz MD, Yusuf S, Eikelboom J, Oldgren J, Parekh A, et al. Dabigatran versus warfarin in patients with atrial fibrillation. N Engl J Med. 2009;361(12):1139-51.
15. Granger CB, Alexander JH, McMurray JJ, Lopes RD, Hylek EM, Hanna M, et al. Apixaban versus warfarin in patients with atrial fibrillation. N Engl J Med. 2011;365(11):981-92.
16. Granger CB AJ, McMurray JJ, Lopes RD, Hylek EM, Hanna M,, Al-Khalidi HR AJ, Atar D, Avezum A, Bahit MC, Diaz R, Easton JD,, Ezekowitz JA FG, Garcia D, Geraldes M, Gersh BJ, Golitsyn S, Goto S,, Hermosillo AG HS, Horowitz J, Mohan P, Jansky P, Lewis BS,, Lopez-Sendon JL PP, Parkhomenko A, Verheugt FW, et al:. Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. N Engl J Med 2011, 365:883–891.
17. Gray LJ, Bath PM, Collier T. Should stroke trials adjust functional outcome for baseline prognostic factors? Stroke. 2009;40(3):888-94.
18. Kahan BC, Jairath V, Dore CJ, Morris TP. The risks and rewards of covariate adjustment in randomized trials: an assessment of 12 outcomes from 8 studies. Trials. 2014;15:139.
19. Johnston KC, Connors AF, Jr., Wagner DP, Knaus WA, Wang X, Haley EC, Jr. A predictive risk model for outcomes of ischemic stroke. Stroke. 2000;31(2):448-55.
20. Melsen WG, Rovers MM, Groenwold RH, Bergmans DC, Camus C, Bauer TT, et al. Attributable mortality of ventilator-associated pneumonia: a meta-analysis of individual patient data from randomised prevention studies. Lancet Infect Dis. 2013;13(8):665-71.
21. Thone-Reineke C, Neumann C, Namsolleck P, Schmerbach K, Krikov M, Schefe JH, et al. The beta-lactam antibiotic, ceftriaxone, dramatically improves survival, increases glutamate uptake and induces neurotrophins in stroke. J Hypertens. 2008;26(12):2426-35.
22. Hawkey PM. The growing burden of antimicrobial resistance. J Antimicrob Chemother. 2008;62 Suppl 1:i1-i9.
23. Baquero F, Negri MC, Morosini MI, Blazquez J. Antibiotic-selective environments. Clin Infect Dis. 1998;27 Suppl 1:S5-11.
24. Schechner V, Temkin E, Harbarth S, Carmeli Y, Schwaber MJ. Epidemiological interpretation of studies examining the effect of antibiotic usage on resistance. Clinical microbiology reviews. 2013;26(2):289-307.

25. Harms H, Prass K, Meisel C, Klehmet J, Rogge W, Drenckhahn C, et al. Preventive antibacterial therapy in acute ischemic stroke: a randomized controlled trial. PLoS One. 2008;3(5):e2158.
26. de Smet AM, Kluytmans JA, Blok HE, Mascini EM, Benus RF, Bernards AT, et al. Selective digestive tract decontamination and selective oropharyngeal decontamination and antibiotic resistance in patients in intensive-care units: an open-label, clustered group-randomised, crossover study. Lancet Infect Dis. 2011;11(5):372-80.
27. McHugh GS, Butcher I, Steyerberg EW, Marmarou A, Lu J, Lingsma HF, et al. A simulation study evaluating approaches to the analysis of ordinal outcome data in randomized controlled trials in traumatic brain injury: results from the IMPACT Project. Clin Trials. 2010;7(1):44-57.

CHAPTER 5

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

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Summary

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 multicentre, 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 2550 patients from 30 sites in the Netherlands, including academic and non-academic medical centres, were randomly assigned to the two treatment groups: 1275 patients to ceftriaxone and 1275 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 2538 patients available for the intention-to-treat-analysis (1268 in the ceftriaxone group and 1270 in the control group). 2514 (99%) of 2538 patients (1257 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.

Funding

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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.

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 centres, 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 centre (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 3200 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 2410 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 2531 patients; we therefore set the sample size as 2550 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 centre. 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 in the regression models were obtained by averaging with use of Rubin's rule.(20) Variables in the imputation model were stratification factors for rando-

misation (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 appendix (table 2 and 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, 2550 adult patients from 30 Dutch sites were enrolled and randomly assigned in a 1:1 ratio to the two study groups: 1275 patients to receive ceftriaxone and 1275 patients to receive standard stroke unit treatment without ceftriaxone (the control group) (figure 1). 12 patients (seven in the ceftriaxone group and fi ve in the control group) withdrew consent immediately after randomisation, leaving 2538 patients available for the intention-to-treat-analysis (1268 in the ceftriaxone group and 1270 in the control group). Preventive antimicrobial treatment was started in 1242 (98%) of 1268 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 2514 (99%) of 2538 patients (1257 in each group). 24 patients were lost to follow-up (11 in the ceftriaxone group and 13 in the control group; appendix table 2). The baseline demographic and clinical characteristics of the two groups were similar (table 1). The definite diagnosis was assessed at discharge in 2538 patients: 2125 (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 table 3). Intravenous thrombolytic therapy was given to 836 (33%) of 2538 patients.

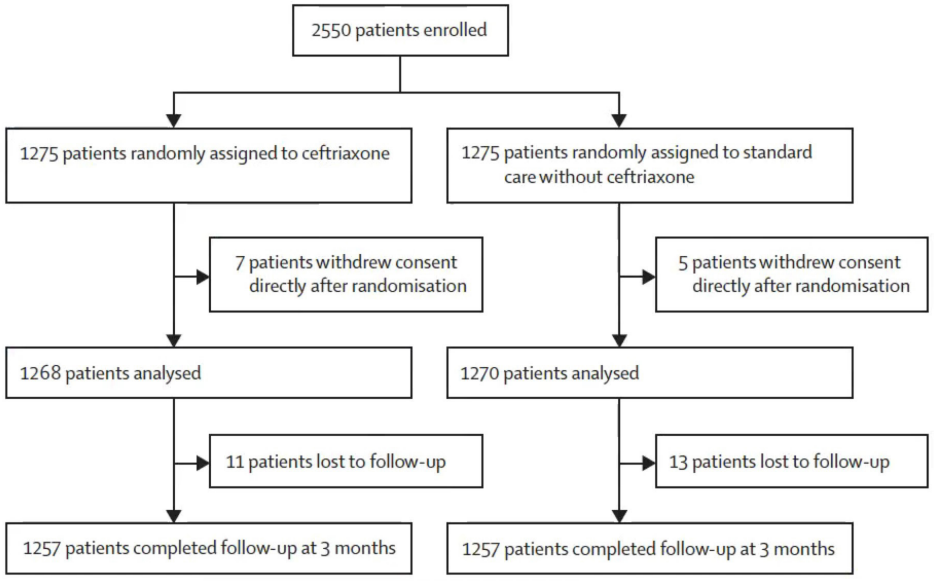

Figure 1. Trial profile

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 table 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).

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). During hospital stay, physicians diagnosed an infection in 348 (14%) of 2538 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 2538 patients: urinary tract infection in 76 patients (3%), pneumonia in 57 patients (2%), and other infections in nine patients (<1%).

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 2538 patients (1268 in the ceftriaxone group and 1270 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 2538 patients (1268 in the ceftriaxone group and 1270 in the standard treatment group).

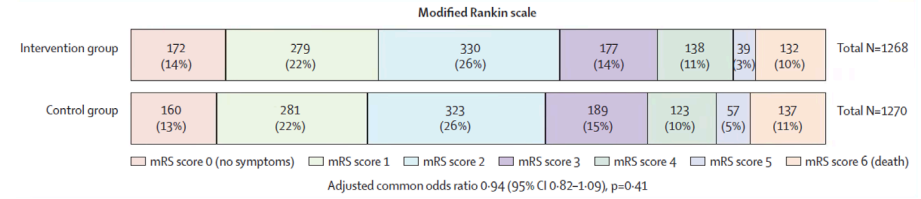

Figure 2. Distribution of modified Rankin Scale scores 3 months after randomisation. mRS=-modified Rankin Scale. Scores on the scale range from 0 to 6, with 0 indicating no symptoms and 6 indicating death. Numbers in the intervention group do not add up to the total number of patients because of the used imputation techniques of outcome in 24 patients.

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 table 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 table 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]).

Table 2. Outcomes

	Ceftriaxone group (n=1268)	Control group (n=1270)	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 1257 patients in the ceftriaxone group and in 1257 in the control group; 24 outcomes were imputed according to the protocol. †Length of hospital stay was assessed in 1267 patients in the ceftriaxone group and in 1268 patients in the control group.

The total antimicrobial use during hospital stay, as measured by defined daily doses, was higher in the ceftriaxone group than in the control group (4979 defined daily doses vs 2120 defined daily doses). In the ceftriaxone group, 4075 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 thegroups (table 2). 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 demo graphic 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 table 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.

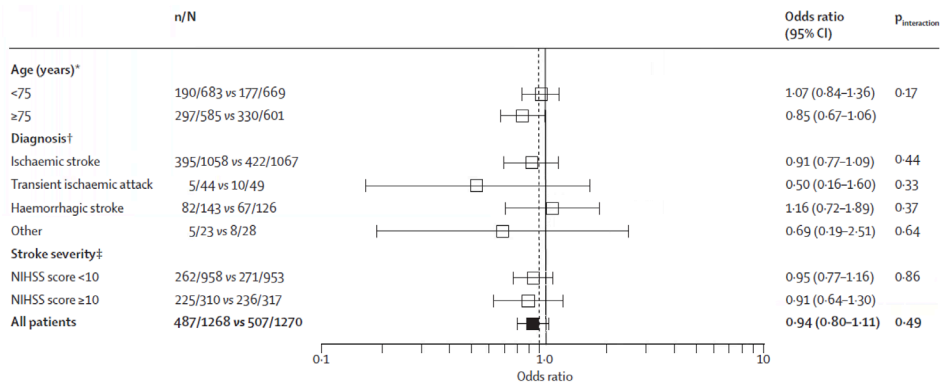

Figure 3. Subgroup analyses on unfavourable outcome 3 months after randomisation Subgroups are age, stroke type, and stroke severity. *Age was dichotomised at 75 years; the median age was 73 years (IQR 63–81) in the ceftriaxone group and 74 years (IQR 63–81) in the control group. †Stroke type was defined 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.

Table 3. Adverse events

	Ceftriaxone group (n=1242)	Control group (n=1270)	p-value
Diarrhoea caused by *Clostridium difficile**	2 (<0.2%)	0	..
Allergic reaction causing cessation of ceftriaxone	7 (0.6%)	0	..
Infection by a ceftriaxoneresistant 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 defned 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%). 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=1200; 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.

Contributors

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.

Declaration of interests

We declare no competing interests.

Acknowledgments

The centres and investigators participating in the Preventive Antibiotics in Stroke Study are listed in the appendix (table 1). Data safety monitoring board: G J Hankey, A Algra, and M J M Bonten; advisory board: R J de Haan and M Vermeulen; writing committee: W F Westendorp, J-D Vermeij, M C Brouwer, P J Nederkoorn, and D van de Beek. This trial was funded by The Netherlands Organization for Health Research and Development (grant no. 171002302 to DvdB and PJN; grant no. 016116358 to DvdB), The Netherlands Heart Foundation (grant no. 2009B095 to DvdB and PJN), and the European Research Council (ERC Starting Grant to DvdB). We thank Graeme Hankey and Ale Algra for their comments on the report.

References

1. Chamorro A, Meisel A, Planas AM, Urra X, van de Beek D, Veltkamp R. The immunology of acute stroke. Nature reviews Neurology. 2012;8(7):401-10.
2. Westendorp WF, Nederkoorn PJ, Vermeij JD, Dijkgraaf MG, van de Beek D. Post-stroke infection: A systematic review and meta-analysis. BMC Neurol. 2011;11:110.
3. Finlayson O, Kapral M, Hall R, Asllani E, Selchen D, Saposnik G. Risk factors, inpatient care, and outcomes of pneumonia after ischemic stroke. Neurology. 2011;77(14):1338-45.
4. Popovic N, Stefanovic-Budimkic M, Mitrovic N, Urosevic A, Milosevic B, Pelemis M, et al. The frequency of poststroke infections and their impact on early stroke outcome. Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association. 2013;22(4):424-9.
5. Vargas M, Horcajada JP, Obach V, Revilla M, Cervera A, Torres F, et al. Clinical consequences of infection in patients with acute stroke: is it prime time for further antibiotic trials? Stroke. 2006;37(2):461-5.
6. Chamorro A, Horcajada JP, Obach V, Vargas M, Revilla M, Torres F, et al. The Early Systemic Prophylaxis of Infection After Stroke study: a randomized clinical trial. Stroke. 2005;36(7):1495-500.
7. Harms H, Prass K, Meisel C, Klehmet J, Rogge W, Drenckhahn C, et al. Preventive antibacterial therapy in acute ischemic stroke: a randomized controlled trial. PLoS One. 2008;3(5):e2158.
8. Lampl Y, Boaz M, Gilad R, Lorberboym M, Dabby R, Rapoport A, et al. Minocycline treatment in acute stroke: an open-label, evaluator-blinded study. Neurology. 2007;69(14):1404-10.
9. P. DFFSRMLA. Antimicrobial prophylaxis in the management of ischemic stroke. Rivista di Neurobiologia 1998;44(1):63–7.1998.
10. Schwarz S, Al-Shajlawi F, Sick C, Meairs S, Hennerici MG. Effects of prophylactic antibiotic therapy with mezlocillin plus sulbactam on the incidence and height of fever after severe acute ischemic stroke: the Mannheim infection in stroke study (MISS). Stroke. 2008;39(4):1220-7.
11. Westendorp WF, Vermeij JD, Vermeij F, den Hertog HM, Dippel DW, van de Beek D, et al. Antibiotic therapy for preventing infections in patients with acute stroke. Cochrane Database Syst Rev. 2012;1:CD008530.
12. Adams HP, Jr., del ZG, Alberts MJ, Bhatt DL, Brass L, Furlan A, et al. Guidelines for the early management of adults with ischemic stroke: a guideline from the American Heart Association/American Stroke Association Stroke Council, Clinical Cardiology Council, Cardiovascular Radiology and Intervention Council, and the Atherosclerotic Peripheral Vascular Disease and Quality of Care Outcomes in Research Interdisciplinary Working Groups: The American Academy of Neurology affirms the value of this guideline as an educational tool for neurologists. Circulation. 2007;115(20):e478-e534.
13. Nederkoorn PJ, Westendorp WF, Hooijenga IJ, de Haan RJ, Dippel DW, Vermeij FH, et al. Preventive antibiotics in stroke study: rationale and protocol for a randomised trial. Int J Stroke. 2011;6(2):159-63.
14. Westendorp WF, Vermeij JD, Dippel DW, Dijkgraaf MG, van der Poll T, Prins JM, et al. Update of the Preventive Antibiotics in Stroke Study (PASS): statistical analysis plan. Trials. 2014;15:382.
15. Westendorp WF, Vermeij JD, van GN, Dippel DW, Dijkgraaf MG, van der Poll T, et al. Update on the Preventive Antibiotics in Stroke Study (PASS): a randomised controlled phase 3 clinical trial. Trials. 2014;15:133.
16. Janssen PM, Visser NA, Dorhout Mees SM, Klijn CJ, Algra A, Rinkel GJ. Comparison of telephone and face-to-face assessment of the modified Rankin Scale. Cerebrovasc Dis. 2010;29(2):137-9.
17. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 2008;36(5):309-32.
18. World Health Organization: ATC/DDD Index. 2014. http://www.whocc.no/atc_ddd_index/, last updated 2013-12-19.
19. Whitehead J. Sample size calculations for ordered categorical data. Stat Med. 1993;12(24):2257-71.
20. DB R. Multiple imputation for nonresponse in surveys. . New York: John Wiley & Sons; 1987.
21. van de Beek D, Wijdicks EF, Vermeij FH, de Haan RJ, Prins JM, Spanjaard L, et al. Preventive antibiotics for infections in acute stroke: a systematic review and meta-analysis. Arch Neurol. 2009;66(9):1076-81.
22. Wechsler LR. Intravenous thrombolytic therapy for acute ischemic stroke. The New England journal of medicine. 2011;364(22):2138-46.
23. Chu K, Lee ST, Sinn DI, Ko SY, Kim EH, Kim JM, et al. Pharmacological Induction of Ischemic Tolerance by Glutamate Transporter-1 (EAAT2) Upregulation. Stroke. 2007;38(1):177-82.
24. Rothstein JD, Patel S, Regan MR, Haenggeli C, Huang YH, Bergles DE, et al. Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. Nature. 2005;433(7021):73-7.
25. Machado LS, Sazonova IY, Kozak A, Wiley DC, El-Remessy AB, Ergul A, et al. Minocycline and tissue-type plasminogen activator for stroke: assessment of interaction potential. Stroke. 2009;40(9):3028-33.

Appendix 'Preventive Antibiotics in Stroke Study'

Table 1. 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.

Table 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

Table 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

Table 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=1268)	**Control group (n=1270)**	**Odds Ratio**	**95 % confidence interval**	**P value**
Unfavourable outcome*					
All patients†	489 (39)	508 (40)	0.94	0.80-1.10	0.47
Ischaemic stroke	395 (38)	419 (40)	0.82	0.77-1.09	0.348
Transient ischaemic attack	5 (11)	10 (20)	0.49	0.15-1.56	0.267
Haemorrhagic stroke	81 (57)	68 (54)	1.11	0.69-1.81	0.712
Other	8	12	0.92	0.32-2.65	1.000
Mortality‡					
Discharge	57 (5)	61 (5)	0.933	0.645-1.350	0.777
3 months	131 (10)	136 (11)	0.961	0.745- 1.238	0.796

*Unfavourable outcome at 3 months was evaluated in 1257 patients in the ceftriaxone group and 1257 patients in the control group; 24 outcomes were imputed according to protocol.
†No. of patients with characteristic (percentage).
‡Mortality was evaluated at discharge in 1267 patients in the ceftriaxone group and 1269 patients in the control group; mortality at 3 months was evaluated in 1268 patients in the ceftriaxone group and 1270 patients in the control group.

Table 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 ischaemic attack	3 (1)	7 (2)
Cerebral haemorrhage	0	0
Other	8 (1)	12 (3)

ACE denotes Angiotensin-converting enzyme.

Table 6. Patients with allergic reaction as noted by physician that caused cessation of ceftriaxone

PASS no.	Allergic reaction
1091	Skin rash during 2nd dose ceftriaxone
10111	Skin rash after 2nd dose ceftriaxone
12063	Dizziness, chest pain, sweating, nausea and vomiting, no cardiac cause was found
12064	Skin rash during 1st dose ceftriaxone
14237	Itching without skin rash during 2nd dose ceftriaxone
15039	Swelling of tongue after ceftriaxone, possibly also due to thrombolysis
20058	Cessation after 1st dose ceftriaxone because of allergic reaction to penicillin in medical history which was not noted at time of inclusion
59038	Skin rash after 2nd dose ceftriaxone

Table 7. Infections with ceftriaxone resistant organisms

PASS no.	Infection
2001	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.
5069	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
12005	Diagnosis urinary tract infection and pneumonia by physician and panel. ESBL producing *E. coli* cultured from urine, no symptoms, no treatment initiated.
13025	Other infection by physician, no other infection by panel
14195	Other infection by physician and panel: sepsis by candida. No antibiotic resistance reported.
14386	Urinary tract infection by physician, not by panel
14439	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)
18003	Pneumonia by physician, not by panel. In discharge letter no resistant micro-organism reported and treatment with amoxicillin/clavulanate potassium was started
19111	Pneumonia by physician and panel. No resistant microorganism reported.
20037	Urinary tract infection by physician during treatment with ceftriaxone, treatment with gentamycin and tobramycin was started (not scored as infection by panel)
20049	Pneumonia by physician, urinary tract infection by panel. No data on resistant micro-organisms reported

Table 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

Table 9. Explanation of protocol violations in eligibility

PASS no.	Explanation of protocol violation in eligibility
11160	Score of 0 on NIHSS and diagnosis subdural hematoma
12050	Score of 0 on NIHSS
12136	Infection and use of antibiotic at admission
14038	Score of 0 on NIHSS
14105	Patient was already randomised in another intervention trial
14221	Use of antibiotics < 24 hours of admission
15057	Onset of stroke > 24 hours ago
16020	Onset of stroke > 24 hours ago
16046	Infection at admission
19105	Patient was already randomised in another intervention trial

Table 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)

Table 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)

CHAPTER 6

Preventive Antibiotics in Stroke Study (PASS) A cost-effectiveness study

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Abstract

Objective

To evaluate the cost-effectiveness of preventive ceftriaxone vs standard stroke unit care without preventive antimicrobial therapy in acute stroke patients.

Methods

In this multicentre, randomised, open-label trial with masked endpoint assessment, 2,550 patients with acute stroke were included between 2010 and 2014. Economic evaluation was performed from a societal perspective with a time horizon of 3 months. Volumes and costs of direct, indirect, medical, and nonmedical care were assessed. Primary outcome was cost per unit of the modified Rankin Scale (mRS) and per quality-adjusted life year (QALY) for costeffectiveness and cost-utility analysis. Incremental cost-effectiveness analyses were performed.

Results

A total of 2,538 patients were available for the intention-to-treat analysis. For the cost-effectiveness analysis, 2,538 patients were available for in-hospital resource use and 1,453 for other resource use. Use of institutional care resources, out-of-pocket expenses, and productivity losses was comparable between treatment groups. The mean score on mRS was 2.38 (95% confidence interval [CI] 2.31–2.44) vs 2.44 (95% CI 2.37–2.51) in the ceftriaxone vs control group, the decrease by 0.06 (95% CI –0.04 to 0.16) in favor of ceftriaxone treatment being nonsignificant. However, the number of QALYs was 0.163 (95% CI 0.159–0.166) vs 0.155 (95% CI 0.152–0.158) in the ceftriaxone vs control group, with the difference of 0.008 (95% CI 0.003–0.012) in favor of ceftriaxone ($p = 0.006$) at 3 months. The probability of ceftriaxone being cost-effective ranged between 0.67 and 0.89. Probability of 0.75 was attained at a willing-to-pay level of €2,290 per unit decrease in the mRS score and of €12,200 per QALY.

Conclusions

Preventive ceftriaxone has a probability of 0.7 of being less costly than standard treatment per unit decrease in mRS and per QALY gained.

Introduction

Stroke is associated with high annual costs for society, which have been estimated at €64.1 billion for Europe.(1) Of these costs, about two-thirds are direct health care costs of stroke care.(1) A common complication in patients with acute stroke is infection.(2) We performed a meta-analysis of 87 studies including 137,817 patients, showing an overall pooled rate of infection of 30% (95% confidence interval [CI] 24%–36%), mainly pneumonia and urinary tract infections.(3) A retrospective cohort study including 8,251 patients showed that post-stroke pneumonia increased 30-day mortality (odds ratio 2.2, 95% CI 1.8–2.7) and 1-year mortality (odds ratio 3.0, 95% CI 2.5–3.7).(4) In the United States, the annual cost of post-stroke pneumonia has been estimated to be $459 million USD.(5) The Preventive Antibiotics in Stroke Study (PASS) investigated the effect of preventive treatment with the antibiotic ceftriaxone on functional outcome in acute stroke patients.(6) In this study, preventive treatment with ceftriaxone did not improve functional outcome, but did reduce the proportion of patients with post-stroke infection from 18% to 10%.(6) Here, we report an economic evaluation of data from the randomised controlled trial comparing preventive ceftriaxone vs standard care in adults with acute stroke.

Methods

Details of PASS design and main results were described previously.(6-8) The institutional review board of the Academic Medical Center (Amsterdam, the Netherlands) approved the study protocol (ISRCTN 66140176). The economic evaluation of preventive antibiotic therapy with ceftriaxone vs standard care without ceftriaxone after acute stroke was performed from a societal perspective with a time horizon of 3 months. Cost-effectiveness analysis (CEA) and cost-utility analysis (CUA) were performed. The costs per unit of the modified Rankin Scale (mRS) was taken as the primary outcome for the CEA with mRS operationalized as an ordinal outcome.(6-8) Costs per quality-adjusted life year (QALY) were the primary outcome in the CUA. All relevant health care costs, out-of-pocket expenses by patients, and costs for the employer resulting from productivity loss were assessed. Incremental cost-effectiveness analyses were performed to determine the extra costs per unit decrease in the mRS and the extra costs per additional QALY.

The volumes of used resources included hospital inpatient stays, the use of ceftriaxone and other antibiotics, diagnostic and therapeutic procedures, out-of-hospital consultations, health-related transport, institutional stay other than the hospital (such as nursing care and rehabilitation), durable medical equipment, home care, informal care, out-of-pocket expenses, and lost working hours. Data on volumes of inhospital medical care were

gathered from case record forms completed during hospital stay by the treating physician. Data on volume of out-of-hospital care, out-of-pocket expenses, and productivity loss were gathered at 3 months after stroke by asking each patient or patient's primary caregiver to complete a cost-effectiveness questionnaire (CEQ), which combined the EQ-5D-3L(9) and a version of the Dutch Health and Labour Questionnaire(10) adjusted for the study population. All data were collected prospectively.(7, 8)

Unit costs of used resources were based on the Dutch Costing Manual (DCM) for health care research and the hospital ledger from the Academic Medical Center in Amsterdam if costs were not described in the DCM.(10) 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. (11) Costs for antibiotic therapy were according to medicijnkosten.nl, and unit costs of medical devices according to gipdatabank.nl (Appendix table 1 and table 6). Out-of-pocket expenses other than health-related transport were based on patient or primary caregiver report. The friction cost method was applied in case of costs of production loss, with the friction period of 85 days falling just short of the follow-up period of 3 months.

Costs were calculated as the sum product of volumes of resources used and their respective unit costs. Considering the restricted time horizon of 3 months, no discounting of costs and health outcomes to account for time preference was applied. Costs were expressed in euros and price-indexed for the base year 2014, the last year of enrollment in PASS.

The health outcome for the CEA was functional outcome at 3 months, defined by them RS, ranging from0 (no symptoms) to 6 (death). Health utilities for CUA were based on the EQ-5D-3L health status profiles from the CEQ. The EQ-5D-3L contains 5 items on health status: mobility, self-care, usual activities, pain/discomfort, and mood (anxiety/depression).(12) Patients responded to each item by stating whether they experienced (1) no problems, (2) some problems, or (3) extreme problems. Health utilities associated with each response pattern were derived by applying an existing Dutch health valuation algorithm based on time trade off–based elicitation of preferences from the Dutch general population.9We divided derived health utilities by 4 to represent the number of QALYs achieved, considering the 3-month follow-up.

When a patient or caregiver returned the CEQ with questions left unanswered, context information on routing or response behavior ("effective" scoring) was used to decide upon assigning zero frequencies or imputing the mean frequency of the patients from the same randomisation group who answered those questions. For example, patients who

indicated that they visited a neurologist, general practitioner, physiotherapist, occupational therapist, or other health professional were subsequently routed to a subquestion on frequency. If a main question was checked for having visited a professional without a frequency provided on the subquestion, the frequency was considered missing and imputed with the mean frequency per randomisation group. The decision how to deal with unanswered frequency questions given the contextual information available followed a standard operating procedure to rule out ascertainment bias. In case of missing CEQs, no volume data on the use of out-of-hospital resources, nonreimbursables, and productivity losses were available. Hence, no costs could be calculated for these patients.

To account for missing cost estimates during follow-up at the major component level—for example, costs of consultations, institutional care elsewhere, home and informal care, devices,transport, production losses, and out-of-pocket expenses—the following scenario was developed. Costs during follow-up beyond hospital discharge were set to zero by definition in patients who died at hospital discharge (n = 118). Missing cost data during follow-up after hospital discharge alive were once imputed based on linear associations with patients' sex, age (dichotomized at 65 years of age), mRS at month 3, and randomisation group. Backward stepwise linear regressions were applied, excluding variables with $p > 0.1$ in order to derive a reasonably parsimonious model for each costs component. The associations to derive the cost estimates can be found in the appendix (table 5).

For all patients without CEQ, including the deceased ones, the number of QALYs was estimated by applying a backward stepwise linear regression with sex, mRS at month 3, total in-hospital costs, age (dichotomized at 65 years), and randomisation group as variable set, with age eventually excluded ($p > 0.1$).

We report the use of resources based on available, nonimputed data. Use of resources is reported as mean (SD) and as proportion of patients using the resource. In an explorative analysis, we compared in-hospital costs, known for all included patients, for 4 groups of patients with varying degrees of completeness of follow-up data (Appendix table 4). The size and direction of nonsignificant differences were varying between the treatment groups, indicative for potential biases that might occur if a particular subset of patients would be selected for the final analyses. We therefore decided not to limit the analysis to patients with complete data but to include data from 2516 patients after the imputation procedure.

