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Cerebrolysin for acute ischaemic stroke (Review)

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TABLE OF CONTENTS

[Intervention Review]

Cerebrolysin for acute ischaemic stroke

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ABSTRACT

Background

Cerebrolysin is a mixture of low-molecular-weight peptides and amino acids derived from porcine brain, which has potential neuroprotective properties. It is widely used in the treatment of acute ischaemic stroke in Russia, Eastern Europe, China, and other Asian and post-Soviet countries. This is an update of a review first published in 2010 and last updated in 2020.

Objectives

To assess the benefits and harms of Cerebrolysin or Cerebrolysin-like agents for treating acute ischaemic stroke.

Search methods

We searched the Cochrane Stroke Trials Register, CENTRAL, MEDLINE, Embase, Web of Science Core Collection, with Science Citation Index, and LILACS in May 2022 and a number of Russian databases in June 2022. We also searched reference lists, ongoing trials registers, and conference proceedings.

Selection criteria

Randomised controlled trials (RCTs) comparing Cerebrolysin or Cerebrolysin-like agents started within 48 hours of stroke onset and continued for any length of time, with placebo or no treatment in people with acute ischaemic stroke.

Data collection and analysis

Three review authors independently applied the inclusion criteria, assessed trial quality and risk of bias, extracted data, and applied GRADE criteria to the evidence.

Main results

Seven RCTs (1773 participants) met the inclusion criteria of the review. In this update we added one RCT of Cerebrolysin-like agent Cortexin, which contributed 272 participants.

We used the same approach for risk of bias assessment that was re-evaluated for the previous update: we added consideration of the public availability of study protocols and reported outcomes to the selective outcome reporting judgement, through identification, examination, and evaluation of study protocols.

For the Cerebrolysin studies, we judged the risk of bias for selective outcome reporting to be unclear across all studies; for blinding of participants and personnel to be low in three studies and unclear in the remaining four; and for blinding of outcome assessors to be low in three studies and unclear in four studies. We judged the risk of bias for generation of allocation sequence to be low in one study and unclear in the remaining six studies; for allocation concealment to be low in one study and unclear in six studies; and for incomplete outcome data to be low in three studies and high in the remaining four studies. The manufacturer of Cerebrolysin supported three multicentre studies, either totally, or by providing Cerebrolysin and placebo, randomisation codes, research grants, or statisticians. We judged two studies to be at high risk of other bias and the remaining five studies to be at unclear risk of other bias. We judged the study of Cortexin to be at low risk of bias for incomplete outcome data and at unclear risk of bias for all other domains.

All-cause death: Cerebrolysin or Cortexin probably result in little to no difference in all-cause death (risk ratio (RR) 0.96, 95% confidence interval (CI) 0.65 to 1.41; 6 trials, 1689 participants; moderate-certainty evidence).

None of the included studies reported on poor functional outcome, defined as death or dependence at the end of the follow-up period, early death (within two weeks of stroke onset), quality of life, or time to restoration of capacity for work.

Only one study clearly reported on the cause of death: cerebral infarct (four in the Cerebrolysin and two in the placebo group), heart failure (two in the Cerebrolysin and one in the placebo group), pulmonary embolism (two in the placebo group), and pneumonia (one in the placebo group).

Non-death attrition (secondary outcome): Cerebrolysin or similar peptide mixtures may result in little to no difference in non-death attrition, but the evidence is very uncertain, with a considerable level of heterogeneity (RR 0.72, 95% CI 0.38 to 1.39; 6 trials, 1689 participants; very low-certainty evidence).

Serious adverse events (SAEs): Cerebrolysin probably results in little to no difference in the total number of people with SAEs (RR 1.16, 95% CI 0.81 to 1.66; 3 trials, 1335 participants; moderate-certainty evidence). This comprised fatal SAEs (RR 0.90, 95% CI 0.59 to 1.38; 3 trials, 1335 participants; moderate-certainty evidence) and an increase in the total number of people with non-fatal SAEs (RR 2.39, 95% CI 1.10 to 5.23; 3 trials, 1335 participants; moderate-certainty evidence). In the subgroup of dosing schedule 30 mL for 10 days (cumulative dose 300 mL), the increase was more prominent (RR 2.87, 95% CI 1.24 to 6.69; 2 trials, 1189 participants).

Total number of people with adverse events: Cerebrolysin or similar peptide mixtures may result in little to no difference in the total number of people with adverse events (RR 1.03, 95% CI 0.92 to 1.14; 4 trials, 1607 participants; low-certainty evidence).

Authors' conclusions

Moderate-certainty evidence indicates that Cerebrolysin or Cerebrolysin-like peptide mixtures derived from cattle brain probably have no beneficial effect on preventing all-cause death in acute ischaemic stroke. Moderate-certainty evidence suggests that Cerebrolysin probably has no beneficial effect on the total number of people with serious adverse events. Moderate-certainty evidence also indicates a potential increase in non-fatal serious adverse events with Cerebrolysin use.

PLAIN LANGUAGE SUMMARY

Cerebrolysin for acute ischaemic stroke

What did we want to know?

In this Cochrane Review, we wanted to find out how well a medicine called Cerebrolysin or other Cerebrolysin-like agents work to treat a stroke.

What is a stroke?

A stroke is a sudden attack of weakness that usually affects one side of the body. It happens when the flow of blood to part of the brain is cut off, stopping the supply of oxygen and nutrients to the brain cells, which is called ischaemia. If the supply of blood to the brain is stopped, brain cells begin to die. This can lead to brain injury, disability, and possibly death.

Ischaemic strokes are the most common type of stroke. An ischaemic stroke happens when the flow of blood is blocked by a blood clot or a piece of fatty material in an artery.

Why is this review important?

Strokes are a medical emergency, and urgent treatment is essential. Ischaemic strokes are usually treated with a combination of medicines to prevent and dissolve blood clots, reduce blood pressure, and lower cholesterol levels.

Cerebrolysin, and the Cerebrolysin-like agent Cortexin, are mixtures of proteins, peptides (short chains of amino acids) and amino acids (small molecules that combine to form a protein) purified from animal brains (cows and pigs). Some of the proteins in Cerebrolysin or Cortexin are found naturally in the human brain and may help to protect and repair brain cells. Cerebrolysin and Cortexin are commonly used in some countries as a treatment for stroke.

What did we do?

We searched for studies looking at the use of Cerebrolysin or Cerebrolysin-like agents to treat acute ischaemic stroke. We searched for randomised controlled studies, in which the treatment people receive is randomly decided, because these studies give the most reliable evidence about treatments.

Search date: we included evidence published up to June 2022.

What we found

We found seven studies in 1773 people who had had an acute ischaemic stroke. The studies looked at the effect of giving Cerebrolysin alongside medicines to prevent and dissolve blood clots (standard therapy) during the first 48 hours after a stroke. The studies compared this treatment with standard therapy alone or standard therapy plus a dummy treatment (placebo).

The studies were conducted in hospitals in Austria, Croatia, the Czech Republic, Hungary, Russia, Slovakia, Slovenia, China, Hong Kong, Iran, Myanmar, and South Korea, and lasted from 28 days to 90 days.

Results of our review

Adding Cerebrolysin or a Cerebrolysin-like agent, Cortexin, to standard therapy probably adds no benefit to the risk of dying from any cause after a stroke (6 studies, 1689 people).

We did not find enough evidence about how Cerebrolysin or the Cerebrolysin-like agent Cortexin affected:

• the risk of dying or needing continuing care at the end of the study;
• the risk of dying within two weeks of having a stroke;
• the time taken for people to be able to go back to work; or
• people's well-being (quality of life).

We are uncertain whether adding Cerebrolysin to standard therapy made any difference to the numbers of people who dropped out of studies (6 studies, 1689 people).

Cerebrolysin added to standard therapy probably made little or no difference to:

• the total number of people who had serious unwanted effects (life-threatening effects that could result in death, disability, or a longer hospital stay) (3 studies, 1335 people);
• the number of serious unwanted effects that caused death (3 studies, 1335 people).

However, more people given Cerebrolysin plus standard therapy probably had serious unwanted effects that did not kill them than those who were given standard therapy (alone or with placebo) (3 studies, 1335 people).

Cerebrolysin or the Cerebrolysin-like agent Cortexin may make little or no difference to the total number of people who had any unwanted effects (4 studies, 1607 people).

Our confidence in the results

We are moderately confident (certain) in the results of this review. However, the evidence comes from a small number of studies. Three studies involved a pharmaceutical company that makes Cerebrolysin, which may have affected how those studies were designed, carried out, and reported. Our conclusions are likely to change if results from further studies become available.

Conclusions

Adding Cerebrolysin or a Cerebrolysin-like agent, Cortexin, to standard therapy after an acute ischaemic stroke probably:

• does not reduce the risk of dying.

Adding Cerebrolysin to standard therapy after an ischaemic stroke probably:

• does not affect how many people have serious unwanted effects overall; but
• increases the number of people with serious, non-fatal unwanted effects.

SUMMARY OF FINDINGS

Summary of findings 1. Cerebrolysin or Cerebrolysin-like agents compared to placebo for acute ischaemic stroke

Cerebrolysin or Cerebrolysin-like agents compared to placebo for acute ischaemic stroke

Patient or population: people with acute ischaemic stroke
Settings: inpatient health facilities
Intervention: Cerebrolysin or Cortexin added to standard therapy
Comparison: placebo added to standard therapy

Outcomes		Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	Number of participants (studies)	Certainty of the evidence (GRADE)
		Assumed risk	Corresponding risk			
		Placebo	Cerebrolysin/Cortexin			
All-cause death (follow-up period up to 90 days)		**61 per 1000** 47/767 (6.1%)	**58 per 1000** 53/922 (5.7%) **3 fewer per 1000** (from 22 fewer to 22 more)	**RR 0.96** (0.65 to 1.41)	1689 (6 RCTs)	⊕⊕⊕⊖ Moderate[a]
Non-death attrition		**145 per 1000** 111/767 (14.5%)	**87 per 1000** 80/922 (8.7%) **58 fewer per 1000** (from 39 fewer to 152 more)	**RR 0.72** (0.38 to 1.39)	1689 (6 RCTs)	⊕⊖⊖⊖ Very low[a,c]
Total number of people with SAEs**	**Follow-up period up to 90 days**	**75 per 1000** 50/668 (7.5%)	**87 per 1000** 58/667 (8.7%) **12 more per 1000** (from 14 fewer to 47 more)	**RR 1.16** (0.81 to 1.66)	1335 (3 RCTs)	⊕⊕⊕⊖ Moderate[a]
	Fatal, follow-up period up to 90 days	**63 per 1000** 42/668 (6.3%)	**57 per 1000** 38/667 (5.7%) **6 fewer per 1000**	**RR 0.90** (0.59 to 1.38)	1335 (3 RCTs)	⊕⊕⊕⊖ Moderate[a]

		(from 26 fewer to 24 more)			
Non-fatal, follow-up period up to 90 days	**12 per 1000** 8/668 (1.2%)	**30 per 1000** 20/667 (3.0%) **18 more per 1 000** (from 0 fewer to 49 more)	**RR 2.39** (1.10 to 5.23)	1335 (3 RCTs)	⊕⊕⊕⊖ Moderate[a]
Total number of people with adverse events, follow-up period up to 90 days	**429 per 1000** 314/732 (42.9%)	**387 per 1000** 339/875 (38.7%) **42 fewer per 1000** (from 38 fewer to 55 more)	**RR 1.03** (0.92 to 1.14)	1607 (4 RCTs)	⊕⊕⊖⊖ Low[a,b]
Death or dependence, follow-up period up to 90 days	Not reported	Not reported	-	-	-
Early death (within 2 weeks of stroke onset)	Not reported	Not reported	-	-	-
Quality of life	Not reported	Not reported	-	-	-

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: confidence interval; **RCT:** randomised controlled trial: **RR:** risk ratio; **SAE:** serious adverse event

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

****Results of the subgroup analysis:**
Total number of people with SAEs, non-fatal
A subgroup by Cerebrolysin dose and length of treatment (30 mL for 10 days), at the end of the follow-up period: assumed risk 12 per 1000 7/600 (1.2%); corresponding risk 33 per 1000 20/589 (3.4%), 22 more per 1000 (from 3 more to 66 more)
RR 2.86 (1.23 to 6.66); number of participants (studies) 1189 (2 RCTs)
Certainty of the evidence ⊕⊕⊕⊖ **Moderate**[a]

[a]We downgraded by one level for risk of bias because most information came from studies at low or unclear risk of bias, with high levels of exclusion from the final analyses, retrospective registration, and other methodological flaws as described in Assessment of risk of bias in included studies. The manufacturer of Cerebrolysin supported CASTA 2012 and CERE-LYSE-1 2012 by providing services including: provision of Cerebrolysin and placebo, randomisation codes, and statisticians.

[b]We downgraded by one level for inconsistency: heterogeneity with I^2 = 37% for the overall effect estimate owing to the opposite direction of effect estimate in the Ladurner 2005 study (high cumulative dose of Cerebrolysin), and heterogeneity with I^2 = 65% in the subgroup of two multicentre studies with the same dosing schedule (CASTA 2012; CERE-LYSE-1 2012).

[c]We downgraded by one level for inconsistency and by one level for imprecision. Five trials contributed to the outcome non-death attrition; we detected heterogeneity, with I^2 = 57% for the overall effect estimate and I^2 = 66% for subgroup differences, and heterogeneity with I^2 = 47% in the subgroup of two multicentre studies with the same dosing schedule (CASTA 2012; CERE-LYSE-1 2012). The confidence intervals were wide.

BACKGROUND

Effective, simple, and reliable treatment methods are urgently needed to reduce stroke mortality and disability. Many clinical trials and Cochrane Reviews have addressed the question of benefits and risks of potential pharmacological treatment options for acute ischaemic stroke. However, strategies with proven therapeutic effects and an acceptable benefit-to-risk ratio are still lacking. Potential strategies can be grouped according to the existing evidence of their benefits and harms determining their role in clinical practice.

Evidence of benefit

Aspirin at a dose of 160 mg to 300 mg daily (orally or per rectum), started within 48 hours of onset of presumed ischaemic stroke, appears to be the only effective treatment for early secondary prevention, reducing the risk of early recurrent ischaemic stroke without a major risk of early haemorrhagic complications, and improving long-term outcomes (Minhas 2022; Sandercock 2014). Despite the positive overall conclusions of a Cochrane Review, Wardlaw 2014, and individual patient data meta-analysis, Emberson 2014, of thrombolysis in acute ischaemic stroke, there is still some debate regarding the optimal use of intravenous recombinant tissue plasminogen activators (rtPA) (Alper 2015). It is estimated that for every person with a good stroke outcome at six months, another person would have symptomatic intracranial bleeding, and for every three to four people without neurological deficits at six months, there is an excess of one death after thrombolysis (Appelros 2015; Brunström 2015). The evidence is inadequate to conclude whether lower doses of thrombolytic agents are more effective than higher doses, whether one agent is better than another, or which route of administration is the best for treatment of people who have had an acute ischaemic stroke (Wardlaw 2013), or whether percutaneous vascular interventions offer any advantages over intravenous thrombolysis in terms of patient-oriented outcomes (Lindekleiv 2018).

Evidence of harm

Glycoprotein IIb-IIIa inhibitors (abciximab and tirofiban) increase the risk of intracranial haemorrhage without evidence of any reduction in death or disability in stroke survivors (Ciccone 2014). These data do not support their routine use in clinical practice. Abciximab contributed 89% of the total number of participants in the Cochrane Review (Ciccone 2014). Anticoagulants (standard unfractionated heparin, low-molecular-weight heparins, heparinoids, oral anticoagulants, and thrombin inhibitors) as immediate therapy for acute ischaemic stroke are not associated with net short- or long-term benefit. Reduced rate of recurrent stroke, deep vein thrombosis, and pulmonary embolism with anticoagulant therapy is offset by the increased risk of intracranial haemorrhage and extracranial bleeding. The data do not support the routine use of any of the currently available anticoagulants in acute ischaemic stroke (Berge 2002; Sandercock 2015; Sandercock 2017; Wang 2021). Long-term anticoagulant therapy in people with presumed non-cardioembolic ischaemic stroke or transient ischaemic attack is not associated with any benefit, but there is a significant risk of bleeding (Sandercock 2009).

Tirilazad, an amino steroid inhibitor of lipid peroxidation, increases the combined endpoint of 'death or disability' in people with acute ischaemic stroke (TISC 2001). Lubeluzole, an ion channel modulator of glutamate release that has a benzothiazole structure with potential neuroprotective properties, does not reduce death or dependency in acute ischaemic stroke patients; in contrast, it increases heart-conduction disorders (Q-T prolongation) (Gandolfo 2002).

Lack of evidence of benefit

Several treatment options that have been tested in clinical trials have not shown any evidence of benefit. The results of these trials have been systematically reviewed: corticosteroids (Sandercock 2011), calcium antagonists (Zhang 2019), haemodilution (Chang 2014), excitatory amino acid antagonists (including ion channel modulators and N-methyl-D-aspartic acid; NMDA) (Muir 2003), piracetam (Ricci 2012a), a free radical trapping agent NXY-059 (Shuaib 2007), and Cerebrolysin (Ziganshina 2020). There is no evidence that colloids lead to lower odds of death or dependence after stroke compared with crystalloids (Visvanathan 2015).

Role in clinical practice

There is still inadequate evidence from randomised controlled trials for the following antithrombotic agents: oral antiplatelet drugs other than aspirin (clopidogrel, ticlopidine, cilostazol, satigrel, sarpolgrelate, KBT 3022, isbogrel) (Minhas 2022; Sandercock 2014), and the fibrinogen-depleting agents ancrod and defibrase (Hao 2012).

The list of interventions of agents tested in clinical trials with subsequent Cochrane Reviews of results that document inadequate evidence to establish a role in clinical practice includes: ginkgo biloba (Zeng 2005); gamma aminobutyric acid (GABA) receptor agonists (Liu 2018); sonothrombolysis (Ricci 2012b); glycerol (Righetti 2004); mannitol (Bereczki 2007); naftidrofuryl, a 5-HT2 serotonergic antagonist (Leonardi-Bee 2007); theophylline or methylxanthine derivatives (Bath 2004a; Bath 2004b); nitric oxide donors (Bath 2017); blood pressure-altering interventions (Bath 2014; Geeganage 2010); prostacyclin and its analogues (Bath 2004c); buflomedil (Wu 2015); vinpocetine (Bereczki 2008); gangliosides (Candelise 2001); colony-stimulating factors (Bath 2013); stem cells (Boncoraglio 2019); Chinese herbal medicines such as sanchi (Chen 2008), puerarin (Liu 2016), mailuoning (Yang 2015), and tongxinluo (Zhuo 2008); and the neuroprotective agent edaravone (Feng 2011).

Description of the condition

Ischaemic stroke occurs when the brain loses its blood and energy supply, resulting in damage to brain tissue; it is the brain equivalent of a heart attack. Most strokes (87%) are ischaemic as confirmed by computerised tomography (CT) scan (AHA 2019; AHA 2022). Worldwide 15 million people suffer a stroke every year; five-and-a-half million people die, and another five million are left permanently disabled, placing a burden on family and community (WHO 2019a). Stroke is one of the major causes of disability and mortality (AHA 2019; AHA 2022; GBD Stroke Collaborators 2019; WHO 2019a). It is the third most common cause of death after coronary disease and cancer. In 2014, the World Health Organization (WHO) stroke statistics registered the number of deaths from stroke to be more than 200,000 in the Russian Federation, as well as in China and in India, with the highest number of 1,652,885 in China and 517,424 in Russia in 2002 (WHO 2019a). According to the Russian data, there were on average 3.52 and 3.27 cases per 1000 population registered in the Russian Federation

in 2009 and 2010, respectively, and mortality was 1.19 and 0.96 per 1000 population in 2009 and 2010, with significant differences between different regions (Gusev 2013). Standardised incidence was 2.39 (3.24 in men and 2.24 in women) per 1000 population (Gusev 2013). In 2016 in Russia there were 345,861 stroke deaths (95% confidence interval (CI) 267,315 to 444,861), 676,846 incident cases (95% CI 607,894 to 746,828), and 6,082,727 disability-adjusted life-years (DALYs) (95% CI 4,773,920 to 7,736,480) (GBD Stroke Collaborators 2019). The case fatality rate of stroke is 40.4% (61.4% for haemorrhagic stroke and 21.8% for ischaemic stroke). The northwest regions of Russia had the highest stroke incidence of 7.43 per 1000, followed by some cities in mid areas of the country (5.37 per 1000) and the far east (4.41 per 1000) (Gusev 2003; Vilenskiĭ 2006). The rate of recurrence of stroke was 30% (Suslina 2009). Stroke survivors experience serious neurological disorders (loss of vision or speech, or both; paralysis; confusion), and in 30% to 66% of cases these are not restored six months after a stroke (French 2007; French 2016). In Russia, stroke is the primary cause of death and disability in adults: 32 cases per 100,000 population. Twenty-five per cent to 30% of stroke survivors develop dementia by the end of one year. Stroke presents a huge financial burden for the health system (Martynchik 2013). The burden of stroke is projected to rise globally to 61 million DALYs in 2020 (WHO 2019a).

Description of the intervention

Cerebrolysin is a mixture of low-molecular-weight peptides and amino acids derived from porcine brain, and has potential neuroprotective and neurotrophic properties. The manufacturer of Cerebrolysin promotes it for multiple neurological conditions, and it is widely used in the treatment of acute ischaemic stroke in Russia, China, and other Asian, Eastern-European and post-Soviet countries. Cortexin is a Russian-made medicine, positioned by the manufacturer Geropharm as a Cerebrolysin-like agent: a lyophilised extract of cerebral cortex of cattle (cows and pigs), a peptide mixture comprising polypeptides and amino acids, considered to be a bioregulator. It is used in Russia and the Commonwealth of Independent States (CIS) countries.