Estimates of the mean costs for major cost components and estimates of mean QALYs were based on imputed datasets, along with bias-corrected and accelerated 95% CIs after nonparametric bootstrapping, drawing 5,000 samples of the same size as the original

samples and with replacement (seeded by code 11111). The bootstrapping procedure was stratified by randomisation group and by follow-up type. Follow-up types were as follows: alive with completed followup (n = 1,451), deceased at hospital discharge (n = 118), alive without completed follow-up (n = 798), deceased without completed follow-up (n = 149). By doing so, the proportions of patients with imputed data were kept constant, while comparing the randomisation groups.

Incremental cost-effectiveness analyses were performed for the extra costs per additional unit decrease on the mRS and the extra costs per additional QALY. A cost-effectiveness plane showing differences in costs in the Y-axis and differences in effect on the X-axis visualized the results. Results of bootstrapping are reported with quadrants of the differences in costs against the differences in effectiveness. Cost-effectiveness acceptability curves were drawn showing the probability of ceftriaxone being more efficient than standard treatment for different levels of willingness to pay (WTP) per unit decrease on the mRS or per additional QALY. Results are represented for the societal costs with and without the costs of production loss due to illness leave from work. Anonymized data will be shared by request from any qualified investigator.

Results

Between July 6, 2010, and March 23, 2014, a total of 2550 patients from 30 sites in the Netherlands were randomly assigned to the 2 treatment groups: 1275 patients to ceftriaxone and 1275 patients to standard treatment (control group). A total of 2538 patients were available for the intention-to-treat analysis. For the CEA, 2538 patients were available for in-hospital resource use and 1,453 for other resource use. The CEQ was available for 1,451 patients and 2 proxies of deceased patients. Missing cost data for patients with a missing mRS at month 3 (n = 22, equally divided between the study groups) were not imputed and the main cost analyses were restricted to the remaining 2516 patients. In-hospital volume of resources was available for 2538 patients (table 1). Number of hospital days was similar between treatment groups. Use of defined daily doses of ceftriaxone was higher in the ceftriaxone than in the control group, while defined daily doses of other antibiotics tended to be lower in the ceftriaxone group. Urinary sediment and urinary cultures were less often performed in the ceftriaxone group compared to the control group. After hospital discharge, there were no differences between groups with respect to consultation of paramedics or a general practitioner, or the use of institutional care resources, out-of-pocket expenses, and productivity losses. Use of informal care was higher in the ceftriaxone group as compared to the control group.

There were no differences for various cost components including the overall societal costs without or with the indirect costs of productivity loss between the ceftriaxone and control groups (table 2).Overall, the societal costs of ceftriaxone were only marginally lower than those in the control group, with ceftriaxone providing per participant savings of €271 and €359 without and with assumed productivity losses. In the primary analysis, preventive ceftriaxone was not associated with a shift on the mRS score distribution (adjusted common odds ratio 0.94 [95% CI 0.82–1.09], p = 0.41) at 3 months.6 The mean score on mRS was 2.38 (95% CI 2.31–2.44) in the ceftriaxone group and 2.44 (95% CI 2.37–2.51) in the control group, the decrease by 0.06 (95% CI –0.04 to 0.16) in favor of ceftriaxone treatment being nonsignificant. However, the number of QALYs was 0.163 (95% CI 0.159–0.166) in the ceftriaxone group and 0.155 (95% CI 0.152–0.158) in the control group, with the difference of 0.008 (95% CI 0.003–0.012) in favor of ceftriaxone (p = 0.006).

With respect to the societal costs with and without the costs of productivity loss, ceftriaxone saved €5,983, respectively €4,517, per unit decrease in mRS and saved €44,875, respectively €33,875, per additional QALY. Based on the point estimates, the cost savings and health gains suggest dominance of ceftriaxone over standard stroke unit care. The costeffectiveness planes based on the societal costs including productivity loss showed point clusters in the lower right quadrants after 5,000 bootstrap draws (figure). The corresponding cost-effectiveness acceptability curve for the WTP per a unit decrease in mRS shows that the probability of ceftriaxone being cost-effective ranges from 0.697, if society is not willing to pay extra money, to 0.89, if society is willing to pay whatever the costs are as long as mRS score is decreased. The probability of ceftriaxone being cost-effective ranged from 0.697 to 0.999 when the willingness to pay per additional QALY was considered (figure). If productivity losses were ignored, then the probability of ceftriaxone being costeffective ranged from 0.658 to 0.89 and from 0.658 to 0.999 considering the willingness to pay per unit decrease in mRS or per additional QALY, respectively.

These results show that preventive ceftriaxone is less costly than standard treatment (controls) per unit decrease of the mRS score and per QALY gained in almost 70% of all bootstraps.

Table 1. Volume analysis

	Ceftriaxone (n=1268)	Control (n=1270)	p-value
IN HOSPITAL CARE[a]			
Length of stay (mean, ±SD)			
In-patient days	8.35 (10.23)	9.02 (12.48)	0.635
In-patient days Neurology Ward	8.20 (9.99)	8.91 (12.35)	0.596
In-patient days ICU	0.15 (1.14)	0.12 (0.99)	0.636
Antibiotic therapy (mean, ±SD)			
No. DDD (ceftriaxone)	3.23 (1.12)	0.03 (0.32)	<0.001
No. DDD (other antibiotic)	0.69 (4.61)	1.30 (5.51)	0.107
Major procedures (mean, ±SD)			
Chest X-ray	0.09 (0.30)	0.09 (0.33)	0.782
Leukocyte count	0.12 (0.36)	0.14 (0.40)	0.192
CRP count	0.11 (0.35)	0.13 (0.38)	0.156
Urine sediment	0.09 (0.33)	0.13 (0.36)	0.009
Pos. culture	0.02 (0.19)	0.08 (0.41)	0.015
Neg. culture	0.01 (0.19)	0.02 (0.23)	0.462
RE-ADMISSION	Ceftriaxone (n=753)	Standard care (n=700)	
Re-admission for stroke (mean, ±SD)			
In-patient days Neurology ward	0.96 (4.41)	1.02 (4.00)	0.599
In-patient days ICU	0.12 (1.04)	0.12 (0.96)	0.878
OUT OF HOSPITAL CARE[b]			
Consultations			
Neurologist (no. of visits (mean, ±SD))	46% (349/753) 0.63 (1.05)	44% (308/700) 0.64 (1.23)	0.939
General practitioner (no. of visits (mean, ±SD))	42% (314/753) 0.82 (1.66)	42% (296/700) 0.87 (1.57)	0.530
Physiotherapist (no. of visits (mean, ± SD))	48% (358/753) 7.16 (12.46)	45% (315/700) 6.78 (12.26)	0.982
Occupational therapist (no. of visits (mean, ± SD))	37% (279/753) 3.85 (9.23)	35% (247/700) 3.48 (8.39)	0.729
Speech therapist (no. of visits (mean, ± SD))	25% (187/753) 2.65 (8.06)	19% (130/700) 2.20 (7.23)	0.347
Psychologist (no. of visits (mean, ± SD))	19% (141/753) 0.64 (2.06)	17% (122/700) 0.60 (2.40)	0.908
Social worker (no. of visits (mean, ± SD))	18% (138/753) 0.53 (1.53)	16% (113/700) 0.58 (1.99)	0.251
Transport by ambulance	9% (64/753)	6% (45/700)	0.137
Transport			
No transport	22% (169/753)	23% (163/700)	0.708
Car	47% (357/753)	44% (308/700)	0.206
Public transport	7% (49/753)	6% (40/700)	0.585

Table 1. Continued

	Ceftriaxone (n=1268)	Control (n=1270)	p-value
Taxi	13% (101/753)	14% (99/700)	0.704
Other	24% (178/753)	25% (177/700)	
Institutional care			
Nursing home admission (no of days (mean, ± SD))	10% (77/753) 4.46 (16.27)	12% (81/700) 4.74 (16.46)	0.476
Admission to rehabilitation (no of days (mean, ± SD))	26% (198/753) 12.65 (25.17)	27% (189/700) 13.24 (26.18)	0.461
Day admission nursing home (no of days (mean, ± SD))	4% (26/753) 1.15 (7.61)	4% (28/700) 0.89 (5.69)	0.370
Day admission rehabilitation (no of days (mean, ± SD))	16% (118/753) 4.67 (14.99)	15% (108/700) 4.42 (13.95)	0.621
Medical devices, house adaptations			
Wheel chair	10% (76/753)	11% (74/700)	0.796
Walker	21% (160/753)	18% (127/700)	0.147
Home care			
House hold (no of hours/wk (mean, ± SD))	13% (100/753) 0.41 (1.33)	12% (86/700) 0.41 (1.62)	0.996
Personal care (no of hours/wk (mean, ± SD))	7% (49/753) 0.27 (1.32)	7% (46/700) 0.34 (2.22)	0.310
Home nursing (no of hours/wk (mean, ± SD))	3% (22/753) 0.10 (0.65)	2% (13/700) 0.09 (0.77)	0.853
Informal care (no of hours/wk (mean, ± SD))	38% (287/753) 4.27 (8.79)	33% (234/700) 3.12 (6.74)	0.005
No home care	52% (394/753)	58% (408/700)	0.023
Out of pocket-expenses			
Transport	25% (188/753)	26% (184/700)	0.588
Home care	5% (36/753)	5% (38/700)	0.633
Medication	9% (69/753)	8% (54/700)	0.346
Other	12% (88/753)	13% (92/700)	0.426
PRODUCTIVITY			
Currently employed	16% (122/753)	17% (120/700)	0.673
No change	7% (50/753)	5.4% (38/700)	0.379
I now work more (stroke)	0% (0/753)	0.1% (1/700)	0.482
I now work more (other)	0% (0/753)	0.1% (1/700)	0.482
I now work less (stroke)	5% (34/753)	4% (30/700)	0.898
I now work less (other)	0.4% (3/753)	0.4% (3/700)	0.929
I do not work (stroke)	5% (63/753)	7% (57/700)	0.924

a: volume of care during hospital stay
b: volume of care after discharge from the hospital until 3 months after admission to the hospital
Abbreviations: CRP = C-reactive protein; ICU = intensive care unit. DDD = defined daily dosis

Table 2. Costs in 2014 euro (imputation after linear regression)

Component	Ceftriaxone n=1257	Control n=1259	Difference	p-value
In-hospital care[a]	3538 (3300-3801)	3710 (3445-3994)	-171 (-549 to 251)	0.391
Readmission	591 (503-688)	618 (538-700)	-27 (-158 to 113)	0.705
Consultation	615 (573-663)	603 (562-642)	12 (-53 to 79)	0.675
Institutional care	9399 (8756-10097)	9653 (9024-10336)	-254 (-1214 to 751)	0.577
Devices	24 (22-26)	23 (21-25)	1 (-2 to 4)	0.491
Home & informal care	89 (82-97)	81 (74-89)	8 (-3 to 18)	0.115
Transport	722 (560-932)	562 (464-666)	160 (-37 to 396)	0.139
Out-of-pocket	68 (59-78)	64 (57-71)	4 (-8 to 18)	0.537
Societal costs excl. prod.loss	**14979 (14136-15878)**	**15250 (14412-16074)**	**-271 (-1607 to 1078)**	**0.657**
Production loss	1215 (1037-1396)	1307 (1126-1491)	-92 (-341 to 157)	0.481
Societal costs incl. prod. loss	**16262 (15350-17209)**	**16620 (15766-17483)**	**-359 (-1804 to 1075)**	**0.574**

a: costs made during hospital stay, eg costs for inpatient days, investigations, medication

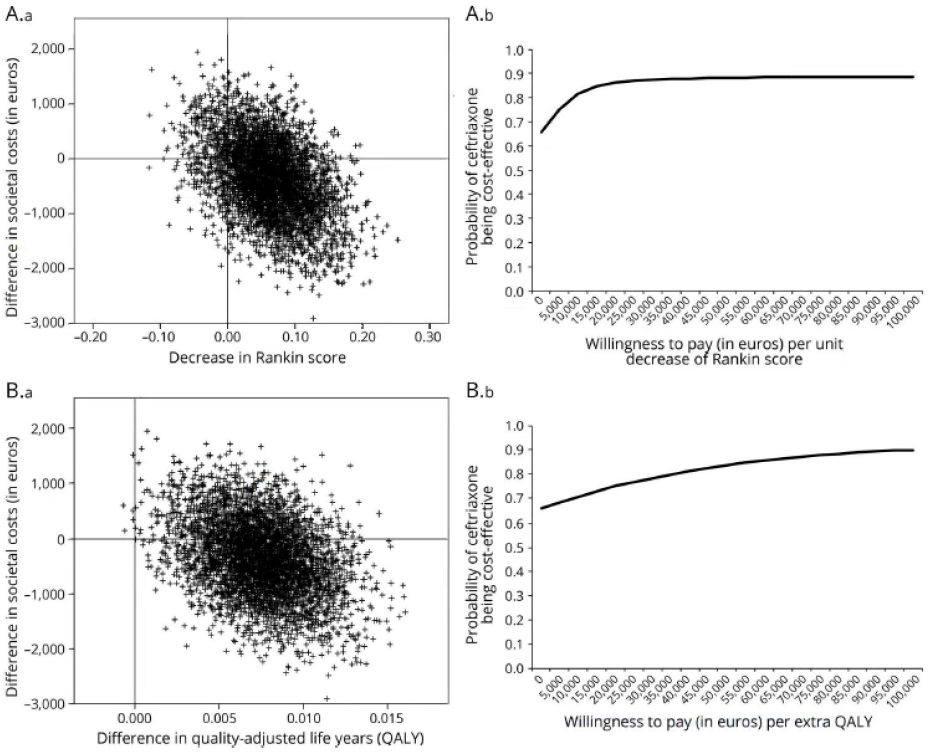

Figure. Incremental cost-effectiveness ratio analyses of preventive ceftriaxone per unit decrease in modified Rankin Scale (mRS) score and additional quality-adjusted life years (QALY)

(A.a) The difference in societal costs including the costs of productivity loss (Y-axis) vs the difference in mRS (X-axis) for ceftriaxone against standard treatment after 5,000 bootstraps. The upper right quadrant contained 23.7% of all bootstrap results, indicating extra costs per unit decrease in mRS score; 6.6% of all bootstraps fell in the upper left quadrant, indicating extra costs and an increase in mRS score on the negative side; 4.4% fell in the lower left quadrant, indicating cost savings with increases in mRS score as the negative tradeoff; finally, 65.3% fell in the lower right quadrant, indicating both cost savings and decreases in mRS score. The corresponding cost-effectiveness acceptability curve (A.b) shows that the probability of ceftriaxone being cost-effective ranges from 0.697 if society is not willing to pay extra money for a unit decrease in mRS to 0.89 if society is willing to pay whatever the costs are as long as mRS is decreased. (B.a) The difference in societal costs including the costs of production loss (Y-axis) vs the difference in QALYs (X-axis) for ceftriaxone against standard treatment after 5,000 bootstraps.Of all bootstraps, 30.2% fell in the upper right and 69.7% in the lower right quadrant. The probability of ceftriaxone being cost-effective (B.b) ranges from 0.697 to 0.999.

Discussion

Our findings show that preventive ceftriaxone in adults with acute stroke is cost-effective. We found a small gain in favor of ceftriaxone of 0.008 QALY in the first quarter following the intervention. The estimated incremental cost-effectiveness ratio remained below the €20000 per QALY threshold used by the National Institute for Health and Care Excellence. (13) However, whether costs justify the use of preventive ceftriaxone in patients with acute stroke, increasing antibiotic pressure with the potential of increasing antibiotic resistance on a population level, remains to be decided.

Preventive ceftriaxone resulted in a nonsignificant improvement on mRS scores at 3 months. It reduced the proportion of patients with post-stroke infection from 18% to 10% in our clinical trial.(6) This small improvement in functional outcome in combination with prevention of infections might explain the cost-effectiveness of preventive ceftriaxone. Among 2550 included patients with acute stroke, the most common infections were urinary tract infections and pneumonia, concordant with previous reports.(3)

The use of preventive antibiotics has potential side effects. On an individual level, increased use of antibiotics may induce a bacterial overgrowth syndrome by antibiotic-resistant pathogens. In PASS, the occurrence of an overgrowth infection with *Clostridium difficile* or infection with ceftriaxone resistant microorganism was rare (<1%).(6) This may lead to economic effect; for example, extension of hospitalization. These economic effects of overgrowth infection due to ceftriaxone-resistant microorganism were included in our analysis. On a population level, increased use of antibiotics may lead to selective antibiotic pressure, which is an important determinant of emergence of antibiotic resistance. (14, 15) Interestingly, a previous study on the implementation of preventive antibiotics in intensive care unit patients did not result in increased resistance rates.(16) Nevertheless, potential increase of antibiotic resistance on a population level was not weighted in our analysis. The economic benefit as shown by our study must be weighed carefully against potential risk of antibiotic resistance development.

The sample size of this study was large and was set in 30 Dutch academic and nonacademic hospitals. The study was large enough to differentiate between the cost savings generated by preventive ceftriaxone and background noise. Inhospital costs were complete for all patients and provide important data on future cost-effectiveness in stroke. Cost estimates during follow-up were known for the majority of patients. For missing cost data, we estimated actual costs by using linear regression. Generally, using (backward stepwise) linear regression for the estimation of costs of missing data is not a sensible approach as predictions may suggest negative costs or values beyond a realistic range.(17)

Negative costs indeed were predicted, accounting for 0.26% of the total societal costs. Likewise, QALYs for individual patients above a realistic upper limit given the length of the follow-up period of 3 months accounted for 0.56 per thousand of the total number of QALYs generated. These low figures indicate that the restriction by applying a simple linear regression model for missing data imputation in this study seems negligible. Another limitation is that QALYs were based on health status profiles at a single point in time, at 3 months of follow-up. It is common to apply an area under the curve approach after interpolation between measurements over time including a baseline assessment. Empirical baseline data were lacking because of the acute care setting and no such approach was feasible. Because of the randomised design, which minimizes baseline differences in study groups, and because QALYs hardly differed at 3 months, we expect that the difference in QALYs will be accurate despite possible overestimation of the absolute numbers of QALYs in both groups. We found a small difference in the number of QALY in the ceftriaxone group compared to the control group. Whether this small difference is a clinically meaningful change remains unclear. Nevertheless, our findings stress that further investigations on preventive antibiotics are needed for subgroups of stroke patients at high risk for infection.

Although preventive antibiotic therapy does not improve functional outcome, it represents an intervention that seems cost-effective in at least 2 out of 3 (65.8%) to perhaps even in 9 out of 10 (>89%) patients. Further research is suggested for the cost-effectiveness of preventive antibiotics in acute stroke patients with high risk for infection. The economic benefit must be weighed against the potential risk of antibiotic resistance development.

Author contributions

W.F.W., E.Z., H.K., P.J.N., M.G.W.D., D.v.d.B.: study concept and design, analysis and interpretation. W.F.W., E.Z., J.-D.V., H.K., P.J.N., D.v.d.B.: acquisition of data. M.G.W.D., D.v.d.B.: study supervision.

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Disclosure

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References

1. Feigin VL, Mensah GA, Norrving B, Murray CJ, Roth GA. Atlas of the Global Burden of Stroke (1990-2013): The GBD 2013 Study. Neuroepidemiology. 2015;45(3):230-6.
2. Chamorro A, Meisel A, Planas AM, Urra X, van de Beek D, Veltkamp R. The immunology of acute stroke. Nature reviews Neurology. 2012;8(7):401-10.
3. Westendorp WF, Nederkoorn PJ, Vermeij JD, Dijkgraaf MG, van de Beek D. Post-stroke infection: A systematic review and meta-analysis. BMC Neurol. 2011;11:110.
4. Finlayson O, Kapral M, Hall R, Asllani E, Selchen D, Saposnik G. Risk factors, inpatient care, and outcomes of pneumonia after ischemic stroke. Neurology. 2011;77(14):1338-45.
5. Katzan IL, Dawson NV, Thomas CL, Votruba ME, Cebul RD. The cost of pneumonia after acute stroke. Neurology. 2007;68(22):1938-43.
6. Westendorp WF, Vermeij JD, Zock E, Hooijenga IJ, Kruyt ND, Bosboom HJ, et al. The Preventive Antibiotics in Stroke Study (PASS): a pragmatic randomised open-label masked endpoint clinical trial. Lancet. 2015;385(9977):1519-26.
7. Nederkoorn PJ, Westendorp WF, Hooijenga IJ, de Haan RJ, Dippel DW, Vermeij FH, et al. Preventive antibiotics in stroke study: rationale and protocol for a randomised trial. Int J Stroke. 2011;6(2):159-63.
8. Westendorp WF, Vermeij JD, Dippel DW, Dijkgraaf MG, van der Poll T, Prins JM, et al. Update of the Preventive Antibiotics in Stroke Study (PASS): statistical analysis plan. Trials. 2014;15:382.
9. Lamers LM, Stalmeier PF, McDonnell J, Krabbe PF, van Busschbach JJ. [Measuring the quality of life in economic evaluations: the Dutch EQ-5D tariff]. Nederlands tijdschrift voor geneeskunde. 2005;149(28):1574-8.
10. Hakkaart-van Roijen LB, C.A.M.; Kanters, T.; Swan Tan, S. Guideline for Costing Research. Methods and Standard Unit Costs for Economic Evaluation in Health Care; Actualized Version. Rotterdam: Erasmus University; 2015.
11. Organization WH. WHOanatomical therapeutic chemical classification system with defined daily doses index (ATC/DDD). Available at: who.int/classifications/atcddd/en. Accessed June 16, 2017.
12. Fish J. EuroQol/EQ-5D. In: Kreutzer JS, DeLuca J, Caplan B, editors. Encyclopedia of Clinical Neuropsychology. New York: Springer; 2011:981–983.
13. National Institute for Health NC. Incorporating health economics: process and methods. Available at: nice.org.uk/process/pmg4/chapter/incorporating-healtheconomics. Accessed June 16, 2017.
14. Hawkey PM. The growing burden of antimicrobial resistance. J Antimicrob Chemother. 2008;62 Suppl 1:i1-i9.
15. Schechner V, Temkin E, Harbarth S, Carmeli Y, Schwaber MJ. Epidemiological interpretation of studies examining the effect of antibiotic usage on resistance. Clin Microbiol Rev. 2013;26(2):289-307.
16. Plantinga NL, Bonten MJ. Selective decontamination and antibiotic resistance in ICUs. Critical care (London, England). 2015;19:259.
17. Harkanen T, Maljanen T, Lindfors O, Virtala E, Knekt P. Confounding and missing data in cost-effectiveness analysis: comparing different methods. Health economics review. 2013;3(1):8.

Appendix Data Cost-effectiveness PASS

Table 1. Unit costing and source

	Unit costs (euro)	Source
In hospital care		
Length of stay (days)		
Neurology ward	392	DCM 2015*
Intensive Care Unit	1·178	DCM 2015
Antibiotic therapy (defined daily dose)		
Study medication (ceftriaxone)	17·46	medicijnkosten.nl
Other antibiotics	suppl. app. table 4	medicijnkosten.nl
Procedures		
Chest X-ray	126·11	HL AMC
Leukocyte count	3·83	HL AMC
C-reactive protein	4·7	DCM 2015
Urine chemical analysis	1·73	HL AMC
Blood, sputum, or urine culture with negative result	48·36	DCM 2015
Blood, sputum, or urine culture with positive result	68·36	DCM 2015
Out of hospital care		
Consultations		
General practitioner	33	DCM 2015
Neurologist	98	DCM 2015
Physiotherapist	33	DCM 2015
Occupational therapist	33	DCM 2015
Speech therapist	30	DCM 2015
Psychologist	64	DCM 2015
Social worker	65	DCM 2015
General practitioner	33	DCM 2015
Neurologist	98	DCM 2015
Transport* **		
Ambulance ordered in advance	270	DCM 2015
Car	3 + 0·19/km	DCM 2015
Public transport	0·19/km	DCM 2015
Taxi	2·93+ 2·64/km	DCM 2015
Ambulance ordered in advance	270	DCM 2015

Table 1. Continued.

	Unit costs (euro)	Source
Institutional care		
Admission nursing home	167	DCM 2015
Admission rehabilitation clinic	457	DCM 2015
Day admission nursing home	134	DCM 2015
Day admission rehabilitation	460	DCM 2015
Medical devices, house adaptations		
Walker	102	gipdatabank.nl
Wheel chair	67	gipdatabank.nl
Home care		
Household (per hour)	20	DCM 2015
Personal care (per hour)	50	DCM 2015
Home nursing	72	DCM 2015
Informal care	14	DCM 2015
Out of pocket expenses (transport, home care, medication, other)	-	CEQ
Productivity loss	34·50	DCM 2015

* DCM = Dutch Costing Manual. ** HL AMC = Hospital Ledger Academic Medical Center Amsterdam. *** mean distances: to hospital 7·0 km, general practitioner 1·1 km, physiotherapist/ occupational/speech therapist/ social worker/psychologist 2·2 km, nursing home 3.· km.

Table 2. Baseline characteristics of patients from whom cost-effectiveness questionnaire was obtained vs· patients from who this was not obtained

	CEQ obtained (n=1453)	CEQ not obtained (n=1085)	p-value
Age – year (med IQR)	71 (63-80)	75 (63-83)	<0·01
Male sex – no (%)	59% (861/1453)	54% (583/1085)	<0·01
History – no (%)			
Atrial fibrillation/flutter	14% (207/1453)	17% (184/1085)	0·07
Stroke	30% (443/1453)	35% (384/1085)	0·01
Hypercholesterolemia	26% (384/1453)	26% (281/1085)	0·82
Hypertension	53% (773/1453)	58% (627/1085)	0·02
Myocardial infarction	12% (173/1453)	15% (158/1085)	0·06
Cardiac valve disease	6% (94/1453)	7% (79/1085)	0·43
Peripheral vascular disease	7% (104/1453)	8% (86/1085)	0·45
Obstructive pulmonary disease	7% (105/1453)	9% (103/1085)	0·04
Immunocompromise	3% (47/1453)	3% (37/1085)	0·67
Current smoker – no (%)	24% (353/1453)	25% (267/1085)	<0·01
Prior medication – no (%)			
Anticoagulants	10% (143/1453)	13% (140/1085)	0·02
Antiplatelet	38% (553/1453)	43% (465/1085)	0·02
Statin	37% (539/1453)	38% (410/1085)	0·74
ACE-inhibitor	24% (352/1453)	27% (292/1085)	0·14
Beta-blocker	34% (492/1448)	37% (393/1058)	0·26
Protonpompinhibitor	25% (358/1453)	27% (297/1085)	0·14
Modified Rankin Scale score	0 (0-1)	0 (0-2)	<0·01
NIHSS	4 (3-7)	6 (3-12)	<0·01
Dysphagia – no (%)	18% (265/1453)	33% (358/1085)	<0·01
Randomisation to ceftriaxone	52% (753/1453)	47% (515/1085)	0·03
Discharge diagnosis – no (%)			
Cerebral infarction	85% (1241/1453)	81% (884/1085)	<0·01
Transient ischaemic attack	4% (60/1453)	3% (33/1085)	0·17
Cerebral haemorrhage	9% (124/1453)	13% (145/1085)	<0·01
Other	2% (28/1453)	2% (23/1085)	0·78
Mortality at discharge	0% (1/1449)	11% (117/1085)	<0·01
Mortality at 3 months	0·1% (2/1449)	24% (265/1085)	<0·01

Table 3. Baseline characteristics of patients from whom cost-effectiveness questionnaire was obtained vs· patients from who this was not obtained, all deceased patients excluded

	CEA obtained (n=1451)	CEA not obtained (n=820)	p-value
Age – year (med IQR)	72 (63-80)	75 (63-83)	0·21
Male sex – no (%)	59% (861/1451)	55% (451/820)	0·05
History – no (%)			
Atrial fibrillation/flutter	14% (207/1451)	14% (113/820)	0·75
Stroke	31% (443/1451)	35% (287/820)	0·03
Hypercholesterolemia	26% (384/1451)	27% (220/820)	0·84
Hypertension	53% (771/1451)	56% (460/820)	0·17
Myocardial infarction	12% (173/1451)	14% (116/820)	0·13
Cardiac valve disease	6% (94/1451)	7% (55/820)	0·86
Peripheral vascular disease	7% (104/1451)	8% (67/820)	0·41
Obstructive pulmonary disease	7% (105/1451)	9% (73/820)	0·17
Immunocompromise	3% (46/1451)	3% (25/820)	0·90
Current smoker – no (%)	24% (353/1451)	28% (233/820)	0·03
Prior medication – no (%)			
Anticoagulants	10% (143/1451)	11% (93/820)	0·28
Antiplatelet	38% (553/1451)	42% (347/820)	0·05
Statin	37% (539/1451)	39% (318/820)	0·44
ACE-inhibitor	24% (351/1451)	26% (217/820)	0·27
Beta-blocker	34% (490/1446)	33% (272/820)	0·75
Protonpompinhibitor	25% (357/1451)	26% (211/820)	0·61
Modified Rankin Scale score (med IQR)	0 (0-1)	0 (0-2)	
NIHSS (med IQR)	4 (3-7)	6 (3-12)	<0·01
Dysphagia – no (%)	18% (264/1451)	25% (203/820)	<0·01
Randomisation to ceftriaxone	52% (751/1451)	47% (386/820)	0·03
Discharge diagnosis – no (%)			
Cerebral infarction	85% (1239/1451)	81% (667/820)	0·01
Transient ischaemic attack	4% (60/1451)	4% (32/820)	0·83
Cerebral haemorrhage	9% (124/1451)	12% (100/820)	0·01
Other	2% (28/1451)	3% (21/820)	0·37

Explorative analysis

With regard to the economic evaluation, four groups of patients were distinguished to explore the feasibility of restricting the analyses to patients with complete data: group 1 (A3Q): alive at 3 months with EQ-5D and cost Questionnaires (N=1451) group 2 (A3): alive at 3 months without EQ-5D and cost questionnaires (N=820) group 3 (DD):

deceased at discharge without EQ-5D and cost questionnaires (N=118) group 4 (D3): deceased at 3 months without EQ-5D and cost questionnaires (N=149 with, considering the analysis being explorative, two available posthume proxy measurements included for programming convenience)· For all four groups, data on in-hospital care were available, consisting of antibiotic therapy, in-hospital stay (neurology ward, intensive care unit) and major diagnostic and therapeutic procedures· Table 4 shows the mean costs of in-hospital care excluding readmissions for the four groups and by treatment allocation, following intention-to-treat· Between brackets, bias corrected and accelerated 95% confidence intervals are provided after non-parametric bootstrapping, drawing 5000 samples of the same size as the original samples and with replacement (seeded by code 11111)·

Table 4. In-hospital costs (not including readmissions) in 2014 €

Group	Ceftriaxone	Control	Difference	P-value
1 A3Q	3141 (2878-3408) n=751 (59·2%)	3196 (2900-3521) n=700 (55·1%)	-55 (-487 to 373)	0·810
2 A3	3729 (3286-4236) n=386 (30·4%)	4270 (3721-4880) n=434 (34·2%)	-541 (-1344 to 288)	0·185
3 DD	4065 (3013-5341) n=57 (4·5%)	3749 (2925-4691) n=61 (4·8%)	316 (-1170 to 1865)	0·713
4 D3	6014 (4798-7601) n=74 (5·8%)	5187 (4242-6219) n=75 (5·9%)	827 (-752 to 2642)	0·422
All	3529 (3289-3783) n=1268	3707 (3447-3975) n-1270	-178 (-569 to 217)	0·365
1&2 A	3341 (3105-3590) n=1137	3607 (3325-3903) n=1134	-267 (-671 to 140)	0·189
3&4 D	5166 (4288-6111) n=131	4542 (3886-5247) n=136	624 (-503 to 1856)	0·344
1&3	3206 (2930-3470) n=808	3241 (2962-3539) n=761	-35 (-442 to 368)	0·879
2&4	4097 (3653-4589) n=460	4405 (3913-4946) n=509	-308 (-1037 to 433)	0·418

Overall, in-hospital costs did not differ by treatment group. Ceftriaxone non-significantly saved in-hospital costs by €178. In the surviving A3Q group, the margin in favor of ceftriaxone even further diminished to a savings by €55. In the surviving A3 group, although still not significant, the savings under ceftriaxone by €541 were almost ten-fold the margin for the A3Q group. Among patients (A) who eventually survived beyond the first quarter, the non-significant cost savings in favor of ceftriaxone added up to €267. Among deceased patients (D) opposite non-significant results were noted. Standard treatment was non-significantly cost saving in the DD group by €316 and in the D3 group by €827, or €624 among all diseased. Among patients with available follow-up data (A3Q and DD)

the mean in-hospital costs savings were €35 in favor of ceftriaxone. Among patients with unavailable follow-up, the savings in favor of ceftriaxone were €308. All comparisons were non-significant but it should be noted that the sample size calculation was not made to detect a difference in costs and insufficient power to detect these differences was likely. Further, subgroups were under scrutiny here, resulting in additional loss of power. So, considering that the relative sizes and directions of the observed differences might still be indicative of potential biases that would occur if a particular subset of patients was selected for the final analyses including the other cost components. The analyses suggested that ceftriaxone lowered the in-hospital costs in patients that eventually survived the first quarter after the incident, ranging from -€55 to -€541. In contrast, ceftriaxone might increase the in-hospital costs among the deceased, ranging from €316 to €827. The analyses further suggested that for patients with available follow-up data as well as for patients with unavailable follow-up data ceftriaxone reduced in-hospital costs, but that the savings were increased ninefold in case of unavailable follow-up. If we would have restricted the final incremental cost-effectiveness analysis to patients with available follow-up data, ceftriaxone would have been at a disadvantage, because cost savings of in-hospitals care would have been slightly underestimated by -€143 (-€178 versus -€35).