How the intervention might work

The term 'neuroprotection' is used to describe the putative effect of interventions protecting the brain from pathological damage. In ischaemic stroke, the concept of neuroprotection includes inhibition of pathological molecular events leading to calcium influx, activation of free radical reactions, and cell death. Knowledge of pathophysiology in acute ischaemic stroke stimulated the development of a number of potential neuroprotective agents. Many neuroprotective agents have proven to be efficacious in animal studies. Cerebrolysin is a mixture of low-molecular-weight peptides (80%) and free amino acids (20%) derived from porcine brain, with proposed neuroprotective and neurotrophic properties similar to naturally occurring growth factors such as nerve growth factor and brain-derived neurotrophic factor (Alvarez 2000; Fragoso 2002). In a study that identified 638 unique peptides in Cerebrolysin, none appeared to be related to any known trophic factor or trophic factor precursor, and it was suggested that the active peptides belong to proteins containing hidden functional peptide sequences (Gevaert 2015). Cortexin, similar to Cerebrolysin, is a mixture of 90% oligo- and short-chain peptides and 10% amino acids (Gomazkov 2015). There is no clear understanding of the molecular mechanism of its action (Gulyaeva 2019).

Results of in vitro and animal studies of Cerebrolysin have traditionally been used to suggest its potential for treating acute ischaemic neuronal damage (Masliah 2012). For example, Cerebrolysin has been shown to be effective in tissue culture models of neuronal ischaemia, dose-dependently increasing neuronal survival (Schauer 2006). In brain slices it counteracts necrotic and apoptotic cell death induced by glutamate (Riley 2006). Cerebrolysin also demonstrates neuroprotective activity in rat models of haemorrhagic stroke (Makarenko 2005) and ischaemic stroke (Zhang 2010), as well as in spinal cord trauma (Sapronov 2005). One randomised, double-blind, placebo-controlled trial showed no effect of Cerebrolysin in acute haemorrhagic stroke on chosen efficacy measures including the Barthel Index, Unified Neurological Stroke Scale, and Syndrome Short Test (Bajenaru 2010).

Why it is important to do this review

Despite the effectiveness of neuroprotective agents in animal models of stroke, the results of clinical trials of neuroprotective agents in humans have been disappointing (European Ad Hoc Consensus 1998; Ginsberg 2016; Goenka 2019). Cochrane Reviews of the effects of individual neuroprotective agents and pharmacological groups confirm this (Gandolfo 2002; Muir 2003; Ricci 2012a; TISC 2001). Yet, other means of neuroprotection are being sought. Cerebrolysin is well accepted by Russian, Eastern European, and Asian physicians, and is widely used in the treatment of acute ischaemic stroke and other neurological disorders (Chukanova 2005; Gromova 2006; Onishchenko 2006). Research data from observational studies and clinical trials of Cerebrolysin in acute stroke or head injury, most of which have been performed in Russia and China, have accumulated (Chukanova 2005; Gafurov 2004; Gromova 2006; Ladurner 2005; Skvortsova 2004; Wong 2005).

As assessed in a Cochrane Review for vascular dementia, Cerebrolysin may have positive effects on cognitive function and global function in elderly people with mild to moderate dementia, but the review authors did not recommend it for routine use in vascular dementia owing to the limitations of the studies in the resulting review, small number of included trials, wide variety of treatment durations, short-term follow-up, and high risk of bias of the included studies (Cui 2019). Cerebrolysin has also been proposed as a treatment for people with Alzheimer's disease (Fragoso 2002). Trials of Cerebrolysin in acute haemorrhagic stroke have been assessed in a meta-analysis (Shu 2012), which concluded on its safety and supported implementation of new trials for definitive efficacy assessment.

Previous versions of this Cochrane Review did not find evidence of clinical benefit of Cerebrolysin for treating acute ischaemic stroke (Ziganshina 2010a; Ziganshina 2015; Ziganshina 2016; Ziganshina 2017), and provoked a number of published papers, particularly in Russian language academic media, in favour of using Cerebrolysin for treating acute ischaemic stroke, which we illustrate in the PRISMA flow diagram developed for the 2020 update (Ziganshina 2020). Ziganshina 2017 created heated debate in the journal *Stroke* (Bereczki 2017). However, the debate did not address the challenges of dealing with potential risk of bias in clinical trials, which in our view reflects an important contribution of Cochrane Reviews.

The most recent update provided moderate-certainty evidence of an increase in non-fatal serious adverse events with Cerebrolysin use (Ziganshina 2020). It is important to evaluate the data that have accumulated since then in order to provide better-certainty evidence.

Amongst the English language publications, there is a meta-analysis of nine clinical trials (Bornstein 2018), presenting a critique of the findings of the Cochrane Review (Ziganshina 2017). We critically appraise Bornstein 2018 in the Agreements and disagreements with other studies or reviews in the Discussion section. The last update of this review, Ziganshina 2020, received extensive comments from the manufacturer of Cerebrolysin, all of which are included in the Comments section with our detailed replies. The 2020 update, Ziganshina 2020, was used to inform two joint guidelines from the European Stroke Organisation and the European Academy of Neurology on post-stroke cognitive impairment (Quinn 2021a; Quinn 2021b). Both guidelines advise against Cerebrolysin use.

This interest in and attention to the research question of our Cochrane Review, particularly in view of the debate around reliable evidence (Horton 2019), encouraged us to update the review once again and revisit the question of reliability of evidence.

This review update is particularly pertinent in view of the continuous presence of Cerebrolysin and the Cerebrolysin-like agent, Cortexin, on the national Essential Medicines List of the Russian Federation (GovRu 2019; GovRu 2022). Both peptide mixtures of the cattle cerebral cortex are recommended for use in acute ischaemic stroke by the national clinical practice guidelines of Russia (MinHealthRu 2021). Cerebrolysin is also listed on the national Essential Medicines Lists of Slovakia, Romania, Vietnam, Uganda, and the Syrian Arab Republic (WHO 2019b; WHO 2022), with uses including acute ischaemic stroke.

In this review update we followed all the methodological approaches refined in the previous update, and once again re-assessed our judgements of the risk of bias for uniformity of judgements across all included studies.

Studies reporting on our outcome measures was not an inclusion criterion for this review; changes in the reporting of outcomes in our data synthesis depended on data reported by the authors of eligible included trials in their trial reports.

The aim of this update was to establish whether the new search and inclusion of data from a newly identified trial would affect the conclusions of the former version of the review, in view of the thorough re-assessment of the risk of bias in the included studies through identification, examination, and evaluation of study protocols, and careful data extraction.

OBJECTIVES

To assess the benefits and harms of Cerebrolysin or Cerebrolysin-like agents for treating acute ischaemic stroke.

METHODS

Criteria for considering studies for this review

Types of studies

We included all published randomised controlled trials (RCTs) comparing Cerebrolysin or Cerebrolysin-like agents with placebo or no treatment in people with acute ischaemic stroke. We excluded uncontrolled studies, as well as quasi-RCTs where allocation to treatment or control was not concealed (e.g. allocation by alteration, open random number list, date of birth, day of the week, or hospital number).

Types of participants

People with acute ischaemic stroke, confirmed by neuroimaging, irrespective of age, sex, or social status, whose symptom onset was less than 48 hours previously. Stroke symptoms include: sudden weakness or numbness of the face, arm, or leg, often unilateral; confusion; difficulties in speaking or seeing with one or both eyes; difficulties walking; loss of balance or co-ordination; severe no-cause headache; fainting or loss of consciousness. Confirmation of stroke diagnosis with neuroimaging was not an inclusion criterion for the earlier versions of this review. However, confirmation of stroke diagnosis with neuroimaging is now mandatory, as we stated in the last update.

The condition of interest in this review is acute ischaemic stroke, as defined above, therefore methods to deal with studies that include only a subset of eligible participants are not required, as such studies would not be conducted.

Types of interventions

We compared Cerebrolysin or Cerebrolysin-like agents added to standard treatment against either placebo or no treatment added to standard treatment.

Standard treatment is not defined precisely and differs between studies. Study medication must have been started within 48 hours of onset of stroke and continued for any period of time.

We planned to add a separate analysis for the comparison 'Cerebrolysin versus other neuroprotective agents (peptide mixtures)', but the available studies did not permit this. We identified in the searches for this update a single eligible trial of a newer peptide mixture, which we have termed a 'Cerebrolysin-like agent', a Russian-produced medicine, Cortexin. The trial provided data for Cortexin versus placebo only. We combined outcome data for Cerebrolysin with data for the newer peptide mixture, Cortexin.

Types of outcome measures

We used one primary outcome and six secondary outcomes, with special attention to adverse events and effects.

We were interested in outcomes measured up to 90 days.

Primary outcomes

- All-cause death, to be measured as the number of people who died from the start of tested treatment to the end of the follow-up period.

Secondary outcomes

Poor functional outcome, defined as death or dependence at the end of the follow-up period: various scales, such as the National Institutes of Health Stroke Scale (NIHSS), the modified Rankin Scale (mRS), and the Barthel Scale/Index (BI) can be used to evaluate impairment brought about by stroke. The mRS is commonly used and is a scale from 0 to 6, with 0 being no symptoms; 1, no significant disability; 2, slight disability; 3, moderate disability; 4, moderate to severe disability; 5, severe disability; and 6, death.

- Early death (within two weeks of stroke onset).
- Quality of life, if assessed in the included studies.
- Time to restoration of capacity for work, either as a time-to-event outcome (e.g. analysed as a hazard ratio) or as a continuous outcome, depending on study data.
- Cause of death.
- Non-death attrition. After identifying and evaluating available trial registration protocols, we added this outcome to the previous update (Ziganshina 2020) as a measure not only of attrition per se, but also as a grey zone in the presentation of trial populations, allowing us to characterise attrition and reporting bias better.

Adverse events and effects

A serious adverse event (SAE), as defined according to the International Council for Harmonisation guideline, is "any untoward medical occurrence that, at any dose, results in death, is life-threatening, requires inpatient hospitalisation or results in prolongation of existing hospitalisation, results in persistent or significant disability/incapacity, is a congenital anomaly/birth defect, or is a medically important event or reaction" (ICH 2003). We confirmed the definition of SAE used by researchers and the numbers of people with SAEs in the CASTA 2012 trial through correspondence with the manufacturer of Cerebrolysin and the lead author of this trial, and we extracted data from the CERE-LYSE-1 2012 trial report that used Medical Dictionary for Regulatory Activities (MedDRA) coded SOC (System Organ Class) and Preferred Term (PT) (MedDRA 2011), developed under the auspices of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH 2003).

We used the following outcomes for SAEs:

- Total number of people with SAEs.
- Total number of people with fatal SAEs.
- Total number of people with non-fatal SAEs.
- Total number of people with adverse events.

Search methods for identification of studies

We attempted to identify all relevant trials regardless of language or publication status, and arranged for the translation of relevant papers where necessary.

Electronic searches

We searched the following databases:

- the Cochrane Central Register of Controlled Trials (CENTRAL 2022, Issue 5) (last searched 9 May 2022; Appendix 1);
- MEDLINE Ovid (from 1946; last searched 9 May 2022; Appendix 2);
- Embase Ovid (from 1980; last searched 9 May 2022; Appendix 3);
- Science Citation Index Expanded Indexes and Conference Proceedings Citation Index - Science – Web of Science Core Collection (last searched 9 May 2022; Appendix 4);
- LILACS (Latin American and Caribbean Health Sciences Literature database) (1982 to 7 June 2022; Appendix 5);
- OpenGrey (System for Information on Grey Literature in Europe; www.opengrey.eu; 1980 to 24 October 2019; Appendix 6, used in the previous version of the review);
- the following Russian Databases: e-library (elibrary.ru; 1998 to 7 June 2022) and EastView (online.ebiblioteka.ru/index.jsp; 2006 to 7 June 2022; Appendix 7).

The Cochrane Stroke Information Specialist developed the search strategies for CENTRAL, MEDLINE, Embase, Web of Science indexes, and trial registers. We then adapted the MEDLINE strategy for the additional Russian language databases.

Searching other resources

We also searched the following ongoing trials and research registers:

- US National Institutes of Health Ongoing Trials Register ClinicalTrials.gov (www.clinicaltrials.gov) (last searched 9 May 2022; Appendix 8);
- World Health Organization International Clinical Trials Registry Platform (apps.who.int/trialsearch) (last searched 9 May 2022; Appendix 9);
- Russian State Register of Approved Medicines (grls.rosminzdrav.ru) (last searched 8 June 2022).

In an effort to identify further published, unpublished, and ongoing trials and to obtain additional trial information, we checked the reference lists of all trials identified by the above methods, and searched the following neurology conference proceedings held in Russia: *Chelovek i Lekarstvo* [Man and Medicine] (2019 to 2022), *National'niy congress cardiologov* [The National Congress of Cardiology] (2019 to 2022), *XI Vserossiyskiy s'ezd nevrologov i IV kongress Natsional'noy assotsiatsii po bor'be s insultom* [XI All-Russian Congress of Neurologists and IV Congress of the National Stroke Association] (2019), *Mezhdunarodniy kongress "Neiroreabilitatsiya-16"* [International Congress "Neurorehabilitation-16"] (2019-2022).

For this update we did not contact the pharmaceutical company EVER Neuro Pharma GmbH, the manufacturer of Cerebrolysin, because we did not identify any new trials of Cerebrolysin. We did not contact the pharmaceutical company Geropharm, the manufacturer of Cortexin, because we included only one trial, which had duplicate publications, and in neither of them did the authors refer in any form to the manufacturer of Cortexin.

We cross-referenced all studies included in this review with Retraction Watch (both the Retraction Watch site and the Retraction Watch Database); last searched June 2022; Appendix 10).

Data collection and analysis

Selection of studies

All review authors (LEZ, DN, KI and TRA) independently examined the titles and abstracts of records from the electronic searches and excluded those studies that were obviously irrelevant. We used the results of our work in Covidence for the previous version of the review, and added the newly identified RCT (Figure 1). We obtained the full texts of all eligible papers, and the same review authors independently selected studies for inclusion based on the predetermined inclusion criteria refined for the last update. Any disagreements were resolved through discussion. We excluded studies that did not meet the inclusion criteria, providing reasons for their exclusion in the Characteristics of excluded studies table.

Figure 1. Study flow diagram

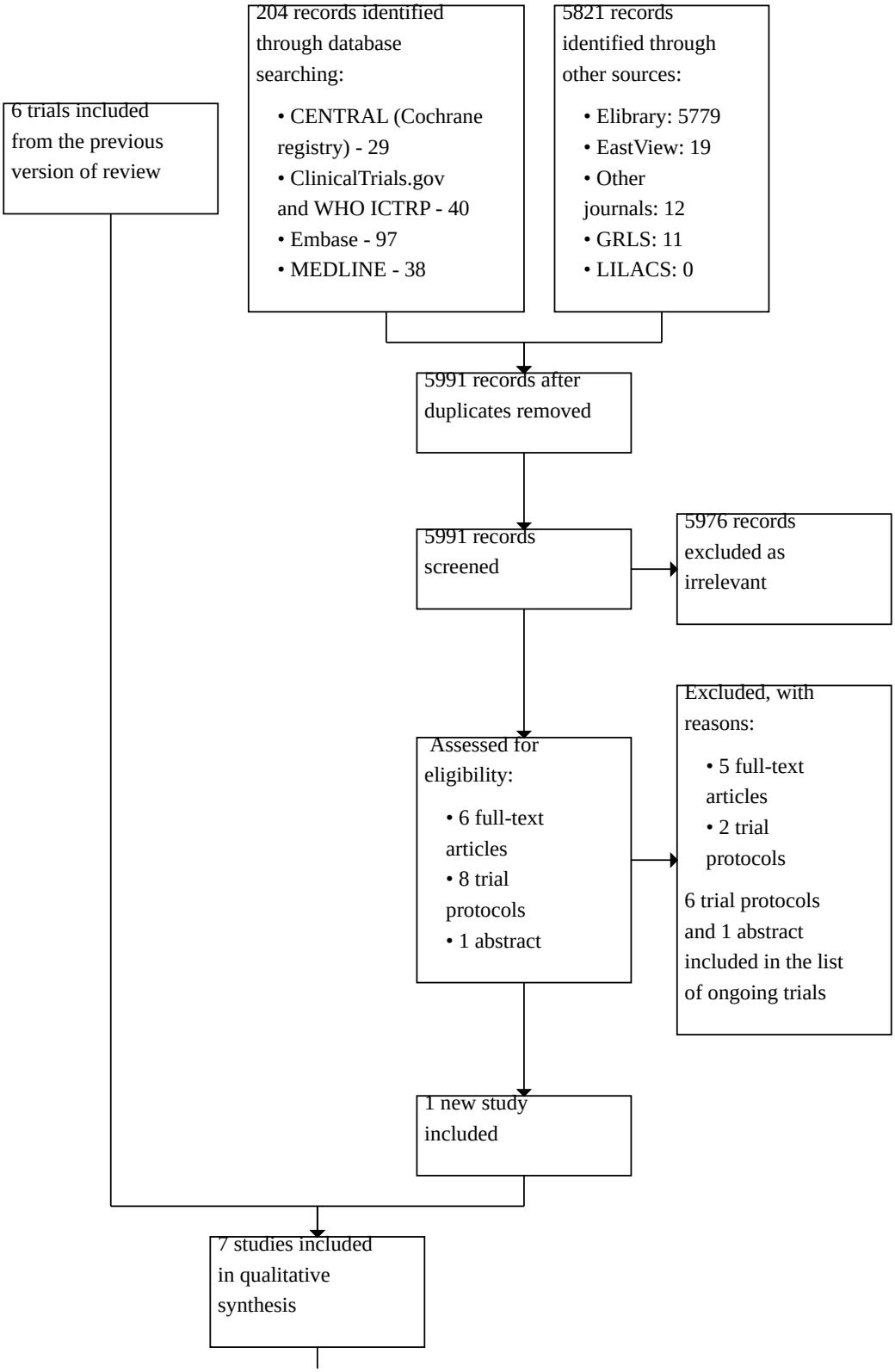
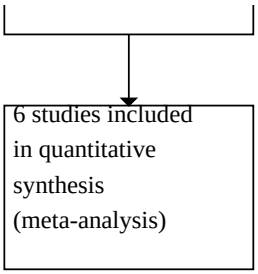


Figure 1. (Continued)



Data extraction and management

All review authors (LEZ, DN, KI, and TRA) independently extracted data on the methods of the studies, participants, interventions, and outcomes. We resolved any differences in the extracted data by referring to the original articles and through discussion. We extracted data to allow an intention-to-treat (ITT) analysis (including all participants in the groups to which they had been randomly allocated). We used these data for the outcome 'all-cause death' in the worst-/best-case analyses we used as sensitivity analyses. We presented the data in the Characteristics of included studies table, generated by Covidence. For all included trials we calculated the percentage loss to follow-up and presented this information in the risk of bias tables.

For binary outcomes, we extracted the number of participants with the event in each group. For continuous outcomes, we planned to use arithmetic means and standard deviations for each group.

Assessment of risk of bias in included studies

Three review authors (DN, KI and LEZ) independently evaluated the methodological quality of studies with regard to the generation of allocation sequence, allocation concealment, blinding, loss to follow-up, and other risk of bias using the Cochrane risk of bias assessment tool (Higgins 2011).

We followed the Cochrane guidance to assess whether adequate steps had been taken to reduce the risk of bias across seven domains: generation of allocation sequence; allocation concealment; blinding of participants and personnel; blinding of outcome assessors; incomplete outcome data (attrition bias); selective outcome reporting; and other sources of bias. We assigned judgements of 'low', 'high', or 'unclear' risk of bias for these domains. We considered loss to follow-up to be acceptable (low risk of bias) if it was less than 10%. We thoroughly re-assessed risk of bias for all included studies across all domains to ensure uniformity of our judgements.

In this update we used the same approach for risk of bias assessment that was re-evaluated for the previous update. We added consideration of public availability of study protocols and reported outcomes to the selective outcome reporting judgement through identification, examination, and evaluation of study protocols.

For the assessment of other sources of bias, we evaluated how study authors described funding sources for their trials and how conflict of interest statements were presented, if presented at all.

We judged the risk of bias to be high in cases of clear sponsorship by the manufacturers of Cerebrolysin or Cortexin, involvement of the manufacturer with trial planning and design, sequence generation, medication provision, statistical procedures, blinding of personnel and outcome assessors, and involvement in reporting, as well as in cases of declared relationship of study authors to the manufacturer of Cerebrolysin. Where there was no mention of funding sources and there were no conflict of interest statements, we judged the risk of bias to be unclear.

We resolved any disagreements arising at any stage by discussion.

We planned to use funnel plots to examine asymmetry, which may be caused by publication bias or heterogeneity.

Measures of treatment effect

We presented dichotomous data and combined them using risk ratios (RRs). We showed RRs accompanied by 95% confidence intervals (CIs). We planned to present continuous outcomes, if identified, as means accompanied by standard deviations (SDs) or as the standardised mean difference (SMD).

Unit of analysis issues

We only included studies that randomised individual participants. We did not identify in the searches any cluster-randomised or cross-over trials, and we did not have multiple time points.

For studies with multiple groups we split the 'shared' group into two or more groups with smaller sample sizes, and included two or more (reasonably independent) comparisons.

Non-standard study designs such as cluster-RCTs and cross-over trials would be inapplicable owing to the nature of the condition of interest of this review. Acute ischaemic stroke is an emergency condition and the eligibility criteria for this review specify participants with stroke symptom onset less than 48 hours before starting study medicines.