Missing cost data during follow-up after hospital discharge alive were once imputed based on linear associations with patients' sex, age (dichotomized at 65 years of age), Rankin score at month 3, and randomisation group· Backward linear regressions were applied. Table 5 shows the used resulting associations to derive the cost estimates.

Table 5. Associations of cost components with predictors to impute missing cost estimates

Cost component	Sex	Age	Rankin	Randomisation
In-hospital care		not applicable		
Readmissions	x	>0·1	x	>0·1
Consultations	>0·1	x	x	>0·1
Institutional care	>0·1	x	x	>0·1
Home & informal care	x	>0·1	x	>0·1
Devices	x	>0·1	x	>0·1
Transport	>0·1	x	x	>0·1
Out-of-pocket	>0·1	x	>0·1	>0·1
Work	>0·1	x	x	>0·1

Table 6. Costs of antibiotic therapy

Amoxicilline/clavulaanzuur oral	0·17	HL AMC **
Amoxicilline/clavulaanzuur iv	3·21	medicijnkosten·nl
Cefazoline iv	7·71	medicijnkosten·nl
Cefotaxim iv	18·83	medicijnkosten·nl
Cefotaxim oraal Ceftazidim iv	33·34	medicijnkosten·nl
Ceftriaxon iv	17·46	medicijnkosten·nl
Cefuroxim oral	0·64	medicijnkosten·nl
Cefuroxim iv	10·00	medicijnkosten·nl
Ciprofloxaxine oral	0·23	medicijnkosten·nl
Ciprofloxacine iv	32·24	medicijnkosten·nl
Clarithromycine oral	0·16	medicijnkosten·nl
Clarithromycine iv	2·86	medicijnkosten·nl
Clindamycine oral	1·55	medicijnkosten·nl
Clindamycine iv	14·67	medicijnkosten·nl
Cotrimoxazol oral	0·11	medicijnkosten·nl
Erythromycine iv	13·28	medicijnkosten·nl
Flucloxacilline iv	8·78	medicijnkosten·nl
Flucloxacilline oraal	0·44	medicijnkosten·nl
Gentamicine iv	7·57	medicijnkosten·nl
Meropenem iv	43·17	medicijnkosten·nl
Metronidazol iv	11·07	medicijnkosten·nl
Metronidazol oraal	0·67	medicijnkosten·nl
Moxifloxacine oral	2·39	medicijnkosten·nl
Nitrofurantoine oral	0·13	medicijnkosten·nl
Ofloxacin oral	0·40	medicijnkosten·nl
Ofloxacin eyedroplets		medicijnkosten·nl
Piperacilline / Tazobactam iv	14·29	medicijnkosten·nl
Tobramycine iv	13·16	medicijnkosten·nl
Trimethoprim oral	0·27	medicijnkosten·nl
Vancomycine iv	29·42	medicijnkosten·nl

CHAPTER 7

Pre-stroke use of beta-blockers does not lower poststroke infection rate: an exploratory analysis of the preventive antibiotics in stroke study

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Abstract

Background

Stroke-associated infections occur frequently and are associated with unfavorable outcome. Previous cohort studies suggest a protective effect of beta-blockers against infections. A sympathetic drive may increase immune suppression and infections.

Aim

To investigate the association between beta-blocker treatment at baseline and post-stroke infection in the Preventive Antibiotics in Stroke Study, a prospective clinical trial.

Methods

We performed an exploratory analysis in PASS, 2538 patients with acute phase of stroke (24 hours after onset) were randomised to ceftriaxone (intravenous, 2 g per day for 4 days) in addition to stroke unit care, or standard stroke unit care without preventive antibiotic treatment. All clinical data including use of beta-blockers was prospectively collected. Infection was diagnosed by the treating physician, and independently by an expert panel blinded for all other data. Multivariable analysis was performed to investigate associations between beta-blocker treatment and infection rate.

Results

Infection as defined by the physician occurred in 348 of 2538 patients (14%). Multivariable analysis showed that use of beta-blockers at baseline was associated with development of infection during clinical course (aOR 1.61; 95%CI 1.19-2.18; p<0.01). Beta-blocker use at baseline was also associated with development of pneumonia (aOR 1.56 95%CI 1.05-2.30; p=0.03). Baseline beta-blocker use was not associated to mortality (aOR 1.14; 95%CI 0.84-1.53; p=0.41) or unfavourable outcome at 3 months (aOR 1.10; 95%CI 0.89-1.35; p=0.39).

Conclusions

Patients treated with beta-blockers prior to a stroke have a higher rate of infection and pneumonia.

Introduction

Infections frequently complicate the acute phase of stroke and have been associated with unfavorable outcome in stroke patients.(1) The high risk for post stroke infection is at least partly driven by a stroke-induced immune suppression, which is hypothesized to be caused by increased sympathetic activity.(2) In an experimental study, administration of beta-blockers after the onset of stroke was found to decrease the risk of infection. (3) It has been suggested that in stroke patients administration of beta blockers in the acute phase after stroke could influence the immune suppression associated with acute stroke and decrease the risk of infections after stroke. Two recent cohort studies reported conflicting results on association between beta-blockers use and occurrence of infections in patients with acute stroke.(4, 5)

Aim

Our aim is to analyze whether beta-blocker treatment influenced post-stroke infection in patients included in the Preventive Antibiotics in Stroke Study (PASS), a randomised open-label masked endpoint clinical trial on the efficacy and safety of preventive ceftriaxone in adults with acute stroke.(6)

Methods

We investigated whether infection risk differs between patients treated with beta-blocker prior to stroke and beta-blocker naive patients. Therefore, all patients included in the intention-to-treat population of PASS were included in the current study. In PASS, adult patients in the acute phase of ischaemic or haemorrhagic stroke (within 24 hours after onset) with an NIHSS-score of 1 or higher, were randomised to receive ceftriaxone (intravenous, 2 g per day for 4 days) in addition to stroke unit care, or standard stroke unit care without preventive antibiotic treatment. We excluded patients with an infection at admission, use of antibiotics within 24 hours of randomisation, with a known allergy to antibiotics, and patients in whom death was imminent. The trial protocol, statistical analysis plan and main article of the study results were have been published before.(6) Since the current analysis was not pre-planned in the PASS statistical analysis plan, it should be regarded as an exploratory analysis.

Baseline characteristics, clinical parameters and endpoints were prospectively collected in case record forms that were filled out by the treating physician. Pneumonia, urinary tract infection and other infection in the PASS were diagnosed by the treating physician, but also by a and scored by an expert panel of 2 independent experts who were blinded for treatment allocation and adhered to the Centers for Disease Control and Prevention criteria.(7) Pre-stroke use of beta-blockers was prospectively recorded at baseline for all patients based on the observational studies and hypotheses mentioned in the introduction. In the Netherlands, it is standard care to continue antihypertensive medication used at home during hospital admission for acute stroke.

Differences in baseline characteristics of patients with or without beta-blocker therapy prior to stroke are shown as percentages or mean (with standard deviation) or median values (with interquartile range). We tested whether baseline characteristics were associated with infection overall and pneumonia and UTI separately by T-test, Mann-Whitney U or Chi-square when appropriate. Included baseline characteristics were: age, sex, ethnicity, medical history prior to stroke (atrial fibrillation/flutter, stroke, myocardial infarction, cardiac valve disease, peripheral vascular disease, hypertension, hypercholesterolemia, pulmonary obstructive disease, diabetes mellitus, alcoholism, malignancy), medication use prior to stroke (anticoagulants, antiplatelet therapy, statins, ACE-inhibitors, beta blockers, proton pump inhibitors), smoking status, disability prior to stroke, physical examination at admission (heart rate, systolic/diastolic RR, temperature), stroke severity (NIHSS), dysphagia, use or urinary catheter, stroke type, acute treatment (intravenous or intra-arterial thrombolysis, coagulant therapy) and randomisation. Characteristics with an association in univariate analysis ($p<0.05$) were included in multivariate analysis. Variables known to have a strong association with infection were a priori included in multivariate analysis; these were: age, stroke severity, presence of dysphagia and urinary catheterization. Association of beta-blockers therapy and mortality at discharge and 3 months, and unfavorable functional outcome on the modified Rankin Scale (defined as mRS 3-6) was estimated in univariate analysis and subsequent regression analysis including strong prognostic baseline variables, as described in the PASS protocol (age, stroke severity, history of stroke or diabetes, prior disability on modified Rankin Scale at admission). Because immune suppression is most pronounced and infection rate higher in the first days after stroke we performed a subgroup analysis on infections within the first week. All analyses were performed with IBM SPSS statistics version 22.

Results

From July 6, 2010, to March 23, 2014, 2538 patients were included in PASS: 84% of patients had ischaemic stroke, 11% haemorrhagic stroke, 4% transient ischaemic attack end 2% had another diagnosis. At baseline, 885 of 2538 patients (35%) used beta-blockers. Baseline characteristics of these patients are shown in table 1. Patients using beta-blockers prior to stroke were older; more often had a history of atrial fibrillation, stroke, hypercholesterolemia, hypertension, myocardial infarction, cardiac valve disease and peripheral vascular disease and used more medication prior to stroke. Disability prior to stroke and stroke severity was similar between patients who did and those who did not use beta-blockers.

Infection as defined by the physician occurred in 348 patients, 130 (10%) in the ceftriaxone group and 218 (17%) in the control group. In one of these patients baseline use of beta-blockers was unknown, this patient was excluded from analysis. Infection was diagnosed within the first week in 270 patients by the physician and in 98 patients according to expert panel.[6]

Infection rates were higher in patients using beta-blockers at baseline, as compared to patients not using beta-blockers (table 2). Adjusted OR for the use of beta-blockers at baseline and post-stroke infection was 1.61 (95%CI 1.19-2.18; p<0.01; table 3); for the expert panel definition for infection the adjusted OR was 1.64 (95%CI 1.08-2.50; p=0.02). Additional analyses restricted to infection occurring in the first week after stroke showed similar results (data not shown).

Beta-blocker use at baseline was associated with stroke-associated pneumonia, as defined by physician (crude OR 1.92; 95%CI; 1.39-2.65; p<0.001). Advanced age, ethnicity, history of atrial fibrillation/flutter, obstructive pulmonary disease, malignancy, current smoking status, diastolic and systolic blood pressure, score on NIHSS, disability prior to symptoms (mRS), dysphagia, coagulant therapy and stroke type were also associated with stroke-associated pneumonia in univariate analysis. After correction for these factors in a multivariable analysis the aOR for beta-blockers and pneumonia was 1.56 (95%CI 1.05-2.30; p=0.03). Analyses using the expert panel definition for pneumonia showed a similar trend for beta-blockers use and increased risk of stroke-associated pneumonia (aOR 1.76, 95%CI 0.92-3.36; p=0.09).

Table 1. Baseline characteristics of patients treated with beta blockers pre-stroke vs patients not treated with BB before stroke

	BB before stroke (n=885)	No BB before stroke (n=1648)	p-value
Age – year (interquartile range)	77 (69-83)	71 (60-80)	<0.01
Male sex – no. (%)	476/885 (5%)	965/1648 (6%)	0.02
History – no. (%)			
Atrial fibrillation/flutter	248/884 (28%)	143/1648 (9%)	<0.01
Stroke	357/884 (40%)	468/1648 (28%)	<0.01
Hypercholesterolemia	320/875 (37%)	343/1640 (21%)	<0.01
Hypertension	695/881 (79%)	700/1648 (42%)	<0.01
Myocardial infarction	207/884 (23%)	123/1648 (7%)	<0.01
Cardiac valve disease	104/885 (12%)	68/1646 (4%)	<0.01
Peripheral vascular disease	95/883 (11%)	93/1642 (6%)	<0.01
Obstructive pulmonary disease	79/885 (9%)	129/1644 (8%)	0.36
Immunocompromise	25/885 (3%)	59/1648 (4%)	0.35
Current smoker – no. (%)	157/871 (18%)	462/1634 (28%)	<0.01
Prior medication – no. (%)			
Anticoagulants	177/885 (20%)	105/1647 (6%)	<0.01
Antiplatelet	491/885 (55%)	524/1647 (32%)	<0.01
Statin	480/884 (54%)	464/1647 (28%)	<0.01
ACE-inhibitor	339/882 (38%)	304/1648 (18%)	<0.01
Protonpompinhibitor	347/883 (39%)	308/1647 (19%)	<0.01
Modified Rankin Scale score	0 (0-1)	0 (0-1)	<0.01
NIHSS	5 (3-10)	5 (3-9)	<0.01
Dysphagia – no. (%)	237/832 (28%)	384/1534 (25%)	0.07
Bladder catheter	176/881 (20%)	277/1645 (17%)	0.06
Thrombolysis – no (%)	284/885 (32%)	550/1648 (33%)	0.54
Randomisation to ceftriaxone	428/885 (48%)	838/1648 (51%)	0.24
Stroke type (haemorrhagic stroke vs. other)	76/885 (9%)	192/1648 (12%)	0.02

The crude OR for beta-blockers and diagnosis of urinary tract infection as defined by the physician was 1.64 (95%CI 1.20-2.34; p<0.01). Advance age, male sex, atrial fibrillation/flutter, hypertension and hypercholesterolemia, current smoking status, stroke severity, presence of bladder catheter, treatment with thrombolysis, stroke type and randomisation were included in the multivariate analysis, showing an aOR for beta-blockers and urinary tract infection of 1.25 (95%CI 0.85-1.83; p=0.25).

Baseline beta-blocker use was associated with mortality and unfavorable outcome (table 2), but these associations did not remain significant after correction for other prognostic variables as mentioned in the methods section (aOR for mortality at 3 months 1.14; 95%CI 0.84-1.53; p=0.41; aOR for unfavorable outcome at 3 months 1.10; 95%CI 0.89-1.35; p=0.39).

Table 2. Infection rate and outcome in patients using BB before stroke vs. patients not using BB before stroke

	BB before stroke (n=885)	No BB before stroke (n=1648)	OR (95% CI)
Physician diagnosis			
Infection	168/885 (19%)	179/1648 (11%)	1.92 (1.53-2.42)
- Pneumonia	79/885 (9%)	80/1648 (5%)	1.92 (1.39-2.65)
- Urinary tract infection	79/885 (9%)	93/1848 (5%)	1.64 (1.20-2.24)
- Other infection	23/885 (3%)	27/1648 (2%)	1.60 (0.91-2.81)
Expert panel diagnosis			
Infection	62/885 (7%)	67/1648 (4%)	1.78 (1.25-2.54)
- Pneumonia	30/885 (3%)	27/1648 (2%)	2.11 (1.24-3.57)
- Urinary tract infection	33/885 (4%)	43/1648 (3%)	1.45 (0.91-2.29)
- Other infection	4/885 (0.5%)	5/1648 (0.3%)	1.49 (0.40-5.57)
Outcome			
Mortality at discharge	85/884 (9.6%)	60/1647 (3.6%)	1.85 (1.28-2.70)
Mortality at 3 months	123/880 (14.0%)	144/1639 (8.8%)	1.67 (1.30-2.17)
Unfavourable outcome at 3 months	392/880 (45%)	592/1629 (36%)	1.41 (1.19-1.66)

Table 3. Risk factors for infection (physician diagnosis)

Characteristic	Infection (n=348)	No infection (n=2190)	Multivariate analysis aOR (95%CI)	p value
Age (median IQR)	79.5 (72-86)	72 (62-80)	1.04 (1.02-1.05)	<0.01
Male sex	46% (160/348)	59% (1284/2190)		
History				
Atrial fibrillation/flutter	23% (81/348)	14% (310/2190)		
Stroke	34% (119/348)	32% (708/2190)		
Hypercholesterolemia	22% (78/348)	27% (587/2190)		
Hypertension	63% (219/348)	54% (1181/2190)		
Myocardial infarction	15% (53/348)	13% (278/2190)		
Cardiac valve disease	7% (24/348)	7% (149/2190)		
Peripheral vascular disease	11% (37/348)	7% (153/2190)	1.76 (1.09-2.84)	0.02
Obstructive pulmonary disease	11% (38/348)	8% (170/2190)		
Diabetes mellitus	23% (81/348)	19% (421/2190)		

Table 3. Continued

Characteristic	Infection (n=348)	No infection (n=2190)	Multivariate analysis aOR (95%CI)	p value
Alcoholism	4% (14/348)	5% (107/2190)		
Malignancy	11% (38/348)	9% (196/2190)		
Immunocompromise	3% (12/348)	3% (72/2190)		
Current smoker	15% (51/348)	26% (569/2190)		
Prior medication				
Anticoagulants	15% (52/348)	11% (231/2190)		
Antiplatelet therapy	44% (153/348)	39% (865/2190)		
Statins	39% (134/348)	37% (815/2190)		
ACE-inhibitors	29% (100/348)	25% (544/2190)		
Beta blockers	48% (168/348)	33% (717/2190)	1.61 (1.19-2.18)	<0.01
Proton pump inhibitors	27% (95/348)	26% (560/2190)		
Modified Rankin Scale	0 (0-2)	0 (0-1)		
NIHSS	11 (6-16)	4 (3-8)	1.09 (1.06-1.12)	<0.01
Dysphagia	60% (186/310)	21% (437/2061)	2.90 (2.10-4.01)	<0.01
Bladder catheter	52% (181/347)	12% (272/2184)	3.95 (2.91-5.36)	<0.01
Stroke type (bleeding vs other)	17% (60/348)	10% (209/2190)	1.79 (1.14-2.82)	0.01
Randomisation to ceftriaxone	37% (130/348)	52% (1138/2190)	0.53 (0.40-0.70)	<0.01

Discussion

In our analysis beta-blockers were not protective for post-stroke infection. In contrast to previous studies, we found that baseline use of beta-blockers was associated with a higher risk for infection. The previous four studies on beta-blocker treatment and infection risk reported beta-blockers either to be associated with decreased infection risk, or found no association.(4, 5, 8, 9) All studies had a retrospective study design and were heterogeneous with respect to stroke type (ischaemic or haemorrhagic) and definitions on beta-blockers use (prior to stroke or after stroke admission) and infections. In the previous 4 studies definition of infection was not described in 2 studies, was based on adverse event recording in one study, and one study used modified CDC-criteria. Our study had a prospective study design and predefined definition of infection according to international consensus.(10) Data on beta-blocker treatment was prospectively collected and our large sample gave us the statistical power to perform multivariable analysis.

Baseline use of beta-blockers was associated with a higher risk for infection. However, patients on beta-blockers were older, more often had comorbidities, and used more medication than patients not on beta-blockers. It has been well-recognized that patients with advance age are more vulnerable for infections than previously healthy patients, also, some comorbidities and medications are associated with infection.(11, 12) By performing multivariate analysis we tried to correct for this higher baseline infection risk in beta-blocker treated patients, but it is still possible that confounding by indication influenced results. Also, mortality rate was higher in patients treated with beta-blockers. This introduces a competing risk bias: deceased patients are not at risk of infection.

The etiology of stroke-associated infection is multifactorial. Infection occurs more often in patients with more severe stroke and higher age; dysphagic patients are at high risk for pneumonia, and patients with indwelling catheter for urinary tract infection. Also, post-stroke immunodepression, which could be mediated by hypothalamo-pituitary-adrenal axis (HPAA) and sympathetic nervous system (SNS) activation, increases infection risk.(2, 3, 11) Stroke-associated respiratory syndrome includes pneumonia, but also respiratory tract infections without chest-X-ray abnormalities and even a subset of these syndromes could be inflammatory rather than infective.(13) In a recent consensus of the 'Pneumonia in Stroke Consensus Group' (PISCES), it was agreed that the spectrum of lower-respiratory-tract-infections in the first seven days after acute stroke are named as stroke-associated-pneumonia, in the current study these criteria for diagnosis were used. From previous experimental and clinical studies it is thought that adrenergic effects on peripheral blood immune cells could enhance immune suppression and increase infection risk, and beta-blockers have the potential to diminish these effects.(14) The results of the current study do not support such an effect for pre-stroke use of beta-blockers. Yet, effects of beta-blockers have been shown to be dose dependent and dosage dependent effects could have been missed since dosage of beta-blocker therapy was not controlled in this study.(15) Also, effects might differ between beta-blockers already used prior to stroke, as compared to beta-blockers started directly after stroke.(5) Only a randomised clinical trial could investigate the true potential of beta-blocker treatment for reducing stroke-associated infections, but the results of the current study are not encouraging.

This study has several limitations. Firstly, only pre-stroke beta-blocker use was investigated. In previous studies stronger associations were found for on-stroke treatment with beta-blocker. Secondly, use of beta-blockers was strictly defined, but class of drug, dose and compliance of beta-blocker use at baseline was not. This treatment was recorded by the physician in a prospective manner, but the treatment itself, including dosage, was not recorded. In the Netherlands it is standard practice to continue antihypertensive medication used prior to stroke during hospital stay after stroke, and in the PASS no standard

protocol was used for discontinuation of antihypertensive treatment during admission. Any discontinuation of treatment after randomisation could theoretically have lead to an underestimation of the effect of beta-blockers, and dosage dependent effects could have been missed. Also we did not distinguish between selective or non-selective beta-blockers. These different classes could have differing working mechanisms, however, for both groups effects associations with immune response and infection have been described.(4) Thirdly, the current study is a cohort study, which contains the risk of selection bias. The population of the PASS had relatively mild stroke and a low rate of infection. This could theoretically have diminished the potential of effect of beta-blocker therapy, but, since infection rate was unchanged or even higher in beta-blocker treated patients, such an effect is unlikely. Finally, we were able to perform multivariable analysis because of the large study population, however because diagnosis of pneumonia by expert panel was made in a limited number of patients, this multivariable analysis contained more variables than statistically appropriate and should therefore be interpret with caution.

Conclusion

Patients treated with beta-blockers prior to a stroke have a higher rate of infection and pneumonia, but not of UTI.

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Authors' disclosures

None

Authors contributions

WW wrote this manuscript together with DvdB and JDV, MCB, YBR and PJN commented on the paper. All authors agreed to the final version.

References

1. Popovic N, Stefanovic-Budimkic M, Mitrovic N, Urosevic A, Milosevic B, Pelemis M, et al. The frequency of poststroke infections and their impact on early stroke outcome. Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association. 2013;22(4):424-9.
2. Chamorro A, Meisel A, Planas AM, Urra X, van de Beek D, Veltkamp R. The immunology of acute stroke. Nature reviews Neurology. 2012;8(7):401-10.
3. Wong CH, Jenne CN, Lee WY, Leger C, Kubes P. Functional innervation of hepatic iNKT cells is immunosuppressive following stroke. Science (New York, NY). 2011;334(6052):101-5.
4. Maier IL, Karch A, Mikolajczyk R, Bahr M, Liman J. Effect of beta-blocker therapy on the risk of infections and death after acute stroke--a historical cohort study. PloS one. 2015;10(2):e0116836.
5. Sykora M, Siarnik P, Diedler J. beta-Blockers, Pneumonia, and Outcome After Ischemic Stroke: Evidence From Virtual International Stroke Trials Archive. Stroke. 2015;46(5):1269-74.
6. Westendorp WF, Vermeij JD, Zock E, Hooijenga IJ, Kruyt ND, Bosboom HJ, et al. The Preventive Antibiotics in Stroke Study (PASS): a pragmatic randomised open-label masked endpoint clinical trial. Lancet. 2015;385(9977):1519-26.
7. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 2008;36(5):309-32.
8. Dziedzic T, Slowik A, Pera J, Szczudlik A. Beta-blockers reduce the risk of early death in ischemic stroke. Journal of the neurological sciences. 2007;252(1):53-6.
9. Kalita J, Misra UK, Kumar B. Is beta-blocker (atenolol) a preferred antihypertensive in acute intracerebral hemorrhage? Neurol Sci. 2013;34(7):1099-104.
10. Smith CJ, Kishore AK, Vail A, Chamorro A, Garau J, Hopkins SJ, et al. Diagnosis of Stroke-Associated Pneumonia: Recommendations From the Pneumonia in Stroke Consensus Group. Stroke. 2015;46(8):2335-40.
11. Emsley HC, Hopkins SJ. Post-stroke immunodepression and infection: an emerging concept. Infectious disorders drug targets. 2010;10(2):91-7.
12. Kwan J, Hand P. Infection after acute stroke is associated with poor short-term outcome. Acta Neurol Scand. 2007;115(5):331-8.
13. Marik PE. Aspiration pneumonitis and aspiration pneumonia. N Engl J Med. 2001;344(9):665-71.
14. Griffin GD. Stroke, mTBI, infection, antibiotics and beta blockade: Connecting the dots. Medical hypotheses. 2015;85(2):224-9.
15. Prass K, Meisel C, Hoflich C, Braun J, Halle E, Wolf T, et al. Stroke-induced immunodeficiency promotes spontaneous bacterial infections and is mediated by sympathetic activation reversal by poststroke T helper cell type 1-like immunostimulation. J Exp Med. 2003;198(5):725-36.