Dealing with missing data

Where data from the trial reports were insufficient, unclear, or missing, we attempted to contact the trial authors for additional information. We aimed to carry out an intention-to-treat (ITT) analysis, but as there were missing data we did a complete case analysis (i.e. including all patients with a measured outcome as per trial authors). The complete case analysis does not make an assumption about the outcome of missing patients.

We explored the potential effects of missing data through a series of sensitivity analyses (Table 1). As a sensitivity analysis, we did a best-/worst-case analysis. The best-case analysis assumed missing patients had a positive outcome (survived acute ischaemic stroke); the worst-case analysis assumed they had a negative outcome (died). We conducted a sensitivity analysis that aimed to restore the integrity of the randomisation process (as is usual in trial analysis) and test the robustness of the results to this methodology. For a summary of the methodology and sensitivity analysis see Table 1.

Assessment of heterogeneity

We tested for heterogeneity of effect sizes between studies by inspecting the forest plots and using the I^2 statistic (Higgins 2003), considering a value of 30% to 60% as denoting moderate levels of heterogeneity (Deeks 2011). If there was clinical heterogeneity, we planned to explore it in subgroup analysis if the amount of data permitted or to describe results narratively rather than pooling heterogeneous data.

Assessment of reporting biases

If there was a sufficient number of studies (10 or more), we planned to use funnel plots to examine asymmetry that may have been caused by publication bias or heterogeneity.

We compared the outcomes predefined in study protocols with those reported in the published manuscripts to detect potential selective reporting.

Data synthesis

We used the ITT principle for data synthesis. We used RevMan Web to analyse the data. We used the RR as the measure of effect for binary outcomes, and we used a fixed-effect model for pooling the data in cases of no or a low level of heterogeneity.

Where we detected heterogeneity (forest plot inspection and an I^2 statistic > 30%), and it was still appropriate to pool the data, we used the random-effects model.

We used and presented 95% CIs for the RRs of all studied outcomes.

Subgroup analysis and investigation of heterogeneity

We investigated potential sources of heterogeneity for all outcomes using the following criteria for subgroups.

- Cerebrolysin or Cortexin dose.
- Length of treatment.

We identified the following subgroups by Cerebrolysin dose and the length of treatment.

- 30 mL for 10 days: cumulative dose 300 mL over 10 days.
- 50 mL for 21 days: cumulative dose 1050 mL over 21 days.
- 10 mL and 50 mL for 10 days: cumulative dose 100 mL and 500 mL over 10 days.

We identified the following subgroups by Cortexin dose and the length of treatment.

- 20 mg for 10 days, 10 days rest, then 20 mg for 10 days: cumulative dose 400 mg over 20 days (200 mg over 10 days - rest - 200 mg over 10 days).
- 20 mg for 10 days, 10 days rest, then placebo for 10 days: cumulative dose 200 mg over 10 days.

Sensitivity analysis

We performed a sensitivity analysis to test the robustness of the results for the outcome all-cause death. We explored the effect of missing data by carrying out a best-/worst-case analysis (Table 1). We investigated the effect of methodological study quality ('low', 'high', or 'unclear' risk of bias) by comparing the results of studies with low and unclear risk of bias with no losses to follow-up (no attrition) to the results of studies with high risk of bias for selective outcome reporting (attrition bias). We compared the results obtained with the use of either a fixed-effect or random-effects model to test the robustness of the results.

Summary of findings and assessment of the certainty of the evidence

We used the GRADE approach to interpret findings (Schünemann 2011). We employed GRADEpro GDT, and imported data from Review Manager 5 to create Summary of findings 1 for the following outcomes: the primary outcome of all-cause death at the end of the follow-up period; the total number of people with SAEs at the end of the follow-up period, comprising fatal and non-fatal SAEs, and a subgroup by Cerebrolysin dose and length of treatment, at the end of the follow-up period (follow-up period in the included studies varied from 28 days (four weeks) to 90 days); the total number of people with adverse events at the end of the follow-up period; non-death attrition; death or dependence at the end of the follow-up period; early death (within two weeks of stroke onset); and quality of life (Review Manager 2014).

Summary of findings 1 includes information on the overall certainty of the evidence from the trials and information of importance for healthcare decision-making. The GRADE approach determines the certainty of the evidence based on an evaluation of eight criteria (risk of bias, inconsistency, indirectness, imprecision, publication bias, effect size, presence of plausible confounding that will change effect, and dose-response gradient). We used the criteria of risk of bias, inconsistency, indirectness, and imprecision to guide our conclusions and recommendations.

RESULTS

Description of studies

We report here on seven trials, which met the inclusion criteria, and how we identified these trials.

Results of the search

In the new searches we identified:

- 204 records through database and trial registration platform searches, out of which 172 were left after duplicate removal;
- 5821 records through Russian database searches (two duplicates were removed);
- nothing through our search of LILACS;
- nothing through Retraction Watch.

The OpenGrey database was not available for the current search.

After duplicate removal, we screened 5991 records and excluded 5976 as irrelevant.

We assessed 15 records for eligibility (six full-text articles, eight trial protocols, and one abstract).

We excluded (with reasons) seven studies, which were presented in nine publications (six full-text articles, three trial protocols), which we grouped in the Excluded studies section. Reasons for the exclusion of studies are shown in Characteristics of excluded studies.

Among the remaining six records, five studies were included in the list of ongoing trials.

We included one new study. Thus, seven studies in total are included in the qualitative synthesis and six studies are included in the quantitative synthesis.

For details, see Characteristics of included studies and Characteristics of ongoing studies.

The results of the search are illustrated in the study flow diagram (Figure 1). We designed an additional table for ongoing trials identified through clinical trials registries searches (Table 2). This demonstrates the intensity of clinical research in the field of potential use of peptide mixtures in people with acute ischaemic stroke, despite the fact that we advocate for no more trials of Cerebrolysin or Cerebrolysin-like agents as it is unethical for patients to be recruited into a study without potential benefit, as found in the previous update of this review (Ziganshina 2020).

Included studies

Seven trials met the published inclusion criteria.

Amiri Nikpour 2014 was performed in the Islamic Republic of Iran. The trial compared Cerebrolysin with placebo (normal saline) in 46 people (23 participants in each group) with acute ischaemic stroke confirmed by computed tomography (CT) scan or magnetic resonance imaging (MRI), or both. Cerebrolysin was started within 24 hours of stroke onset and continued for 10 days as a once-daily intravenous infusion of 30 mL in addition to standard treatment of 100 mg of aspirin daily. The average age of trial participants was 60 years. There were no significant differences between the two groups in terms of baseline characteristics. The duration of follow-up was 90 days; one participant in the Cerebrolysin group and two participants in the placebo group died within 30 days of trial initiation. The causes of death were not reported; these three people were excluded from the final analyses. The study protocol is not publicly available, and there is no mention of a study protocol in the text of the published trial report. The study authors reported the results of the trial in two publications (Amiri Nikpour 2014).

CASTA 2012 was a multicentre, placebo-controlled trial performed in four countries: China, Hong Kong, South Korea, and Myanmar. The trial compared Cerebrolysin with placebo added to standard baseline therapy in 1070 people with acute ischaemic stroke confirmed with CT or MRI results compatible with a clinical diagnosis of acute hemispheric stroke (529 participants in the Cerebrolysin group and 541 participants in the control group). Cerebrolysin was started within 12 hours of stroke onset and continued for 10 days as a once-daily intravenous infusion of 30 mL diluted in saline (total of 100 mL) in addition to standard treatment of 100 mg of aspirin daily. Placebo was 100 mL saline as a daily intravenous infusion for 10 days starting within 12 hours of stroke onset. The average age of the trial participants was 65 years. The duration of follow-up was 90 days; 162 participants were lost to follow-up (15%). There were differences between the two groups in terms of baseline prognostic variables, having more people with chronic diseases in the placebo group than in the Cerebrolysin group, 293 versus 251 (55% versus 46% of randomised participants). There were more people with diabetes, 117 (21.7%) versus 108 (20.5%); arrhythmia, 90 (16.7%) versus 71 (13.5%); and coronary heart disease, 86 (16.0%) versus 72 (13.7%) in the placebo group compared to the Cerebrolysin group. The trial was supported by the manufacturer of Cerebrolysin, EVER Neuro Pharma GmbH. The study authors reported the results of the trial in five publications (CASTA 2012 with the protocol registered at ClinicalTrials.gov (NCT00868283) and published as a separate paper (Hong 2009), both retrospectively).

CERE-LYSE-1 2012 was a multicentre, placebo-controlled trial performed in five countries: Austria, Croatia, the Czech Republic, Slovakia, and Slovenia. The trial compared Cerebrolysin with placebo in 119 people (60 in the Cerebrolysin group and 59 in the control group) with acute hemispheric ischaemic stroke after exclusion of brain haemorrhage by CT. Cerebrolysin was started within two hours of stroke onset and continued for 10 consecutive days as a once-daily intravenous infusion of 30 mL mixed with 70 mL of normal saline (total volume 100 mL over a time period of 30 minutes), starting immediately one hour after thrombolytic treatment (alteplase). The placebo consisted of 100 mL normal saline. The average age of the trial participants was 66 years. There were no significant differences between treatment groups in terms of baseline prognostic variables. The duration of follow-up was 90 days, and 19 participants of 119 (16%) were lost to follow-up. The study authors did not report any information on funding sources of the trial, including provision of Cerebrolysin. The statistician of the study was contracted by EVER Neuro Pharma GmbH, the manufacturer of Cerebrolysin. The study authors reported the results of the trial in one publication (CERE-LYSE-1 2012), with the protocol registered at ClinicalTrials.gov retrospectively (NCT00840671).

Cortexin-Shamalov 2014 was a randomised, multicentre, prospective, double-blind, placebo-controlled trial performed in Russia. The study compared Cortexin with placebo in 272 people with acute ischaemic stroke in the basin of the internal carotid artery, after exclusion of brain haemorrhage by CT or MRI. Cortexin was started within 24 hours of stroke onset. Patients were randomised into three groups. The first group (136 participants) was treated with Cortexin at a dose of 10 mg two times a day (morning and afternoon) for 10 days; after a 10-day break the same course of treatment was repeated. The authors did not provide any information on the standard baseline therapy. The second group (72 participants) received Cortexin during the first 10 days of the onset of stroke at a dose of 10 mg two times a day (morning and afternoon), then placebo for 10 days after a 10-day break. The third group (64 participants) received placebo in two 10-day courses with a 10-day break between them. The average age of the trial participants was 62 years. The authors reported a lower incidence of hypercholesterolaemia in the Cortexin + Cortexin group compared to patients in the Cortexin + placebo group. Other baseline characteristics were not significantly different between the groups. The median NIH score at admission in all groups was 6 (the mean NIH score was 7.03 in the Cortexin group, 7.68 in the Cortexin + placebo group, and 7.94 in the placebo + placebo group), which is lower than in most of the included studies of

Cerebrolysin (Table 3). The duration of follow-up was 60 to 70 days (two months). The authors reported no losses to follow-up. There were seven deaths in the Cortexin groups (4/136 and 3/72), no deaths in the placebo group and no other losses to follow-up (non-death attrition). The authors did not provide any information on funding sources for the trial or a conflict of interest statement. The study authors reported the results of the trial in two publications (Cortexin-Shamalov 2014). We did not find a trial protocol, either published or registered with any of trial registration databases. The study contributed data only on three outcomes of interest in this review: all-cause death, total number of people with adverse events, and non-death attrition.

Ladurner 2005 was a multicentre, placebo-controlled trial conducted in Austria, the Czech Republic, and Hungary. The trial compared Cerebrolysin with placebo (100 mL normal saline) added to standard baseline therapy in 146 people with acute ischaemic stroke with clinical symptoms of the middle cerebral artery area after exclusion of brain haemorrhage by CT. Cerebrolysin (50 mL mixed with 50 mL of normal saline) and placebo were started within 24 hours of stroke onset and continued for 21 days as a once-daily intravenous infusion over a period of 20 minutes. The same basic therapy was used in the treatment group and the control group (pentoxifylline and acetylsalicylic acid): Cerebrolysin plus basic therapy, 78 participants and placebo plus basic therapy, 68 participants. The average age of the trial participants was 65 years. The duration of follow-up was 90 days. Twenty-five participants (17%) were lost to follow-up, nine in the treatment group and 16 in the control group. There were no significant differences between the two groups in terms of baseline characteristics. The trial was supported by the manufacturer of Cerebrolysin, EVER Neuro Pharma GmbH, who also provided the study centres with Cerebrolysin. The study authors reported the results of the trial in three publications (Ladurner 2005).

Skvortsova 2004 was performed in Russia. The trial compared Cerebrolysin with placebo added to standard baseline therapy in 36 people with acute ischaemic stroke in the territory of the internal carotid artery, confirmed by CT or MRI. Cerebrolysin was started within 12 hours of stroke onset and was continued for 10 days as a once-daily intravenous infusion of either 10 mL or 50 mL. There were three groups, 12 participants in each, treated with 10 mL Cerebrolysin, 50 mL Cerebrolysin, or placebo. Standard baseline therapy consisted of aspirin 100 mg per day, haemodilution, pentoxifylline, and heparin (when needed). There were no significant differences in baseline characteristics between groups. The average age of the trial participants was 69 years. The duration of follow-up was 30 days, and there were no losses to follow-up. No information on funding sources for the trial and no conflict of interest statements were provided. The study authors reported the results of the trial in three publications (Skvortsova 2004).

Xue 2016 was performed in China. The trial compared Cerebrolysin with placebo and another neuroprotective agent (DL-3-n-butylphthalide; NBP) in 60 people with acute ischaemic stroke, confirmed by CT or MRI (20 participants each). There were no significant differences in baseline characteristics between the Cerebrolysin and placebo groups. Cerebrolysin was administered for 10 days as a once-daily intravenous infusion of 30 mL mixed with 70 mL of normal saline; the infusions lasted for 50 to 70 minutes. Participants in the control group received intravenous infusions of 100 mL of normal saline, whilst the Cerebrolysin group received an intravenous infusion of 100 mL of 25 mg NBP in normal saline, twice daily for 10 days starting within 12 hours after stroke onset. Standard baseline therapy consisted of antithrombotics, hypoglycaemics, antilipaemic agents, antihypertensives, and dehydration, according to local current guidelines for the management of ischaemic stroke in neurological intensive care units, and 100 mg aspirin orally. The duration of follow-up was 90 days. The study authors reported the results of the trial in one publication (Xue 2016), with the protocol registered at ClinicalTrials.gov retrospectively (NCT02149875).

For details of the included trials, see Characteristics of included studies.

There are no trials awaiting classification.

Excluded studies

In this update of the review we present only the results of the latest search. We excluded one study from the previous list of included trials as not meeting the updated eligibility criteria (Gharagozli 2017). For details of all studies found, screened, and excluded since the first publication (Ziganshina 2010a), please see the previous versions of the review in the version history section, which serves as one particular source of studies (Ziganshina 2020).

With the current search we excluded seven studies reported in nine publications/records, because of:

- ineligible study design, including lack of randomisation or control arm;
- ineligible patient population, including participants with treatment initiation exceeding the protocol-specified 48 hours after stroke onset and stroke diagnosis not confirmed by neuroimaging.

The reasons for exclusion of these studies are detailed in the Characteristics of excluded studies table.

Risk of bias in included studies

Seven RCTs met the inclusion criteria.

Allocation

For sequence generation, we judged one trial to be at low risk of bias (Ladurner 2005), and six trials to be at unclear risk of bias because the study authors did not provide any information on sequence generation (Amiri Nikpour 2014; CASTA 2012; CERE-LYSE-1 2012; Cortexin-Shamalov 2014; Skvortsova 2004; Xue 2016).

In Ladurner 2005, the manufacturer of Cerebrolysin, EVER Neuro Pharma GmbH, provided the randomisation method, which was a computer-generated randomisation code; we judged this to fit the criteria for low risk of bias. However, we noted the direct involvement of EVER Neuro Pharma with regard to the randomisation codes and the unavailability of the study protocol.

In Amiri Nikpour 2014 and Skvortsova 2004, no information was provided on sequence generation procedures which, combined with the unavailability of a study protocol, resulted in a judgement of unclear risk of bias.

We carefully reviewed the published protocol of the CASTA 2012 study, which was published retrospectively to participant enrolment as Hong 2009, and did not find a description of the procedure for sequence generation, resulting in a judgement of unclear risk of bias.

In CERE-LYSE-1 2012, the described procedure for sequence generation did not fit the criteria for an assessment of low risk of bias. There was no information about the actual process of generation of a randomisation sequence. In addition, there was a retrospective protocol registration and a statistician contracted by the manufacturer of Cerebrolysin, EVER Neuro Pharma, resulting in a judgement of unclear risk of bias.

In Xue 2016, the sequence generation was performed with computer-generated numbers by a third party; however, it was unclear who the third party was and this, together with the retrospective nature of the trial registration, resulted in a judgement of unclear risk of bias.

In Cortexin-Shamalov 2014, the authors used simple randomisation, however they did not provide details of the sequence generation method. Combined with an unavailable registered study protocol, this resulted in a judgement of unclear risk of bias.

For allocation concealment, we judged one trial to be at low risk of bias because they used identical vials (CERE-LYSE-1 2012), and the remaining six included trials to be at unclear risk of bias because the study authors did not provide a clear description of concealment. The exception was Ladurner 2005, in which the trial authors used sealed envelopes with information on the actual treatment dispensed, and provided these envelopes to the investigator in case of emergency. The published report describes how all envelopes remained sealed throughout the study. However, as the trial authors did not describe the envelopes as opaque, and the trial protocol was unavailable, we judged Ladurner 2005 to be at unclear risk of bias for allocation concealment.

Blinding

For blinding of participants and personnel (performance bias), we judged three trials to be at low risk of bias (CASTA 2012; CERE-LYSE-1 2012; Ladurner 2005), and the remaining four trials, which did not provide clear information on blinding, as at unclear risk of bias (Amiri Nikpour 2014; Cortexin-Shamalov 2014; Skvortsova 2004; Xue 2016). For blinding of outcome assessors (detection bias), we judged three studies to be at low risk of bias (CASTA 2012; CERE-LYSE-1 2012; Ladurner 2005), and the remaining four studies to have an unclear risk of bias owing to no or insufficient information to judge low or high risk of bias (Amiri Nikpour 2014; Cortexin-Shamalov 2014; Skvortsova 2004; Xue 2016).

Incomplete outcome data

Amiri Nikpour 2014, Cortexin-Shamalov 2014, and Skvortsova 2004 reported no losses to follow-up and we therefore judged them as having a low risk of attrition bias. The four remaining studies all reported participant losses in excess of 10% (between 16% and 29%, Table 4), and we therefore judged them to be at high risk of attrition bias (CASTA 2012; CERE-LYSE-1 2012; Ladurner 2005; Xue 2016). According to publicly available information, all trials included in this meta-analysis received either unclear or considerable support from the pharmaceutical company that manufactures Cerebrolysin. We judged two studies to be at high risk of other bias owing to the direct involvement of the manufacturer (CASTA 2012; CERE-LYSE-1 2012).

The authors of CERE-LYSE-1 2012 used the 'last observation carried forward' (LOCF) method for their National Institutes of Health Stroke Scale (NIHSS) analysis to fill in their missing data points. There was a 16% loss of participants, but there is no indication as to when these participants were lost, nor for any of the time points is there any indication as to when or how many virtual (i.e. imputed) data were used. It is well understood that using LOCF can introduce bias that may exaggerate the effectiveness of a drug (Molnar 2008; Salim 2008): "The only condition where LOCF is unbiased is when the missing data occurs completely by chance and the data used as the basis for the LOCF imputation has exactly the same distribution as does the unknown missing data. Since it can never be proven that these distributions are exactly the same, all LOCF analyses are suspect and should be dismissed" (Lachin 2016). LOCF provides biased results and its use is to be deprecated (Lachin 2016; Molnar 2008; Salim 2008).

Ladurner 2005 also applied LOCF analysis. In this study, 146 participants were randomised, of whom 119 completed the study; 27 participants were therefore lost to follow-up, but the study authors state that there were only 25 cases lost. Either way, this is a 17% to 18% loss, greater than the 10% that we would find acceptable. The trial authors studied six time points but are silent as to which time points include virtual data or how much virtual data, claiming a complete cohort of N = 146 (despite losing 25 or 27 participants).

Xue 2016 was the only study that compared Cerebrolysin and another neuroprotective agent (NBP). There were 84 participants at the trial initiation; however, data are presented for only 60 participants (20 participants in each of the three comparison groups) without any explanation for the loss of 24 participants (29% attrition). We could not include any data from this study in the quantitative synthesis.

Selective reporting

We judged the risk of bias for selective outcome reporting to be unclear for all seven included studies.

For four studies there were no protocols in the public domain, with no mention of protocols in the texts of the reports (Amiri Nikpour 2014; Cortexin-Shamalov 2014; Ladurner 2005; Skvortsova 2004). This made it impossible to assess whether the study authors had reported on all of their predefined outcomes. Three studies published their protocols retrospectively (CASTA 2012; CERE-LYSE-1 2012; Xue 2016).

The study protocol for CASTA 2012 was available, and all of the prespecified (primary and secondary) outcomes, which were of interest to the review, were reported accordingly. However, the study authors did not describe the causes of the deaths, and the Kaplan-Meier mortality curve presented only the subgroup of trial participants with an NIHSS score greater than 12. We judged this study to be at an unclear risk of reporting bias. In their 'Analyses of Mortality', the study authors declared 28 and 32 deaths in the Cerebrolysin and placebo groups, respectively. The hazard ratio is given as 1.26 with a probability of 0.19. The study authors describe this as showing "a small superiority for the Cerebrolysin group". At

this level of probability these data show nothing except that there is no difference between groups. Elsewhere in the study the authors claim that probabilities of 0.16 and 0.28 provide evidence in favour of Cerebrolysin in the treatment of ischaemic stroke. The study authors used NIHSS scores and stratified the participants according to scores > 12 and ≤ 12. In their > 12 group, of 252 participants, 12 and 22 Cerebrolysin- and placebo-treated participants died, respectively, with a hazard ratio of 1.9661 and a probability of 0.02485 (notably quoted to five decimal places). It should be noted that among the remaining 815 participants in the ≤ 12 group, 16 and 10 participants in the Cerebrolysin and placebo groups died, respectively. The study authors do not report how many participants were treated with Cerebrolysin or placebo in either the > 12 group or the ≤ 12 group to permit calculation of a hazard ratio, but even so, in a hugely larger number of participants, there is a result that does not favour Cerebrolysin, about which the study authors are silent.

Ladurner 2005 did not report on the time when the deaths of participants in their trial occurred, and did not assess potential causality with administered medicines. Using the ITT principle, we compared the number of deaths extracted from the safety section of the trial report and presented data as all-cause death.

Skvortsova 2004 described the causes of deaths (pulmonary embolism, pneumonia, pyelonephritis, and brainstem syndrome secondary to the brain oedema), but without a precise indication of the time when the deaths occurred and a clear indication as to which study group the participants belonged, nor the confirmed cause of death. The study authors did not report on adverse events. The timing of the outcomes presented in a table and a graph in the publication was also unclear.

Cortexin-Shamalov 2014 reported four deaths among participants who received two courses of Cortexin (4/136), and three deaths in the group of people who received one course of Cortexin and one course of placebo (3/72). There were no deaths in the placebo group (0/64). The authors described the causes of deaths in one of the two identified publications of the trial results: repeated stroke (two), pulmonary embolism (one), polysegmental pneumonia (one) in the Cortexin + Cortexin group; acute intestinal obstruction (one), sudden death (two) in the Cortexin + placebo group. The authors did not indicate the time when the deaths occurred. In all cases, according to the researchers, the deaths were not associated with the study drug. The authors reported the following numbers of adverse events between the groups - 20/136 (14.7%) in the Cortexin + placebo group, 11/72 (15.3%) in the Cortexin + placebo group, and 7/64 (10.9%) in the placebo group. The authors presented their results on post-stroke functioning using three approaches: Rankin scale, Bartel and Rivermead indices without providing baseline data, and presenting their data only in graphs, which do not allow any extraction of either binary or continuous functional outcomes. We contacted the corresponding author asking for clarification, but did not receive any reply. We made a judgement of unclear risk of bias for selective outcome reporting.

Other potential sources of bias

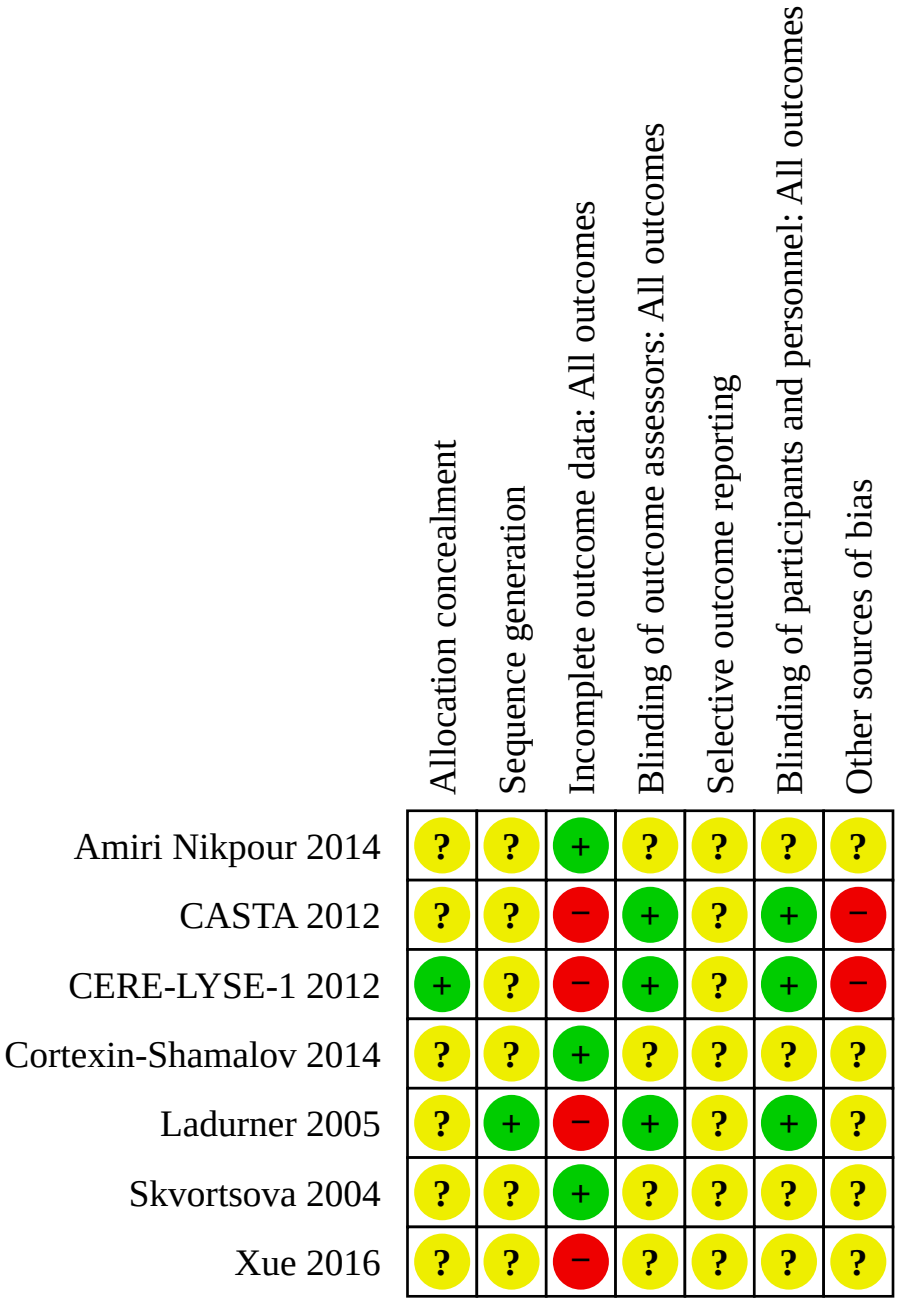
CERE-LYSE-1 2012 was stopped because no significant result for the main study outcome was reached.

We did not identify any protocol, published or registered, for the trial of the Cerebrolysin-like agent Cortexin, and the authors did not provide any information on conflicts of interest (Cortexin-Shamalov 2014).

In both trials, according to the study authors, there was no causal relationship between Cerebrolysin or the Cerebrolysin-like agent Cortexin and any of the deaths observed (CERE-LYSE-1 2012; Cortexin-Shamalov 2014). Neither the reasons for nor the timing of the deaths was presented. The timing of adverse events and serious adverse events was also not presented. For details, see the risk of bias section of the Characteristics of included studies table.

These judgements are illustrated in the risk of bias summary plot (Figure 2).

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



Effects of interventions

See: **Summary of findings 1** Cerebrolysin or Cerebrolysin-like agents compared to placebo for acute ischaemic stroke

Primary outcomes

All-cause death

The included studies reported on the numbers of deaths in various sections of their trial reports, including in the description of adverse events. We used these data on the numbers of deaths in the

comparison groups to generate the primary outcome of all-cause death.

Compared to placebo Cerebrolysin probably results in little to no difference in all-cause death: there were 46 deaths in the 714 Cerebrolysin-treated participants and 47 deaths in the 703 placebo-treated participants: risk ratio (RR) 0.91, 95% confidence interval (CI) 0.61 to 1.34 (5 trials, 1417 participants; moderate-certainty evidence). The I^2 statistic revealed no heterogeneity: I^2 = 0% (Analysis 1.1).

With the addition of the newly identified trial of the Cerebrolysin-like agent Cortexin, we still found no difference in all-cause death between the peptide mixture groups and placebo: there were 53 deaths in the 922 peptide-treated participants and 47 deaths in the 767 placebo-treated participants (RR 0.96, 95% CI 0.65 to 1.41; 6 trials, 1689 participants; moderate-certainty evidence). The I^2 statistic revealed no heterogeneity: I^2 = 0% (Analysis 1.1). Sensitivity analysis for worst-case and best-case scenarios did not alter the finding of no difference (6 trials, 1689 participants; moderate-certainty evidence; Analysis 2.1; Analysis 2.2; Analysis 2.3).

Secondary outcomes

None of the included trials reported on the following clinically important secondary outcomes: poor functional outcome (defined as death or dependence at the end of the follow-up period), early death (within two weeks of stroke onset), quality of life, or the time to restoration of capacity for work.

Cause of death

Amiri Nikpour 2014: the causes of death are not described; the authors only mention that one participant in the Cerebrolysin group and two participants in the placebo group died before day 30. The study authors excluded these three participants from their final analysis; we used these data for the all-cause death assessment.

CASTA 2012: 28/529 participants randomised to the Cerebrolysin group and 32/541 participants randomised to the placebo group died. The study authors described neither the causes of death nor the times when the deaths occurred.

CERE-LYSE-1 2012: four participants died in each group: 4/60 in the Cerebrolysin group and 4/59 in the placebo group. The study authors described neither the causes of death nor the times when the deaths occurred, and did not find any relationship in any of the cases to the study medication.

Cortexin-Shamalov 2014: four out of 136 participants randomised to the first group (Cortexin + Cortexin) and three out of 72 participants randomised to the Cortexin + placebo group died. There were no deaths in the placebo + placebo group (0/64). The study authors reported the following causes of death: repeated stroke (two), pulmonary embolism (one), and polysegmental pneumonia (one) in the Cortexin + Cortexin group; acute intestinal obstruction (one) and sudden death (two) in the Cortexin + placebo group. The authors did not report on the time when the deaths occurred.

Ladurner 2005: 6/78 participants in the Cerebrolysin group and 6/68 participants in the placebo group died. The study authors reported on the following causes of death: cerebral infarct (four in the Cerebrolysin group and two in the placebo group), heart failure (two in the Cerebrolysin group and one in the placebo group), pulmonary embolism (two in the placebo group), and pneumonia (one in the placebo group). The trial authors did not report on the times when the deaths occurred.

Skvortsova 2004: the study authors described the causes of death. They reported the following causes of death not attributed to the stroke: pulmonary embolism, pneumonia, and pyelonephritis in three participants in the Cerebrolysin group and one in the placebo group (not clear which of these), and the causes of death associated with the stroke: brain oedema with secondary brainstem syndrome, which occurred in two participants in both the Cerebrolysin and placebo groups. The deaths occurred within 30 days after the stroke onset, but the study authors did not report precisely on the time of each death. It was unclear to which Cerebrolysin subgroup by dose these participants belonged, 10 mL or 50 mL.

Xue 2016: one death occurred in the DL-3-n-butylphthalide (NBP) group.

Non-death attrition

We included all six studies that provided numerical results for analysis of non-death attrition (Amiri Nikpour 2014; CASTA 2012; CERE-LYSE-1 2012; Cortexin-Shamalov 2014; Ladurner 2005; Skvortsova 2004). In the Cerebrolysin only subset of data, we found that Cerebrolysin or similar peptide mixtures may result in little to no difference in non-death attrition, but the evidence is very uncertain: 80 out of 714 Cerebrolysin-treated participants and 111 out of 703 placebo-treated participants were lost to follow-up for reasons other than death (RR 0.72, 95% CI 0.38 to 1.39; 5 trials, 1417 participants; Analysis 1.2). Combining the Cerebrolysin data with the new Cortexin-Shamalov 2014 study data did not change the results since the study reported no loss to follow-up (RR 0.72, 95% CI 0.38 to 1.39; 6 trials, 1689 participants; very low-certainty evidence) (Analysis 1.2). Three studies reported no loss to follow-up (no non-death attrition) (Amiri Nikpour 2014; Cortexin-Shamalov 2014; Skvortsova 2004). There were substantial differences amongst the trials when grouped by Cerebrolysin dose regimen (subgroup differences: P = 0.006) and there was a considerable level of heterogeneity (I^2 = 76%).

One study stands out in this analysis. In Ladurner 2005, we found a lower rate of non-death attrition in the Cerebrolysin group, with 3 of 78 Cerebrolysin-treated participants and 10 of 68 placebo-treated participants being lost to follow-up (RR 0.26, 95% CI 0.08 to 0.91; P = 0.04; 1 trial, 146 participants; Analysis 1.2).

Adverse events and effects

Serious adverse events (SAEs)

Three trials with a total of 1335 participants contributed to this outcome. We found that Cerebrolysin probably results in little to no difference in the total number of people with SAEs: 58 out of 667 Cerebrolysin-treated participants and 50 out of 668 placebo-treated participants (RR 1.16, 95% CI 0.81 to 1.66; 3 trials, 1335 participants; moderate-certainty evidence; Analysis 1.3).

Similarly, we found that Cerebrolysin probably results in little to no difference in the total number of people with fatal SAEs. For fatal SAEs, there were 38/667 and 42/668 in the Cerebrolysin and placebo groups, respectively (RR 0.90, 95% CI 0.59 to 1.38; 3 trials, 1335

participants; moderate-certainty evidence; Analysis 1.4) and for non-fatal SAEs: 20/667 and 8/668 participants in the Cerebrolysin and placebo groups, respectively (RR 2.39, 95% CI 1.10 to 5.23; P = 0.03; I^2 = 0%; 3 trials, 1335 participants; moderate-certainty evidence; Analysis 1.5). Examination of the resulting forest plot revealed opposite directions of effect estimates in the subgroups of different Cerebrolysin dosing regimens (30 mL for 10 days and 50 mL for 21 days) despite a low level of subgroup differences in the overall data synthesis (Analysis 1.5). In the subgroup of the dosing regimen 30 mL for 10 days we found a large difference in the total numbers of people with non-fatal SAEs treated with Cerebrolysin: 20 out of 589 participants randomised to Cerebrolysin and 7 out of 600 participants randomised to placebo suffered a non-fatal SAE (RR 2.87, 95% CI 1.24 to 6.69; P = 0.01; 2 trials, 1189 participants; Analysis 1.5).

The authors of CASTA 2012 do not describe the nature of adverse events. In CERE-LYSE-1 2012, the study authors did describe SAEs. For the Cerebrolysin-treated participants these included: acute coronary syndrome, atrial fibrillation, cardiac failure, gastric ulcer, pneumonia (three cases), rectal cancer, coma, pleural effusion, aspiration pneumonia (two cases), cerebral haematoma, and pulmonary embolism. For the placebo-treated participants these included: cardiac arrest, cardiac failure, hepatic cirrhosis, infective arthritis, pneumonia, sepsis, renal failure, respiratory failure, cerebral haemorrhage, and haemorrhagic stroke (one case each).

The Ladurner 2005 study authors reported only one serious non-fatal adverse event in the placebo group (haematemesis).

The authors of Cortexin-Shamalov 2014 did not provide details of how they monitored and registered adverse events. The authors did not specify the numbers of people with certain adverse events (AEs) for the first group; for the second and third groups the authors provided the numbers of occurrences, but it was not clear how many people suffered a specific AE. It was not clear which AEs could be classified as serious AEs, since the authors did not differentiate between AEs of various severities, did not report AEs by severity level, and did not reply to our request for clarification. Thus, we decided not to include Cortexin-Shamalov 2014 in the analysis of SAEs, although some of the reported AEs could be classified as serious. Adverse events in people in the Cortexin + Cortexin group (20 participants) included: recurrent stroke, focal epilepsy, cardiac arrhythmias, acute intestinal obstruction, acute cholecystitis, and urological infection. The authors did not provide the numbers of people with each of the reported AEs. In the Cortexin + placebo group, the authors reported AEs in 11 people, which included cardiac arrhythmias (one), decompensation of coronary heart disease (three), thromboembolic complications (one), pneumonia (one), oncological disease (one), increased activity of liver enzymes in the blood (three), and respiratory infection (one). In the placebo + placebo group the authors described AEs in seven people: progressive course of stroke (two), cardiac arrhythmias (three), mental disorders (two), and subfebrile hyperthermia (one).

Total number of people with adverse events

We found information on this outcome in four included studies (CASTA 2012; CERE-LYSE-1 2012; Cortexin-Shamalov 2014; Ladurner 2005). The synthesis of the data from these studies revealed no difference between the Cerebrolysin and placebo groups, with 308 of 667 Cerebrolysin-treated participants and 307 of 668 placebo-treated participants suffering one or more AEs (RR 1.01, 95% CI 0.91 to 1.13; 3 trials, 1335 participants; Analysis 1.6). Similarly, when including data from Cortexin-Shamalov 2014, we found that Cerebrolysin or similar peptide mixtures may result in little to no difference in the total number of people with adverse events (RR 1.03, 95% CI 0.92 to 1.14; P = 0.65; 4 trials, 1607 participants; low-certainty evidence; Analysis 1.6).

In CASTA 2012, the study authors reported that 242/529 participants in the Cerebrolysin group and 243/541 participants in the placebo group experienced adverse events (RR 1.02, 95% CI 0.89 to 1.16; 1 trial, 1070 participants).

CERE-LYSE-1 2012 described the overall evaluation of safety, stating that 88% of Cerebrolysin-treated participants and 97% of placebo-treated participants reported at least one adverse event. We recalculated from this for the outcome total number of people with adverse events: 53/60 participants in the Cerebrolysin group and 57/59 participants in the placebo group (RR 0.91, 95% CI 0.82 to 1.01; 1 trial, 119 participants).

In Cortexin-Shamalov 2014, the study authors reported AEs in 20 of 136 participants in the Cortexin + Cortexin group, in 11 of 72 participants in the Cortexin + placebo group, and in 7 of 64 patients (eight occurrences) in the placebo + placebo group (RR 1.38, 95% CI 0.63 to 3.03; 1 trial, 272 participants). Adverse events included: isolated cases of recurrent stroke, focal epilepsy, cardiac arrhythmias, urological infection, acute intestinal obstruction, acute cholecystitis, cardiac arrhythmias, decompensation of coronary heart disease, thromboembolic complications, increased activity of liver enzymes in the blood, pneumonia, respiratory infection, oncological disease, progressive course of stroke, mental disorders, cardiac arrhythmias, and subfebrile hyperthermia. The authors stated that the AEs were not related to the treatment.

In Ladurner 2005, the study authors reported the overall incidence of adverse events: 16.4% in the Cerebrolysin group and 10.3% in the placebo group. We recalculated from this for the outcome total number of people with adverse events: 13/78 participants in the Cerebrolysin group and 7/68 participants in the placebo group (RR 1.62, 95% CI 0.69 to 3.82; 1 trial, 146 participants). The trial authors did not report on any adverse effects specifically associated with Cerebrolysin, for example hypersensitivity reactions.

Subgroup analysis and investigation of heterogeneity

We investigated potential sources of heterogeneity using the following subgroups of evaluated treatment regimens, which differ by Cerebrolysin dose and length of treatment, for the outcomes all-cause death, total number of people with adverse events, and total number of people with non-fatal SAEs.

- Cerebrolysin dose 30 mL for 10 days: cumulative dose 300 mL over 10 days (Amiri Nikpour 2014; CASTA 2012; CERE-LYSE-1 2012; Xue 2016). Xue 2016 did not contribute data to the quantitative analyses.
- Cerebrolysin dose 50 mL for 21 days: cumulative dose 1050 mL over 21 days (Ladurner 2005).
- Cerebrolysin dose 10 mL or 50 mL for 10 days: cumulative dose 100 mL or 500 mL over 10 days (Skvortsova 2004).

In the Cerebrolysin-only subset of data for the outcomes all-cause death, total number of people with SAEs, and total number of

people with fatal SAEs, we found no heterogeneity between the subgroups: I^2 = 0% in each case (Analysis 1.1; Analysis 1.3; Analysis 1.4). In the combined Cerebrolysin or Cortexin analysis we also found no heterogeneity between subgroups for the outcomes all-cause death or total number of people with AEs (Analysis 1.1; Analysis 1.6).

For the outcome total number of people with non-fatal SAEs in the Cerebrolysin-only subset of data we observed opposing directions of effect estimates in Subgroup 1 (Cerebrolysin dose 30 mL for 10 days: cumulative dose 300 mL over 10 days; CASTA 2012; CEREGYSE-1 2012) versus Subgroup 2 (Cerebrolysin dose 50 mL for 21 days: cumulative dose 1050 mL over 21 days; Ladurner 2005), with the test for subgroup differences revealing an I^2 value of 46.1% (Analysis 1.5). In Subgroup 1 (the lowest dose amongst all tested doses in the included trials), we found a nearly threefold increase in the incidence of non-fatal SAEs (RR 2.87, 95% CI 1.24 to 6.69, P = 0.01, I^2 = 0%; 2 trials, 1189 participants). In Subgroup 2 (the highest dose, cumulatively more than three times that of Subgroup 1), the RR was 0.29 with a large range of the confidence interval (95% CI 0.01 to 7.03; P = 0.45; 1 trial, 146 participants).

In the Cerebrolysin-only subset of data we found substantial heterogeneity (I^2 = 60%) for the outcome total number of people with adverse events amongst the two subgroups of Cerebrolysin dose regimen (30 mL for 10 days; 50 mL for 21 days). It was suggested that the highest dose (cumulatively 1050 mL of Cerebrolysin) and the 21-day duration might be associated with a higher risk of adverse events, but with an RR of 1.62 (95% CI 0.69 to 3.82; 1 trial, 146 participants), this did not achieve conventional levels of statistical significance (P = 0.27; Analysis 1.6).