CHAPTER 8

Development and internal validation of a prediction rule for post-stroke infection and post-stroke pneumonia in acute stroke patients

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Abstract

Introduction

Patients with acute stroke are at high risk for infection. These infections are associated with unfavourable outcome after stroke. A prediction rule can identify the patients at the highest risk for strategies to prevent infection. We aim to develop a prediction rule for post-stroke pneumonia and other infections in patients with acute stroke.

Patients and methods

We used data from the Preventive Antibiotics in Stroke Study, a multicentre randomised trial comparing preventive ceftriaxone vs. standard stroke care in patients with acute stroke. Possible predictors for post-stroke pneumonia or infection were selected from the literature. Backward elimination logistic regression analysis was used to construct prediction rules for pneumonia or infection. Internal validation was performed and a risk chart was constructed. We adjusted for preventive antibiotic use.

Results

Pneumonia was diagnosed in 159 of the 2538 included patients, and infection in 348. Pneumonia was predicted by higher age, male sex, pre-stroke disability, medical history of chronic obstructive pulmonary disease, more severe stroke, dysphagia and intracerebral haemorrhage (rather than ischaemic stroke). Infections were predicted by higher age, male sex, history of diabetes, chronic obstructive pulmonary disease, more severe stroke, dysphagia, use of bladder catheter, preventive antibiotic use and intracerebral haemorrhage. With the prediction rule developed, risks for pneumonia ranged from 0.4% to 56.2% and from 1.8% to 88.0% for infection. Discrimination of the score was good (C-statistic, 0.84; 95% CI: 0.81–0.87 and 0.82; 95% CI: 0.79–0.84 for pneumonia and infection).

Conclusion

The Preventive Antibiotics in Stroke Study pneumonia and infection rule identify patients at the highest risk for post-stroke pneumonia or infection and may be used for future studies and novel therapies, after confirmation in an external population.

Background

Post-stroke infections occur in 30% of the patients with acute stroke and have a strong relation with unfavourable outcome.(1-3) In two recent large randomised trials, preventive antibiotic therapy did not improve functional outcome in relatively unselected patients with acute stroke.(4, 5) New approaches are needed to prevent infection and thereby improve outcome in acute stroke patients. One option would be to prevent infection only in those with high risk of developing these infections.

Risk scores aiming to predict pneumonia in patients with acute stroke have previously been developed. These were mostly derived from retrospective cohort studies or large stroke registries and often were not specifically designed to predict post-stroke infections in general.(6-11) In contrast to many models aimed at predicting post-stroke pneumonia, only one study in 568 stroke patients aimed at predicting post-stroke infection in general. The model developed in this study was neither internally nor externally validated. Predictors for pneumonia from these studies were higher age, sex, more severe stroke, history of congestive heart failure, history of pneumonia or chronic obstructive pulmonary disease (COPD), current smoking, alcoholism, prestroke dependence, reduced level of consciousness, intracerebral haemorrhage (rather than ischaemic stroke), higher systolic arterial blood pressure, higher blood glucose, higher white blood cell (WBC) count and infratentorial location, intraventricular extension, and volume of the hematoma. For any infection, higher age, diabetes, and more severe stroke were predictors.

Aims

The current study aimed to construct a prediction rule both for pneumonia and for any post-stroke infection in patients with acute ischaemic stroke or intracerebral haemorrhage, with data from the 'Preventive Antibiotics in Stroke Study' (PASS).

Methods

Patients and definitions of outcome variables

Data were used from PASS, a multicentre randomised controlled trial including 2550 patients in the acute phase (<24h) of ischaemic stroke or intracerebral haemorrhage. Patients with signs of an infection at study inclusion were excluded. In this trial, preventive treatment with intravenous ceftriaxone during 4 days in addition to standard stroke care was compared with standard stroke care alone. Baseline characteristics and outcome parameters were prospectively collected. Dysphagia was assessed by performing a swallowing test according to national guidelines.(12) We assessed for each patient whether

the swallowing test was performed, the result of the test or the reason why it was not performed (good recovery, reduced consciousness or other reasons). A patient was considered dysphagic in case of an abnormal swallowing function on the test. A patient was considered not dysphagic in case of a normal swallowing function on the test, or when the test was not performed because of good recovery. When the test was not performed due to lowered consciousness or other reasons, dysphagic status was considered unavailable.

Definition of post-stroke infections

In the PASS post-stroke infections were categorized as diagnosed by the clinician, and as judged by an independent adjudication committee blinded for treatment allocation with modified Centres for Disease Control and Prevention criteria, as described in the study protocol.(5, 13) In the current analyses the occurrence of pneumonia or infection as assessed by the clinician during admission was used.

Selection of candidate predictors

Previously described risk factors for post-stroke pneumonia and infection were identified by literature search (search strategy see supplemental appendix). To be considered as candidate predictors, risk factors had to be frequently described in literature and had to be collected in the PASS dataset. The maximum number of candidate predictors should range between the numbers of pneumonia or infection divided by 10-15 according to the events-per-variable rule.(14) For example, if the maximum number of candidate predictors was 10, we chose the 10 most frequently reported risk factors from literature. Each factor had to be known at admission, since the aim of the prediction rule is to assess infection risk early in clinical course. Dysphagia assessment was performed at admission by a trained nurse before any intake by a water swallowing test according to the Dutch Stroke Guideline.(12) Since pneumonia is a subcategory of infection, predictors of pneumonia were also used for prediction of infection. We also considered treatment allocation as a candidate predictor in both prediction rules.

Statistical analysis

All statistical analyses were performed in R version 3.2.4.(15) We developed two prediction rules; one for post-stroke pneumonia and on for post-stroke infections. For each variable the proportion of missingness was assessed. Missing data was imputed with single imputation with 20 iterations using baseline and outcome variables and the Mice Package. (16) For continuous variables linearity was assessed by plotting the data and examining the risk for each decile. Logistic regression analyses were performed to study the association between candidate predictors as defined beforehand (literature search) and the outcomes. Predictors for pneumonia or infection were selected with backward selection, using the Akaike Information Criterion as stopping rule.(14) Next, the performance of

both prediction rules was assessed by investigating the explained variance, calibration curve and Hosmer-Lemeshow test, and discrimination by c-statistic. Both prediction rules were internally validated to prevent overfitting. Regression coefficients were corrected for optimism by a shrinkage factor obtained by bootstrapping (250 samples) including the full backward model. When treatment allocation was selected as independent predictor, we adjusted the intercept and presented the predicted risks based on the standard care subset of the data. We constructed prediction rules based on the regression coefficients of the two models. For the performance of this study, we adhered to the Tripod statement and completed the checklist (supplemental appendix). (17)

Results

Patients

Between July 2010 and March 2014, 2550 patients were enrolled. Twelve patients withdrew consent immediately after randomisation, leaving 2538 patients available for the analysis; 57% of patients were male, the median NIHSS was 5 (IQR 3–9) and 26% of patients were dysphagic (for all baseline characteristics see table 1). During admission, pneumonia was diagnosed in 159 and any infection in 348 patients (159 patients were diagnosed with pneumonia, 173 patients with urinary tract infections, 50 with other infections; see main article PASS for specification of other infections (5); 306 patients were diagnosed with a single infection, and 48 with more than one infection: 29 patients had two infections, 11 patients had 3 infections and 2 patients had 4 infections as diagnosed by the treating physician.

Candidate predictors

The search strategy resulted in 2706 articles. After screening titles, abstracts and full-text, 46 articles were used for the extraction of risk factors for pneumonia and infection (see Supplemental Appendix). For pneumonia, the most consistently reported risk factors (>13 studies) were higher age, male sex, more severe stroke and dysphagia. Other frequently reported risk factors were decreased level of consciousness at admission, pre-stroke dependence, medical history of diabetes, COPD, atrial fibrillation and intracerebral haemorrhage. For infection, the most consistently reported risk factors were higher age, male sex, more severe stroke and a reduced consciousness on admission. Other relatively frequently reported risk factors were pre-stroke dependence, medical history of diabetes, previous stroke, atrial fibrillation, malignancy, immune suppression (HIV, immunosuppressive medication, splenectomy) and urinary catheterisation. The aforementioned risk factors were used as candidate predictors. Age and stroke severity were the only continuous variables and had a linear association with pneumonia and infection. The pre-stroke

Modified Rankin Scale Score (mRS) and GCS were a priori categorized in three groups (mRS: 0, 1–2, 3–5; GCS <13, 13–14 and 15).

Handling of missing data

There were no missing data for the candidate predictors age, sex, intracerebral haemorrhage, pre-stroke dependence, stroke severity and immune suppression. For dysphagia, data were missing in 176 (6.9%) patients, and in 199 patients (7.8%), the verbal score of the GCS was missing (mostly because these patients were aphasic). For all other candidate predictors, data were missing in less than 0.3%.

Prediction rule

Post-stroke pneumonia was predicted by higher age, male sex, pre-stroke disability, medical history of COPD, more severe stroke, dysphagia and intracerebral haemorrhage. Post-stroke infections were predicted by higher age, male sex, medical history of diabetes, COPD, more severe stroke, dysphagia, use of bladder catheter, preventive antibiotic use and intracerebral haemorrhage. Because of selection of preventive antibiotic use as predictor for infection, the linear predictor for infection was adjusted to the placebo population. The linear predictors for pneumonia and infection are shown in table 4 of supplementary appendix, together with the performance measures. After internal validation by bootstrapping, the linear predictor for pneumonia discriminated well between patients with and those without pneumonia (C-statistic 0.83 95%CI 0.80–0.86). The study was well calibrated (Hosmer–Lemeshow test p=0.94). The linear predictor for infection discriminated well between patients with and those without infection (c-statistic 0.82 95% CI 0.79–0.84) and had a good calibration (Hosmer–Lemeshow test p=0.44). The calibration plots for pneumonia and infection are shown in figure 1.

Derivation of a prediction rule and chart

We derived the PASS pneumonia rule and the PASS infection rule, a 22 and 24 points score respectively, which were both subdivided in 4 categories (figure 2). Predicted risk for the lowest and highest score on the prediction rule ranged from 0.4 to 56.2% for pneumonia and 1.8% to 88.0% for infection. In figure 2, the observed risks in the PASS population are shown for each point on the PASS pneumonia and infection rule. Discrimination of the derived scores remained good (C-statistic 0.84 95%CI 0.81–0.87 and 0.82 95% CI 0.79–0.84 for pneumonia and infection, respectively).

Table 1. Prediction chart for PASS Pneumonia Rule and PASS Infection Rule

PASS pneumonia rule			PASS infection rule		
Characteristic		Points	Characteristic		Points
Age			Age		
- 51-60		1	- 51-60		1
- 61-70		2	- 61-70		2
- 71-80		3	- 71-80		3
- >80		4	- >80		4
Male sex		2	Male sex		-1
History of			History of		
- COPD		2	- COPD		2
			- diabetes		1
Disability prior to stroke					
- mRS 1-2		2			
- mRS > 2		1			
Intracerebral haemorrhage		1	Intracerebral haemorrhage		1
Stroke severity (NIHSS)			Stroke severity (NIHSS)		
- 6-10		2	- 6-10		2
- 11-20		4	- 11-20		4
- 21-30		6	- 21-30		6
			Use of bladder catheter		6
Dysphagia		5	Dysphagia		4
Score	Risk category	Pneumonia % (n/N)	Score	Risk category	Infection % (n/N)
0-5	Low	1 (7/1072)	0-5	Low	4 (47/1315)
6-10	Moderate	4 (34/855)	6-10	Moderate	11 (72/633)
11-15	High	17 (88/507)	11-16	High	31 (116/379)
16-22	Very high	29 (30/104)	17-24	Very high	54 (113/211)

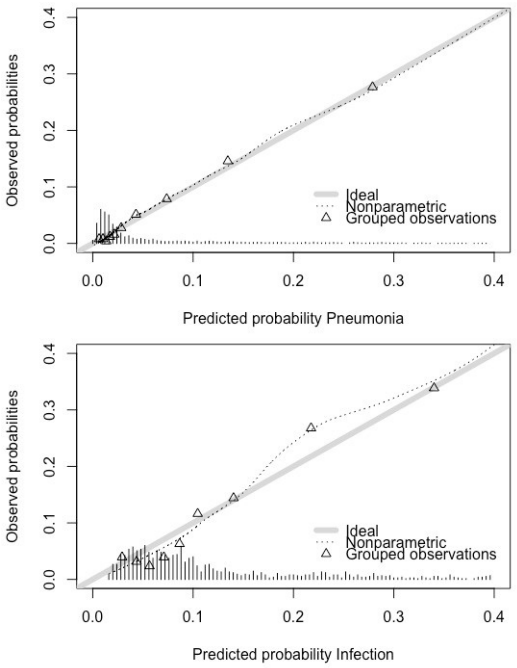

Figure 1. Calibration plot for pneumonia and infection. *Legend Ideal*: line drawn for the situation in which predicted probabilities perfectly match the observed probabilities. *Nonparametric*: line displaying observed probabilities. *Grouped observations*: observed probabilities for 10 groups of equal size.

Discussion

Post-stroke infections were predicted by higher age, male sex, history of diabetes, history of COPD, more severe stroke, dysphagia, use of bladder catheter and intracerebral haemorrhage. Post-stroke pneumonia was predicted by higher age, male sex, prior disability, history of COPD, more severe stroke, dysphagia and intracerebral haemorrhage.

Our study is the first to develop and internally validate a prediction score for post-stroke infection. Post-stroke pneumonia is the most studied infection because of the strongest association with mortality and unfavourable functional outcome. However, other infections such as urinary tract infection, phlebitis, gastro-intestinal infections, sepsis and infections without determined focus can complicate and influence the clinical course after stroke as well. Infections have been shown to complicate the clinical course in 30%, whilst pneumonia occurs in 10% of stroke patients.(3) Because infections are also associated with mortality and unfavourable functional outcome, it seems necessary to aim therapies not only at prevention of pneumonia, but at preventing all infections.(2, 3) In

PASS, preventive antibiotic therapy did not have an effect on functional outcome in the overall population, but did reduce infection rate. Whether preventive antibiotic therapy in a high risk subgroup does improve functional outcome remains to be investigated. The PASS prediction rule has the potential, after external validation, to identify the patients at high risk of infections and could be an important first step for selection of patients for future trials or intensive monitoring for the development of an infection during admission.

Previous models have been developed to predict pneumonia after stroke (see table 2 for overview of all models). Four scores were also externally validated: the A^2DS^2 and AIS-APS scores for ischaemic stroke, the ICH-APS for intracerebral haemorrhage and the ISAN-score for both ischaemic and intracerebral haemorrhage.(7-9, 11) These four scores were developed from and mostly validated in large stroke registries. The other available prediction models for prediction of pneumonia after stroke were mostly based on a smaller number of patients and often too many predictors were included according to the events-per-variable rule, which can lead to overfitting and poorer performance of the model.(6, 10, 14, 18) The present study adds to previous models because it was constructed from data from a large prospective randomised trial in which infection and pneumonia were predefined outcome parameters and might therefore have been more rigorously detected. Also, patients with infection at admission were excluded, which is difficult to control in stroke registries. After external validation, the current study might therefore be better applicable when selection of patients in a randomised trial is considered. Finally, this study includes patients with either ischaemic stroke or intracerebral haemorrhage. The A^2DS^2 score does not include intracerebral haemorrhage patients, the ISAN score was shown to perform less well in intracerebral haemorrhage patients and other scores were made for either ischaemic or intracerebral haemorrhage.(19) If our results are confirmed in an independent population, the PASS pneumonia rule and infection rule could be applicable to patients with ischaemic stroke and intracerebral haemorrhage.

This study has limitations. First, the PASS dataset did not contain all characteristics reported in previous studies as possible risk factors for infection or pneumonia. For example, history of dementia, 'being found down at symptom onset', location of stroke, 'tong pressure movements' or laboratory markers such as monocytic HLA-DR, IL-1 or IL-6, were not prospectively collected in the PASS. Also, CT- or MRI-characteristics of an infarct or bleeding were not used for the development of this model. Since the strongest predictors for infection – as described in literature – were included, it is unlikely that this strongly affected the results and that these factors would otherwise have been included in the model. Also, patients included in PASS had a relatively mild stroke severity that decreased infection and pneumonia rates. External validation should be performed to assess the performance of the model in stroke patients with higher stroke severity and infection rates. Next, the

predictive value of the use of a bladder catheter could be overestimated, since it cannot be excluded that asymptomatic bacteriuria was interpreted as bladder infection in patients with a bladder catheter. Also, in standard stroke practice in the Netherlands, the majority of patients will receive a bladder catheter within the first 24 h when needed, but we did not collect the exact date and time of placement of the catheter. Finally, intracerebral haemorrhage was selected as a predictor for both infections and pneumonia, but this might be explained by increased length of stay. Patients with intracerebral haemorrhage had a longer length of stay than patients with ischaemic stroke, and we used the length of admission as exposure time for infections and pneumonia.

In conclusion, we present an internally validated risk score for post-stroke infection and pneumonia. After external validation, this score can be used for selection of patients at high risk for infection and pneumonia after stroke.

Declaration of competing interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval and informed consent
Ethics approval was obtained from the local institutional review board and all patients or their legal representatives provided written informed consent.

Guarantor
PJN – Paul Nederkoorn.

Contributorship
WW, PN and DvdB conceived the study. WW, JV, MB PN, DvdB, DWJD, BvdW were involved in patient recruitment and data acquisition. WW, NH and AA performed the data analysis. WF wrote the first draft of the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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None.

Supplementary material

Online supplementary appendix (Figure 1 search terms; Table 1 inclusion and exclusion criteria; Table 2 risk factors for pneumonia after stroke; Table 3 risk factors for infection after stroke; Table 4 test characteristics after internal validation).

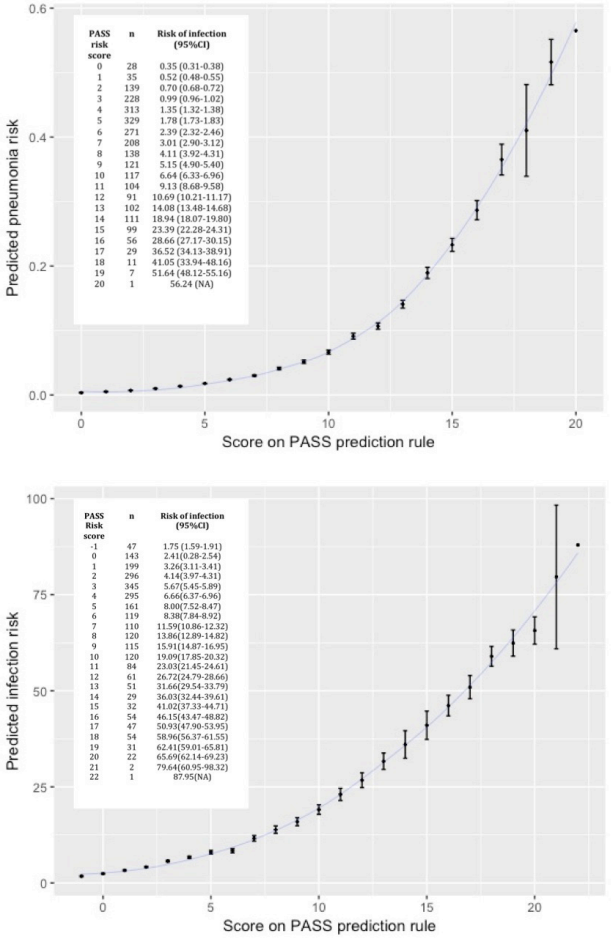

Figure 2. Score on PASS prediction rule and predicted risks for pneumonia and infection.

Table 2. Models to predict pneumonia and infection in acute ischaemic and/or intracerebral haemorrhage patients

Pneumonia			
Author, year, name	**Stroke type***	**Study design**	**No. of patients in derivation group**
Kwon et al, 2006 *The pneumonia score*	I&H	Cohort study	286
Chumbler et al, 2009	I&H	Retrospective cohort	926
Hoffmann et al, 2012 *A2DS2-score*	I	Registry	15335
Ji et al, 2013 *AIS-APS*	I	Registry	8820
Ji et al, 2014 *ICH-APS*	H	Registry	2998
Harms et al, 2013 *Pantheris-score*	I on ICU	RCT	114
Smith et al, 2015 *ISAN-score*	I&H	Registry	11551
Kumar et al, 2016 *ACDD4-score*	I&H	Retrospective cohort	1644
Westendorp et al, 2017 *PASS score*	I&H	RCT	2538
Infection			
Friedant et al, 2015	I	Retrospective cohort	568
Westendorp et al, 2017 *PASS score*	I&H	RCT	2538

* I= ischaemic; H=haemorrhagic; WBC=white blood cell; COPD=chronic obstructive pulmonary disease; NIHSS= National Institutes of Health Stroke Scale score; GCS=Glasgow coma scale; NR= not reported; D=derivation; V=validation; IV=internal validation

Predictors	C-statistic	Validation
age, sex NIHSS, dysphagia, mechanical ventilation	NR	None
age, stroke severity, dysphagia, history of pneumonia, patient being ' found down' at symptom onset	0.78 (D); 0.76 (V)	Internal
age, sex, stroke severity, dysphagia, atrial fibrillation	0.84 (D); 0.84 (V)	External
age, history of atrial fibrillation, congestive heart failure, COPD, current smoking, prestroke dependence, dysphagia, NIHSS, GCS, stroke subtype, blood glucose	0.79 (D); 0.79 (V)	External
age, NIHSS, prestroke dependence, GCS, dysphagia, current smoking, alcoholism, COPD, infratentorial location of ICH, intraventricular extension, hematoma volume.	0.75 (D); 0.76 (V)	Internal
age, GCS, systolic arterial blood pressure, WBC count	0.85 (D); 0.88 (int. V)	Internal
age, sex, nihss, prestroke independence	0.79 (D); 0.78 (V)	External
age, congestive heart failure, dysarthria, dysphagia	0.82 (D); 0.81 (V)	Internal
age, sex, prior disability, medical history of COPD, stroke severity, dysphagia, intracerebral haemorrhage	0.82 (IV)	Internal
age, diabetes, stroke severity	NR	None
age, male sex, diabetes, medical history of COPD, stroke severity, dysphagia, bladder catheter , intracerebral haemorrhage	0.84 (IV)	Internal

References

1. Katzan IL, Cebul RD, Husak SH, Dawson NV, Baker DW. The effect of pneumonia on mortality among patients hospitalized for acute stroke. Neurology. 2003;60(4):620-5.
2. Vermeij FH, Scholte op Reimer WJ, de Man P, van Oostenbrugge RJ, Franke CL, de Jong G, et al. Stroke-associated infection is an independent risk factor for poor outcome after acute ischemic stroke: data from the Netherlands Stroke Survey. Cerebrovasc Dis. 2009;27(5):465-71.
3. Westendorp WF, Nederkoorn PJ, Vermeij JD, Dijkgraaf MG, van de Beek D. Post-stroke infection: A systematic review and meta-analysis. BMC Neurol. 2011;11:110.
4. Kalra L, Irshad S, Hodsoll J, Simpson M, Gulliford M, Smithard D, et al. Prophylactic antibiotics after acute stroke for reducing pneumonia in patients with dysphagia (STROKE-INF): a prospective, cluster-randomised, open-label, masked endpoint, controlled clinical trial. Lancet. 2015;386(10006):1835-44.
5. Westendorp WF, Vermeij JD, Zock E, Hooijenga IJ, Kruyt ND, Bosboom HJ, et al. The Preventive Antibiotics in Stroke Study (PASS): a pragmatic randomised open-label masked endpoint clinical trial. Lancet. 2015;385(9977):1519-26.
6. Chumbler NR, Williams LS, Wells CK, Lo AC, Nadeau S, Peixoto AJ, et al. Derivation and validation of a clinical system for predicting pneumonia in acute stroke. Neuroepidemiology. 2010;34(4):193-9.
7. Hoffmann S, Malzahn U, Harms H, Koennecke HC, Berger K, Kalic M, et al. Development of a clinical score (A2DS2) to predict pneumonia in acute ischemic stroke. Stroke. 2012;43(10):2617-23.
8. Ji R, Shen H, Pan Y, Du W, Wang P, Liu G, et al. Risk score to predict hospital-acquired pneumonia after spontaneous intracerebral hemorrhage. Stroke. 2014;45(9):2620-8.
9. Ji R, Shen H, Pan Y, Wang P, Liu G, Wang Y, et al. Novel risk score to predict pneumonia after acute ischemic stroke. Stroke. 2013;44(5):1303-9.
10. Kwon HM, Jeong SW, Lee SH, Yoon BW. The pneumonia score: a simple grading scale for prediction of pneumonia after acute stroke. Am J Infect Control. 2006;34(2):64-8.
11. Smith CJ, Bray BD, Hoffman A, Meisel A, Heuschmann PU, Wolfe CD, et al. Can a novel clinical risk score improve pneumonia prediction in acute stroke care? A UK multicenter cohort study. Journal of the American Heart Association. 2015;4(1):e001307.
12. Neurologie NVv. Richtlijn diagnostiek, behandeling en zorg voor patiënten met een beroerte 2009. 2009.
13. Nederkoorn PJ, Westendorp WF, Hooijenga IJ, de Haan RJ, Dippel DW, Vermeij FH, et al. Preventive antibiotics in stroke study: rationale and protocol for a randomised trial. Int J Stroke. 2011;6(2):159-63.
14. Steyerberg EW. Clinical Prediction Models. A Practical Approach to Development, Validation, and updating: Springer-Verlag new York; 2009.
15. R Core Team Rffsc. R: a language and environment for statistical computing. . 2016.
16. Groothuis-Oudshoorn SvBK. mice: Multivariate Imputation by Chained Equations in R. Journal of Statistical Software2011. p. 1-67.
17. Collins GS, Reitsma JB, Altman DG, Moons KG. Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD): the TRIPOD Statement. The British journal of surgery. 2015;102(3):148-58.
18. Harms H, Grittner U, Droge H, Meisel A. Predicting post-stroke pneumonia: the PANTHERIS score. Acta neurologica Scandinavica. 2013;128(3):178-84.
19. Papavasileiou V, Milionis H, Smith CJ, Makaritsis K, Bray BD, Michel P, et al. External Validation of the Prestroke Independence, Sex, Age, National Institutes of Health Stroke Scale (ISAN) Score for Predicting Stroke-Associated Pneumonia in the Athens Stroke Registry. Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association. 2015;24(11):2619-24.