Heterogeneity was high in the analysis of the outcome non-death attrition, with I^2 = 57% and I^2 = 66.4% for subgroup differences (Analysis 1.2). No one study affected this heterogeneity substantially, and even removing the outermost group from the analysis (Ladurner 2005), there is little change of note in either the heterogeneity (which becomes moderate, I^2 = 47%) or the effect of Cerebrolysin (RR 0.87, 95% CI 0.50 to 1.52; P = 0.63; 5 trials, 1543 participants; Analysis 1.2).

Sensitivity analyses

We conducted the sensitivity analyses for the outcome all cause-death to test the robustness of our methodology and results.

To explore the potential effects of missing data we performed a series of sensitivity analyses for worst-case and best-case scenarios (as described in Table 1). These analyses with corresponding assumptions did not alter the finding of no difference (6 trials, 1689 participants; moderate-certainty evidence; Analysis 2.1; Analysis 2.2; Analysis 2.3).

To explore the potential effect of risk of bias we performed the following analysis. Our judgements of risk of bias both across studies and across risk of bias domains were low, unclear, and high. We were unable to identify studies being at overall high risk of bias. However, we could single out three trials with low risk of attrition bias and unclear risk of bias across the remaining domains (Amiri Nikpour 2014; Cortexin-Shamalov 2014; Skvortsova 2004) and in this way investigate the effect of study robustness on our primary outcome all-cause death. Synthesising the outcome data for all-cause death in these three trials we found a change in the direction of effect to more deaths in people treated with Cerebrolysin or Cerebrolysin-like peptide mixture Cortexin: there were 15 deaths in the 255 peptide-treated participants and 5 deaths in the 99 placebo-treated participants (RR 1.28, 95% CI 0.51 to 3.21; 3 trials, 354 participants; fixed-effect model). There was no statistical heterogeneity (I^2 = 0%) and the test for subgroup differences also showed no statistical subgroup differences. Changing the fixed-effect model to the random-effects model did not affect the result (RR 1.20, 95% CI 0.47 to 3.04; 3 trials, 354 participants; random-effects model) (Analysis 2.4). These three studies had common characteristics: they had no missing data (loss to follow-up, non-death attrition) and they did not report on direct manufacturer involvement. The direction of the effect of peptide mixtures on all-cause death was reversed in these three trials, favouring placebo.

We could not use funnel plots to examine asymmetry and small-study effects because there were only seven included studies, with six at most contributing data to the quantitative analysis for any given outcome.

The use of either a fixed-effect or random-effects model made no difference to either direction or value of the effect estimates in any of the analyses, although it might have made a difference to the level of statistical probability of the findings.

DISCUSSION

The World Health Organization (WHO) collection of national Essential Medicines Lists (EMLs) includes the latest acting country editions that recommend Cerebrolysin for treating various neurological conditions including acute ischaemic stroke. These include the national EMLs of the Russian Federation (GovRu 2019; GovRu 2022), Slovakia, Romania, Vietnam, Uganda, and the Syrian Arab Republic (WHO 2019b; WHO 2022). Cortexin is listed in the national EML (GovRu 2019; GovRu 2022) and in the clinical guidelines of the Russian Federation together with Cerebrolysin for treating acute ischaemic stroke (MinHealthRu 2021). However, the potential benefits of Cerebrolysin or Cortexin for improving clinical outcomes in people with acute ischaemic stroke and the risks of its use have not been demonstrated on the basis of research synthesis of randomised controlled trials (RCTs) of acceptable quality. In this Cochrane Review we have assessed the benefits and harms of Cerebrolysin and the Cerebrolysin-like agent Cortexin, when added to standard treatment for acute ischaemic stroke, focusing on clinically relevant and widely accepted outcomes, and specifically excluding assessment methods with numerous varying scales.

Summary of main results

Seven RCTs involving 1773 participants met our inclusion criteria. Six studies contributed to the quantitative analyses.

In this review update we again confirmed with moderate-certainty evidence that Cerebrolysin does not substantially alter the risk of death (Analysis 1.1). We also note that the only RCT eligible for inclusion on the Cerebrolysin-like agent Cortexin presented data on deaths in people treated with Cortexin, while documenting no deaths in participants in the placebo group, despite the less severe stroke in all participants in the trial. When we combined Cerebrolysin and Cortexin data for all-cause death we established with moderate-certainty evidence that peptide mixtures do not substantially alter the risk of death (Analysis 1.1). Our sensitivity analysis indicated that the exclusion of trials without a single low

risk of bias domain judgement resulted in a change in the direction of effect of peptide mixtures on all-cause death, with more deaths in the combined Cerebrolysin and Cortexin group.

None of the seven included trials provided sufficient evidence of the effects of Cerebrolysin or Cortexin on clinically relevant outcome measures in acute ischaemic stroke, such as poor functional outcome (death or dependence by the end of the follow-up period) and early death (within two weeks of stroke onset), or time to restoration of capacity for work and quality of life.

The authors of only three of the included trials described the causes of death, which could potentially be used for analysis (Cortexin-Shamalov 2014; Ladurner 2005; Skvortsova 2004). In Ladurner 2005, the causes of death included: cerebral infarct (four in the Cerebrolysin group and two in the placebo group), heart failure (two in the Cerebrolysin group and one in the placebo group), pulmonary embolism (two in the placebo group), and pneumonia (one in the placebo group), with 6/78 deaths in the Cerebrolysin group and 6/68 deaths in the placebo group, although they did not report the time of death. In the newly added study Cortexin-Shamalov 2014, the authors reported the following causes of deaths: two cases of repeated stroke, one case of pulmonary embolism, and one of polysegmental pneumonia (4/136 deaths) in the Cortexin + Cortexin group, and one acute intestinal obstruction and two sudden deaths (3/72 deaths) in the Cortexin + placebo group; no deaths occurred in placebo + placebo group. The authors did not report the time of the deaths. In Skvortsova 2004, the authors reported the following causes of death not attributed to the stroke: pulmonary embolism, pneumonia, and pyelonephritis (three in the Cerebrolysin group and one in the placebo group), and the causes of death associated with the stroke: brain oedema with secondary brainstem syndrome (two participants in the Cerebrolysin and placebo groups). Owing to unclear numbers we could not synthesise these data.

For the outcome non-death attrition, we did not find a statistical difference between comparison groups. However, the Ladurner 2005 trial stands out in this analysis with a substantially lower rate of non-death attrition in the Cerebrolysin group (Analysis 1.2). The obvious main reason is that Ladurner 2005 used a much higher dose of Cerebrolysin (50 mL for 21 days; cumulative dose 1050 mL) than all other trials (cumulative doses ranging from 100 mL to 500 mL over 10 days). Hence, it is not surprising that the sensitivity analyses, which we performed to explore the potential effect of attrition (missing data) on the outcome all-cause death, did not alter the overall finding of no difference in the outcome all cause-death between participants with acute ischaemic stroke treated with cattle brain peptide mixtures or placebo (6 trials, 1689 participants; moderate-certainty evidence). However, the difference in Cerebrolysin dosing regimen does not help us to understand the potential origins of the contrasting non-death attrition patterns in these two trials.

Noteworthy are the results of sensitivity analysis performed to explore the potential effect of risk of bias on the outcome all-cause death: there was a change in the direction of effect in the three trials with low risk of attrition bias and unclear risk of bias across the remaining domains (Amiri Nikpour 2014; Cortexin-Shamalov 2014; Skvortsova 2004), compared to the trials with high risk of attrition bias (CASTA 2012; CERE-LYSE-1 2012; Ladurner 2005), to more deaths in people treated with Cerebrolysin or Cerebrolysin-like peptide mixture Cortexin. There were 15 deaths in the 255 peptide-treated participants and 5 deaths in the 99 placebo-treated participants (risk ratio (RR) 1.28, 95% confidence interval (CI) 0.51 to 3.21; 3 trials, 354 participants; fixed-effect model). The direction of the effect of peptide mixtures on all-cause death in this sensitivity analysis in the three non-high risk of bias trials was opposite to the high risk of attrition bias trials favouring placebo. The three non-high risk of bias studies had no missing data (no non-death attrition) and their authors did not report on direct manufacturer involvement.

We confirmed with moderate-certainty evidence that Cerebrolysin probably makes little to no difference to fatal serious adverse events (SAEs) (Analysis 1.4) and total SAEs (Analysis 1.3). We also found moderate-certainty evidence that Cerebrolysin likely increases the number of participants with non-fatal SAEs (Analysis 1.5).

By subgrouping two studies with the same dosing schedule (30 mL for 10 days), which contributed data on adverse events and were both multicentre (CASTA 2012; CERE-LYSE-1 2012), we confirmed an almost threefold increase in the incidence of non-fatal SAEs in participants treated with Cerebrolysin, with a resulting number needed to treat to harm (NNTH) of 45, which means that in every 45 acute ischaemic stroke patients treated with Cerebrolysin, one will experience a non-fatal SAE (Summary of findings 1; moderate-certainty evidence).

For the total number of people with adverse events, we did not find a statistical difference between the Cerebrolysin and placebo groups, but identified substantial heterogeneity (I^2 = 60%) amongst the three Cerebrolysin trials contributing to this outcome (Analysis 1.6). Combining these with the Cortexin data contributed to a lower level of heterogeneity (I^2 = 38%; Analysis 1.6).

Overall completeness and applicability of evidence

In this update we included one new study of the Cerebrolysin-like agent Cortexin. We followed the protocol in all respects. The new study evaluated participants with acute ischaemic stroke who had been assessed clinically, and the diagnosis was confirmed by neuroimaging (Cortexin-Shamalov 2014).

The seven eligible studies, four of which were multicentre studies, were carried out in multiple clinical centres in Europe (seven countries): Austria, Croatia, the Czech Republic, Hungary, Russia, Slovakia, and Slovenia; and in Asia (five countries): China, Hong Kong, Iran, Myanmar, and South Korea. The participant populations were geographically diverse. The included studies were conducted in high-, middle-, and low-income countries, which means the results of this Cochrane Review are likely to be applicable to settings where the burden of stroke and stroke deaths is high. Of particular importance is the fact that the results of this update are likely to be applicable to the settings of low-income countries, where the burden of stroke deaths and disability is even higher (WHO 2019a), and poses a huge financial demand on health systems and society (Martynchik 2013), and where Cerebrolysin or Cerebrolysin-like agents are in widespread use. The included studies tested various doses of Cerebrolysin (10 mL, 30 mL, and 50 mL) and treatment duration with Cerebrolysin varied from 10 days to four weeks. One study tested Cortexin 10 mg twice a day administered in two 10-day courses with a 10-day break between the courses. We did not find any clear evidence that Cerebrolysin or Cortexin improves clinical outcomes in acute ischaemic stroke

with any of the tested treatment regimens. Treatment strategies for acute ischaemic stroke should be reviewed in light of this evidence.

Reporting of data on death and safety parameters without clarification of the time of death or the time of development of adverse events, and the loss of data for many enrolled participants owing to attrition, hampered meaningful interpretation of these data. However, it is apparent that treatment with Cerebrolysin or Cortexin had no beneficial effect on the incidence of death, and may even have had a harmful effect, as demonstrated by the number of deaths in people treated with Cortexin, while Cerebrolysin increased the incidence of non-fatal SAEs.

None of the included studies reported on Cerebrolysin (Cortexin)-specific, or peptide mixture-specific, adverse events. These may include: hypersensitivity or emotional disturbance, arousal and aggression; fatigue, tiredness and apathy or sleeplessness; convulsions; rise or fall in blood pressure; shortness of breath; flu-like syndromes; and reactions on immediate intravenous administration, such as feelings of chills or heat, cold sweats, dizziness and tachycardia, or redness and itching at the site of administration; and gastrointestinal disturbance (Registry of Medicines 2019).

Quality of the evidence

We assessed the certainty of the evidence using the GRADE approach (Guyatt 2008), and presented the results in Summary of findings 1. For this review we asked the question: should Cerebrolysin or Cerebrolysin-like agents be used in acute ischaemic stroke to improve clinical outcomes?

Based on the six studies that contributed to quantitative analysis and the one trial contributing to qualitative synthesis, there is no evidence that Cerebrolysin or Cerebrolysin-like agents added to standard therapy reduce deaths in people with acute ischaemic stroke (Amiri Nikpour 2014; CASTA 2012; CERE-LYSE-1 2012; Cortexin-Shamalov 2014; Ladurner 2005; Skvortsova 2004). There is moderate-certainty evidence that Cerebrolysin or Cerebrolysin-like agents perform no better or worse than placebo in preventing all-cause death in people with acute ischaemic stroke if started within 48 hours of stroke onset and continued for 10 days to four weeks (Summary of findings 1). There is concern from one trial on the Cerebrolysin-like agent Cortexin that the use of peptide mixtures in acute ischaemic stroke may contribute to deaths.

Three studies with a total of 1335 participants, two of which were multicentre, contributed to the outcomes total number of people with SAEs, total number of people with non-fatal SAEs, and total number of people with adverse events. These studies found 58 SAEs in the Cerebrolysin group (667 randomised participants) and 50 SAEs in the placebo group (668 randomised participants); 20 non-fatal SAEs in the Cerebrolysin group (667 randomised participants) and 8 non-fatal SAEs in the placebo group (668 randomised participants); and 308 people with adverse events in the Cerebrolysin group (667 randomised participants) and 307 people with adverse events in the placebo group (668 randomised participants). Although the confidence intervals in Ladurner 2005 were wide and the direction of effect was opposite, this did not result in statistical heterogeneity.

Three studies contributed to the outcome total number of people with non-fatal SAEs, and there is moderate-certainty evidence that Cerebrolysin likely increases non-fatal SAEs (but not total SAEs) in people with acute ischaemic stroke (Analysis 1.5).

Based on four studies, Cerebrolysin or Cortexin added to standard therapy for acute ischaemic stroke may be no different from placebo in the total number of people with adverse events (CASTA 2012; CERE-LYSE-1 2012; Cortexin-Shamalov 2014; Ladurner 2005). There is low-certainty evidence that Cerebrolysin and similar peptide mixtures perform no better or worse than placebo in terms of the total number of people with adverse events (Summary of findings 1).

We assessed none of the included studies as being at high risk of bias for all domains. For the majority of the risk of bias domains, such as sequence generation, allocation concealment, blinding of participants and personnel, and blinding of outcome assessors, we judged the risk of bias to be low or unclear. Selective outcome reporting was unclear for all seven included studies. High levels of exclusions from the final analyses caused us to assess four studies at high risk of bias for incomplete outcome reporting; three of these studies contributed to quantitative synthesis and all three were multicentre studies, as described in Assessment of risk of bias in included studies (CASTA 2012; CERE-LYSE-1 2012; Ladurner 2005). We judged that these potential limitations were unlikely to lower confidence in the estimate of effect. One of the reasons for judging incomplete outcome reporting as at high risk of bias was the high rate of attrition (Table 4); however, despite this, large numbers of participants remained in the trials, and large numbers of effects were reported by the study authors, both for all-cause death and adverse events. Furthermore, we found no difference between the comparison groups in the numbers of participants lost to follow-up for reasons other than death, which we analysed as the outcome non-death attrition, although there was a considerable level of heterogeneity between the subgroups (Analysis 1.2). The new multicentre RCT on the Cerebrolysin-like agent Cortexin had no losses to follow-up; it adds to the concerns over the overall safety of peptide mixtures derived from animal brains in people with acute ischaemic stroke.

Potential biases in the review process

We performed the data extraction unblinded.

The included trials are published, but we obtained unpublished data on SAEs through feedback received from the manufacturer of Cerebrolysin, EVER Neuro Pharma GmbH (formerly EBEWE Pharma). We were unable to obtain sufficient data on important outcomes such as death or dependency.

Agreements and disagreements with other studies or reviews

We asked whether Cerebrolysin or Cerebrolysin-like agents have a role in improving treatment outcomes for people diagnosed with acute ischaemic stroke. The original version of this review did not provide evidence that Cerebrolysin was effective (Ziganshina 2010a), and none of the updates since then have shown effectiveness (Ziganshina 2013; Ziganshina 2015; Ziganshina 2016; Ziganshina 2017; Ziganshina 2020).

These unfavourable results caution against the widespread use of Cerebrolysin and its inclusion in national Essential Medicines Lists (EMLs) in Russia (GovRu 2019; GovRu 2022), Ukraine, Romania, Slovakia, Vietnam, Uganda, and the Syrian Arab Republic (WHO

2019b; WHO 2022). As new research data have accumulated, we have updated the review several times since 2010 after performing new literature searches. The conclusions of the previous version of this Cochrane Review, Ziganshina 2020, have remained largely unchanged in this update, with the inclusion of a newly identified trial testing the Cerebrolysin-like agent Cortexin.

In contrast to our findings is a recent meta-analysis of nine trials, which concluded that the safety of Cerebrolysin was comparable to placebo and that Cerebrolysin has a beneficial effect on early global neurological deficits in people with acute ischaemic stroke (Bornstein 2018). Six trials that Bornstein 2018 included in their meta-analysis are also included in this Cochrane Review (Amiri Nikpour 2014; CASTA 2012; CERE-LYSE-1 2012; Gharagozli 2017; Skvortsova 2004; Xue 2016). The three studies included in Bornstein 2018 that we excluded from our meta-analysis did not meet our inclusion criteria (Guekht 2015; Muresanu 2016; Shamalov 2010). The Ladurner 2005 trial, which we included in our review, was excluded from Bornstein 2018.

An earlier meta-analysis of nine trials testing Cerebrolysin, Cortexin, and Cellex in ischaemic stroke, vascular dementia, and Alzheimer's disease, published in Russian, also presented contrasting results for Cerebrolysin reducing mortality in stroke, with ambiguous concluding remarks about not being able to "exclude some possible positive effect" of Cerebrolysin (Plavinski 2016). Five trials that Plavinski 2016 included in their meta-analysis are included in this Cochrane Review (Amiri Nikpour 2014; CASTA 2012; CERE-LYSE-1 2012; Ladurner 2005; Skvortsova 2004). Of the four studies included in Plavinski 2016 that we excluded from our meta-analysis, three did not meet our eligibility criteria (Muresanu 2016; Shamalov 2010; Skvortsova 2006), and one was an economic evaluation.

In addition to contrasting with our results, the meta-analysis Bornstein 2018 differs from another recent meta-analysis that showed lack of benefit from Cerebrolysin treatment for ischaemic stroke compared to placebo for functional recovery at day 90 (Wang 2017). Of the six studies included in Wang 2017, four overlapped with those included in this Cochrane Review (Amiri Nikpour 2014; CASTA 2012; CERE-LYSE-1 2012; Ladurner 2005). Wang 2017 also included studies that we had excluded in a previous revision, as they dealt with different research questions and did not meet our eligibility criteria (CARS study - Chang 2016; Muresanu 2016).

AUTHORS' CONCLUSIONS

Implications for practice

This review indicates that Cerebrolysin and the Cerebrolysin-like agent Cortexin probably result in little to no difference in deaths after acute ischaemic stroke.

We found moderate-certainty evidence that there is probably little to no difference between groups in the total number of people with serious adverse events, but there is a possible increase in the total number of people with non-fatal serious adverse events with Cerebrolysin use.

We note that there were deaths with the use of the Cerebrolysin-like agent Cortexin in people with mild acute ischaemic stroke, while there were no deaths in the placebo group.

Implications for research

Future research should focus on systematic review of the harms of Cerebrolysin and Cerebrolysin-like agents in acute ischaemic stroke and in its other potential uses.