Appendix

Date search: January 10 2017

Figure 1. Search terms (medline)

((((((((((cerebrovascular disorders/ or exp basal ganglia cerebrovascular disease/ or exp brain ischemia/ or stroke/ or))) OR "basal ganglia cerebrovascular disease") OR "brain ischemia") OR "stroke") OR "brain infarction") OR "hypoxia ischemia brain") OR "intracranial arterial diseases") OR (("intracranial embolism and thrombosis"))) OR "intracranial hemorrhages" OR (((((ischemi$ OR infarct$ OR emboli$ OR oculus OR hypox$ OR obstruction OR vasculopathy)) .tw.))) AND ((((brain$ or cerebr$ or cerebell$ or cortical or vertebrobasilar or hemispher$ or intracran$ or intracerebral or infratentorial or supratentorial or MCA or anterior circulation or posterior circulation or basal ganglia))) OR ((lacunar or cortical) AND infarct$).tw. or ((brain$ or cerebr$ or cerebell$ or intracerebral or intracran$ or parenchymal or intraventricular or infratentorial or supratentorial or basal gangli$ or subarachnoid or putaminal or putamen or posterior fossa) AND (haemorrhage$ or hemorrhage$ or haematoma$ or hematoma$ or bleed$)).tw. or ((brain or intracranial or basal ganglia or lenticulostriate) AND (vascular AND (disease$ or disorder or event))).tw. or ((ischemic or apoplectic) adj5 (event or events or insult or attack$)).tw. or ((intracranial or cerebral art$ or basilar art$ or vertebral art$ or vertebrobasilar or vertebral basilar) AND (stenosis or ischemia or insufficiency or arteriosclero$ or atherosclero$ or occlus$)).tw. AND Search (((((((("sepsis") OR sepsis[Title/Abstract]) OR (((pulmonary[Title/Abstract] OR lung[Title/Abstract] OR airway[Title/Abstract] OR chest)[Title/Abstract] AND (infection[Title/Abstract] OR inflammation)[Title/Abstract]))) OR pneumonia[Title/Abstract]) OR "pneumonia") OR cystitis[Title/Abstract]) OR "cystitis") OR UTI[Title/Abstract]) OR "urinary tract infections"AND Risk OR hazard OR predict$ OR associate$

Table 1. Inclusion- and exclusion criteria

Inclusion:
- cohortstudies, RCT's, meta-analysis (these studies were cross-checked for individual cohort studies)
- ischaemic and/or haemorrhagic stroke patients
- risk factors present/assessable at admission
- multivariate analysis reported
- in-hospital diagnosis of infection
- English, Dutch, French, German language
Exclusion:
- no multivariate analysis performed
- only one risk factor reported (not total multivariate model)
- studies on patients with subarachnoidal haemorrhage

Table 2a. Risk factors for pneumonia after stroke (table 2b)

Demographics	No. of studies
Age	1,3,5,7, 8,9, 10, 14, 16, 17, 18, 19, 20, 21, 22, 24, 25, 26, 27, 29, 31
Male sex	1, 5, 7,9,10,11, 14, 19, 21, 22, 24, 29, 31
Medical history	
Pre-stroke dependence	9, 16, 24
Previous stroke	8,11
COPD	8, 24
Atrial fibrillation	5, 8,10, 22, 26
Congestive heart failure	3, 7, 26
Coronary heart disease	8, 24
Diabetes	8, 21, 31
Smoking	16
Alcoholism	5, 16
No dyslipidemie	19
Hypertension	8
renal failure	7
pneumonia	15, 25
Charlson comorbidity index	14
History of dementia	26 (lower risk), 27
Baseline medication	
Ace-inhibitors	
Acid-supressive drugs	14, 15
Treatment lipid-lowering drugs	5 (lower risk)
Pre-stroke beta-blocker therapy	

Table 2a. Continued

Demographics	No. of studies
Clinical assessment at admission	
GCS at admission	8, 16, 18, 25, 30
Stroke severity (NIHSS score)	1, 2,5, 8,9,10,11, 12, 16, 17, 19, 20, 21, 22, 24, 25, 27, 28
Dysarthria	3, 27
Dysphagia	2, 3, 4, 6, 8,10, 15, 16, 20, 21, 22, 24, 25, 27, 28, 29
Haemorrhagic stroke	38, 43
Aphasia / no speech	38, 27
Severe facial palsy	30, 41
Hypertension	18 (higher), 19 (lower risk), 23 (higher risk)
Requiring full assistance with mobility	13
Urinary incontinence	13
Left hemispheric stroke	5 (lower risk)
Patient 'found down' at symptom onset	25
Laboratory tests at admission	
CRP at admission	10, 20, 23
white blood cell count >11.000/µl [no = 0, yes = 3].	18, 23
Radiology	
Hematoma: intraventricular extension, volume, infratentorial location	16
Bilateral laesions	17
Non-lacunar ischeemic stroke	24
Non-lacunar basal ganglia infarction	28
Stroke treatment	
Total time intra-arterial treatment	4
Oral hygienic care	6
Total time intra-arterial treatment	4

Table 2b. Studies describing risk factors for pneumonia after stroke

No.	Study, year	Type of study	No. of patients
1	Nakamuri et al 2016 (1)	cohort	220
2	Sari et al, 2016 (2)	retrospective cohort	210
3	Kumar et al 2017 (3)	cohort	1644
4	Liu et al, 2016 (4)	cohort	165
5	Matz et al, 2016 (5)	registry	59558
6	Wagner et al 2016 (6)	cohort	1656
7	Colbert et al, 2016 (7)	retrospective cohort	91643
8	Yuan et al, 2015 (8)	Meta-analysis	Different per risk factor
9	Papavasileiou et al, 2015 (9)	External validation of ISAN prediction score	204
10	Lu et al, 2015 (10)	Prospective cohort	101
11	Bruening et al, 2015 (11)	Prospective cohort	538
12	Almeida et al, 2015 (12)	Retrospective cohort	159
13	Brogan et al, 2014 (13)	Retrospective cohort	536
14	Ho et al, 2014 (14)	Retrospective cohort	2170
15	Herzig et al, 2014 (15)	Retrospective cohort	1676
16	Ji et al, 2014 (16)	Chinese stroke registry prediction score	4998

Type of stroke	Risk factors
stroke	age, sex, NIHSS, tong pressure movement
stroke	severe neurological deficit, dysphagia
stroke	age, congestive heart failure, dysarthrie, dysphagia
ischaemic stroke	time IAT treatment, dysphagia
ischaemic stroke	age, stroke severity, chronic alcohol consumption, atrial fibrillation. Lower risk: female seks, left hemispheric stroke, cryptogenic stroke, treatment lipid lowering drugs
stroke	oral hygienic care, dysphagia
ischaemic stroke	male seks, age, hispinic race asian race, CHF, renal failiure
' stroke'	age >65 years, atrial fibrillation, heart disease, coronary heart disease, diabetes, chronic obstructive pulmonary disease (COPD), stroke, hyper- tension, National Institute of Health Stroke Scale (NIHSS) 5–15 points, NIHSS >15 points, dysphagia, Glasgow coma scale <=8, length of hospital stay >20 days, use of mechanical ventilation, use of nasogastric tubes, tracheal incision, use of antibiotic prophylactics, - ing pump inhibitors, multiple verte- brobasilar stroke, multiple hemispheric stroke and >66% of the middle cerebral artery territory affected by stroke.
AIS: 2732, ICH: 472	ISAN score: prestroke independence, sex, age, National Institutes of Health Stroke Scale score
Stroke	dysphagia, NIHSS score, A(2)DS(2) score, CURB-65 score, serum iron, serum ferritin, PCT and CRP
Ischaemic stroke patients who received IV-thrombolysis	male sex (odds ratio [OR], 1.9; 95% CI, 1.2-3.1; P = .006), neurologic deficit severity (NIHSS score ≥10; OR, 4.4; 95% CI, 2.5-7.4; P < .0019), previous stroke (OR, 1.5; 95% CI, 1.0-2.2; P = .06), and occurrence of symptomatic intracerebral haemorrhage (OR, 1.6; 95% CI, 1.0-3.2; P = .048)
Stroke	Multivariable logistic regression analysis identified NIHSS as an independent predictor of pneumonia (95%CI: 1.049-1.246, p = 0.002).
stroke	requiring full assistance with mobility [OR 6.48, 95% CI 1.35, 31.16] and urinary incontinence [OR 3.21, 95% CI 1.16, 8.87] were associated with respiratory infections
non-traumatic ICH	PPI, age, men, charlson comorbidity index
acute ischaemic stroke or intracerebral haemorrhage	Acid-suppressive medication, history of pneumonia, dysphagia
Intracerebral haemorrhage	Age (1-y increase) Current smoking (yes) Excess alcohol consumption (yes) Prestroke dependence (mRS ≥3) (yes) Admission NIHSS score (1 increase) Admission GCS score (1 decrease) Dysphagia (yes) Infratentorial location of ICH (yes) Intraventricular extension (yes) Hematoma volume (1 mL increase)

Table 2b. Continued

No.	Study, year	Type of study	No. of patients
17	Maeshima et al, (17)	Cohort	292
18	Harms, 2013 (18)	Pantheris score	335
19	Masrur et al, 2013 (19)	Stroke registry	314,007
20	Zhang et al, 2012 (20)	Cohort	106
21	Scheitz et al, 2015 (21)	Registry	481
22	Hoffman et al , 2012 (22)	Prediction score	15335
23	Ishigami, 2012 (23)	Cohort	53
24	Finlayson, 2011 (24)	retrospective cohort study	8,251
25	Chumbler et al, 2010 (25)	Retrospective cohort	
26	Ovbiagele (26)	California Acute Stroke Prototype Registry.	663
27	Sellars et al, 2007 (27)	series of consecutive patients	412
28	Walter, 2007 (28)		236
29	Kwon et al, 2005 (29)	Consecutive cohort	382
30	Dziewas, 2004 (30)	Prospective cohort	100
31	Aslanyan, 2004 (31)	Data from a prospective trial	1455

Type of stroke	Risk factors
Ischaemic stroke	elderly age, bilateral lesions, and severe neurological deficit were significantly associated with pneumonia.
MCA infarction	Glasgow Coma Scale (GCS) [GCS < 9 = 5, GCS 9-12 = 2, GCS > 12 = 0], age [<60 = 0, 60-80 = 1, >80 = 2], increase in systolic arterial blood pressure >200 mmHg within the first 24 h after admission [no = 0, yes = 2], and white blood cell count >11.000/µl [no = 0, yes = 3].
ischaemic stroke patients	patients with HAP were older, had admission National Institutes of Health Stroke Scale (NIHSS) score (median NIHSS score: 10 versus 4), were more likely to undergo DS (75.5% versus 68.5%), and had increased length of stay and in-hospital mortality (12.4% versus 2.3%). In multivariable analyses, factors independently associated with a lower risk of HAP were female gender (odds ratio [OR] 0.84), dyslipidemia (OR 0.84), and hypertension (OR 0.94).
acute ischaemic stroke with diabetes	Raised levels of IL-6 and CRP, older age, more severe stroke, longer duration of hospitalization and dysphagia were significantly associated with the development of pneumonia.
ischaemic stroke patients	After multivariable adjustment for known risk factors for poststroke pneumonia (age, stroke severity, dysphagia, male sex and diabetes), statin treatment was negatively associated with pneumonia (OR 0·31; 95% CI 0·10-0·94).
Ischaemic	age, sex, stroke severity, dysphagia, atrial fibrillation
elderly patients with acute ischaemic stroke.	CRP, WBC, hypertension
Ischaemic stroke	Older age, male sex, stroke severity, dysphagia, chronic obstructive pulmonary disease, coronary artery disease, nonlacunar ischaemic stroke, and preadmission dependency were independent predictors of pneumonia.
Ischaemic stroke	age, stroke severity, dysphagia, history of pneumonia, patient being ' found down' at symptom onset
ischaemic stroke in the	Older age, atrial fibrillation, and congestive heart failure history of dementia associated with lesser risk.
acute stroke	Older age, dysarthria/no speech due to aphasia, severity of post-stroke disability, cognitive impairment, and an abnormal water swallow test result.
acute ischaemic stroke admitted to the neurological intensive care unit	dysphagia (RR, 9.92; 95% CI, 5.28-18.7), National Institute of Health Stroke Scale > or = 10 (RR, 6.57; CI, 3.36-12.9), non-lacunar basal-ganglia infarction (RR, 3.10; CI, 1.17-5.62), and any other infection present on admission (RR, 3.78; CI, 2.45-5.83).
Ischaemic and haemorrhagic	age, sex, NIHSS, dysphagia, mechanical ventilation
Acute stroke	Decreased level of consciousness and severe facial palsy.
Acute ischaemic stroke	Higher baseline National Institute of Health Stroke Scale (NIHSS) and age, male gender, history of diabetes

Table 3a. Risk factors for infection (1-10, table 3b), sepsis (11,12 table 3c), urinary tract infection (13-15, table 3d) after stroke

Demographics	No. of studies
Age	4,5,7, 10, 11, 13, 15
Sex	5, 10, 11, 14, 15
Etnicity: black race	6, 11
Medical history	
Pre-stroke dependence	5, 13
Diabetes	4
Congestive heart failure	11
Renal disease	11
Previous stroke	8, 14
Atrial fibrillation	8
Barthel-index < 5	9
Malignancy	11
Immunesuppression	12
Clinical assessment at admission	
GCS at admission	6,8,9
Stroke severity (NIHSS score)	3,4,5,7, 10, 15
Large-vessel disease / thrombo-embolic infarction	8
MCA territory infarcts	9
Urinary catheterization	13
Laboratory assessment at admission	
Baseline IL-10	1,3
CRP	3
Radiological assessment	
Size infarct	1
Size hematoma	2,6
Deep location hematoma	6
Intraventricular hematoma	2

Table 3b. Studies describing risk factors for infection after stroke

No.	Study, year	Type of study	No. of patients
1	Ashour et al, 2016 (32)	cohort	60
2	Vial et al, 2016 (33)	prospective cohort	222
3	Worthmann et al, 2015 (34)	Prospective cohort	56
4	Friedant et al, 2015 (35)	Retrospective cohort	568
5	Smith et al, 2015 (36)	Registry, design of prediction score (ISAN)	derivation (n=11 551) and validation (n=11 648) samples
6	Lord et al, 2014 (37)	Prospective cohort	800
7	Wartenberg et al , 2011 (38)	cohort	
8	Hanchaiphiboolkul S1, 2005 (39)	Retrospective cohort	332
9	Hamidon, 2005 (40)	Prospective cohort	163
10	Kammersgaard, 2001 (41)	cohort	1156

Table 3c. Studies describing risk factors for sepsis after stroke

No.	Study, year	Type of study	No. of patients
11	Colbert et al, 2016 (7)	Retrospective	91643
12	Berger et al, 2014 (42)	Retrospective	238

Table 3d. Studies describing risk factors for urinary tract infection after stroke

No.	Study, year	Type of study	No. of patients
13	Stott et al , 2009 (43)	Prospective study	412
14	Ovbiagele (26)	California Acute Stroke Prototype Registry.	663
15	Aslanyan, 2004 (31)	Data from a prospective trial	1455

Type of stroke	Risk factors
ischaemic stroke	Baseline IL10 en size infarct
haemorrhagic stroke	ICH score, size hematoma, intraventricular hematoma
ischaemic stroke and TIA	IL-10 at 6 hours, CRP at 6 hours and NIHSS on admission were identified as independent predictors of infection (IL-10: P = 0.009; CRP: P = 0.018; NIHSS: P = 0.041)
In-hospital ischaemic stroke	Patients who developed infection were older (73 versus 64, P < .0001), more frequently diabetic (43.9% versus 29.1%, P = .0077), and had more severe strokes on admission (National Institutes of Health Stroke Scale [NIHSS] score 12 versus 5, P < .0001).
ischaemic stroke or intracerebral haemorrhage	prestroke Independence [modified Rankin scale], Sex, Age, National Institutes of Health Stroke Scale
intracerebral haemorrhage	Admission characteristics associated with infection in multivariable models were ICH volume (odds ratio [OR], 1.02/mL; 95% confidence interval [CI], 1.01-1.03), lower Glasgow Coma Scale (OR, 0.91 per point; 95% CI, 0.87-0.95), deep location (reference lobar: OR, 1.90; 95% CI, 1.28-2.88), and black race (reference white: OR, 1.53; 95% CI, 1.01-2.32)
acute iscahemic stroke	Age and NIHSS predicted the development of infections.
cerebral infarct	Atrial fibrillation, thromboembolic infarction (large vessel disease), admission conscious level (subconscious or unconscious/ coma), and previous stroke were independent risk factors for development of early infection.
Acute ischaemic stroke	Barthel index (BI) less than 5 (OR 4.23; 95% CI 1.70 to 5.11), middle cerebral artery (MCA) territory infarcts (OR 4.91; 95%CI 1.57 to 8.82), and a Glasgow coma score (GCS) less than 9 (OR 5.12; 95% CI 2.98 to 15.52)
stroke	Advanced age, female gender, decreased SS score admission

Type of stroke	Risk factors
ischaemic stroke	male sex, age, ethnicity, cancer, CHF, renal failure
ICU admitted ischaemic or haemorrhagic stroke	Comorbidities (chronic obstructive pulmonary disease and immunosuppressive disorders) and Simplified Acute Physiology Score II but none of the factors describing stroke severity were independent predictors of sepsis acquisition

Type of stroke	Risk factors
consecutive acute stroke patients	UTI was associated with urinary catheterization (OR = 3.03, 95% CI 1.41-6.52), higher mRS (OR = 1.85, 1.29-2.64) and increasing age (OR = 1.51, 1.13-2.00 for each decade).
ischaemic stroke in the	Women and patients with a history of cerebrovascular events were significantly more likely to experience a UTI.
Acute ischaemic stroke	Female gender and higher baseline NIHSS and age predicted UTI, which occurred in 17.2% of patients.

Table 4. Test characteristics after internal validation

Linear predictor		Area under the ROC curve	Hosmer-Leme show test (p-value)
Pneumonia			
- 7.43185000 + 0.03619129 * age (years) + 0.66757292 * male sex (yes=1, no=0) + 0.51496703 * prior disability (mRS=1 or mRS==2) + 0.30302425 * prior disability (mRS=>3) + 0.66445794 * COPD (yes=1, no=0) + 0.07731002 * stroke severity (points on NIHSS) + 1.43901004 * dysphagia (yes=1, no=0) + 0.43500888 * haemorrhagic stroke (yes=1, no=0)	0.288	0.83 (0.80-0.86)	0.94
Infection			
-5.2469 +0.03396999 * age (years) - 0.21755542 * male sex (yes=1, no=0) + 0.31346007 * diabetes (yes=1, no=0) + 0.44140730 * COPD (yes=1, no=0) + 0.04893813 * stroke severity (points on NIHSS) + 0.88103705 * dysphagia (yes=1, no=0) + 1.35096805 * bladder catheter (yes=1, no=0) + 0.29529332 * haemorrhagic stroke (yes=1, no=0)	0.327	0.82 (0.79-0.84)	0.44

References Appendix

1. Nakamori M, Hosomi N, Ishikawa K, Imamura E, Shishido T, Ohshita T, et al. Prediction of Pneumonia in Acute Stroke Patients Using Tongue Pressure Measurements. PloS one. 2016;11(11):e0165837.
2. Sari IM, Soertidewi L, Yokota C, Kikuno M, Koga M, Toyoda K. Comparison of Characteristics of Stroke-Associated Pneumonia in Stroke Care Units in Indonesia and Japan. Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association. 2017;26(2):280-5.
3. Kumar S, Marchina S, Massaro J, Feng W, Lahoti S, Selim M, et al. ACDD4 score: A simple tool for assessing risk of pneumonia after stroke. Journal of the neurological sciences. 2017;372:399-402.
4. Liu R, Li W, Li Y, Han Y, Ma M, Zhu W, et al. Total time of operation is a risk factor of stroke-associated pneumonia in acute ischemic stroke patients with intra-arterial treatment. Medicine. 2016;95(29):e3958.
5. Matz K, Seyfang L, Dachenhausen A, Teuschl Y, Tuomilehto J, Brainin M. Post-stroke pneumonia at the stroke unit - a registry based analysis of contributing and protective factors. BMC neurology. 2016;16:107.
6. Wagner C, Marchina S, Deveau JA, Frayne C, Sulmonte K, Kumar S. Risk of Stroke-Associated Pneumonia and Oral Hygiene. Cerebrovascular diseases (Basel, Switzerland). 2016;41(1-2):35-9.
7. Colbert JF, Traystman RJ, Poisson SN, Herson PS, Ginde AA. Sex-Related Differences in the Risk of Hospital-Acquired Sepsis and Pneumonia Post Acute Ischemic Stroke. Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association. 2016;25(10):2399-404.
8. Yuan MZ, Li F, Tian X, Wang W, Jia M, Wang XF, et al. Risk factors for lung infection in stroke patients: a meta-analysis of observational studies. Expert review of anti-infective therapy. 2015;13(10):1289-98.
9. Papavasileiou V, Milionis H, Smith CJ, Makaritsis K, Bray BD, Michel P, et al. External Validation of the Prestroke Independence, Sex, Age, National Institutes of Health Stroke Scale (ISAN) Score for Predicting Stroke-Associated Pneumonia in the Athens Stroke Registry. Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association. 2015;24(11):2619-24.
10. Lu Y, Liu XY, Chen YJ, Yu J, Yin SJ. Serum iron and A(2)DS(2) score in stroke-associated pneumonia. International journal of clinical and experimental medicine. 2015;8(4):6163-70.
11. Bruening T, Al-Khaled M. Stroke-Associated Pneumonia in Thrombolyzed Patients: Incidence and Outcome. Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association. 2015;24(8):1724-9.
12. Almeida SR, Bahia MM, Lima FO, Paschoal IA, Cardoso TA, Li LM. Predictors of pneumonia in acute stroke in patients in an emergency unit. Arquivos de neuro-psiquiatria. 2015;73(5):415-9.
13. Brogan E, Langdon C, Brookes K, Budgeon C, Blacker D. Dysphagia and factors associated with respiratory infections in the first week post stroke. Neuroepidemiology. 2014;43(2):140-4.
14. Ho SW, Tsai MC, Teng YH, Yeh YT, Wang YH, Yang SF, et al. Population-based cohort study on the risk of pneumonia in patients with non-traumatic intracranial haemorrhage who use proton pump inhibitors. BMJ open. 2014;4(11):e006710.
15. Herzig SJ, Doughty C, Lahoti S, Marchina S, Sanan N, Feng W, et al. Acid-suppressive medication use in acute stroke and hospital-acquired pneumonia. Annals of neurology. 2014;76(5):712-8.
16. Ji R, Shen H, Pan Y, Du W, Wang P, Liu G, et al. Risk score to predict hospital-acquired pneumonia after spontaneous intracerebral hemorrhage. Stroke. 2014;45(9):2620-8.
17. Maeshima S, Osawa A, Hayashi T, Tanahashi N. Elderly age, bilateral lesions, and severe neurological deficit are correlated with stroke-associated pneumonia. Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association. 2014;23(3):484-9.
18. Harms H, Grittner U, Droge H, Meisel A. Predicting post-stroke pneumonia: the PANTHERIS score. Acta neurologica Scandinavica. 2013;128(3):178-84.
19. Masrur S, Smith EE, Saver JL, Reeves MJ, Bhatt DL, Zhao X, et al. Dysphagia screening and hospital-acquired pneumonia in patients with acute ischemic stroke: findings from Get with the Guidelines--Stroke. Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association. 2013;22(8):e301-9.
20. Zhang X, Wang F, Zhang Y, Ge Z. Risk factors for developing pneumonia in patients with diabetes mellitus following acute ischaemic stroke. The Journal of international medical research. 2012;40(5):1860-5.
21. Scheitz JF, Endres M, Heuschmann PU, Audebert HJ, Nolte CH. Reduced risk of poststroke pneumonia in thrombolyzed stroke patients with continued statin treatment. International journal of stroke : official journal of the International Stroke Society. 2015;10(1):61-6.
22. Hoffmann S, Malzahn U, Harms H, Koennecke HC, Berger K, Kalic M, et al. Development of a clinical score (A2DS2) to predict pneumonia in acute ischemic stroke. Stroke. 2012;43(10):2617-23.

23. Ishigami K, Okuro M, Koizumi Y, Satoh K, Iritani O, Yano H, et al. Association of severe hypertension with pneumonia in elderly patients with acute ischemic stroke. Hypertension research : official journal of the Japanese Society of Hypertension. 2012;35(6):648-53.
24. Finlayson O, Kapral M, Hall R, Asllani E, Selchen D, Saposnik G. Risk factors, inpatient care, and outcomes of pneumonia after ischemic stroke. Neurology. 2011;77(14):1338-45.
25. Chumbler NR, Williams LS, Wells CK, Lo AC, Nadeau S, Peixoto AJ, et al. Derivation and validation of a clinical system for predicting pneumonia in acute stroke. Neuroepidemiology. 2010;34(4):193-9.
26. Ovbiagele B, Hills NK, Saver JL, Johnston SC. Frequency and determinants of pneumonia and urinary tract infection during stroke hospitalization. J Stroke Cerebrovasc Dis. 2006;15(5):209-13.
27. Sellars C, Bowie L, Bagg J, Sweeney MP, Miller H, Tilston J, et al. Risk factors for chest infection in acute stroke: a prospective cohort study. Stroke. 2007;38(8):2284-91.
28. Walter U, Knoblich R, Steinhagen V, Donat M, Benecke R, Kloth A. Predictors of pneumonia in acute stroke patients admitted to a neurological intensive care unit. J Neurol. 2007;254(10):1323-9.
29. Kwon HM, Jeong SW, Lee SH, Yoon BW. The pneumonia score: a simple grading scale for prediction of pneumonia after acute stroke. Am J Infect Control. 2006;34(2):64-8.
30. Dziewas R, Ritter M, Schilling M, Konrad C, Oelenberg S, Nabavi DG, et al. Pneumonia in acute stroke patients fed by nasogastric tube. Journal of neurology, neurosurgery, and psychiatry. 2004;75(6):852-6.
31. Aslanyan S, Weir CJ, Diener HC, Kaste M, Lees KR. Pneumonia and urinary tract infection after acute ischaemic stroke: a tertiary analysis of the GAIN International trial. Eur J Neurol. 2004;11(1):49-53.
32. Ashour W, Al-Anwar AD, Kamel AE, Aidaros MA. Predictors of early infection in cerebral ischemic stroke. Journal of medicine and life. 2016;9(2):163-9.
33. Vial F, Brunser A, Lavados P, Illanes S. Intraventricular Bleeding and Hematoma Size as Predictors of Infection Development in Intracerebral Hemorrhage: A Prospective Cohort Study. Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association. 2016;25(11):2708-11.
34. Worthmann H, Tryc AB, Dirks M, Schuppner R, Brand K, Klawonn F, et al. Lipopolysaccharide binding protein, interleukin-10, interleukin-6 and C-reactive protein blood levels in acute ischemic stroke patients with post-stroke infection. Journal of neuroinflammation. 2015;12:13.
35. Friedant AJ, Gouse BM, Boehme AK, Siegler JE, Albright KC, Monlezun DJ, et al. A simple prediction score for developing a hospital-acquired infection after acute ischemic stroke. Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association. 2015;24(3):680-6.
36. Smith CJ, Kishore AK, Vail A, Chamorro A, Garau J, Hopkins SJ, et al. Diagnosis of Stroke-Associated Pneumonia: Recommendations From the Pneumonia in Stroke Consensus Group. Stroke. 2015;46(8):2335-40.
37. Lord AS, Langefeld CD, Sekar P, Moomaw CJ, Badjatia N, Vashkevich A, et al. Infection after intracerebral hemorrhage: risk factors and association with outcomes in the ethnic/racial variations of intracerebral hemorrhage study. Stroke. 2014;45(12):3535-42.
38. Wartenberg KE, Stoll A, Funk A, Meyer A, Schmidt JM, Berrouschot J. Infection after acute ischemic stroke: risk factors, biomarkers, and outcome. Stroke research and treatment. 2011;2011:830614.
39. Hanchaiphiboolkul S. Risk factors for early infection after an acute cerebral infarction. Journal of the Medical Association of Thailand = Chotmaihet thangphaet. 2005;88(2):150-5.
40. Hamidon BB, Raymond AA, Norlinah MI, Jefferelli SB. The predictors of early infection after an acute ischaemic stroke. Singapore Med J. 2003;44(7):344-6.
41. Kammersgaard LP, Jorgensen HS, Reith J, Nakayama H, Houth JG, Weber UJ, et al. Early infection and prognosis after acute stroke: the Copenhagen Stroke Study. J Stroke Cerebrovasc Dis. 2001;10(5):217-21.
42. Berger B, Gumbinger C, Steiner T, Sykora M. Epidemiologic features, risk factors, and outcome of sepsis in stroke patients treated on a neurologic intensive care unit. Journal of critical care. 2014;29(2):241-8.
43. Stott DJ, Falconer A, Miller H, Tilston JC, Langhorne P. Urinary tract infection after stroke. QJM. 2009;102(4):243-9.

CHAPTER 9

Summary and general discussion

Summary and general discussion

This thesis deals with the important clinical question if prevention of infections with antibiotic therapy improves functional outcome in acute stroke patients. Main focus of this discussion are the results of the 'Preventive Antibiotics in Stroke Study' (PASS), the randomised controlled trial described in chapter 5; and all its consequences. In this summary and discussion we will in-depth discuss the findings of this trial, as well as the results of the side studies described in the other chapters, which will put the PASS results in a broader perspective. Finally, future directions for the prevention of infections after acute stroke will be discussed.

Incidence and type of infection after stroke

Infections after stroke have been the focus of research for many years. In the 90's *Kalra et al* and *Davenport et al* described infection as one of the most common complications after acute stroke.(1, 2) In chapter 2 we describe a systematic review and meta-analysis in which we investigated the frequency of post-stroke infections, as this was unknown and ranged in literature from 5 to 65%. We included 87 studies that reported the frequency of infection in stroke patients in this review, 8 studies were performed on an Intensive Care Unit (ICU). Pooled frequency of infection was 30% (24-36%), frequency of pneumonia and urinary tract infections was both 10% (9-10% and 9-12%). Frequencies were higher in patients admitted on an ICU: pneumonia occurred in 28% (95%CI 18-38%) and urinary tract infections in 20% (95%CI 0-40%) of patients. The rates reported in this study show that pneumonia is more common in stroke patients than in patients admitted to general wards for other diseases: estimated incidence of hospital-acquired pneumonia in general wards ranges between 1.6 and 3.7 cases per 1000 admissions.(3) In ICU patients in Europe incidence of hospital-acquired pneumonia ranges between 19% and 40%; this approximates the incidence found in acute stroke patients admitted on ICU's.(4, 5) We will discuss the reasons for this increased risk for infection in stroke patients below. Phlebitis, sepsis and gastro-intestinal infections are other infection that can occur in the wake of a stroke, but occur far less often than pneumonia and urinary tract infections.

Diagnosis of infection in acute stroke patients

An important limitation of the review described in chapter 2 is that the criteria for diagnosis of infection varied considerably between studies, or were occasionally not reported. Clinical criteria that are often used for diagnosing infection in all patients are described by the Centers for Disease Control and Prevention (CDC), although other criteria also exist.(6) However, a survey in German stroke units showed that diagnosis of pneumonia in stroke patients was mostly based on presence of fever in combination with stroke severity.(7) The chest X-ray, which is an important criterion in CDC-criteria, was of minor importance.

The manner of diagnosing infection after stroke in clinical studies was also assessed in a systematic review of 64 different studies.(8) It appeared that diagnosis was based on published standard criteria in 20 studies, and on unpublished ad hoc criteria in 26 studies. In the other studies the diagnosis was made by 'unspecified clinician-reported diagnosis', initiation of antibiotics or no information was reported. This review showed that the lack of criteria can lead to underreporting of infections, because occurrence of pneumonia was the highest in studies applying standard criteria. On the other hand, when the presence of severe stroke and fever are main predictors of clinical diagnosis of pneumonia, one can imagine that patients could be wrongly diagnosed as having a pneumonia, which could lead to 'over-treatment' and thus possibly decrease the potential effect of therapies for pneumonia tested in trials. In trials on preventive antibiotic therapy in acute stroke patients up until now, diagnosis of infection was mostly based on the strict modified CDC-criteria (9-13), sometimes this was not described(14) or infection rate was not assessed (because the antibiotic therapy was chosen for possible neuroprotective properties). (15)

At least 1 of the following:

1. Fever (>38°C) with no other recognized cause
2. Leukopenia (<4000 WBC/mm^3) or leukocytosis (>12 000 WBC/mm^3)
3. For adults ≥70 y old, altered mental status with no other recognized cause

And at least 2 of the following:

1. New onset of purulent sputum, or change in character of sputum over a 24 h period, or increased respiratory secretions, or increased suctioning requirements
2. New onset or worsening cough, or dyspnea, or tachypnea (respiratory rate>25/min)
3. Rales, crackles, or bronchial breath sounds
4. Worsening gas exchange (eg, O_2 desaturation [eg, $Pao_2/FiO_2 \leq 240$], increased oxygen requirements*)

And ≥2 serial chest radiographs† with at least 1 of the following:

New or progressive and persistent infiltrate, consolidation, or cavitation

Note: In patients without underlying pulmonary or cardiac disease, 1 definitive chest radiograph is acceptable

Probable SAP: all CDC criteria met, BUT initial CXR and serial/repeat CXR nonconfirmatory (or not undertaken), and no alternative diagnosis or explanation. Definite SAP: ALL CDC criteria met, including diagnostic CXR changes (on at least one). CDC indicates Centers for Disease Control and Prevention; CXR, chest x-ray; FIO2, fraction of inspired oxygen; Pao_2, partial pressure oxygen; SAP, stroke-associated pneumonia; and WBC, white blood cell.

*Category of increased ventilator demand removed.

†CDC recommendation is for repeat CXR at days 2±7 if initial CXR negative.

Figure 1. Modified CDC-criteria for diagnosis of stroke-associated pneumonia

In 2015, a recommendation for operational diagnostic criteria has been proposed by the 'Pneumonia in Stroke Consensus Group' to standardize criteria for pneumonia after stroke.(16) The proposed terminology is 'stroke-associated-pneumonia' and the criteria are modified CDC-criteria (figure 1). 'Probable stroke-associated-pneumonia' is diagnosed when CDC criteria are met, but when typical chest x-ray changes are absent even after repeat or serial chest x-ray, definite stroke-associated-pneumonia is diagnosed when CDC criteria met, including typical chest x-ray changes. Reliability, validity, impact on clinician behaviors (including antibiotic prescribing), and clinical outcomes of these criteria have to be assessed in future research.(16) No specific diagnostic criteria have been recommended for urinary tract infections or other infections after stroke.

Since lack of a standard definition of infection can lead to under- or over diagnosis of infections, unnecessary use of antibiotic therapy and heterogeneity in outcomes between randomised clinical trials, we strongly recommend the use of these diagnostic criteria for stroke-associated pneumonia for clinical practice and future research.

Effect of infections after stroke on outcome

Most evidence shows that pneumonia is the infection with the strongest associations with mortality and unfavourable outcome after stroke. In our meta-analysis, described in **chapter 2**, the independent effect of pneumonia on mortality was assessed in 4 studies involving 19971 patients.(17) Corrected odds ratio for in-hospital mortality was 3.62 (2.80-4.68). In 5 studies, involving 3959 patients, pneumonia was also independently associated with unfavourable outcome. After this review, a cohort study on 8251 stroke patients in Canada showed similar results: pneumonia was associated with mortality at 30 days (2.2 [1.8-2.7]) and 1 year (3.0 [2.5-3.7]), longer length of stay (20.8 days [20.5–21.1] vs. 13.3 days [13.3–13.4], $p < 0.001$), and dependency at discharge (38.2% with mRS <3 [37.3%–39.1%] vs. 12.3% [7.0%–17.7%]). (18) Also in the PASS, pneumonia was independently associated with unfavourable outcome at 3 months (9.64 [5.06-18.42]). However in another large trial on preventive antibiotic therapy in acute stroke, the 'Prophylactic antibiotics after acute stroke for reducing pneumonia in patients with dysphagia' (STROKE-INF; this study is discussed into more detail below), only pneumonia according to the physician was associated with worse functional outcome at 90 days (p=0.001), but algorithm-diagnosed pneumonia was not. In a German study involving 16,518 stroke patients attributable risks for mortality were calculated; pneumonia was a modifiable risk factor that contributed to 12.2% of in-hospital deaths.(19)

We also assessed the effect of infections in general after stroke on outcome in **chapter 2**. In a pooled sub analysis of 1839 patients, 48% of patients with an infection died vs. 18% of patients without infection (N = 1839; OR 2.08, 95% CI 1.63 - 2.67). However, this

was not corrected for potential confounders. One of the studies that were included in this meta-analysis did correct for potential confounders, this was a cohort study from 521 Dutch stroke patients.(20) In this study, infection was independently associated with poor outcome at discharge (OR 2.6 [95% CI 0–6.7]) and at 1 year (OR 3.8 [95% CI 1.8–8.9]). In another Spanish cohort study including 229 stroke patients, infection – when promptly treated - was not independently associated to unfavourable outcome. Also in a Danish study, infection did not influence outcome at discharge, but did increase in-hospital stay. (21) In the 2538 patients included in the PASS, infections were independently associated with unfavourable outcome at 3 months, both for infection according to physician and to expert panel (OR 3.48 [95% CI 2.53–4.77], p<0.0001, and 4.37 [95% CI 2.51–7.59], p<0.0001). Also, in a cohort study of 413 stroke patients, stroke-associated infection was associated with mortality within 3 years, although the effect on in-hospital mortality was the most pronounced.(22)

In contrast to pneumonia, the association of urinary tract infection with unfavourable outcome is less consistent. In a cohort study of 412 consecutive stroke patients, urinary tract infection was associated with mortality and disability at 3 months, but this association disappeared after correction for stroke severity and pre-stroke morbidity. In a meta-analysis including this and 3 other studies, urinary tract infection did not have an independent association with death, but 2 of 3 studies reporting functional outcome showed an association. In PASS, urinary tract infection was also independently associated with unfavourable outcome at 3 months in PASS (1.86 [1.24–2.79], p=0.003).

Preventive antibiotics in stroke

In **chapter 3** we describe a systematic review and meta-analysis on preventive antibiotic therapy in acute stroke. At the time of the writing of this review in 2010, 5 randomised trials investigated preventive antibiotic therapy in acute stroke, with 506 patients in total. Trials differed largely in study populations, design, type of antibiotic used and the manner of diagnosing an infection. In a pooled analysis of these 5 studies, preventive antibiotic therapy lowered the frequency of infections but did not lower the number of deceased or dependent patients. No important side-effects of the treatment were reported. Because the number of infections was lowered in these studies, and because these studies were small and heterogeneous, a large phase III trial was urgently needed.

The protocol of PASS, a large randomised trial, designed because of the lack of evidence as described in chapter 3, is described in **the first part of chapter 4**. This study is a multicenter, prospective, randomised open-label phase III trial with a blinded outcome assessment. Preventive antibiotic therapy with ceftriaxone intravenously during 4 days in addition to standard care is compared to standard care alone. Patients with stroke

and a minimal score of 1 on the NIHSS are included. The sample size is 3200 patients. Primary outcome is functional outcome on the mRS at 3 months, dichotomized in favorable outcome (mRS 0-2) vs. unfavorable outcome (mRS 3-6). Secondary outcomes are death at discharge and 3 months, frequency of infection, length of hospital stay, and a cost-effectiveness analysis. In the second part of chapter 4 we describe a change in the study protocol. Because of lower inclusion rate than expected, ending of funding, and because of the more and more customary use of an ordinal analysis of the mRS in stroke trials, we changed the primary analysis of primary outcome.(23-26) The dichotomization of the mRS becomes the secondary analysis of primary outcome, and the ordinal analysis the primary analysis of primary outcome. This change allowed for a smaller sample size of 2550 patients. In the third part of chapter 4 we describe the statistical analysis plan in detail. This plan was designed, and published, before termination of the trial and thus before we knew any of the results. The plan contains the manner of diagnosing infection, by the treating physician and by an independent infection panel. We describe the planned subgroup analyses of patients with severe stroke (NIHSS ≥10), elderly patients (≥75 year) and type of stroke (haemmorhagic or ischaemic stroke, TIA).

In chapter 5 we present the results of the PASS. 2550 patients were randomised 1268 to the ceftriaxone group and 1270 to the standard care group were analysed (12 patients withdrew consent). Preventive antibiotic therapy did not improve functional outcome at 3 months, although it did prevent infections. However, this was mostly caused by a reduction of urinary tract infections, the number of pneumonias was not significantly different between both groups (6% vs. 7%). Side effects were equal in the two treatment groups. The pre-planned subgroup analyses did not show an effect on outcome either. As described above, infections and pneumonia were associated with unfavourable outcome in the PASS.

After the publication of PASS, two other studies on preventive antibiotic therapy were published: the (STROKE-INF), and the 'Procalcitonin-guided antibiotic therapy after stroke' (STRAWINSKI). In the 1224 included patients in STROKE-INF, preventive antibiotic therapy according to local policy did not reduce pneumonia rate and did not improve functional outcome.(11) In the 227 severe ischaemic stroke patients included in STRAWINSKI, procalcitonin-guided antibiotic therapy did not lower pneumonia rate and did not improve functional outcome at 3 months.(14) The only effect of preventive antibiotic therapy on outcome was seen in a post hoc subgroup analysis of PASS in patients receiving thrombolysis, but this was not confirmed in the STROKE-INF.

After publication of these three trials we updated our Cochrane meta-analysis of preventive antibiotic therapy in acute stroke, but the conclusion remained similar as the conclusion in the previous meta-analysis: preventive antibiotic therapy in acute stroke does

not improve functional outcome or decrease mortality, and it does not lower pneumonia rate.(27) Although this result seems somewhat disappointing, it is important to realise that with this update a 'burning clinical question' was finally answered; and that this is of large importance for daily practice on stroke units. Explanations for the lack of effect of preventive antibiotic therapy on pneumonia rate and on outcome are not conclusive, but include the following arguments.

Since pneumonia rate was not lowered in the PASS, the question arises whether the type of antibiotic, ceftriaxone, did cover the causative pathogens of pneumonia after stroke. Microbiologic data of patients with post-stroke pneumonia shows a pattern of mostly early onset nosocomial pneumonia (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli or Enterobacter spp, Staphylococcus aureus*), or a community acquired aspiration syndrome (*streptococcus species*).(17) This agrees with the timing of infections after stroke: in a previous cohort study 75% of infections occurred in the first 3 days after stroke.(20) In the PASS, sputum culture was performed in 21% of patients with physician diagnosis of pneumonia, this yielded a pathogen in 11% (unpublished data, PASS-trial(13)). Most common pathogens were *Streptococcus pneumoniae, Staphylococcus aureus and Haemophilus influenzae*. Also, *enterobacteriaceae*, yeast and *Escherichia coli* were cultured. The microbiological etiologies of pneumonia after stroke were also investigated in a recent systematic review of fifteen studies (including the PASS).(28) Frequency of positive cultures in patients diagnosed with pneumonia varied widely (15-88%) and the most frequent pathogens were: *Enterobacteriacea* (21.8%, *Klebsiella pneumoniae* and *Escherichia coli*), *Staphylococcus aureus* (10.1%), *Pseudomonas aeruginosa* (6%), *Acinetobacter baumanii* (4.6%) and *Streptococcus pneumoniae* (3.5%). For urinary tract infection the most common pathogen is *Escherichia coli*. In 110 consecutive stroke patients, *Escherichia coli* was the most common pathogen in urinary tract infection and *Pseudomonas aeruginosa* was the second cause of urinary tract infection in patients with indwelling catheters.(29) In the PASS, urinary culture was performed in 58% with physician diagnosis of urinary tract infection and a pathogen was detected in 38%. This was *Escherichia coli* in the vast majority, but also *Staphylococcus saprophyticus, Proteus vulgaris* and *mirabilis*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were cultured. Ceftriaxone covers the previously described bacterial pathogens of pneumonia, but is does not cover resistant or anaerobic pathogens. Resistance rates in PASS were low (see section on antimicrobial resistance below) so this does not seem to be an sufficient explanation for the lack of effect. Since aspiration is a well-known cause for pneumonia in stroke, and aspiration pneumonia is caused by a mixture of anaerobic and aerobic pathogens, it is possible that these pathogens were not covered by ceftriaxone. However, in STROKE-INF 78% of patients received the recommended therapy that did cover anaerobic pathogens (amoxicillin or co-amoxiclav and clarithromycin),

and this did not lower pneumonia rate in the treatment group. Therefore, it is not likely that this explains the total lack of effect of preventive antibiotic therapy. (30) None of the studies included in the previous described review on pathogens of post-stroke infection investigated viral or fungal etiologies, although viral (co-) infection is increasingly recognized as a cause for community and hospital acquired pneumonia. (31, 32) Their role in infection after stroke is unclear.

Next, the question arises whether the timing of preventive antibiotic therapy and the route of administration were chosen well in the trials on preventive antibiotic therapy. In STROKE-INF, preventive therapy with antibiotics had to be started within 48 hours. This could have been too late, since many infections occur in the first days after stroke. However, in PASS, antibiotic therapy was initiated within 24 hours after stroke onset and this trial showed similar results. Finally, the route of administration could be further studied. In studies performed on ICU's, topical plus systemic prophylactic antibiotic therapy does reduce respiratory tract infection and reduces mortality rate. The Cochrane review on antibiotic prophylaxis in ICU's shows that incidence of respiratory tract infections was 19% in patients receiving prophylactic antibiotics and 40% in patients not receiving prophylactic antibiotics. (5) In general, ICU-patients have higher infection rates and more opportunity exists to prevent infections than in stroke patients at stroke units or general wards so these populations are not directly comparable. In stroke patients, the combination of topical and systemic antibiotic therapy has not been investigated to date. The effect of selective oral decontamination in stroke patients is investigated in one ongoing trial (ISRCTN14124645). Results of the trials do not strongly warrant a new trial investigating the combination of systemic and topical antibiotic therapy in acute stroke.

Another explanation for the lack of effect in the PASS, is that patients with relatively mild strokes were included. Since patients with more severe disease are at higher risk for infections, this could have led to lower pneumonia rates overall and less power to prevent pneumonia. Yet, preventive antibiotics also did not affect outcome in a predefined subgroup analysis of patients with an NIHSS score of 10 or higher. In this subgroup preventive antibiotic therapy did also not reduce pneumonia rate (17% vs 21% p=0.39; PASS unpublished data). This subgroup analysis is hampered by limited statistical power because of a relatively low number of patients. Nevertheless, in STROKE-INF and STRAW-INSKI patients with more severe strokes and higher pneumonia rates were included, and these trials also showed no effect of preventive antibiotic therapy on pneumonia rate.

Another possibility is that preventive antibiotic therapy does not add up to the high level of care within stroke units. Stroke unit care includes early detection of dysphagia, measures to prevent aspiration and early detection of complications in the acute phase of

stroke. Stroke unit care has been shown to improve outcome in acute stroke patients in a Cochrane meta-analysis of many studies.(33) Stroke unit care lowered the number of infections and pneumonias in a study on the effect of stroke unit care on the prevention of complications. (34) One of the effects of stroke unit care could include early detection of fever or other signs of infection which could prompt the treating physician to start antibiotic therapy in an early phase. An early start of antibiotic therapy in patients suspected of having an infection decreases the potential effect of preventive antibiotic therapy.

Herewith, one could argue that the open-label design of PASS and STROKE-INF, in which the physician was aware of treatment allocation, could lower the threshold for start of antibiotic therapy in the patients randomised to the control group. In PASS, the total antimicrobial use during hospital stay, as measured by defined daily doses (DDD), was higher in the ceftriaxone group than in the control group (4979 DDD vs 2120 DDD doses). In STROKE-INF 98% of patients in the antibiotics group vs 34% of the standard care group received antibiotic therapy at least once, and 87% vs 10% at least three times. Both in PASS and STROKE-INF, more patients received antibiotic therapy in the preventive antibiotics group than in the standard care group. Nevertheless, the possibility remains that therapy was initiated earlier than in standard practice in the control group in patients suspected of having an infection, hereby decreasing the potential effect of preventive antibiotic treatment.

Pneumonia could not be an infection only, but a respiratory syndrome which preventive antibiotic therapy cannot prevent.(109) Marik et al describe aspiration pneumonitis as 'a chemical injury caused by the inhalation of sterile gastric contents, whereas aspiration pneumonia is an infectious process caused by the inhalation of oropharyngeal secretions that are colonized by pathogenic bacteria'.(109) Predisposing factors are a lowered consciousness and dysphagia, which are often present in stroke patients. Antibiotic therapy is not a recommended therapy for aspiration pneumonitis, although secondary infections can emerge. No other therapies are currently available for aspiration pneumonitis.

Finally, whether infection after stroke is causally related to unfavourable outcome or merely a bystander of severe disease remains debated. Possible mechanisms by which infection could contribute to unfavourable outcome are induction of inflammation or autoimmunity by post-stroke infections, or systemic effects like fever, hypotension and hypoxia and deterioration of the clinical condition of a patient. Stroke has been shown to induce an antibody response to brain antigens.(35) As described by *Vogelgesang et al*, infection after stroke could induce autoimmunity to the brain and hereby worsen outcome after stroke. Infections are thought to mediate the upregulation of co-stimula-

tory molecules and to promote antigen presentation, and to cause a shift towards a Th1 response. (36, 37) In a mice model of middle cerebral artery occlusion, administration of bacterial lipopolysaccharide was used to mimick infection and this was shown to negatively affect outcome and increase brain atrophy at follow-up.(36, 38) Also in a mice model, animals with a Th1 reponse to myelin-basic protein had worse 1-month outcomes. (39, 40) However another experimental study shows that the association of autoimmunity with outcome after stroke is not clear: blocking of immune suppression after stroke (this phenomenon is described into more detail below) decreased infection rate and improved outcome, despite increased autoimmunity.(41) The association between infection, autoimmunity and outcome was investigated in a clinical study of 114 stroke patients. Patients with infection more often had a Th1 response to brain antigens after 90 days, but this effect was lost after controlling for stroke severity.(42, 43) An eventual causal relationship between infection and outcome through promotion of autoimmunity to brain antigens after stroke remains to be elucidated.

Other systemic effects after infection such as fever, hypoxia, hypotension and deterioration of the clinical condition of a patient could contribute to unfavourable outcome after post-stroke infection. Fever after stroke has been extensively studied. One third of patients have temperatures exceeding 37.5 degrees Celcius after the onset of stroke.(44) In a study of 390 stroke patients it was shown that relative risk of poor outcome increased with 2.2 (95% CI 1.4-3.5) for each degree Celsius increase in body temperature. (45) A subsequent meta-analysis of 9 studies involving 3790 patients confirmed the effect of raised temperature after stroke.(46) How fever exactly contributes to unfavourable outcome remains speculative. Next, pneumonia can lead to hypoxia. Hypoxia has been shown to occur frequently in acute stroke and it is suggested that this could contribute to unfavourable clinical outcome, although no clinical studies have been performed to shown such an effect.(47) Currently, the Stroke Oxygen Study (SO2S) is investigating the effect on long-term outcome of supplemental oxygen in 8000 acute stroke patients and results are expected soon.(48) Infection could also lead to hypotension, which has been shown to negatively affect outcome after stroke.(49) And finally, infections could deteriorate the clinical condition of a patient and hereby negatively influence the rehabilitation process. Many guidelines recommend early mobilization to aid recovery, infections could counteract an early start of the rehabilitation process.(50)

Since pneumonia rate was not reduced in the previous described trials, the lack of effect on the prevention of pneumonia seems a more logical explanation for the lack of effect on functional outcome, than the hypothesis that no causal relationship exists, although this is still a possibility.

Health-care costs of preventive antibiotic therapy

The costs associated with infection, and especially pneumonia after stroke are substantial. The yearly cost of pneumonia after stroke in the United States was estimated to be 459 million dollars in 2007.(51) We describe the cost-effectiveness analysis of the PASS in chapter 6. In the cost-effectiveness analysis we investigate the cost of an improvement in functional outcome on the mRS; in the cost-utility analysis we investigate the cost of an improvement in Quality Adjusted Life Years (QALY's), one QALY is one year in perfect health. We showed in this thesis that, although preventive antibiotic therapy did not improve outcome, it is still a cost-effective therapy for patients with stroke, due to the low costs and a small favourable shift in mRS scores in the preventive treatment group, based on data from the PASS.(52) However, the cost-effectiveness of this therapy may not outweigh the possible risk and costs of increased antimicrobial resistance.

Development of antimicrobial resistance

Both in PASS and STROKE-INF preventive antibiotic treatment was safe and was not associated with higher incidence of *clostridium difficile* infections or infection with multi-resistant organisms. In PASS 0.5% in the preventive antibiotic treatment group vs. 0.4% in the control group was infected with an organism resistant to ceftriaxone, in STROKE-INF rates of MRSA colonization were 2% in both treatment groups. *Clostridium difficile* infection occurred in 0.2% of patients randomised to ceftriaxone vs. 0% in the standard care group in PASS; and in 0.3% of patients in the preventive antibiotic treatment group vs. 0.7% in the control group in STROKE-INF. Due to the lack of effect of preventive antibiotic therapy on outcome, the pre-planned substudy of PASS on prevalence of extended-spectrum- bètalactamase (ESBL)-producing *Enterobacteriaceae* between patients in the two treatment groups was not executed. ESBL is an enzyme which hydrolyzes bèta-lactam antibiotics by which they become ineffective. According to antimicrobial resistance surveillance data from Europe, this is the most important mechanism involved in the development of resistance to third generation cephalosporins such as ceftriaxone.(53) For potential future studies on preventive treatment with antibiotics the development of antimicrobial resistance remains an important topic, since increased use of antibiotic therapy is associated with increased resistance rates.(54)

Stroke induced immune suppression and use of betablockers to prevent infection

In chapter 7 we aim to investigate another strategy than antibiotic therapy to prevent infection after stroke: the use of betablockers. The patients with stroke that are vulnerable for infection, are patients with more severe disease and with risk factors for this specific infection, such as dysphagia and chronic obstructive pulmonary disease for pneumonia, and female sex and urinary catheterisation for urinary tract infection (an extensive overview of all risk factors is presented in chapter 8). Apart from these clinical characteristics,

it is thought that all stroke patients are at a higher risk for infections due to systemic immune alterations after stroke: so called 'stroke induced immune suppression'. This is a phenomenon that is also seen in other central nervous system diseases or disturbances, such as traumatic brain injury.(55)

Stroke causes local inflammation in the brain with release of danger associated molecular patterns (DAMP's) and cytokines, that also spread systemically. This causes a transient inflammatory response with production of TNF and IL-6 within 24 hours of the stroke. (56) This inflammation in turn induces an anti-inflammatory response that is thought to be mediated through the hypothalamic pituitary adrenal (HPA) axis, the vagus nerve, and the sympathetic nervous system.(57)

Inflammatory mediators stimulate the HPA-axis through the paraventricular nucleus of the hypothalamaus. Via release of CRH the pituitary gland is stimulated to release ACTH, which in turn promotes release of glucocorticoids from the adrenal glands with immunosuppressive effects.(57) Indeed, in 114 acute stroke patients cortisol levels were higher in severe stroke and were associated with established post-stroke leukocyte changes such as neutrophilia and lymphopenia.(58) In another study of 45 stroke patients cortisol and metanephrin levels were higher in patients with stroke associated infections and cortisol levels were also increased in patients who developed infections in a study on 39 stroke patients. (59, 60) However, in another study that also included patients with TIA and haemorrhagic stroke, ACTH and cortisol levels did not predict infection, but lymphocytopenia did.(61)

The second pathway that connects the brain with the immune system and regulates immune depression is the sympathetic nervous system. Sympathetic neurons secrete catecholamines in different tissues, including the lymphoid organs, and into the bloodstream from the adrenal medullary gland.(57) The evidence for sympathetic involvement in immune depression and subsequent post-stroke infections comes from experimental studies and has also been investigated in stroke patients. In a mice model of stroke, bacterial infections could be prevented by blocking the sympathetic nervous system directly after stroke.(62) In 75 acute stroke patients elevated metanephrin, a metabolite of catecholamines secreted in the adrenal medullary gland, was associated with infections, independent of stroke severity.(63) In a post-hoc multivariate analysis of the 80 ischaemic stroke patients of the PANTHERIS trial, elevated norepinephrine levels were associated with reduced HLA-DR expression on monocytes and higher susceptibility to post-stroke infections.(64) Also in patients of the PANTHERIS trial, urinary levels of norepinephrine were higher in infected than non-infected patients of the placebo group at days 1 and 2 after stroke, although this was not corrected for possible confounders such as stroke

severity.(65) Additionally, catecholamines suppressed phagocyte function in vitro in blood samples of 63 patients with ischaemic stroke, and patients .(66)

The third important brain-immune connection is the cholinergic anti-inflammatory pathway.(67) The paraventricular nucleus of the hypothalamus is also linked with the cholinergic anti-inflammatory pathway.(68) Acetylcholin is the main vagus nerve neurotransmitter.(68) In experimental studies vagus nerve stimulation through the α7 nAChR-receptor on macrophages and other cells has been shown to inhibit TNF release and to control cytokine synthesis.(68) In addition, inhibition of cholinergic signaling (by vagotomy or by using α7 nicotinic acetylcholine receptor-deficient mice) prevented pneumonia in mice.(69)

The balance between the sympathetic and parasympathetic nervous system after stroke has been studied by assessing heart rate variability and baroreflex sensitivity in stroke patients and mostly show sympathetic overactivity.(70) Two clinical studies evaluated the association of heart rate variability as marker of autonomic activity with post stroke infection with contradicting results. One study on 103 stroke patients found that heart rate variability, suggestive of parasympathetic overweight, collected within 48 hours was associated with post stroke infections.(71) In contrast, another study found that decreased HF power within the first 24 hours, suggestive of sympathetic overweight, was associated with infections.(72) And another study in haemorrhagic stroke patients showed that decreased baroreflex sensitivity within 24 hours, a marker of sympathetic activity, was associated with infections.

The actions of the HPA-axis, sympathetic nervous system and the vagus nerve result in changes in both the innate and adaptive immune system. Immune alterations in stroke patients that are associated with the development of infections are the following: lymphocytopenia, reduced expression of antigen-presenting molecules on monocytes, impaired T-helper 1 cell activity, increased production of anti-inflammatory cytokines and low production of pro-inflammatory tumour necrosis factor. (36, 59, 66, 73-77)

Although the contribution of the different pathways and their interactions are not completely clear, it is evident that stroke induces alterations to the systemic immune system. Whether these alterations are beneficial for patients because of prevention of autoimmunity to the brain is not certain (as described above), but they do predispose stroke patients to infection. Prevention of immune suppression might improve outcome by decreasing infection rate, although interfering in this complex cascade of events could easily have unpredicted negative side-effects.

As discussed above, immune depression after stroke is hypothesized to be at least partly caused by increased sympathetic activity.(73) In experimental studies, preventive treatment with beta-blocker propranolol reduced the number of bacterial infection after stroke.(62, 78, 79) In clinical stroke studies, 4 studies reported that pre- or on-stroke treatment with beta-blockers was associated with decreased infection risk, or no association was found.(80-83) In **chapter 7**, we investigated in the patients included in PASS whether the patients who used betablockers before stroke had less infections after stroke. In this explorative analysis, we found that the patients using betablockers before stroke had a higher risk for post-stroke infection (aOR 1.61; 95%CI 1.19-2.18; p<0.01) and for post-stroke pneumonia. Patients using betablockers were older and had more comorbidities, however we corrected for these factors in the multivariate analysis. In this study on-stroke treatment was not investigated, and limitations were that pre-stroke treatment was not controlled and no distinction was made between selective and non-selective beta-blockers. Results of this study were confirmed in another study on 1431 patients with ischaemic stroke: use of beta-blockers, especially non-selective beta-blockers, was associated with increased infections (16.4 vs. 10.7%, p = 0.030).(84) Also in 525 haemorrhagic stroke patients, infection rate was increased in patients using labetalol as compared to nicardipine.(85) Another study showed that continuation of pre-stroke use of beta-blockers did not increase, nor reduce, infection rate in severe stroke patients.(86) The only randomised trial on beta-blockade in acute stroke did not evaluate infections.(87) However, these results do not warrant a randomised trial on beta-blocker treatment in stroke for prevention of infections.