We advocate for no further trials of Cerebrolysin and Cerebrolysin-like agents on the grounds that it would be unethical to recruit patients into a study that offers no potential benefit.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Amiri Nikpour 2014

Study characteristics	
Methods	Study design: RCT Study grouping: parallel-group Losses to follow-up: none Trial protocol registration: no protocol identified
Participants	Total number of participants: 46. However, 3 participants died before day 30: 1 participant in the Cerebrolysin group and 2 participants in the placebo group. 43 participants included in the final analysis. **Baseline characteristics:** Cerebrolysin • Participants: 22 • Mean age: 60 year (SD ± 9.6) • Men: 12 (54.5%) • Women: 10 (45.5%) • Risk factor: ischaemic heart disease: 4 (18.2%); diabetes mellitus: 8 (36.4%); hypertension: 13 (59.1%); dyslipidaemia: 11 (50%); smoking: 3 (13.6%)

Amiri Nikpour 2014 *(Continued)*

- Drug histories: beta-blockers: 4 (18.2); ACE-1: 3 (13.6%); angiotensin receptor blocker: 8 (36.4%); calcium channel blocker: 0 (0%); diuretic: 3 (13.6%); statin: 12 (54.5%); antidiabetic: 8 (36.4%); antidiabetic plus statin: 3 (13.6%); antidiabetic plus antihypertensive: 4 (18.2%); antihypertensive plus statin: 4 (18.2%)
- Stroke location: anterior circulation: 14 (63.6%); posterior circulation: 8 (36.4%)

Placebo

- Participants: 21
- Mean age: 60.1 years (SD ± 10)
- Men: 10 (47.6%)
- Women: 11 (52.4%)
- Risk factor: ischaemic heart disease: 3 (14.3%); diabetes mellitus: 10 (47.6%); hypertension: 13 (61.9%); dyslipidaemia: 12 (57.1%); smoking: 3 (14.3%)
- Drug histories: beta-blockers: 5 (23.8%); ACE-1: 2 (9.5%); angiotensin receptor blocker: 5 (23.8%); calcium channel blocker: 1 (4.8%); diuretic: 6 (28.6%); statin: 12 (57.1%); antidiabetic: 10 (47.6%); antidiabetic plus statin: 7 (33.3%); antidiabetic plus antihypertensive: 2 (9.5%); antihypertensive plus statin: 5 (23.8%)
- Stroke location: anterior circulation: 16 (76.2%); posterior circulation: 5 (23.8%)

Inclusion criteria: both sexes, 18 to 85 years; focal neurological injury; ischaemic stroke within 6 to 24 hours before admission; acute focal ischaemic stroke detected by CT or MRI or both; NIHSS score of 6 to 22 at presentation

Exclusion criteria: rapid improvement of signs and symptoms, or complete resolution, or both, within 24 hours; seizure upon the development of stroke; any conditions interfering with neurological examination, such as severe dementia or psychological diseases; severe heart failure; acute myocardial infarction; pregnancy or breastfeeding; significant systemic diseases associated with disability and decreased well-being; systolic and diastolic blood pressure above 220 mmHg and 120 mmHg, respectively; CT or MRI suggesting acute or chronic haemorrhagic stroke or neoplasm, or both; hernia in the brain or increased intracranial pressure; contraindication or sensitivity to aspirin or Cerebrolysin, or both; taking other neuroprotective agents such as piracetam; and taking vasodilators such as nimodipine

Pretreatment: no difference

Interventions	Cerebrolysin • Frequency of dosage: intravenous injection of 30 mL of Cerebrolysin diluted in normal saline once a day for 10 days • Standard treatment: 100 mg of aspirin daily Placebo • Frequency of dosage: normal saline, as placebo, with a prescription order similar to the main drug • Standard treatment: 100 mg of aspirin daily
Outcomes	We extracted data for all-cause death (dichotomous outcome).
Identification	Sponsorship source: Urmia University of Medical Sciences grant Country: Iran Setting: hospital (inpatient setting) Author: Mohammad Reza Amiri-Nikpour Institution: Seyyed-al-Shohada Heart Centre Email: yousefrezaei1986@gmail.com
Notes	No protocol identified

Amiri Nikpour 2014 *(Continued)*

Risk of bias

Bias	Authors' judgement	Support for judgement
Allocation concealment	Unclear risk	**Quote:** "In a randomised, double-blinded, placebo-controlled clinical trial, patients who had signs and symptoms of acute brain stroke were assessed from March 2013 to March 2014." **Comment:** there was insufficient information to permit a judgement of low risk or high risk, so we opted for a judgement of unclear risk.
Sequence generation	Unclear risk	**Quote:** "In a randomised, double-blinded, placebo-controlled clinical trial, patients who had signs and symptoms of acute brain stroke were assessed from March 2013 to March 2014." **Comment:** there was no information on allocation concealment. In addition to the unavailability of a study protocol, we judged this as an unclear risk.
Incomplete outcome data All outcomes	Low risk	**Quote:** "After receiving treatments, one patient in the Cerebrolysin-received group and two patients in the placebo-received group died before day 30 (4.3% versus 8.7%); they were excluded from the final analysis due to lack of measuring their outcomes at 90-day follow-up." **Comment:** no losses to follow-up. However, adverse events and causes of death were not reported, and the study protocol was not available.
Blinding of outcome assessors All outcomes	Unclear risk	**Quote:** "In a randomised, double-blinded, placebo-controlled clinical trial, patients who had signs and symptoms of acute brain stroke were assessed from March 2013 to March 2014." **Comment:** there was no information as to whether outcome assessors were aware of the allocated interventions. No information was provided on allocation concealment. In addition to the unavailability of a study protocol, we judged this as an unclear risk.
Selective outcome reporting	Unclear risk	**Comment:** study protocol not available. Causes of death were not described; there was no information on clinically relevant outcomes.
Blinding of participants and personnel All outcomes	Unclear risk	**Quote:** "All patients who met inclusion criteria were randomly assigned into two groups to receive intravenously either 30 ml of Cerebrolysin diluted in normal saline once a day for 10 days (n = 23) or normal saline, as placebo, with a prescription order similar to the main drug (n = 23)." **Comment:** there was no information on blinding of participants and personnel. In addition to the unavailability of a study protocol, we judged this as an unclear risk.
Other sources of bias	Unclear risk	**Quote:** "We thank the vice-chancellor of research in Urmia University of Medical Sciences for providing the grant of this study. Moreover, we would like to greatly thank all members of emergency department of Imam Khomeini Hospital, Urmia, West Azerbaijan Province, Iran, for helping us in collecting the study data." **Comment:** there was no clear information on funding sources, and all authors declared no conflict of interest; no protocol identified.

CASTA 2012

Study characteristics	
Methods	Study design: phase IV clinical trial designed as a multicentre, randomised, double-blind, placebo-controlled, parallel-group study Study grouping: parallel-group Losses to follow-up: 180 participants (16.8%) Trial protocol registration: retrospective (3 years difference between study start date (2006) and the date registration record posted (2009), study was completed in 2011)
Participants	Total number of participants: 1070 **Baseline characteristics:** Cerebrolysin • Men: 314 (59.6%) • Mean age: 65.0 years (SD 12.22) • Mean body mass index: 23.7 kg/m^2 (SD 3.04) • Mean time until hospital admission: 5.6 hours (SD 3.00) • Mean time until start of treatment, calculated from stroke onset: 7.7 hours (SD 5.97) • Thrombolysis treatment: 50 (9.49%) • Prevalence of risk factors: 582 • ○ Hypertension: 331 (62.8%) ○ Diabetes: 108 (20.5%) ○ Arrhythmia: 71 (13.5%) ○ Coronary heart disease: 72 (13.7%) • Baseline efficacy criteria, median (range) ○ NIHSS maximum (range, 0 to 42 points): 9 (6 to 33) ○ Barthel Index maximum (range, 0 to 100 points): 30 (0 to 100) ○ Modified Rankin Scale maximum (range, 0 to 6 points): 4 (0 to 5) Placebo • Men: 326 (60.4%) • Mean age: 65.5 years (SD 11.71) • Mean body mass index: 24.0 kg/m^2 (SD 3.20) • Mean time until hospital admission: 5.6 hours (SD 3.75) • Mean time until start of treatment, calculated from stroke onset: 7.6 hours (SD 3.69) • Thrombolysis treatment: 44 (8.1%) • Prevalence of risk factors: 625 ○ Hypertension: 332 (61.6%) ○ Diabetes: 117 (21.7%) ○ Arrhythmia: 90 (16.7%) ○ Coronary heart disease: 86 (16%) • Baseline efficacy criteria, median (range) ○ NIHSS maximum (range, 0 to 42 points): 9 (6 to 26) ○ Barthel Index maximum (range, 0 to 100 points): 30 (0 to 100) ○ Modified Rankin Scale maximum (range, 0 to 6 points): 4 (0 to 5) **Inclusion criteria:** men and women, aged 18 to 85 years with focal neurological deficit and a clinical diagnosis of acute hemispheric ischaemic stroke with CT or MRI results compatible with a clinical diagnosis of acute hemispheric stroke, NIHSS score between 6 and 22 (both inclusive), and functionally independent before stroke with a pre-stroke Rankin Scale score of 0 or 1. Randomisation and treatment

CASTA 2012 *(Continued)*

	with the trial medication initiated within 12 hours after stroke onset. Signed informed consent was obtained from the participant or the participant's legally accepted representative. **Exclusion criteria:** evidence on CT/MRI of intracranial haemorrhage, decreased consciousness (defined as score of ≥ 2 on NIHSS Question 1a), neurological signs and symptoms that were likely to resolve completely within 24 hours, systolic blood pressure ≥ 220 mmHg or diastolic blood pressure ≥ 120 mmHg on repeated measurement, severe congestive heart failure or presentation with acute myocardial infarction, pre-existing systemic disease significantly limiting life expectancy, concomitant treatment with other neuroprotective or nootropic drugs, and intolerance or contraindication to aspirin or Cerebrolysin **Pretreatment:** more participants with diabetes (117 (21.7%) versus 108 (20.5%)); arrhythmia (90 (16.7%) versus 71 (13.5%)); and coronary heart disease (86 (16.0%) versus 72 (13.7%)) in the placebo group
Interventions	Cerebrolysin • Frequency of dosage: daily intravenous infusion of 30 mL Cerebrolysin diluted in saline (total of 100 mL) for 10 days starting within 12 hours of stroke onset • Standard treatment: 100 mg aspirin orally as standard treatment every day Placebo • Frequency of dosage: daily intravenous infusion of placebo (100 mL saline) for 10 days starting within 12 hours of stroke onset • Standard treatment: 100 mg aspirin orally as standard treatment every day
Outcomes	• Poor functional outcome defined as death or dependence at the end of the follow-up period (dichotomous outcome) • Early death (dichotomous outcome) • All-cause death (dichotomous outcome) • Adverse effects specifically associated with Cerebrolysin (dichotomous outcome) • Total number of participants with adverse events (dichotomous outcome) • Serious adverse events (dichotomous outcome)
Identification	Sponsorship source: EVER Neuro Pharma GmbH (Oberburgau 3, Austria) Country: China, Hong Kong, South Korea, Myanmar Setting: inpatient (hospital) Comments: all study authors were closely bound with EVER Neuro Pharma. Dr Heiss is an advisor for the company; Dr Brainin has received financial support from EVER Neuro Pharma; Dr Bornstein is a consultant for EVER Neuro Pharma; Dr Tuomilehto is active in the Speakers Bureau of EVER Neuro Pharma; and Dr Hong received a research grant from EVER Neuro Pharma. Authors: Wolf-Dieter Heiss and Zhen Hong Institution: Max-Planck Institut fur Neurologie and Hua Shan Hospital, Department of Neurology Email: wdh@nf.mpg.de; profzhong@sina.com
Notes	No results posted on trial registration platform. **Quote:** "This study was funded by EVER Neuro Pharma GmbH, Oberburgau 3, Austria. The steering committee, safety committee, and other study investigators were working independently. The sponsor assisted in the writing of the protocol, selection of study sites, data collection, and project management. The statistical data analysis was carried out by an independent statistical consultant from Idv Gauting, Germany. The interpretation of results and conclusions are those of the authors, and these and writing of the article were not influenced by the sponsor. The article was reviewed and approved

CASTA 2012 *(Continued)*

by the independent steering committee and safety committee. The authors received an honorarium related to this work from the sponsor and support for travel."

Quote: "Dr Heiss is an advisor for EVER Neuro Pharma and received honoraria for this activity. He is active in the speaker's bureau of EVER Neuro Pharma and CoAxia and he receives support from the Wolf-Dieter Heiss Foundation. Dr Brainin has received financial support for research grants from EVER Neuro Pharma and Boeh- ringer Ingelheim and other research support from the European Research Foundation and Life Science Krems. He is in the speaker's bureau of Allergan, Boehringer Ingelheim, Ferrer, Pfizer, and EVER Neuro Pharma. He is active as a consultant and advisor for Allergan and EVER Neuro Pharma. Dr Bornstein is a consultant for EVER Neuro Pharma and received honoraria for this activity. He is also active in the speaker's bureau of EVER Neuro Pharma. Dr Tuomilehto is active in the speaker's bureau of EVER Neuro Pharma and received honoraria for this activity from EVER Neuro Pharma. Dr Hong received a research grant from EVER Neuro Pharma."

Risk of bias

Bias	Authors' judgement	Support for judgement
Allocation concealment	Unclear risk	**Quote:** "From September 2005 to September 2009, 1070 patients were randomised. Of 1069 patients who received at least 1 infusion of study medication, 529 patients (49.5%) received Cerebrolysin and 540 patients (50.5%) placebo". **Comment:** there was no information on allocation concealment. We searched the published protocol, Hong 2009, for a description of allocation concealment, but this was not reported.
Sequence generation	Unclear risk	**Quote:** "From September 2005 to September 2009, 1070 patients were randomised. Of 1069 patients who received at least 1 infusion of study medication, 529 patients (49.5%) received Cerebrolysin and 540 patients (50.5%) placebo". **Comment**: there was no information on allocation concealment. We searched the published protocol, Hong 2009, for a description of allocation concealment, but this was not reported.
Incomplete outcome data All outcomes	High risk	**Quote:** "Eighty-nine serious adverse events occurred after start of the treatment (Cerebrolysin 50 serious adverse events, placebo 39 serious adverse events). Sixty of 1069 patients sustained fatal adverse events (Cerebrolysin 28 patients [5.3%] and placebo 32 patients [5.9%]). Of 1069 patients, 85 patients (8.0%) discontinued the study due to adverse events, 39 patients in the Cerebrolysin group". **Quote:** "Sixty patients died and 890 (83.2% of all randomised patients) completed the 90-day follow-up ..." **Comment:** 16.8% of participants were lost to follow-up. The proportion of missing outcomes compared with the observed event risk was enough to induce clinically relevant bias in observed intervention effect estimate.
Blinding of outcome assessors All outcomes	Low risk	**Quote:** "Before unblinding the study, a blind review of the data was performed. The review was within the framework of the requirements of the ICH Guideline E9. 17" **Quote:** "Patients and investigators remained strictly blinded to the treatment assignments, and the occurrence or nature of adverse events did not compromise the blinding either." **Comment:** it is impossible to assess blinding by outcome. Described in report as a randomised, double-blind, placebo-controlled, parallel-group study.

CASTA 2012 *(Continued)*

Selective outcome reporting	Unclear risk	**Comment:** the study protocol is available, and all of the study's prespecified (primary and secondary) outcomes that are of interest in the review have been reported in the prespecified way. The protocol was registered retrospectively; results not posted on trial registration platform. No causes of death were described in the trial report; Kaplan-Meier mortality curve presented only for the subgroup of participants NIHSS > 12.
Blinding of participants and personnel All outcomes	Low risk	**Quote:** "Patients and investigators remained strictly blinded to the treatment assignments, and the occurrence or nature of adverse events did not compromise the blinding either. Missing data were handled according to international standards or guidelines." **Comment:** we judged this as low risk, although this statement about strict blinding appeared only in the discussion section of the trial report. Other details were available in the protocol published as Hong 2009, although no information on blinding was provided in the methods or results sections of the trial report.
Other sources of bias	High risk	Protocol registered at ClinicalTrials.gov NCT00868283 and published as a separated paper (Hong 2009), both retrospectively. No results posted on trial registration platform.

CERE-LYSE-1 2012

Study characteristics	
Methods	Study design: prospective, randomised, placebo-controlled, double-blind trial Study grouping: parallel-group Losses to follow-up: 19 (16%) Trial protocol registration: retrospective (4 years difference between study start date (2005) and the date registration record posted (2009), when the trial was already completed in 2008)

Participants	**Baseline characteristics:**
	
	Cerebrolysin
	
	• Participants: 60
	• Mean age: 65.5 years (SD 11.30)
	• Smokers: 15 (25%)
	• Men: 40 (66.7%)
	• Mean time from first symptoms to rtPA infusion: 142.4 minutes (SD 27.39)
	• Mean NIHSS score: 12.3 (SD 5.39)
	• Medical history:
	  ◦ Hypertension: 46 (76.7%)
	  ◦ Hyperlipidaemia: 20 (33.3%)
	  ◦ Arrhythmia: 17 (28.3%)
	  ◦ Coronary heart disease: 15 (25%)
	  ◦ Obesity: 12 (20%)
	  ◦ Diabetes of old age: 10 (16.7%)
	  ◦ Earlier TIA: 6 (10.0%)
	• Mean time from first symptoms to hospital admission: 82.6 minutes (SD 38.91)
	• Mean time from first symptoms to rtPA infusion: 142.4 minutes (SD 27.39)
	• Mean time from hospital admission to rtPA infusion: 59.9 minutes (SD 36.59)

CERE-LYSE-1 2012 *(Continued)*

Placebo

- Participants: 59
- Mean age: 67.0 years (SD 10.56)
- Smokers: 12 (20.7%)
- Men: 37 (62.7%)
- Mean NIHSS score: 11.0 (SD 5.44)
- Medical history:
  - Hypertension: 41 (69.5%)
  - Hyperlipidaemia: 16 (27.1%)
  - Arrhythmia: 17 (28.8%)
  - Coronary heart disease: 12 (20.3%)
  - Obesity: 9 (15.3%)
  - Diabetes of old age: 7 (11.9%)
  - Earlier TIA: 6 (10.2%)
- Mean time from first symptoms to hospital admission: 72.5 minutes (SD 30.86)
- Mean time from first symptoms to rtPA infusion: 133.4 minutes (SD 34.37)
- Mean time from hospital admission to rtPA infusion: 60.9 minutes (SD 29.04)

Inclusion criteria: men and women, 18 to 80 years, who had a clinical diagnosis of acute ischaemic hemispheric stroke that had commenced within 3 hours prior to initiation of administration of rtPA, and had stroke symptoms being present for at least 30 minutes with no significant improvement before treatment, were eligible (further inclusion and exclusion criteria, see Table 1). All participants had to meet the admission standards of the European Medicines Agency (EMA) consensus criteria for the application of thrombolytic therapy with alteplase (rtPA): (1) clinical diagnosis of ischaemic stroke causing a measurable neurological deficit defined as impairment of language, motor function, cognition and/or gaze, vision or neglect. Ischaemic stroke is defined as an event characterised by the sudden onset of an acute focal neurologic deficit presumed to be due to cerebral ischaemia after CT scan excluded haemorrhage, (2) informed consent

Exclusion criteria: evidence of intracranial haemorrhage on the CT scan; participation in another therapeutic clinical trial 3 months before baseline; people with any history of prior stroke and concomitant diabetes; prior stroke within the last 3 months; platelet count below 100 to 103/mm^3; blood glucose < 50 or > 400 mg/dL (< 2.77 or > 22.15 mmol/L); known haemorrhagic diathesis; manifest or recent severe or dangerous bleeding; known bacterial endocarditis, pericarditis; acute pancreatitis; documented ulcerative gastrointestinal disease during the last 3 months, oesophageal varices, arterial-aneurysm, arterial/venous malformation; neoplasm with increased bleeding risk; severe liver disease, including hepatic failure, cirrhosis, portal hypertension, oesophageal varices, and active hepatitis; major surgery or significant trauma in past 3 months; multiple serious drug allergies; hypersensitivity or allergy to 1 of the components of the drug; severe renal impairment; systolic blood pressure > 185 mmHg or diastolic blood pressure > 110 mmHg, or aggressive management (intravenous medication repeatedly) needed to reduce blood pressure to these limits; recent (less than 10 days) traumatic external heart massage, obstetrical delivery, recent puncture of a non-compressible blood vessel (e.g. subclavian or jugular vein puncture); chronic intoxication or chronic substance use disorder with pharmaceuticals, drugs, alcohol, or industrial poisons; symptoms of ischaemic attack began more than 3 hours prior to start of thrombolytic therapy or if time of symptom onset is unknown; minor neurological deficit or symptoms rapidly improving before start of infusion; severe stroke as assessed clinically (e.g. NIHSS > 25) and/or by appropriate imaging techniques; epilepsy; symptoms suggestive of subarachnoid haemorrhage, even if the CT scan is normal; known history of or suspected intracranial haemorrhage; suspected subarachnoid haemorrhage or condition after subarachnoid haemorrhage from aneurysm; any history of CNS damage (i.e. neoplasm, aneurysm, intracranial, or spinal surgery); haemorrhagic retinopathy, e.g. in diabetes (vision disturbances may indicate haemorrhagic retinopathy); administration of heparin within the previous 48 h and a thromboplastin time exceeding the upper limit of normal for laboratory; people receiving oral anticoagulants, e.g. warfarin, sodium; people receiving nifedipine for acute treatment

Pretreatment: the 2 groups were well balanced with respect to baseline prognostic variables, and no significant differences between treatment groups were observed

CERE-LYSE-1 2012 *(Continued)*

Interventions	Cerebrolysin • Frequency of dosage: once daily for 10 consecutive days: intravenous infusion of 30 mL of Cerebrolysin diluted with 70 mL of 0.9% physiological saline to a total volume of 100 mL. Cerebrolysin starting immediately 1 hour after thrombolytic treatment • Standard treatment: the thrombolytic therapy with rtPA was administered as intravenous infusion over 60 minutes. Immediately thereafter, the first intravenous infusion of the study medication (Cerebrolysin/placebo) was administered over a time period of 30 minutes. Placebo • Frequency of dosage: once daily for 10 consecutive days: an identical amount of physiological saline (100 mL) was used as placebo • Standard treatment: the thrombolytic therapy with rtPA was administered as intravenous infusion over 60 minutes. Immediately thereafter, the first intravenous infusion of the study medication (Cerebrolysin/placebo) was administered over a time period of 30 minutes.
Outcomes	• Poor functional outcome defined as death or dependence at the end of the follow-up period (dichotomous outcome) • Early death (dichotomous outcome) • All-cause death (dichotomous outcome) • Serious adverse events (dichotomous outcome) • Adverse effects specifically associated with Cerebrolysin (dichotomous outcome) • Total number of participants with adverse events (dichotomous outcome)
Identification	Sponsorship source: not mentioned. Only the conflict of interest statement: "Wilfried Lang has served as consultant for Bayer, Boehringer Ingelheim, EVER, MSD, Sanofi-Aventis and Pfizer and has received speaking honoraria from these companies. Christian Stadler has received speaker honoraria from EVER. Zdavka Poljakovic received Principal Investigator fee for the clinical study. David Fleet is a freelance consultant statistician undertaking statistical contracts on behalf of pharmaceutical/biotechnology organizations and as such was contracted by EVER. All authors have no other financial interest in the company or its products." Country: 5 countries: Austria, Croatia, the Czech Republic, Slovakia, Slovenia Setting: inpatient (hospital) Author: Wilfried Lang Institution: Department of Neurology, Hospital St John, Austria Email: wilfried.lang@bbwien.at
Notes	No results posted on trial registration platform **Quote:** "Ljubljana, Ljubljana/Slovenia) ClinicalTrials.gov identifier: NCT00840671 Conflicts of interest: Wilfried Lang has served as consultant for Bayer, Boehringer Ingelheim, EVER, MSD, Sanofi-Aventis and Pfizer and has received speaking honoraria from these companies. Christian Stadler has received speaker honoraria from EVER. Zdavka Poljakovic received Principal Investigator fee for the clinical study. David Fleet is a freelance consultant statistician undertaking statistical contracts on behalf of pharmaceutical/ biotechnology organizations and as such was contracted by EVER. All authors have no other financial interest in the company or its products."