Predicting patients at high risk for infection

Identification of patients at high risk for infection is important for early initiation of treatment and for selection of patients for future randomised trials. To be able to select the patients at the highest risk for infection after stroke we constructed a prediction model for infection after stroke in **chapter 8**. Pneumonia was predicted by higher age, male gender, disability prior to stroke, history of COPD, severe stroke, dysphagia (swallowing disturbances) and haemorrhagic stroke (rather than ischaemic stroke). Infections were predicted by higher age, male gender, history of diabetes or COPD, severe stroke, dysphagia, urinary catheterization and haemorrhagic stroke (rather than ischaemic stroke). The lowest score on the prediction rule gave a risk for pneumonia of 0.4% and 1.8% for infection, the highest score 56.2% for pneumonia and 88% for infection. Discrimination (the ability of a prediction rule to distinguish between patients with and without an infection) of the score was good (C-statistic, 0.84; 95%CI, 0.81-0.87 en 0.82; 95% CI, 0.79-0.84 for pneumonia and infection). The prediction rule was internally validated and can be used in other populations after external validation.

For prediction of pneumonia after stroke, other models have been developed; for prediction of infection in general only 2 from which 1 model was not validated (for names and references see chapter 8, table 2). For pneumonia the most extensive validated models are the *A2DS2-score, ISAN-score and AIS-APS*. These scores were mostly derived from stroke registries and were not specifically designed to predict infection. The *A2DS2-score* and *ISAN-score* use age, sex and NIHSS as predictors, in addition the *A2DS2-score* also uses dysphagia and *ISAN-score* uses pre-stroke dependence. The *AIS-APS* uses more predictors: age, history of atrial fibrillation, congestive heart failure, chronic obstructive pulmonary disease and current smoking, pre-stroke dependence, dysphagia, admission NIHSS score, GCS score, stroke subtype (Oxfordshire), and blood glucose. The *Pantheris score* and the *PASS score* (described in chapter 8) are the only two scores derived from randomised trial data with infection as outcome measure. Both of these scores were internally, but not yet externally, validated. In case of use of a prediction rule for selection of patients in future trials, we recommended to check which score predicts infection the best in de population intended for inclusion in future trials, and to use this score for selection of patients.

Future directions

Based on the previously discussed large randomised trials, we can conclude that preventive antibiotic therapy does not improve functional outcome and should not be recommended as standard therapy in *all* stroke patients. However, further research in this area is warranted and should be targeted towards better diagnosis of infection, selection of high risk patients for inclusion in trials, subgroup analysis of previous trials and combined treatment approaches in high risk patients.

Firstly, the exact nature of pneumonia after stroke deserves further investigation. Extensive radiological, microbiological and laboratory research is needed for a better diagnosis of pneumonia. Performance of (serial) CT or MRI-scans of the chest could give more information on the type of lung problem, e.g. infiltrate, fluid overload or inflammatory reaction. A trial of pulmonary CT in stroke patients is currently recruiting patients (ClinicalTrials.gov Identifier: NCT03106909). Serial culturing could increase yield of cultures or give more information on the presence or absence of an infectious cause. The causative pathogens in post-stroke infections should be more rigorously detected. Apart from standard microbiological techniques the yield of PCR-based assays for well-known respiratory viruses could be investigated. All future studies on diagnosis of pneumonia after stroke should aim to use the diagnostic criteria as proposed by PISCES.

For all future research, patients at the highest risk for infection should be selected by using a prediction rule. In the high risk population the largest treatment effects can be obtained. The existing prediction rules use clinical criteria that are present on admission,

possibly, the addition of laboratory markers can increase predictive value. For example, a recent study on 486 ischaemic stroke patients showed that dysphagia and decreased monocytic HLA-DR were independent predictors of stroke-associated pneumonia and patients without dysphagia and a normal monocytic HLA-DR had no pneumonia risk.(88) Addition of laboratory markers to the existing prediction rules might increase performance of these rules.

Preventive antibiotic therapy was not effective in all stroke patients and in predefined subgroups. However, the previous subgroup analysis were limited by statistical power and did not investigate subgroups based on combinations of risk factors. An analysis of the effect of preventive antibiotic therapy in high-risk subgroups is an interesting next step. High-risk subgroups can be defined by combining risk factors for infection, or by using the existing prediction rules. In order to have enough statistical power, this subgroup analysis should preferably be performed on the pooled data of the previous trials on preventive antibiotic therapy in acute stroke.

Another interesting area of ongoing research is a combined treatment approach for complications in the acute phase of stroke. Limitations of the previous trials were for PASS the low infection rate due to low stroke severity due to inclusion of all stroke patients (not a high risk group), and for STROKE-INF the delay in start of antibiotic therapy (within 48 hours) and heterogeneity in antibiotic treatment. These limitations, and the possibility that pneumonia might be a respiratory syndrome which antibiotic therapy alone cannot prevent, have resulted in a new trial, the PREvention of Complications to Improve OUtcome in elderly patients with acute Stroke 'PRECIOUS'. In this trial, a combined preventive treatment approach with ceftriaxone, paracetamol, and/or metoclopramide vs. standard care in elderly (>65 years) stroke (NIHSS>5) patients is investigated.(89) In a subgroup analysis of 787 elderly patients with NIHSS>5 in the PASS we found a trend towards reduction of pneumonia: 12% (47/395) vs. 17% (65/392) (adjusted OR 0.69 (95%CI 0.45-1.05) p-value 0.083). In addition, the PRECIOUS trial aims not only to prevent infection, but also to detect dysphagia in an early stage, and to prevent fever. The prevention of fever by Paracetamol to improve outcome has previously been investigated in the Paracetamol (Acetaminophen) in Stroke trial (PAIS). In this trial 1400 stroke patients were randomised for high-dose paracetamol or standard therapy. In the total group a small shift towards better outcome was seen, this was not significant, in a post-hoc analysis of a subgroup with higher baseline temperature (>36.5 and >37.0) a significant effect was found.(44) The PAIS2 trial aimed to confirm this finding but was stopped prematurely due to lack of funding for inclusion of patients.(90) Aim of the PRECIOUS trial is to include 3800 patients, recruitment has started and results are expected in 2020.

Conclusion

Stroke is a major health problem and infections after stroke are associated with unfavourable outcome. Whether this is a causal relationship or whether infection is a marker of severe disease remains unknown. Trials comparing preventive antibiotic treatment versus standard care did not improve functional outcome after stroke, nor did they reduce pneumonia rate. Future research should be targeted to a better diagnosis of infection, selection of high risk patients, subgroup analysis of high risk patients in the pooled data of previous preventive antibiotic therapy trials and combined treatment approaches.

References

1. Davenport RJ, Dennis MS, Wellwood I, Warlow CP. Complications after acute stroke. Stroke. 1996;27(3):415-20.
2. Kalra L, Yu G, Wilson K, Roots P. Medical complications during stroke rehabilitation. Stroke. 1995;26(6):990-4.
3. Torres A. Clinical Management of Bacterial Pneumonia. In: Cillóniz C, editor.: Springer International Publishing Switzerland 2015; 2015.
4. Koulenti D, Tsigou E, Rello J. Nosocomial pneumonia in 27 ICUs in Europe: perspectives from the EU-VAP/CAP study. European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology. 2016.
5. Liberati A, D'Amico R, Pifferi S, Torri V, Brazzi L, Parmelli E. Antibiotic prophylaxis to reduce respiratory tract infections and mortality in adults receiving intensive care. The Cochrane database of systematic reviews. 2009(4):Cd000022.
6. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 2008;36(5):309-32.
7. Harms H, Hoffmann S, Malzahn U, Ohlraun S, Heuschmann P, Meisel A. Decision-making in the diagnosis and treatment of stroke-associated pneumonia. Journal of neurology, neurosurgery, and psychiatry. 2012;83(12):1225-30.
8. Kishore AK, Vail A, Chamorro A, Garau J, Hopkins SJ, Di Napoli M, et al. How is pneumonia diagnosed in clinical stroke research? A systematic review and meta-analysis. Stroke. 2015;46(5):1202-9.
9. Chamorro A, Horcajada JP, Obach V, Vargas M, Revilla M, Torres F, et al. The Early Systemic Prophylaxis of Infection After Stroke study: a randomized clinical trial. Stroke. 2005;36(7):1495-500.
10. Harms H, Prass K, Meisel C, Klehmet J, Rogge W, Drenckhahn C, et al. Preventive antibacterial therapy in acute ischemic stroke: a randomized controlled trial. PLoS One. 2008;3(5):e2158.
11. Kalra L, Irshad S, Hodsoll J, Simpson M, Gulliford M, Smithard D, et al. Prophylactic antibiotics after acute stroke for reducing pneumonia in patients with dysphagia (STROKE-INF): a prospective, cluster-randomised, open-label, masked endpoint, controlled clinical trial. Lancet. 2015;386(10006):1835-44.
12. Schwarz S, Al-Shajlawi F, Sick C, Meairs S, Hennerici MG. Effects of prophylactic antibiotic therapy with mezlocillin plus sulbactam on the incidence and height of fever after severe acute ischemic stroke: the Mannheim infection in stroke study (MISS). Stroke. 2008;39(4):1220-7.
13. Westendorp WF, Vermeij JD, Zock E, Hooijenga IJ, Kruyt ND, Bosboom HJ, et al. The Preventive Antibiotics in Stroke Study (PASS): a pragmatic randomised open-label masked endpoint clinical trial. Lancet. 2015;385(9977):1519-26.
14. Ulm L, Ohlraun S, Harms H, Hoffmann S, Klehmet J, Ebmeyer S, et al. STRoke Adverse outcome is associated WIth NoSocomial Infections (STRAWINSKI): procalcitonin ultrasensitive-guided antibacterial therapy in severe ischaemic stroke patients - rationale and protocol for a randomized controlled trial. International journal of stroke : official journal of the International Stroke Society. 2013;8(7):598-603.
15. Lampl Y, Boaz M, Gilad R, Lorberboym M, Dabby R, Rapoport A, et al. Minocycline treatment in acute stroke: an open-label, evaluator-blinded study. Neurology. 2007;69(14):1404-10.
16. Smith CJ, Kishore AK, Vail A, Chamorro A, Garau J, Hopkins SJ, et al. Diagnosis of Stroke-Associated Pneumonia: Recommendations From the Pneumonia in Stroke Consensus Group. Stroke. 2015;46(8):2335-40.
17. Westendorp WF, Nederkoorn PJ, Vermeij JD, Dijkgraaf MG, van de Beek D. Post-stroke infection: A systematic review and meta-analysis. BMC Neurol. 2011;11:110.
18. Finlayson O, Kapral M, Hall R, Asllani E, Selchen D, Saposnik G. Risk factors, inpatient care, and outcomes of pneumonia after ischemic stroke. Neurology. 2011;77(14):1338-45.
19. Koennecke HC, Belz W, Berfelde D, Endres M, Fitzek S, Hamilton F, et al. Factors influencing in-hospital mortality and morbidity in patients treated on a stroke unit. Neurology. 2011;77(10):965-72.
20. Vermeij FH, Scholte op Reimer WJ, de Man P, van Oostenbrugge RJ, Franke CL, de Jong G, et al. Stroke-associated infection is an independent risk factor for poor outcome after acute ischemic stroke: data from the Netherlands Stroke Survey. Cerebrovasc Dis. 2009;27(5):465-71.
21. Kammersgaard LP, Jorgensen HS, Reith J, Nakayama H, Houth JG, Weber UJ, et al. Early infection and prognosis after acute stroke: the Copenhagen Stroke Study. Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association. 2001;10(5):217-21.
22. Kwan J, Pickering RM, Kunkel D, Fitton C, Jenkinson D, Perry VH, et al. Impact of stroke-associated infection on long-term survival: a cohort study. Journal of neurology, neurosurgery, and psychiatry. 2013;84(3):297-304.
23. Anderson CS, Heeley E, Huang Y, Wang J, Stapf C, Delcourt C, et al. Rapid blood-pressure lowering in patients with acute intracerebral hemorrhage. N Engl J Med. 2013;368(25):2355-65.

24. Bath PM, Gray LJ, Collier T, Pocock S, Carpenter J. Can we improve the statistical analysis of stroke trials? Statistical reanalysis of functional outcomes in stroke trials. Stroke. 2007;38(6):1911-5.
25. den Hertog HM, van der Worp HB, van Gemert HM, Algra A, Kappelle LJ, van GJ, et al. Correction: PAIS: paracetamol (acetaminophen) in stroke; protocol for a randomized, double blind clinical trial. [ISCRTN74418480]. BMC Cardiovasc Disord. 2008;8:29.
26. Miller SW, Palesch YY. Comments regarding the recent OAST article. Stroke. 2008;39(1):e14.
27. 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.
28. Kishore AK, Vail A, Jeans AR, Chamorro A, Di Napoli M, Kalra L, et al. Microbiological Etiologies of Pneumonia Complicating Stroke: A Systematic Review. Stroke. 2018;49(7):1602-9.
29. Ersoz M, Ulusoy H, Oktar MA, Akyuz M. Urinary tract infection and bacteriurua in stroke patients: frequencies, pathogen microorganisms, and risk factors. Am J Phys Med Rehabil. 2007;86(9):734-41.
30. Marik PE, Careau P. The role of anaerobes in patients with ventilator-associated pneumonia and aspiration pneumonia: a prospective study. Chest. 1999;115(1):178-83.
31. Dandachi D, Rodriguez-Barradas MC. Viral pneumonia: etiologies and treatment. Journal of investigative medicine : the official publication of the American Federation for Clinical Research. 2018.
32. Shorr AF, Zilberberg MD, Micek ST, Kollef MH. Viruses are prevalent in non-ventilated hospital-acquired pneumonia. Respiratory medicine. 2017;122:76-80.
33. Organised inpatient (stroke unit) care for stroke. The Cochrane database of systematic reviews. 2013(9):Cd000197.
34. Govan L, Langhorne P, Weir CJ. Does the prevention of complications explain the survival benefit of organized inpatient (stroke unit) care?: further analysis of a systematic review. Stroke. 2007;38(9):2536-40.
35. Bornstein NM, Aronovich B, Korczyn AD, Shavit S, Michaelson DM, Chapman J. Antibodies to brain antigens following stroke. Neurology. 2001;56(4):529-30.
36. Iadecola C, Anrather J. The immunology of stroke: from mechanisms to translation. Nature medicine. 2011;17(7):796-808.
37. Vogelgesang A, Becker KJ, Dressel A. Immunological consequences of ischemic stroke. Acta neurologica Scandinavica. 2014;129(1):1-12.
38. McColl BW, Rothwell NJ, Allan SM. Systemic inflammatory stimulus potentiates the acute phase and CXC chemokine responses to experimental stroke and exacerbates brain damage via interleukin-1- and neutrophil-dependent mechanisms. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2007;27(16):4403-12.
39. Becker KJ, Kindrick DL, Lester MP, Shea C, Ye ZC. Sensitization to brain antigens after stroke is augmented by lipopolysaccharide. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2005;25(12):1634-44.
40. Zierath D, Thullbery M, Hadwin J, Gee JM, Savos A, Kalil A, et al. CNS immune responses following experimental stroke. Neurocritical care. 2010;12(2):274-84.
41. Romer C, Engel O, Winek K, Hochmeister S, Zhang T, Royl G, et al. Blocking stroke-induced immunodeficiency increases CNS antigen-specific autoreactivity but does not worsen functional outcome after experimental stroke. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2015;35(20):7777-94.
42. Becker KJ, Kalil AJ, Tanzi P, Zierath DK, Savos AV, Gee JM, et al. Autoimmune responses to the brain after stroke are associated with worse outcome. Stroke. 2011;42(10):2763-9.
43. Urra X, Planas AM, Chamorro A. Letter by Urra et al regarding article, "Autoimmune responses to the brain after stroke are associated with worse outcome". Stroke. 2012;43(2):e26; author reply e7-8.
44. den Hertog HM, van der Worp HB, van Gemert HM, Algra A, Kappelle LJ, van GJ, et al. The Paracetamol (Acetaminophen) In Stroke (PAIS) trial: a multicentre, randomised, placebo-controlled, phase III trial. Lancet Neurol. 2009;8(5):434-40.
45. Reith J, Jorgensen HS, Pedersen PM, Nakayama H, Raaschou HO, Jeppesen LL, et al. Body temperature in acute stroke: relation to stroke severity, infarct size, mortality, and outcome. Lancet. 1996;347(8999):422-5.
46. Hajat C, Hajat S, Sharma P. Effects of poststroke pyrexia on stroke outcome : a meta-analysis of studies in patients. Stroke. 2000;31(2):410-4.
47. Ferdinand P, Roffe C. Hypoxia after stroke: a review of experimental and clinical evidence. Experimental & translational stroke medicine. 2016;8:9.
48. Roffe C, Nevatte T, Crome P, Gray R, Sim J, Pountain S, et al. The Stroke Oxygen Study (SO2S) - a multi-center, study to assess whether routine oxygen treatment in the first 72 hours after a stroke improves long-term outcome: study protocol for a randomized controlled trial. Trials. 2014;15:99.

49. Leonardi-Bee J, Bath PM, Phillips SJ, Sandercock PA. Blood pressure and clinical outcomes in the International Stroke Trial. Stroke. 2002;33(5):1315-20.
50. Bernhardt J, Godecke E, Johnson L, Langhorne P. Early rehabilitation after stroke. Current opinion in neurology. 2017;30(1):48-54.
51. Katzan IL, Dawson NV, Thomas CL, Votruba ME, Cebul RD. The cost of pneumonia after acute stroke. Neurology. 2007;68(22):1938-43.
52. Westendorp WF, Zock E, Vermeij JD, Kerkhoff H, Nederkoorn PJ, Dijkgraaf MGW, et al. Preventive Antibiotics in Stroke Study (PASS): A cost-effectiveness study. Neurology. 2018;90(18):e1553-e60.
53. EARSS. European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2015. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net).Stockholm: ECDC; 2017.
54. Hawkey PM. The growing burden of antimicrobial resistance. J Antimicrob Chemother. 2008;62 Suppl 1:i1-i9.
55. Meisel C, Schwab JM, Prass K, Meisel A, Dirnagl U. Central nervous system injury-induced immune deficiency syndrome. Nature reviews Neuroscience. 2005;6(10):775-86.
56. Anrather J, Iadecola C. Inflammation and Stroke: An Overview. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics. 2016;13(4):661-70.
57. Chamorro A, Urra X, Planas AM. Infection after acute ischemic stroke: a manifestation of brain-induced immunodepression. Stroke. 2007;38(3):1097-103.
58. Zierath D, Tanzi P, Shibata D, Becker KJ. Cortisol is More Important than Metanephrines in Driving Changes in Leukocyte Counts after Stroke. Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association. 2017.
59. Urra X, Cervera A, Obach V, Climent N, Planas AM, Chamorro A. Monocytes are major players in the prognosis and risk of infection after acute stroke. Stroke. 2009;40(4):1262-8.
60. Kumar D, Rasool R, Masoodi KZ, Bhat IA, Verma S, Saleem S. Stroke-induced immune depression-a randomized case control study in Kashmiri population of North India. Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association. 2014;23(8):2041-6.
61. Liesz A, Ruger H, Purrucker J, Zorn M, Dalpke A, Mohlenbruch M, et al. Stress mediators and immune dysfunction in patients with acute cerebrovascular diseases. PloS one. 2013;8(9):e74839.
62. Prass K, Meisel C, Hoflich C, Braun J, Halle E, Wolf T, et al. Stroke-induced immunodeficiency promotes spontaneous bacterial infections and is mediated by sympathetic activation reversal by poststroke T helper cell type 1-like immunostimulation. J Exp Med. 2003;198(5):725-36.
63. Chamorro A, Amaro S, Vargas M, Obach V, Cervera A, Gomez-Choco M, et al. Catecholamines, infection, and death in acute ischemic stroke. J Neurol Sci. 2007;252(1):29-35.
64. Harms H, Reimnitz P, Bohner G, Werich T, Klingebiel R, Meisel C, et al. Influence of stroke localization on autonomic activation, immunodepression, and post-stroke infection. Cerebrovascular diseases (Basel, Switzerland). 2011;32(6):552-60.
65. Klehmet J, Harms H, Richter M, Prass K, Volk HD, Dirnagl U, et al. Stroke-induced immunodepression and post-stroke infections: lessons from the preventive antibacterial therapy in stroke trial. Neuroscience. 2009;158(3):1184-93.
66. Ruhnau J, Schulze K, Gaida B, Langner S, Kessler C, Broker B, et al. Stroke alters respiratory burst in neutrophils and monocytes. Stroke. 2014;45(3):794-800.
67. Rosas-Ballina M, Tracey KJ. Cholinergic control of inflammation. Journal of internal medicine. 2009;265(6):663-79.
68. Tracey KJ. Physiology and immunology of the cholinergic antiinflammatory pathway. The Journal of clinical investigation. 2007;117(2):289-96.
69. Engel O, Akyuz L, da Costa Goncalves AC, Winek K, Dames C, Thielke M, et al. Cholinergic Pathway Suppresses Pulmonary Innate Immunity Facilitating Pneumonia After Stroke. Stroke. 2015;46(11):3232-40.
70. De Raedt S, De Vos A, De Keyser J. Autonomic dysfunction in acute ischemic stroke: an underexplored therapeutic area? Journal of the neurological sciences. 2015;348(1-2):24-34.
71. Gunther A, Salzmann I, Nowack S, Schwab M, Surber R, Hoyer H, et al. Heart rate variability - a potential early marker of sub-acute post-stroke infections. Acta neurologica Scandinavica. 2012;126(3):189-96.
72. De Raedt S, De Vos A, Van Binst AM, De Waele M, Coomans D, Buyl R, et al. High natural killer cell number might identify stroke patients at risk of developing infections. Neurology(R) neuroimmunology & neuroinflammation. 2015;2(2):e71.
73. Chamorro A, Meisel A, Planas AM, Urra X, van de Beek D, Veltkamp R. The immunology of acute stroke. Nature reviews Neurology. 2012;8(7):401-10.
74. Dirnagl U, Klehmet J, Braun JS, Harms H, Meisel C, Ziemssen T, et al. Stroke-induced immunodepression: experimental evidence and clinical relevance. Stroke. 2007;38(2 Suppl):770-3.

75. Haeusler KG, Schmidt WU, Fohring F, Meisel C, Helms T, Jungehulsing GJ, et al. Cellular immunodepression preceding infectious complications after acute ischemic stroke in humans. Cerebrovasc Dis. 2008;25(1-2):50-8.
76. Morotti A, Marini S, Jessel MJ, Schwab K, Kourkoulis C, Ayres AM, et al. Lymphopenia, Infectious Complications, and Outcome in Spontaneous Intracerebral Hemorrhage. Neurocritical care. 2017;26(2):160-6.
77. Vogelgesang A, Grunwald U, Langner S, Jack R, Broker BM, Kessler C, et al. Analysis of lymphocyte subsets in patients with stroke and their influence on infection after stroke. Stroke. 2008;39(1):237-41.
78. Stanley D, Mason LJ, Mackin KE, Srikhanta YN, Lyras D, Prakash MD, et al. Translocation and dissemination of commensal bacteria in post-stroke infection. Nature medicine. 2016;22(11):1277-84.
79. Wong CH, Jenne CN, Lee WY, Leger C, Kubes P. Functional innervation of hepatic iNKT cells is immunosuppressive following stroke. Science (New York, NY). 2011;334(6052):101-5.
80. Dziedzic T, Slowik A, Pera J, Szczudlik A. Beta-blockers reduce the risk of early death in ischemic stroke. Journal of the neurological sciences. 2007;252(1):53-6.
81. Kalita J, Misra UK, Kumar B. Is beta-blocker (atenolol) a preferred antihypertensive in acute intracerebral hemorrhage? Neurol Sci. 2013;34(7):1099-104.
82. Maier IL, Karch A, Mikolajczyk R, Bahr M, Liman J. Effect of beta-blocker therapy on the risk of infections and death after acute stroke--a historical cohort study. PloS one. 2015;10(2):e0116836.
83. Sykora M, Siarnik P, Diedler J. beta-Blockers, Pneumonia, and Outcome After Ischemic Stroke: Evidence From Virtual International Stroke Trials Archive. Stroke. 2015;46(5):1269-74.
84. Starr JB, Tirschwell DL, Becker KJ. Increased infections with beta-blocker use in ischemic stroke, a beta2-receptor mediated process? Neurol Sci. 2017;38(6):967-74.
85. Starr JB, Tirschwell DL, Becker KJ. Labetalol Use Is Associated With Increased In-Hospital Infection Compared With Nicardipine Use in Intracerebral Hemorrhage. Stroke. 2017;48(10):2693-8.
86. Maier IL, Becker JC, Leyhe JR, Schnieder M, Behme D, Psychogios MN, et al. Influence of beta-blocker therapy on the risk of infections and death in patients at high risk for stroke induced immunodepression. PloS one. 2018;13(4):e0196174.
87. Barer DH, Cruickshank JM, Ebrahim SB, Mitchell JR. Low dose beta blockade in acute stroke ("BEST" trial): an evaluation. British medical journal (Clinical research ed). 1988;296(6624):737-41.
88. Hoffmann S, Harms H, Ulm L, Nabavi DG, Mackert BM, Schmehl I, et al. Stroke-induced immunodepression and dysphagia independently predict stroke-associated pneumonia - The PREDICT study. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2016.
89. PRECIOUS: PREvention of Complications to Improve OUtcome in elderly patients with acute Stroke. www.precious-trial.eu (last accessed May 18th 2017).
90. de Ridder IR, de Jong FJ, den Hertog HM, Lingsma HF, van Gemert HM, Schreuder AH, et al. Paracetamol (Acetaminophen) in stroke 2 (PAIS 2): protocol for a randomized, placebo-controlled, double-blind clinical trial to assess the effect of high-dose paracetamol on functional outcome in patients with acute stroke and a body temperature of 36.5 degrees C or above. International journal of stroke : official journal of the International Stroke Society. 2015;10(3):457-62.

APPENDIX

Nederlandse samenvatting /
Summary (Dutch)
NIHSS, GCS and mRS
List of abbreviations
Contributing authors and affiliations
PhD Portfolio
About the author
List of publications
Dankwoord

Nederlandse samenvatting

Hoofdstuk 1. Beroerte is wereldwijd de op één na grootste doodsoorzaak en staat in de top drie van belangrijkste oorzaken van handicap. Ook in Nederland komt beroerte vaak voor. In 2015 waren er 41300 nieuwe patiënten met een beroerte en waren er 437100 mensen in leven die ooit een beroerte hadden gehad. Beroerte is een verzamelnaam voor zowel een herseninfarct, een hersenbloeding en een 'transient ischaemic attack' ofwel TIA. Bij een herseninfarct en een TIA sluit een bloedpropje een bloedvat in de hersenen af, bij een hersenbloeding is er sprake van een bloeding uit een bloedvat in de hersenen. Bij alle drie de vormen krijgt een gedeelte van de hersenen geen zuurstof meer waardoor er klachten bij de patiënt ontstaan, bijvoorbeeld (eenzijdig) krachtsverlies, gevoelsstoornissen, problemen met spreken of met zien of een afhangende mondhoek. Deze 'uitvalsverschijnselen' kunnen gescoord worden op de 'National Institutes of Health Stroke Scale' (NIHSS, zie Appendix), en eventueel bijkomend bewustzijnsverlies op de Glasgow Coma Scale (GCS, zie Appendix). Bij een TIA zijn de symptomen per definitie na 24 uur over.

Eén maand na de beroerte is ongeveer 15% van de patiënten overleden, en is 40% van de patiënten die de beroerte overleefden gehandicapt. Na 1 jaar is ongeveer een kwart van de patiënten met een herseninfarct overleden, en 55% van de patiënten met een hersenbloeding. Prognose na een beroerte wordt meestal uitgedrukt in mortaliteit en in functionele uitkomst op de 'modified Rankin Scale (mRS, Appendix). In het algemeen wordt een score op de mRS van 0-2 gezien als onafhankelijkheid of gunstige uitkomst.

De behandeling van beroerte kan worden ingedeeld naar acute behandeling, preventie van een nieuwe beroerte en de behandeling van complicaties direct na de beroerte. Intraveneuze trombolyse en intra-arteriële trombectomie zijn acute behandelingen voor een herseninfarct. Bij trombolyse wordt geprobeerd het bloedpropje op te lossen. Deze behandeling voorkomt dood of afhankelijkheid bij 41 op de 1000 mensen bij behandeling binnen 6 uur, en bij 95 op de 1000 mensen bij behandeling binnen 3 uur. Bij intra-arteriële trombectomie wordt geprobeerd het bloedpropje door middel van een endovasculaire behandeling te verwijderen. Deze behandeling vergroot de kans op 1 punt verbetering op de mRS 2.5 keer. Voor hersenbloedingen bestaat behandeling uit het eventueel couperen van antistolling, bloeddruk verlagende therapie en soms neurochirurgisch ingrijpen.

Verdere behandeling van beroerte is gericht op het voorkomen van complicaties direct na de beroerte. Een van de meest voorkomende complicaties zijn infecties. Pneumonie (longontsteking) en urineweginfectie zijn de twee meest frequente infecties. Patiënten die ouder zijn een ernstigere beroerte of slikstoornissen hebben krijgen vaker infecties.

In een Nederlandse studie van 521 patiënten met beroerte werd gezien dat 78 patiënten een infectie kregen na de beroerte. Deze patiënten herstelden slechter dan patiënten zonder infectie, zowel bij ontslag uit het ziekenhuis als bij evaluatie 1 jaar na de beroerte. Vooral de mensen met een pneumonie hadden een grote kans op slechtere uitkomst na de beroerte. De basis voor dit proefschrift was een subsidie die werd toegekend door ZonMw en de Hartstichting voor een groot gerandomiseerd onderzoek naar preventieve antibiotica bij beroerte, met als doel infecties na beroerte te voorkomen en de uitkomst bij patiënten te verbeteren. In dit proefschrift beschrijven we dit onderzoek en verschillende andere artikelen over dit onderwerp.

Hoofdstuk 2. In het tweede hoofdstuk beschrijven we een systematische review en meta-analyse naar de frequentie van infecties na beroerte. In dit review includeerden we 87 studies die de frequentie van infectie bij patiënten met een beroerte rapporteerden, waarvan 8 studies op een Intensive Care waren uitgevoerd. De gepoolde frequentie van infecties in al deze studies was 30% (95%CI 24-36%), waarbij pneumonie en urineweg-infecties allebei bij 10% (95%CI 9-10% en 9-12%) van de patiënten voorkwamen. Bij patiënten opgenomen op een Intensive Care lagen deze frequenties hoger: infecties kwamen voor bij 45% (95% CI 38-52%), pneumonie bij 28% (95%CI 18-38%) en urine-weginfecties bij 20% (95%CI 0-40%) van de patiënten. Het vóórkomen van pneumonie was (onafhankelijk) geassocieerd met overlijden (odds ratio 3.62 (95%CI 2.80-4.68)) in een gepoolde analyse van 4 studies met in totaal 19971 patiënten waarin dit was onderzocht.

In **hoofdstuk 3** beschrijven we een systematische review en meta-analyse naar preventieve antibiotica bij beroerte. Er waren ten tijde van het schrijven van dit review in 2010 vijf gerandomiseerde beroerte trials waarin preventieve antibiotica was onderzocht, met in totaal 506 patiënten. Deze trials verschilden van elkaar in studie populatie, studieontwerp, het type antibiotica dat werd gebruikt en de manier van diagnosticeren van een infectie. In een gepoolde analyse van deze 5 studies verlaagde preventieve antibiotica het aantal infecties van 36% naar 22%, maar het aantal overleden patiënten en het aantal afhankelijke patiënten was niet-significant minder (13% vs. 15% en 47% vs. 61%). Er waren geen belangrijke bijwerkingen van de behandeling. Omdat het aantal infecties wel verlaagd werd en omdat de studies klein en heterogeen waren, concludeerden we dat een groter onderzoek naar functionele uitkomst van patiënten na preventieve antibiotica nodig was.

Het protocol voor deze grote gerandomiseerde trial, genaamd de 'Preventive Antibiotics in Stroke Study' (PASS) beschrijven we in **het eerste deel van hoofdstuk 4**. Dit onderzoek is een multicenter, prospectieve, gerandomiseerde, open-label trial met geblindeerde uitkomst meting waarin preventieve ceftriaxon intraveneus eenmaal daags gedurende 4

dagen samen met standaard zorg wordt vergeleken met standaard zorg alleen. Patiënten met een beroerte (herseninfarct of bloeding), een score van minimaal 1 op de NIHSS worden geïncludeerd. De beoogde grootte van de trial is 3200 patiënten. De primaire uitkomst is functionele uitkomst op de mRS bij 3 maanden, gedichotomiseerd in gunstige uitkomst (mRS 0-2) en ongunstige uitkomst (3-6). Secundaire uitkomsten zijn overlijden bij ontslag en 3 maanden, infectie frequentie, opnameduur en een kosteneffectiviteitsanalyse. In het **tweede deel van hoofdstuk 4** beschrijven we een aanpassing in het protocol. Vanwege tegenvallende inclusiesnelheid en het steeds gebruikelijker worden van een ordinale analyse van de mRS, waardoor je minder patiënten nodig hebt voor dezelfde onderzoeksvraag, veranderen we hierin de primaire analyse van primaire uitkomst. De dichotomisatie van de mRS wordt nu een secundaire analyse van primaire uitkomst en de ordinale analyse van de mRS de primaire analyse van de primaire uitkomst. In het **derde deel van hoofdstuk 4** beschrijven we het statistisch analyse plan tot in detail. Hierin wordt onder andere de manier van infecties diagnosticeren beschreven, enerzijds door de behandelend arts en anderzijds door een onafhankelijk infectiepanel. Ook beschrijven we de geplande subgroep analyses van patiënten met ernstige beroerte (NIHSS ≥10), oudere patiënten (≥75 jaar) en van type beroerte (bloeding, infarct, TIA, andere diagnose).

In **hoofdstuk 5** presenteren we de resultaten van de PASS. Er werden uiteindelijk 2550 patiënten gerandomiseerd, waarvan 1268 patiënten in de ceftriaxon groep en 1270 in de standaard zorg groep konden worden geanalyseerd (12 patiënten trokken hun eerdere toestemming in). Preventieve antibiotica verbeterde de functionele uitkomst bij drie maanden niet. Er waren niet meer bijwerkingen in de preventieve antibiotica groep. Preventieve antibiotica voorkwam wel infecties, maar dit kwam voornamelijk doordat er minder urineweginfecties voorkwamen in de antibiotica groep, het aantal patiënten met een pneumonie was niet significant verschillend tussen beide groepen. Dit gold zowel voor diagnose van infecties door de behandelend arts als diagnose door het expert panel. Ook in de subgroep analyses werd geen effect op functionele uitkomst van de behandeling gevonden. Wel viel op dat infecties opnieuw geassocieerd waren met ongunstige uitkomst. Er zijn verschillende verklaringen voor het niet vinden van een effect op uitkomst, zie hiervoor het gedeelte over de samenvatting van hoofdstuk 9.

De kosteneffectiviteitsanalyse van de PASS wordt beschreven in **hoofdstuk 6**. In de kosteneffectiviteitsanalyse wordt berekend hoeveel het kost om een verbetering in functionele uitkomst te krijgen (op de mRS), in de kostenutiliteitsanalyse gaat het om een verbetering gemeten in Quality Adjusted Life Years (QALY's), kort gezegd een jaar doorgebracht in goede gezondheid. Voor deze analyse waren de gegevens van 2538 patiënten beschikbaar voor analyse van volume en kosten van zorg in het ziekenhuis, en voor 1453 patiënten na ontslag uit het ziekenhuis tot de follow-up bij drie maanden. Dit laatste werd tijdens de

studie verzameld door een vragenlijst naar de patiënten toe te sturen, waarin ook vragen stonden waarmee de Quality-Adjusted-Life-Years (QALY's) berekend konden worden. Het volume en de kosten van zorg waren vergelijkbaar in de ceftriaxon groep en de standaard zorg groep. Het gemiddelde verschil in functionele uitkomst was 0.06 op de mRS en het aantal QALY's 0.008, beide in het voordeel van de ceftriaxon groep. Uit de uiteindelijke analyse bleek dat de kans dat ceftriaxon kosteneffectief was varieerde tussen 0·67-0·89. Bij een kans van 0.75 kost het de maatschappij €2290 per punt vermindering op de mRS en €12200 per QALY. Hieruit blijkt dat ceftriaxon, ook al had het niet het beoogde effect op de functionele uitkomst, wel een kosteneffectieve behandeling zou kunnen zijn. Belangrijk hierbij is dat eventuele kosten van toekomstige antimicrobiële resistentie niet meegenomen zijn in deze analyse.

In **hoofdstuk 7** gaan we in op een andere manier van het voorkomen van infecties na beroerte. In een experimentele studie werd gezien dat het krijgen van een pneumonie na beroerte kon worden voorkomen door toediening van een betablokker. In de studies die hiernaar bij patiënten zijn gedaan werden tegenstrijdige resultaten gevonden. We hebben daarom gekeken of de patiënten uit de PASS die betablokkers gebruikten voorafgaand aan de beroerte een lagere kans hadden op het krijgen van een infectie. In deze exploratieve analyse zagen we dat patiënten die betablokkers gebruiken juist een hogere kans hadden op het krijgen van een infectie en het krijgen van een pneumonie. Het was wel zo dat de patiënten die betablokkers gebruikten vaak ouder waren en meer comorbiditeit hadden, maar hiervoor werd gecorrigeerd in de analyse. Verder hebben we alleen gekeken naar patiënten die al betablokkers gebruikten voorafgaand aan de beroerte, en niet naar patiënten waarbij betablokkers na de beroerte gestart werden. Ook was deze behandeling niet gecontroleerd en werden er verschillende soorten van betablokkers gebruikt. Deze resultaten ontmoedigen een eventueel vervolgonderzoek naar betablokkers ter preventie van infecties bij patiënten met een beroerte.

Om voor toekomstige studies juist de patiënten te kunnen selecteren die het hoogste risico hebben om een infectie te krijgen beschrijven we in **hoofdstuk 8** een predictiemodel naar het krijgen van infectie en pneumonie na beroerte. Het krijgen van een pneumonie werd voorspeld door hogere leeftijd, mannelijk geslacht, handicap voorafgaand aan beroerte, voorgeschiedenis van COPD, ernstigere beroerte, dysfagie (slikstoornissen) en het hebben van een hersenbloeding (in vergelijking met een herseninfarct). Infecties werden voorspeld door een hogere leeftijd, mannelijk geslacht, voorgeschiedenis van diabetes of COPD, ernstigere beroerte, dysfagie, gebruik van een blaaskatheter en het hebben van een hersenbloeding (in vergelijking met een herseninfarct). Bij de laagste score op de ontworpen predictieregel was het risico 0.4% voor pneumonie en 1.8% voor infectie, en voor de hoogste score 56.2% voor pneumonie en 88% voor infectie. Discriminatie (dit geeft aan

hoe goed het model onderscheid maakt tussen patiënten met en zonder infectie) van de beide scores was goed. De predictieregel werd intern gevalideerd en kan verder in andere populaties gebruikt worden na externe validatie. Voor toekomstig onderzoek is het belangrijk een predictieregel te gebruiken voor de selectie van patiënten.

Hoofdstuk 9 bevat de discussie van dit proefschrift. Hierin geven we een overzicht van de karakteristieken van infectie na beroerte, vatten we het bewijs over preventieve antibiotica samen, bediscussiëren we dit bewijs en gaan we in op toekomstige onderzoeksstrategieën. In deze samenvatting lichten we het bewijs tot nu toe over preventieve antibiotica bij beroerte toe. Na de in hoofdstuk 3 beschreven systematische review en meta-analyse zijn er 3 gerandomiseerde trials gepubliceerd waarin preventieve antibiotica onderzocht werd bij beroerte. De PASS trial wordt beschreven in hoofdstuk 5 (zie boven). De Engelse 'Prophylactic antibiotics after acute stroke for reducing pneumonia in patients with dysphagia' (STROKE-INF) randomiseerde 1224 patiënten voor behandeling met preventieve antibiotica (volgens lokaal protocol) naast standaard zorg versus standard zorg alleen. (13) In dit onderzoek was de frequentie van pneumonie in de antibiotica groep niet lager en verbeterde deze behandeling ook niet de uitkomst. In de Duitse 'Procalcitonin-guided antibiotic therapy after stroke' (STRAWINSKI) verlaagde procalcitonine-geleide antibiotische therapie het aantal patiënten met pneumonie niet en verbeterde het ook niet de uitkomst bij 3 maanden. Verklaringen voor het niet kunnen voorkomen van pneumonie en het niet verbeteren van de functionele uitkomst zijn als volgt.

Allereerst werden in de PASS patiënten met minder ernstige beroerte geïncludeerd, waardoor de frequentie van infectie relatief laag was. Bij een lage infectie frequentie is het potentiele effect van de behandeling op de uitkomst kleiner. Hiertegen pleit echter dat in de subgroep analyse van de PASS van patiënten met ernstige beroerte er ook geen effect op uitkomst werd gevonden. Daarnaast werden in STROKE-INF en STRAWINSKI wel patiënten met ernstigere beroerte geïncludeerd, en werd er in deze studies ook geen effect aangetoond.

Een andere verklaring is het hoge niveau van de zorg op stroke-units in Nederland. Mogelijk is behandeling met preventieve antibiotica niet beter in vergelijking met stroke-unit zorg, waarin goed wordt gelet op het ontwikkelen van complicaties zoals infecties, en zo nodig in een vroeg stadium gestart wordt met antibiotica. Wanneer in de controlegroep ook veel mensen vroeg met antibiotica behandeld worden is het effect van een preventieve therapie veel minder groot. Het is in een eerdere studie inderdaad aangetoond dat stroke-unit zorg het aantal infecties verlaagt.

De twee grootste trials, de PASS en STROKE-INF, hadden een open-label design, waardoor de behandelend arts wist voor welke behandeling een patiënt was gerandomiseerd. Hierdoor zou het kunnen dat de drempel voor het behandelen van een infectie in de controle groep ook lager werd. Het totale gebruik van antibiotica was hoger in de groep die had gerandomiseerd voor preventief antibiotica in beide trials, maar dit sluit niet uit dat er toch sneller behandeld werd in de standaard zorg groep. Hierdoor zou het potentiele effect van preventieve behandeling met antibiotica kleiner kunnen zijn geworden.

Vervolgens is het de vraag of het tijdstip van toedienen van preventieve antibiotica, het type antibioticum en de manier van toedienen goed gekozen waren. In de STROKE-INF moest antibiotica binnen 48 uur gestart worden, wat vrij laat is aangezien de meeste infecties in de eerste dagen na beroerte voorkomen. In de PASS werd een grens van 24 uur aangehouden, en werd ook geen effect gevonden in de subgroep analyse naar tijd tot behandeling, wat tegen deze verklaring pleit. Het is verder ook mogelijk dat niet alle pathogenen die de infecties na beroerte veroorzaken goed behandeld werden door het gekozen antibioticum. In de PASS werden bijvoorbeeld anaerobe verwekkers niet gedekt. Echter dit was wel het geval in de STROKE-INF, en de uitkomsten van beide onderzoeken zijn gelijk. Ook is het mogelijk dat er niet zozeer sprake is van een infectie, maar meer een ontsteking, een pneumonitis, waar behandeling met antibiotica niet voor helpt. Verder zou het kunnen dat intraveneuze toediening alleen niet voldoende is, maar dat je antibiotica juist ook lokaal moet geven, zoals gebeurt op Intensive Care Afdelingen waar ook preventieve antibiotica wordt gebruikt. Dit is bij beroerte patiënten nog niet onderzocht. Als laatste mogelijkheid zou het kunnen dat infectie en pneumonie geen onafhankelijk effect op de uitkomst hebben. Infectie en pneumonie komen inderdaad voor bij patiënten met hogere leeftijd, meer co-morbiditeit en ernstigere beroerte. In dit geval zal het behandelen van deze infecties geen effect hebben op de uitkomst. Echter, het niet kunnen voorkomen van een pneumonie lijkt een meer voor de hand liggende verklaring dan dat er geen onafhankelijk effect is van pneumonie, ook al is dit nog steeds mogelijk.

Voor toekomstig onderzoek is het belangrijk patiënten te selecteren met een hoog risico op infectie, door gebruik van een predictieregel. Een subgroep analyse van deze hoog risico patiënten in de samengevoegde gegevens van eerdere trials is een interessante volgende stap. Vervolgens moet beter diagnostisch onderzoek naar de ware aard van de infecties na beroerte worden verricht. Een andere interessante richting voor onderzoek is een gecombineerde behandeling van infectie, aspiratie en koorts. Er wordt momenteel geen grote Europese studie gedaan die niet alleen preventieve antibiotica, maar ook middelen tegen misselijkheid (en zo mogelijk aspiratie) en koorts onderzoekt.

Conclusie

Beroerte is een groot gezondheidsprobleem en infecties na beroerte zijn geassocieerd met ongunstige uitkomst. Preventieve antibiotica bij patiënten met een beroerte verbetert de functionele uitkomst niet. Ook krijgen patiënten hierdoor niet minder vaak een pneumonie. Toekomstig onderzoek moet zich richten op selectie van hoog-risico patiënten, subgroep analyse van hoog risico patiënten in data van de eerder verrichte onderzoeken, diagnostisch onderzoek naar infecties na beroerte en gecombineerde behandelingen van infecties, aspiratie en koorts.

NIHSS, GCS and mRS

National Institutes of Health Stroke Scale (NIHSS), Glasgow Coma Scale (GCS) and modified Rankin Scale (mRS)

NIH-Stroke Scale			
1a.	Level of consciousness (LOC)	9 = Alert, keenly responsive 1 = Not alert, but arousable by minor stimulation 2 = Not alert, requires repeated stimulation to attend 3 = No response, other than reflexive posturing	__
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 2 = Forced deviation	__
3.	Visual fields	0 = No visual loss 1 = Partial hemianopia 2 = Complete hemianopia 3 = Bilateral hemianopia (including (cortical) blindness)	__
4.	Facial palsy	0 = Normal symmetrical movements 1 = Minor paralysis (flattened nasolabial fold) 2 = Partial paralysis (total lower face paralysis) 3 = Complete paralysis (upper and lower face)	__
5.	Motor arm	0 = No drift 1 = Drift, drifts down before full 10 sec, does not hit bed 2 = Some effort against gravity, hits bed before 10 sec 3 = No effort against gravity, arm falls 4 = No movement UN = Amputation or joint fusion	L __ R __
6.	Motor leg	0 = No drift 1 = Drift, drifts down before full 5 sec, does not hit bed 2 = Some effort against gravity, hits bed before 5 sec 3 = No effort against gravity, leg falls 4 = No movement UN = Amputation or joint fusion	L __ R __
7.	Limb ataxia	0 = Absent 1 = Present in one limb 2 = Present in two limbs UN = Amputation or joint fusion	__
8.	Sensory	0 = Normal 1 = Mild-to-moderate sensory loss 2 = Severe to total sensory loss	__

NIH-Stroke Scale (Continued)			
9.	Best language	0 = Normal 1 = Mild-to-moderate aphasia 2 = Severe aphasia 3 = Mute, global aphasia	__
10.	Dysarthria	0 = Normal 1 = Mild-to-moderate dysarthria 2 = Severe dysarthria UN = Intubated, other physical barrier	__
11.	Extinction and inattention	0 = No abnormality 1 = Visual, tactile, auditory, spatial or personal inattention 2 = Profound hemi-inattention or more modalities	__
Total score			__

Glasgow Coma Scale		
Eyes	Motor	Verbal
4. Spontaneous	6. Obey commands	5. Orientated
3. To sound	5. Localising	4. Confused
2. To pressure	4. Normal flexion	3. Words
1. None	3. Abnormal flexion	2. Sounds
	2. Extension	1. None
	1. None	

Modified Rankin Scale	
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	Moderately 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

BB	beta blocker
CDC	centers for disease control and prevention
CEA	cost-effectiveness analysis
CEQ	cost-effectiveness questionnaire
CI	confidence interval
CONSORT	consolidated standards of reporting trials
CRF	case record form
CUA	cost-utility analysis
DCM	Dutch costing manual
DDD	defined daily dose
DSMB	data safety monitoring board
GCS	Glasgow coma scale
HPAA	hypothalamo-pituitary-adrenal axis
IAT	intra-arterial treatment
ICU	intensive care unit
INR	international normalised ratio
IQR	interquartile range
ISRCTN	international standard randomised controlled trial number
ITT	intention to treat
IVT	intravenous thrombolysis
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
PI	principal investigator
PISCES	pneumonia in stroke consensus group
PP	per protocol
PROBE	prospective randomised open label design with blinded endpoint
RCT	randomised clinical trial
SAE	serious adverse events
SAP	statistical analysis plan
SNS	sympathetic nervous system
SUSAR	suspected unexpected serious adverse reactions
QALY	quality-adjusted life year

TIA	transient ischaemic attack
UTI	urinary tract infection
VAP	ventilator-associated pneumonia
WBC	white blood cell
WTP	willingness to pay

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PhD portfolio

Name PhD student: Willeke Westendorp

PhD period: 2010-2018

Name PhD supervisors: Diederik van de Beek, Paul Nederkoorn

	Year	Workload ECTS
Courses		
• Infectious diseases	2010	1.3
• Biostatistics	2010	1.1
• Scientific writing in English	2010	
• Clinical epidemiology	2011	0.9
• Basic course in legislation and organization for clinical researchers (BROK)	2012	1.0
• Advanced immunology	2012	2.9
• Clinical prediction models	2017	1.0
Seminars, workshops and master classes		
• Weekly research meeting or journal club, Department of Neurology, Academic Medical Center, Amsterdam, the Netherlands	2010-2016	8
• Weekly research meetings cerebrovascular neurology and neurological infectious diseases, Department of Neurology, Academic Medical Center, Amsterdam, the Netherlands	2010-2013	8
Presentations		
• The impact of post-stroke infection: a systematic review and meta-analysis (poster): ICAAC Boston, United States, 2010	2010	0.5
• Infections after stroke (oral): Dutch neurovascular working group, Amsterdam, the Netherlands	2010	0.5
• Post-stroke infection rates, a systematic review and analysis (oral): European Stroke Conference, Hamburg, Germany	2011	0.5
• Preventive Antibiotics in Stroke Study (poster): Scientific Meeting Dutch Conference of Neurology, Garderen, the Netherlands	2011	0.5
• Antibiotic therapy for preventing infections in patients with acute stroke (poster): European Stroke Conference, Lisbon, Portugal	2012	0.5
• Blood pressure lowering therapy before thrombolysis (oral): Dutch Neurovascular working group, Amsterdam, the Netherlands	2012	0.5
• Preventive Antibiotics in Stroke Study (oral): scientific meeting of the Amsterdam neurologists society, Amsterdam, the Netherlands	2014	0.5

• Prediction of stroke-associated infection in the Preventive Antibiotics in Stroke Study (PASS) (oral): ESOC, Barcelona, Spain	2016	0.5
• Use of pre-stroke beta-blockers and stroke-associated infection risk in acute stroke patients (oral): ESOC, Barcelona, Spain	2016	0.5
• Preventive Antibiotics in Stroke Study (oral): Dutch neurovascular working group, Utrecht, the Netherlands	2016	0.5
• Preventive Antibiotics in Stroke Study (oral): each participating centre of PASS, yearly investigator's meeting	2010-2014	0.5
• Preventive Antibiotics in Stroke Study: background, trial design and results (oral): Masterclass Majank Goyal, Amsterdam, the Netherlands	2016	0.5
• Prediction of stroke-associated infection (poster): Amsterdam Neuroscience Kick-off Meeting, Amsterdam, the Netherlands	2016	0.5
• Cost-effectiveness of preventive antibiotics in stroke: an economic evaluation of data from a randomised clinical trial (oral): European Academy of Neurology Congress, Amsterdam, the Netherlands	2017	0.5
(International) conferences and meetings		
• 50th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Boston, United States	2010	1.25
• 20th European Stroke Conference, Hamburg, Germany	2011	1.0
• 21th European Stroke Conference, Lisbon, Portugal	2012	1.0
• Stroke Research Group Seminar, The University of Edinburgh	2012	0.1
• 2nd European Stroke Organisation Conference, Barcelona, Spain	2016	0.75
• 3rd Congress of the European Academy of Neurology, Amsterdam, the Netherlands	2017	1.0
Teaching		
Student coaching and mentoring		
• Bachelor thesis (Medicine)	2011-2012	1
• Bachelor thesis (Biomedical Sciences)	2012-2013	1
• Neurological infectious diseases and cerebrovascular disease course. Nurse training at Amstel Academy.	2010-2013	1
Parameters of esteem		
• Neurovascular working group	2016	

About the author

Willeke Frederieke Westendorp was born on October 17th, 1983 in Oostburg, the Netherlands. In 2002 she finished secondary school at the 'Zwin College' in Oostburg (cum laude). In the same year she started Medical School at the University Utrecht. During her study, she did an internship in Surinam (Academic Hospital Paramaribo) and electives on the Intensive Care Department (UMC Utrecht) and Emergency Department (St Antonius Hospital Nieuwegein). During the internship neurology in Gelre Hospital Apeldoorn her interest in Neurology arose. In the final year of medical school she did her senior internship neurology in the Academic Medical Center and a scientific internship in the meningitis group of the Academic Medical Center (supervisor Dr. de Gans, Prof. van de Beek). In 2008 she finished medical school and started working as resident not in training at the neurology department of the OLVG in Amsterdam (Prof. Portegies) in 2009. In 2010 she started a PhD on preventive antibiotics in stroke at the department of Neurology of the AMC (Prof. van de Beek, Dr. Nederkoorn) and in 2013 she started her residency Neurology in the AMC (Prof. Stam, Prof. Vermeulen, Prof. Roos, Prof. van Schaik, Dr. Koelman). In January 2016 the article of the 'Preventive Antibiotics in Stroke Study' was rewarded with scientific award of the Dutch neurovascular working group. Willeke spent one year of her residency in Flevo Ziekenhuis Almere (Prof. Limburg) and plans to complete her residency in 2019. Willeke lives together with Matthijs and their son Abel in Amsterdam.

List of publications

1. Vermeij JD, Westendorp WF, van de Beek D, Nederkoorn PJ. Post-stroke infections and preventive antibiotics in stroke: Update of clinical evidence. Int J Stroke. 2018 Sep 3
2. de Ridder IR, Dijkland SA, Scheele M, den Hertog HM, Dirks M, Westendorp WF et al; Development and validation of the Dutch Stroke Score for predicting disability and functional outcome after ischemic stroke: A tool to support efficient discharge planning. Eur Stroke J. 2018 Jun;3(2):165-173.
3. Westendorp WF, Vermeij JD, Hilkens NA, Brouwer MC, Algra A, van der Worp HB et al. Development and internal validation of a prediction rule for post-stroke infection and post-stroke pneumonia in acute stroke patients. Eur Stroke J. 2018 Jun;3(2):136-144.
4. Groot AE, Vermeij JM, Westendorp WF, Nederkoorn PJ, van de Beek D, Coutinho JM. Continuation or Discontinuation of Anticoagulation in the Early Phase After Acute Ischemic Stroke. Stroke. Stroke. 2018;49(7):1762-5.
5. Westendorp WF, Zock E, Vermeij JD, Kerkhoff H, Nederkoorn PJ, Dijkgraaf MGW, van de Beek D; PASS investigators. Preventive Antibiotics in Stroke Study (PASS): A cost-effectiveness study. Neurology. 2018 May 1;90(18):e1553-e1560.
6. Vermeij JD, Westendorp WF, Dippel DW, van de Beek D, Nederkoorn PJ. Antibiotic therapy for preventing infections in people with acute stroke. Cochrane Database Syst Rev. 2018 Jan 22;1:CD008530.
7. Zonneveld TP, Nederkoorn PJ, Westendorp WF, Brouwer MC, van de Beek D, Kruyt ND. Hyperglycemia predicts poststroke infections in acute ischemic stroke. Neurology. 2017;88(15):1415-21.
8. Westendorp WF, Vermeij JD, Brouwer MC, Roos YB, Nederkoorn PJ, van de Beek D. Pre-Stroke Use of Beta-Blockers Does Not Lower Post-Stroke Infection Rate: An Exploratory Analysis of the Preventive Antibiotics in Stroke Study. Cerebrovascular diseases. 2016;42(5-6):506-11.
9. 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.
10. Van Montfrans JM, Hartman EA, Braun KP, Hennekam EA, Hak EA, Nederkoorn PJ, et al. Phenotypic variability in patients with ADA2 deficiency due to identical homozygous R169Q mutations. Rheumatology. 2016;55(5):902-10.
11. Westendorp WF, Nederkoorn PJ, Aksentijevich I, Hak AE, Lichtenbelt KD, Braun KP. Unexplained early-onset lacunar stroke and inflammatory skin lesions: Consider ADA2 deficiency. Neurology. 2015;84(20):2092-3.

12. Westendorp WF, Vermeij JD, Zock E, Hooijenga IJ, Kruyt ND, Bosboom HJ, et al. The Preventive Antibiotics in Stroke Study (PASS): a pragmatic randomised open-label masked endpoint clinical trial. Lancet. 2015;385(9977):1519-26.
13. Westendorp WF, Vermeij JD, Dippel DW, Dijkgraaf MG, van der Poll T, Prins JM, et al. Update of the Preventive Antibiotics in Stroke Study (PASS): statistical analysis plan. Trials. 2014;15:382.
14. Westendorp WF, Vermeij JD, van GN, Dippel DW, Dijkgraaf MG, van der Poll T, et al. Update on the Preventive Antibiotics in Stroke Study (PASS): a randomised controlled phase 3 clinical trial. Trials. 2014;15:133.
15. Westendorp WF, Vermeij JD, Vermeij F, den Hertog HM, Dippel DW, van de Beek D, et al. Antibiotic therapy for preventing infections in patients with acute stroke. Cochrane Database Syst Rev. 2012;1:CD008530.
16. Westendorp WF, Nederkoorn PJ, Vermeij JD, Dijkgraaf MG, van de Beek D. Post-stroke infection: A systematic review and meta-analysis. BMC Neurol. 2011;11:110.
17. Nederkoorn PJ, Westendorp WF, Hooijenga IJ, de Haan RJ, Dippel DW, Vermeij FH, et al. Preventive antibiotics in stroke study: rationale and protocol for a randomised trial. Int J Stroke. 2011;6(2):159-63.
18. Schut ES, Westendorp WF, de Gans J, Kruyt ND, Spanjaard L, Reitsma JB, et al. Hyperglycemia in bacterial meningitis: a prospective cohort study. BMC infectious diseases. 2009;9:57.

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