Risk of bias

Bias	Authors' judgement	Support for judgement
Allocation concealment	Low risk	**Quote:** "The vials containing the study drug and the placebo were visually identical."

CERE-LYSE-1 2012 *(Continued)*

		Comment: although the quote refers to potential blinding and not allocation concealment, we judged this as low risk.
Sequence generation	Unclear risk	**Quote:** "according to a pre-compiled 1:1 randomization schedule, stratified by centre." **Comment:** there was not only "insufficient information to permit judgement of low risk or high risk" as the basis for a judgement of unclear risk as per the *Cochrane Handbook*. The described procedure does not fit with any of the criteria for an assessment of low risk of bias, i.e. referring to a random number table; using a computer random number generator; coin tossing; shuffling cards or envelopes; throwing dice; drawing of lots; or minimisation. There is no information about the process of generation of the randomisation sequence. In addition to the retrospective protocol registration and a statistician contracted by the Cerebrolysin manufacture EVER Neuro Pharma, we judged this as an unclear risk.
Incomplete outcome data All outcomes	High risk	**Quote:** "Two patients received the incorrect study medication assignment." **Quote:** "Based on statistical information from the third interim analysis, it was decided to terminate the study, as no significant result for the main outcome criteria was expected to be reached." **Quote:** "All patients were included in the ITT population with 60 patients being assigned to Cerebrolysin and 59 assigned to placebo. In the PP population, 100 patients were included with 49 receiving Cerebrolysin and 51 receiving placebo (Fig. 1)." **Comment:** 19 participants of 119 (16%) were lost to follow-up. Attrition bias. Information not available by outcome. Furthermore, the study authors used the 'last observation carried forward' (LOCF) method to fill in the missing data points. There is not a single peer-reviewed statistical publication that describes general conditions under which LOCF provides a statistically unbiased result.
Blinding of outcome assessors All outcomes	Low risk	**Quote:** "All study personnel and participants were blinded to treatment assignment for the duration of the study." **Comment:** however, there was a retrospective protocol registration, and the statistician was contracted by the Cerebrolysin manufacturer EVER Neuro Pharma.
Selective outcome reporting	Unclear risk	**Quote:** "There were no obvious differences between either treatment arms. In each treatment group, four patients died, but in none of the cases was any relationship to the study medication seen. The number of patients with serious adverse events was slightly higher in the Cerebrolysin group compared to the placebo group (12 vs. 7, respectively). In total, 19 (16%) patients experienced at least one serious adverse event (Table 5). " **Comment:** the study was stopped because of no significant result for the main outcome criteria. According to the study authors, there was no causal relationship with the study drug for any of the deaths observed. Neither reasons for nor timing of deaths is presented. Timing of adverse events and serious adverse events are not presented. Study protocol was registered retrospectively. We judged this to be an unclear risk of bias.
Blinding of participants and personnel All outcomes	Low risk	**Quote:** "All study personnel and participants were blinded to treatment assignment for the duration of the study."

CERE-LYSE-1 2012 *(Continued)*

Other sources of bias	High risk	**Comment:** no information on funding sources for the trial. Statistician was contracted by EVER, the manufacturer of Cerebrolysin. There is no information about the provider of Cerebrolysin. Retrospective protocol NCT00840671 registration. No results posted on trial registration platform. Early stopping of the trial after an interim analysis.

Cortexin-Shamalov 2014

Study characteristics

Methods	Study design: randomised, multicentre, prospective, double-blind, placebo-controlled Study grouping: parallel-group Losses to follow-up: none Trial protocol registration: no protocol identified
Participants	Total number of participants: 272 **Baseline characteristics:** Group 1: Cortexin + Cortexin • 136 participants: 81 (59.6%) males, 55 (40.4) females • Mean age: 62.6 ± 10 • NIH score at admission mean 7.03 ± 3.63, median 6.0 • Risk factors: smoking 47 (34.6%), arterial hypertension 119 (87.5%), hypercholesterolaemia 78 (57.4%), diabetes mellitus 24 (17.6%), ischaemic heart disease 48 (35.3%), atrial fibrillation 21 (15.4%) • Pathogenetic variant of stroke according to TOAST criteria: atherothrombotic 38 (27.9%), cardioembolic 20 (14.7%), lacunar 12 (8.8%), other aetiology 1 (0.7%), unknown aetiology 65 (47.8%) • Mortality 4 (2.9%) Group 2: Cortexin + placebo • 72 participants: 45 (62.5%) males, 27 (37.5) females • Mean age: 62.1 ± 12 • NIH score at admission mean 7.68 ± 4.94, median 6.0 • Risk factors: smoking 26 (36.1%), arterial hypertension 64 (88.9%), hypercholesterolaemia 55 (76.4%), diabetes mellitus 12 (16.7%), ischaemic heart disease 21 (29.2%), atrial fibrillation 16 (22.2%) • Pathogenetic variant of stroke according to TOAST criteria: atherothrombotic 14 (19.4%), cardioembolic 13 (18.1%), lacunar 3 (4.2%), other aetiology 4 (5.6%), unknown aetiology 38 (52.8%) • Mortality 3 (4.2%) Group 3: placebo + placebo • 64 participants: 32 (50.0%) males, 32 (50.0) females • Mean age: 62 ± 9.5 • NIH score at admission mean 7.94 ± 4.58, median 6.0 • Risk factors: smoking 24 (37.5%), arterial hypertension 56 (87.5%), hypercholesterolaemia 43 (67.2%), diabetes mellitus 9 (14.1%), ischaemic heart disease 9 (14.1%), atrial fibrillation 10 (15.6%) • Pathogenetic variant of stroke according to TOAST criteria: atherothrombotic 18 (28.1%), cardioembolic 11 (17.2%), lacunar 4 (6.3%), other aetiology 1 (1.6%), unknown aetiology 30 (46.9%) • Mortality 0 **Inclusion criteria:** patients aged 30 to 80 years with ischaemic stroke in the carotid basin that occurred in the first 24 hours from the development of the disease

Cortexin-Shamalov 2014 *(Continued)*

	Exclusion criteria: • Patients with complete regression of neurological symptoms at the time of inclusion in the study • Ischaemic stroke in the vertebrobasilar system • Signs of any intracranial haemorrhage at the first computed tomography (CT) or magnetic resonance imaging (MRI) study • Severity of neurological deficit of more than 25 points according to the NIH stroke scale at admission • Signs of severe comorbidity • Acute myocardial infarction • Uncontrolled arterial hypertension at the time of inclusion (systolic blood pressure (BP) above 180 mmHg and/or diastolic BP above 110 mmHg) • Prior therapy with cytoprotectors
Interventions	**Pretreatment:** lower incidence of hypercholesterolaemia in Cortexin + Cortexin group compared to patients in Cortexin + placebo group; otherwise the 3 groups were not significantly different Cortexin + Cortexin • Frequency of dosage: daily intramuscular injections of 10 mg Cortexin twice a day (morning and afternoon) (total of 20 mg) for 10 days starting within 24 hours of stroke onset. After 10 days rest, the course repeated 10 mg Cortexin twice a day (morning and afternoon) (total of 20 mg) for 10 days. • Standard treatment: no information Cortexin + placebo • Frequency of dosage: daily intramuscular injections of 10 mg Cortexin twice a day (morning and afternoon) (total of 20 mg) for 10 days starting within 24 hours of stroke onset. After 10 days rest, the course of intramuscular injections of placebo twice a day (morning and afternoon) for 10 days. • Standard treatment: no information Placebo + placebo • Frequency of dosage: daily intramuscular injections of placebo twice a day for 10 days starting within 24 hours of stroke onset. After 10 days rest, the course of intramuscular injections of placebo twice a day (morning and afternoon) repeated for 10 days. • Standard treatment: no information
Outcomes	Primary outcome: number of patients with good recovery of impaired neurological function, determined using: modified Rankin scale, Barthel Index, and Rivermead Mobility Index Outcomes are reported in graphs as percentages of people. Precise percentages and raw numbers, as well as baseline levels for the modified Rankin scale, Barthel Index, and Rivermead Mobility Index, are not provided Secondary outcomes: the severity of cognitive deficit was determined with the MMSE scale, reported in a graph as a percentage of people - precise percentages and raw numbers, as well as baseline levels for MMSE are not provided. Quality of life was determined with the SF-36 questionnaire; results are not reported. Throughout the study, the safety of the drug was assessed in terms of mortality and the number of adverse events. Assessments performed at days 0, 11 to 13 (visit 1), 21 to 28 (visit 2), 35 to 40 (visit 3), 60 to 70 (visit 4)
Identification	Country: Russia (7 regional specialised centres for the treatment of vascular pathology) Author: NA Shamalov Email: shamalovn@gmail.com

Cortexin-Shamalov 2014 *(Continued)*

Phone number: +7-926-211-24-98

Notes

Risk of bias

Bias	Authors' judgement	Support for judgement
Allocation concealment	Unclear risk	**Quote:** "The multicenter, double-blind, placebo-controlled study included patients aged 30 to 80 years with ischaemic stroke in the carotid system that occurred within the first 24 hours of the onset of the disease." "Patients who met the inclusion criteria and did not have the exclusion criteria, after signing the informed consent form, were randomized into one of three groups in a 2:1:1 ratio by simple randomization." **Comment:** there was no information on allocation concealment. In addition to the unavailability of a study protocol, we judged this as an unclear risk.
Sequence generation	Unclear risk	**Quote:** "The multicenter, double-blind, placebo-controlled study included patients aged 30 to 80 years with ischemic stroke in the carotid system that occurred within the first 24 hours of the onset of the disease." "Patients who met the inclusion criteria and did not have the exclusion criteria, after signing the informed consent form, were randomized into one of three groups in a 2:1:1 ratio by simple randomization." **Comment:** there was insufficient information to permit a judgement of low risk or high risk, so we opted for a judgement of unclear risk.
Incomplete outcome data All outcomes	Low risk	**Quote:** "The level of 2-month mortality did not differ significantly between the compared groups and amounted to 4 (2.9%) patients in the 1st group and 3 (4.2%) patients in the 2nd, in the 3rd group there were no lethal outcomes. In all cases, according to the investigators, the deaths were not related to the use of the study drug. There were also no differences in the frequency of other adverse events between the groups. Treatment with study drug did not affect laboratory parameters or vital functions." **Comment:** no losses to follow-up. However, adverse events and causes of death were not reported, and the study protocol was not available.
Blinding of outcome assessors All outcomes	Unclear risk	**Quote:** "The multicenter, double-blind, placebo-controlled study included patients aged 30 to 80 years with ischemic stroke in the carotid system that occurred within the first 24 hours of the onset of the disease." "Patients who met the inclusion criteria and did not have the exclusion criteria, after signing the informed consent form, were randomized into one of three groups in a 2:1:1 ratio by simple randomization." **Comment:** there was no information as to whether outcome assessors were aware of the allocated interventions. No information was provided on allocation concealment. In addition to the unavailability of a study protocol, we judged this as an unclear risk.
Selective outcome reporting	Unclear risk	**Quote:** "The level of 2-month mortality did not differ significantly between the compared groups and amounted to 4 (2.9%) patients in the 1st group and 3 (4.2%) patients in the 2nd, in the 3rd group there were no lethal outcomes. In all cases, according to the investigators, the deaths were not related to the use of the study drug. There were also no differences in the frequency of other adverse events between the groups. Treatment with study drug did not affect laboratory parameters or vital functions."

Cortexin-Shamalov 2014 *(Continued)*

		Comment: study protocol not available. Causes of death and the timing of the deaths were not reported. No losses to follow-up; no non-death attrition. We opted for a judgement of unclear risk.
Blinding of participants and personnel All outcomes	Unclear risk	**Quote:** "Patients who met the inclusion criteria and did not have the exclusion criteria, after signing the informed consent form, were randomized into one of three groups in a 2:1:1 ratio by simple randomization. Patients of the 1st group were injected intramuscularly with Cortexin at a dose of 10 mg 2 times a day (morning and afternoon) for 10 days, with a repeated similar course 10 days after the first. The second group consisted of patients who, during the first 10 days of the disease, were injected with Cortexin at a dose of 10 mg 2 times a day (morning and afternoon) for 10 days, then after a 10-day break, a placebo was administered. In the 3rd group, patients received a placebo in two courses, lasting 10 days each, with a frequency of administration similar to the 1st and 2nd groups." **Comment:** there was no information on blinding of participants and personnel. In addition to the unavailability of a study protocol, we judged this as an unclear risk.
Other sources of bias	Unclear risk	**Comment:** the authors did not provide any information on funding sources for the study or potential conflicts of interest. In addition to the unavailability of a study protocol, we judged this as an unclear risk.

Ladurner 2005

Study characteristics	
Methods	Study design: multicentre, randomised, double-blind controlled trial Mean duration of follow-up: 90 days Study grouping: parallel-group Loss to follow-up: 15 of 146 (10%) Trial protocol registration: no protocol identified
Participants	**Baseline characteristics:** Cerebrolysin • Age: 65 years ± 1.17 • Men: 47 (60.3%) • Women: 31 (39.7%) • Total number: 78 • Handedness: left: 1 (1.3%); right: 77 (98.7%) • Stroke location: left hemisphere: 41 (52.6%); right hemisphere: 37 (47.4%) • Duration of symptoms (values are means ± SEM): 12.3 hours ± 0.73 • CNS (values are means ± SEM): 6.88 ± 0.09 • GCS (values are means ± SEM): 14.1 ± 0.20 Placebo • Age: 65 years ± 1.32 • Male: 38 (55.9%) • Female: 30 (44.1%) • Total number: 68

Ladurner 2005 *(Continued)*

- Handedness: left: 0 (0%); right: 68 (100%)
- Stroke location: left hemisphere: 31 (45.6%); right hemisphere: 37 (54.4%)
- Duration of symptoms (values are means ± SEM): 13.5 hours ± 1.16
- CNS (values are means ± SEM): 6.68 ± 0.14
- GCS (values are means ± SEM): 14.4 ± 0.16

Inclusion criteria: men and women suffering from their first acute ischaemic stroke with clinical symptoms of middle cerebral artery area were enrolled. Patients were eligible if they were admitted to the hospital and received the first dose of study medication within 24 hours of the onset of the stroke and were between 45 and 85 years of age at study entry. Participants were also required to have a GCS score of greater than 10 and a CNS score between 4.5 and 8.0 at baseline.

Exclusion criteria: people with haemorrhagic strokes, transient ischaemic attacks, uncontrollable hypertension, acute myocardial infarction, congestive heart failure, moderate-severe dementia prior to the stroke, coma or stupor, other severe concomitant diseases, impaired renal function, and people with a history of prior stroke

Pretreatment: no significant group differences of the demographic characteristics were observed at baseline, and the severity of the stroke at study entry was comparable between the 2 groups

Interventions	Cerebrolysin • Frequency of dosage: Cerebrolysin 50 mL was administered once daily for 21 days by intravenous infusion in a peripheral vein over a period of 20 minutes. Cerebrolysin mixed with 50 mL of normal saline • Standard treatment: pentoxifylline (300 mg/day, intravenous) and acetylsalicylic acid (250 mg/day, orally) for the first 21 days, and pentoxifylline (2400 mg/day, orally) and acetylsalicylic acid (250 mg/day, orally) from day 22 to the end of the study at day 90 Placebo • Frequency of dosage: placebo was administered once daily for 21 days by intravenous infusion in a peripheral vein over a period of 20 minutes. Placebo contained 100 mL of normal saline. • Standard treatment: pentoxifylline (300 mg per day, intravenous) and acetylsalicylic acid (250 mg/day, orally) for the first 21 days, and pentoxifylline (2400 mg/day, orally) and acetylsalicylic acid (250 mg/day, orally) from day 22 to the end of the study at day 90
Outcomes	• Poor functional outcome defined as death or dependence at the end of the follow-up period (dichotomous outcome) • Early death (dichotomous outcome) • All-cause death (dichotomous outcome) • Serious adverse events (dichotomous outcome) • Adverse effects specifically associated with Cerebrolysin (dichotomous outcome) • Total number of participants with adverse events (dichotomous outcome)
Identification	Sponsorship source: EBEWE Pharma Country: Austria, the Czech Republic, Hungary Setting: inpatient (hospital) Authors: Dr G Ladurner and H Moessler Institution: Department of Neurology, Christian-Doppler Hospital, Salzburg, Austria Email: g.ladurner@lks.at and herbert.moessler@ebewe.com
Notes	Population: concomitant use of nootropic drugs (e.g. piracetam), drugs with dilatating effects on peripheral blood vessels (naftidrofuryl, cinnarizine, flunarizine, nimodipine), as well as chronic intake of antidepressants, tranquillisers, sedatives, or CNS stimulants was prohibited throughout the study

Ladurner 2005 *(Continued)*

No study protocol identified

Risk of bias

Bias	Authors' judgement	Support for judgement
Allocation concealment	Unclear risk	**Quote:** "For each patient a sealed envelope with information on the actual treatment dispensed was provided to the investigator for emergency cases. All envelopes remained sealed throughout the study." **Comment:** sealed envelopes were used to conceal allocation, but it is not mentioned if they were opaque. In addition to the unavailability of a study protocol, we judged this as an unclear risk of bias.
Sequence generation	Low risk	**Quote:** "Patients who met all entry criteria were assigned to the treatment groups in a 1:1 ratio, according to a randomisation code generated by a computer software (EBEWE Pharma, Unterach, Austria). The randomisation was carried out in blocks of 12 patients, stratified by study centre." **Comment:** the computer software used to generate the random numbers was provided by EBEWE Pharma, which is also the provider of Cerebrolysin.
Incomplete outcome data All outcomes	High risk	**Quote:** "146 patients were randomised to two treatment groups and constituted the ITT population: 78 patients to the Cerebrolysin group and 68 patients to the placebo group. Of these patients, 67 of the Cerebrolysin group and 52 of the placebo group completed the study. Reasons for the 25 cases of study discontinuation were death (6 Cerebrolysin, 6 placebo), serious adverse event (1 placebo), and consent withdrawn (3 Cerebrolysin; 9 placebo)." **Comment:** attrition bias - 25 out of 146 randomised participants were lost to follow-up (17%). Information on the outcomes of interest to this review was available only for serious adverse events including death. Furthermore, the study authors used the 'last observation carried forward' (LOCF) method to fill in the missing data points. There is not a single peer-reviewed statistical publication that describes general conditions under which LOCF provides a statistically unbiased result.
Blinding of outcome assessors All outcomes	Low risk	**Quote:** "The investigators and all other study personnel were blind as to the random code assignment until the completion of the statistical analysis."
Selective outcome reporting	Unclear risk	**Quote:** "Twelve patients died during the study: 6 in the Cerebrolysin group (7.69%) and 6 in placebo group (8.83%). None of the deaths was reportedly related to the study drug administration." **Quote:** "With the exception of one SAE (hematemesis) in the placebo group which was rated to be likely related to the study drug, there was no causal relationship to the study drug for any other of the SAEs, as per the investigator's assessment." **Comment:** the trial authors did not report on the time when deaths occurred, and did not assess potential causality with the administered medicines. Furthermore, the authors used the 'last observation carried forward' (LOCF) method to fill in the missing data points. There is not a single peer-reviewed statistical publication that describes general conditions under which LOCF provides a statistically unbiased result.
Blinding of participants and personnel All outcomes	Low risk	**Quote:** "The investigators and all other study personnel were blind as to the random code assignment until the completion of the statistical analysis."

Ladurner 2005 *(Continued)*

		Comment: impossible to assess blinding by outcome.
Other sources of bias	Unclear risk	**Quote:** "The participants of the Cerebrolysin study group were as follows: G. Ladurner, Christian-Doppler Clinic, Salzburg, Austria; K. Niederkorn, University Hospital for Neurology, Graz, Austria; I. Szirmai, Semmelweis University of Medicine, Budapest, Hungaria; P. Kalvach, Charles University, FNKV, Department of Neurology, Prague; F. Stockenhuber, Landeskrankenhaus, Oberpullendorf, Austria; Z. Haffner, Petz Alada ´Megyei Koorha'z, Gyoor, Hungaria; P. Ridzon, Thomayer's Hospital, Praha, Czech Republic; E. Diabl, Linz General Hospital, Linz, Austria." **Quote:** "The study medication was provided to the study centres by EBEWE Pharma in the form of a ready-to-use infusion solution. The active medication contained 50 ml Cerebrolysin mixed with 50 ml of normal saline." **Comment:** there was no information on funding sources for the trial, and no conflict of interest statement was provided. EBEWE Pharma provided the medication and randomisation codes. No study protocol publicly available.

Skvortsova 2004

Study characteristics	
Methods	Study design: RCT Study grouping: parallel-group Losses to follow-up: none Trial protocol registration: no protocol identified
Participants	Cerebrolysin • Participants: 12 • Men: 6 • Women: 6 • Mean age: 68.7 years ± 10.6 • Ratio of participants with lesions of the left and right hemispheres: 8/4 • Period since the stroke to admission in hospital: 9.2 hours ± 2.9 • NIHSS score prior to intervention: 11.2 ± 4.7 • Rankin score prior to intervention: 3.5 ± 1.1 • Number of participants with an NIHSS score more than 14 (severe stroke): 3 (25%); 14 and less: 9 (75%) • Average volume of brain lesions: 17.5 cm^3 ± 14.7 • Number of participants with a lesion volume between 7 cm^3 and 64 cm^3: 8 Placebo • Participants: 12 • Men: 9 • Women: 3 • Mean age: 69.4 years ± 9.5 • Ratio of participants with lesions of the left and right hemispheres: 8/4 • Period since the stroke to admission in hospital: 8.6 hours ± 2.9 • NIHSS score prior to intervention: 12.2 ± 2.8 • Rankin score prior to intervention: 3.8 ± 0.9 • Number of participants with an NIHSS score more than 14 (severe stroke): 3 (25%); 14 and less: 9 (75%)

Skvortsova 2004 *(Continued)*

	• Average volume of brain lesions: 21.7 cm^3 ± 23.1 • Number of participants with a lesion volume between 7 cm^3 and 64 cm^3: 7 **Inclusion criteria:** people with first-in-lifetime ischaemic stroke in the basin of internal carotid artery, aged 45 to 85 years, admitted to the ICU within 12 hours of stroke symptoms onset **Exclusion criteria:** disappearance of symptoms within 4 hours from the beginning of stroke; people with haemorrhagic stroke or stroke in the vertebrobasilar system; people with blood pressure levels higher than 200/100 mmHg; people with acute myocardial infarction, with a priori severe dementia; pregnant women; and participants in other studies **Pretreatment:** no difference
Interventions	Cerebrolysin • Frequency of dosage: diluted with 40 mL of saline infused by slow drip over 1 hour for 10 days after stroke onset (within 12 hours) • Standard treatment: aspirin 100 mg/day, haemodilution, pentoxifylline, heparin (when needed) Placebo • Frequency of dosage: physiological saline • Standard treatment: aspirin 100 mg/day, haemodilution, pentoxifylline, heparin (when needed)
Outcomes	• Poor functional outcome defined as death or dependence at the end of the follow-up period (dichotomous outcome) • Early death (dichotomous outcome) • All-cause death (dichotomous outcome) • Serious adverse events (dichotomous outcome) • Adverse effects specifically associated with Cerebrolysin (dichotomous outcome) • Total number of participants with adverse events (dichotomous outcome)
Identification	Sponsorship source: not reported Country: Russia Setting: inpatient Author's name: Skvortsova Institution: Department of Basic and Clinical Neurology, Russian State Medical University Address: Moscow
Notes	No study protocol identified

Risk of bias

Bias	Authors' judgement	Support for judgement
Allocation concealment	Unclear risk	**Quote:** "Всем пациентам рандомизированно и вслепую было назначено плацебо или церебролизин в дозе 10 либо 50 мл (по 12 человек в каждой группе)." ["Vsem patsiyentam randomizirovanno i vslepuyu bylo naznacheno platsebo ili tserebrolizin v doze 10 libo 50 ml (po 12 chelovek v kazhdoy gruppe)"]: "All patients were randomly and blindly assigned to placebo or Cerebrolysin at 10 or 50 mL (12 in each group)." **Comment:** insufficient information to permit a judgement of low risk or high risk. There was no mention of allocation concealment. In addition to the unavailability of a study protocol, we judged this as an unclear risk.

Skvortsova 2004 *(Continued)*

Sequence generation	Unclear risk	**Quote:** "Всем пациентам рандомизированно и вслепую было назначено плацебо или церебролизин в дозе 10 либо 50 мл (по 12 человек в каждой группе)." ["Vsem patsiyentam randomizirovanno i vslepuyu bylo naznacheno platsebo ili tserebrolizin v doze 10 libo 50 ml (po 12 chelovek v kazhdoy gruppe)"]: "All patients were randomly and blindly assigned to placebo or Cerebrolysin at 10 or 50 mL (12 in each group)." **Comment:** there was no information on allocation concealment. In addition to the unavailability of a study protocol, we judged this as an unclear risk.
Incomplete outcome data All outcomes	Low risk	**Quote:** "Анализ исходов инсульта к 30-м суткам не обнаружил достоверных различий между группами в летальности. Причины смерти 3 из 5 больных, получавших церебролизин, а также одного пациента из группы плацебо не были связаны с инсультом (тромбоэмболия легочной артерии, пневмония, пиелонефрит). У 2 пациентов, получавших церебролизин, и 2 получавших плацебо смерть наступила вследствие отека мозга с развитием вторичного стволового синдрома." ["Analiz iskhodov insul'ta k 30-m sutkam ne obnaruzhil dostovernykh razlichiy mezhdu gruppami v letal'nosti. Prichiny smerti 3 iz 5 bol'nykh, poluchavshikh tserebrolizin, a takzhe odnogo patsiyenta iz gruppy platsebo ne byli svyazany s insul'tom (tromboemboliya legochnoy arterii, pnevmoniya, piyelonefrit). U 2 patsiyentov, poluchavshikh tserebrolizin, i 2 poluchavshikh platsebo smert' nastupila vsledstviye oteka mozga s razvitiyem vtorichnogo stvolovogo sindroma.": "Analysis of stroke outcomes by day-30 did not uncover significant differences between groups in lethality. The causes of death of 3 out of 5 patients, treated with Cerebrolysin, and one patient from the placebo group were not attributed to stroke (pulmonary oedema, pneumonia, pyelonephritis). In 2 patients, treated with Cerebrolysin, and 2 treated with placebo, deaths occurred due to cerebral oedema with development of secondary brain stem syndrome."] **Comment:** no losses to follow-up, causes of death described. However, adverse events were not reported and the study protocol was not available.
Blinding of outcome assessors All outcomes	Unclear risk	**Comment:** there was no information on blinding of outcome assessors. In addition to the unavailability of a study protocol, we judged this as an unclear risk.
Selective outcome reporting	Unclear risk	**Comment:** study protocol not available; we judged this as an unclear risk. **Quote:** "Причины смерти 3 из 5 больных, получавших церебролизин, а также одного пациента из группы плацебо не были связаны с инсультом (тромбоэмболия легочной артерии, пневмония, пиелонефрит)". ["Prichiny smerti 3 iz 5 bol'nykh, poluchavshikh tserebrolizin, a takzhe odnogo patsiyenta iz gruppy platsebo ne byli svyazany s insul'tom (tromboemboliya legochnoy arterii, pnevmoniya, piyelonefrit).": "The causes of death for 3 of 5 patients who received Cerebrolysin and 1 patient in the placebo group were not associated with stroke (pulmonary embolism, pneumonia, pyelonephritis)".] **Comment:** the time when deaths occurred was not reported. Furthermore, the study authors considered that deaths were not drug-related. Adverse events were not reported. The timing was not clear for outcomes presented in a table and a graph, although these outcomes were not those of interest for the review.
Blinding of participants and personnel All outcomes	Unclear risk	**Quote:** "Всем пациентам рандомизированно и вслепую было назначено плацебо или церебролизин в дозе 10 либо 50 мл (по 12 человек в каждой группе)". ["Vsyem patziyentam randomizirovanno i vslyepooyo bilo naznachyeno platzyebo ili tzyeryebrolizin v dozye 10 libo 50 ml (po 12 chyelovyek v kazdoy gurooppye).": "All patients were randomly and blindly assigned to placebo or Cerebrolysin at 10 or 50 mL (12 in each group)."]

Skvortsova 2004 *(Continued)*

		Comment: there was no information on blinding of participants and personnel. In addition to the unavailability of a study protocol, we judged this as an unclear risk.
Other sources of bias	Unclear risk	**Comment:** no information on funding sources for the trial, and no conflict of interest statement was provided. No study protocol available.

Xue 2016

Study characteristics	
Methods	Study design: RCT Study grouping: parallel-group Losses to follow-up: 19 (16%) Trial protocol registration: retrospective (4 years difference between study start date (2010) and the date registration record posted (2014), when the trial was already completed in 2010)
Participants	Cerebrolysin • Participants: 20 • Age: 66.5 years (SD ± 8.1) • Men: 9 • Women: 11 • Time until admission: 5 hours (SD ± 3.3) • Time until treatment: 7.6 hours (SD ± 3.6) • Systolic blood pressure: 150.7 mmHg (SD ± 13.7) • Diastolic blood pressure: 85.1 mmHg (SD ± 13.6) • Thrombolysis treatment: 7 (35%) • Previous history: hypertension: 6 (30%); diabetes: 7 (35%); coronary heart disease: 8 (40%) • NIHSS score: 10.6 (SD ± 4.75) • Barthel Index score: 22.25 (SD ± 7.16) Placebo • Participants: 20 • Age: 68.4 years (SD ± 4.2) • Men: 10 • Women: 10 • Time until admission: 4.8 hours (SD ± 3.7) • Time until treatment: 5.6 hours (SD ± 3.0) • Systolic blood pressure: 152.5 mmHg (SD ± 12.8) • Diastolic blood pressure: 87.2 mmHg (SD ± 12.5) • Thrombolysis treatment: 6 (30%) • Previous history: hypertension: 10 (50%); diabetes: 6 (30%); coronary heart disease: 9 (45%) • NIHSS score: 10.20 (SD ± 3.72) • Barthel Index score: 20.0 (SD ± 6.96) Other neuroprotective agent • Participants: 20 • Age: 67.1 years (SD ± 6.3) • Men: 9

Xue 2016 *(Continued)*

- Women: 11
- Time until admission: 5.4 hours (SD ± 3.0)
- Time until treatment: 7.7 hours (SD ± 5.9)
- Systolic blood pressure: 148.6 mmHg (SD ± 14.6)
- Diastolic blood pressure: 88.7 mmHg (SD ± 10.7)
- Thrombolysis treatment: 5 (25%)
- Previous history: hypertension: 7 (35%); diabetes: 8 (40%); coronary heart disease: 6 (30%)
- NIHSS score: 12.4 (SD ± 4.38)
- Barthel Index score: 19.75 (SD ± 6.38)

Inclusion criteria: acute ischaemic stroke for the first time < 12 h prior to entry into the study, with a score of 6 to 25 on the NIHSS. Prior to randomisation, all participants were evaluated using cranial CT or MRI scanning and were followed with serial neurological examinations to confirm acute ischaemic stroke.

Exclusion criteria: people with lacunar infarction, cerebral haemorrhagic infarction, epilepsy or epileptic seizures, history of neurological diseases, myocardial infarction, renal and hepatic abnormalities, metabolic diseases, and contraindications to antiplatelet treatments

Pretreatment: comparison of baseline characteristics amongst the treatment groups revealed no significant differences (P > 0.05)

Interventions	Cerebrolysin • Frequency of dosage: intravenous infusion of 30 mL Cerebrolysin/day in 100 mL normal saline for 10 days; the infusion lasted 50 to 70 minutes • Standard treatment: routine treatments including antithrombotic drugs, hypoglycaemic agents, antilipaemic agents, antihypertensive(s), and dehydration, according to guidelines for the management of ischaemic stroke in the neurological ICU (14); 100 mg aspirin orally as standard treatment Placebo • Frequency of dosage: 100 mL saline intravenous infusion once daily for 10 days • Standard treatment: routine treatments including antithrombotic drugs, hypoglycaemic agents, antilipaemic agents, antihypertensive(s), and dehydration, according to guidelines for the management of ischaemic stroke in the neurological ICU (14); 100 mg aspirin orally as standard treatment Other neuroprotective agent • Frequency of dosage: intravenous infusion of 100 mL NBP and sodium chloride injection, which contained 25 mg NBP and 0.9 g sodium chloride, twice daily during 10 days starting within 12 hours after stroke onset • Standard treatment: routine treatments including antithrombotic drugs, hypoglycaemic agents, antilipaemic agents, antihypertensive(s), and dehydration, according to guidelines for the management of ischaemic stroke in the neurological ICU (14); 100 mg aspirin orally as standard treatment
Outcomes	• Poor functional outcome defined as death or dependence at the end of the follow-up period (dichotomous outcome) • Early death (dichotomous outcome) • All-cause death (dichotomous outcome) • Serious adverse events (dichotomous outcome) • Adverse effects specifically associated with Cerebrolysin (dichotomous outcome) • Total number of participants with adverse events (dichotomous outcome)
Identification	Sponsorship source: this study was supported by the Shanghai Jiao Tong University Affiliated Sixth People's Hospital (grant nos. 1462 and 1583) and the Shanghai Science and Technology Council (grant no. 13411951401) Country: China

Xue 2016 *(Continued)*

	Setting: "from January 2010 to May 2010, a randomised, double-blind trial was conducted, which involved patients with acute ischaemic stroke in the neurology ward of Shanghai Jiao Tong University Affiliated Sixth People's Hospital (Shanghai, China)" Comments: there were 3 treatment groups: NBP, Cerebrolysin, or placebo. We found the numbers randomised and evaluated to be unclear, thus the numerical results were meaningless for the purposes of this review. Author's name: Dr Hao Chen Institution: Department of Neurosurgery, Shanghai Jiao Tong University, Affiliated Sixth People's Hospital Email: chenhao_316@aliyun.com
Notes	Results posted on trial registration platform

Risk of bias

Bias	Authors' judgement	Support for judgement
Allocation concealment	Unclear risk	**Quote:** "The random numbers were placed in concealed envelopes." **Comment:** concealed envelopes; not clear by whom and from whom the envelopes were concealed, and who might have had access to the envelopes. In addition to the retrospective nature of the trial registration, we judged this as an unclear risk of bias.
Sequence generation	Unclear risk	**Quote:** "Patients were randomly assigned to the NBP group, Cerebrolysin group or placebo group." **Quote:** "Randomization was performed by means of computer-generated numbers through software by a third party who was not involved in patient management." **Comment:** the investigators describe a random component (computer random number generator) in the sequence generation process. Unclear who the third party was; in addition to the retrospective nature of the trial registration, we judged this as an unclear risk of bias.
Incomplete outcome data All outcomes	High risk	**Quote:** "During the trial period, 84 patients with AIS underwent randomization. Among these, 60 patients who received study intervention were included in the efficacy analysis. The NBP group contained 9 male and 11 female patients, whose ages ranged from 53 to 79 years. The Cerebrolysin group contained 9 males and 11 females, and their ages ranged from 54 to 85 years. The placebo group contained 10 males and 10 females, whose ages were from 52 to 87 years." **Comment:** 84 − 60 = 24, which is 29% of randomised participants lost in the trial report, with no description of why only rounded numbers 20, 20, and 20 were included in any data presentation. Furthermore, the authors used the 'last observation carried forward' (LOCF) method to fill in the missing data points. There is not a single peer-reviewed statistical publication that describes general conditions under which LOCF provides a statistically unbiased result.
Blinding of outcome assessors All outcomes	Unclear risk	**Quote:** "Patients and methods: patient selection. From January 2010 to May 2010, a randomised, double-blind trial was conducted, which involved patients with AIS in the Neurology Ward of Shanghai Jiao Tong University Affiliated Sixth People's Hospital (Shanghai, China)."

Xue 2016 *(Continued)*

		Comment: there was no information on blinding of outcome assessors. We looked for specifics on blinding in the trial registration record with results posted, but did not find the relevant information. It was not possible to assess blinding by outcome, therefore we judged this as an unclear risk.
Selective outcome reporting	Unclear risk	**Quote:** "Missing values were substituted by last observation carried forward. P < 0.05 was considered to indicate a statistically significant result." **Comment:** 84 − 60 = 24, which is 29% of randomised participants lost in the trial report, no description of why only rounded numbers 20, 20, and 20 were included in any data presentation. Furthermore, the authors used the 'last observation carried forward' (LOCF) method to fill in the missing data points. There is not a single peer-reviewed statistical publication that describes general conditions under which LOCF provides a statistically unbiased result.
Blinding of participants and personnel All outcomes	Unclear risk	**Quote:** "a randomised, double-blind trial was conducted, ..." **Comment:** no description of blinding; impossible to assess blinding by outcome.
Other sources of bias	Unclear risk	**Comment:** no conflict of interest statement was provided. Retrospective trial registration.

ACE: angiotensin-converting enzyme
AIS: acute ischaemic stroke
ANCOVA: analysis of covariance
CNS: central nervous system
CT: computed tomography
GCS: Glasgow Coma Score
ICU: intensive care unit
ITT: intention-to-treat
MMSE: Mini-Mental State Examination
MRI: magnetic resonance imaging
mRS: modified Rankin Scale
MW: Mann-Whitney
NBP: DL-3-n-butylphthalide
NIH: National Institutes of Health
NIHSS: National Institutes of Health Stroke Scale
RCT: randomised controlled trial
rtPA: recombinant tissue plasminogen activator
SD: standard deviation
SEM: standard error of the mean
TIA: transient ischaemic attack

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Belova 2018	Ineligible intervention: no information about time of administration of Cerebrolysin (only time of hospital admission is provided)
Bogolepova 2019	Meta-analysis of 9 RCTs; ineligible intervention: therapy was started during 72 hours after stroke
CEREC-Stroke	Trial registration record and trial report: completed study with ineligible study design - not an RCT
Gharagozli 2017	Ineligible population: stroke diagnosis not confirmed by MRI

Study	Reason for exclusion
Kurenkova 2014	Ineligible specifics of intervention: treatment started 3 to 5 days after admission to the hospital
NCT04904341	Trial registration record; ineligible study design, not an RCT
Slyusar 2021	Ineligible study design; not an RCT

CT: computed tomography
MRI: magnetic resonance imaging
NIHSS: National Institutes of Health Stroke Scale
RCT: randomised controlled trial

Characteristics of ongoing studies *[ordered by study ID]*

CERE-REHA-RU/01

Study name	CERE-REHA-RU
Methods	Double-blind, placebo-controlled, multicentre, randomised, parallel-group, phase IV clinical study on effectiveness of adding Cerebrolysin to standard rehabilitation complex interventions in patients with ischaemic stroke
Participants	180 - target
Interventions	Cerebrolysin, 30 mL for 20 days, no further details provided
Outcomes	No details provided
Starting date	19 January 2015
Contact information	Company 'Ligand Research': 3/7 Odoevskiy Driveway, Moscow, Russia, 117574
Notes	Stopped, reasons not provided

CEREHETIS

Study name	Cerebrolysin as Early add-on to REperfusion therapy and risk of HEmorrhagic Transformation after Ischemic Stroke (CEREHETIS). A prospective randomized active-control multicenter pilot study
Methods	Prospective, randomised, active-control, multicentre trial in parallel groups
Participants	Participant type(s): patient Age group: mixed Sex: both Target number of participants: 263 Total final enrolment: 341
Interventions	Randomisation in a 1:2 ratio into case or control group by using a random number generating software Control group: intravenous thrombolytic therapy (IV TLT) with recombinant tissue plasminogen activator (alteplase, 0.9 mg/kg)

CEREHETIS *(Continued)*

	Case group: IV TLT + at the same time Cerebrolysin 30 mL diluted in 100 mL of normal saline over 20 min via another IV cubital line. Then, infusions of Cerebrolysin daily for 14 consecutive days. Standard care is allowed for both groups.
Outcomes	Primary outcome measure: Rate of haemorrhagic transformation (any and symptomatic) on any of follow-up non-contrast brain computed tomography (CT) scan. CT is performed 24 h after the IV TLT (visit 1), on day 7 (visit 2), 14 (visit 3) and if required by a treating neurologist. Symptomatic intracranial haemorrhage was defined according the ECASS III study as any apparently extravascular blood in the brain or within the cranium that is associated with clinical deterioration (an increase of ≥ 4 points on the NIHSS), or led to death. Secondary outcome measures: 1. Functional outcome measured using National Institutes of Health Stroke Scale (NIHSS) score at (visits 1, 2, 3 and on day 90 (visit 4) 2. Functional outcome measured using modified Rankin scale score at day 14 and 90 3. Blood-brain barrier permeability measures: fractional anisotropy, axial and radial diffusivity, permeability-surface area product measured using axial diffusion-tensor imaging at 24 h after the IV TLT, on day 14 and brain CT perfusion on day 14 and 90 4. Adverse events are assessed by interview during the follow-up 5. Vital signs (blood pressure, heart rate), standard biochemical panel and complete blood count (blood test) are evaluated at admission and on day 146. C-reactive protein is measured by blood test at visit 1
Starting date	24 April 2018
Contact information	Primary contact: Prof Dina Khasanova Interregional Clinical Diagnostic Center 12A Karbyshev St Kazan 420101 Russian Federation +7 (843) 291 10 16 dhasanova@mail.ru Additional contact: Dr Mikhail Kalinin Interregional Clinical Diagnostic Center 12A Karbyshev St Kazan 420101 Russian Federation +79172959770 ninilak@gmail.com
Notes	Overall study end date: 30 November 2020

GP20011-P4-32

Study name	Multicenter, double-blind, placebo-controlled, randomized, in two parallel groups study of the efficacy of Cortexin®, 10 mg, manufactured by GEROPHARM LLC, Russia, in patients in the acute period of ischemic stroke
Methods	Multicentre, double-blind, placebo-controlled, randomised study, in 2 parallel groups

GP20011-P4-32 *(Continued)*

Participants	320
Interventions	Cortexin®, 10 mg, manufactured by GEROPHARM LLC, lyophilisate for solution for intramuscular injection, 10 mg. No further details provided
Outcomes	No details provided
Starting date	29 August 2019
Contact information	Limited Liability Company "GEROPHARM", 000000, St. Petersburg, 191119, St. Petersburg, st. Zvenigorodskaya, 9, Russia
Notes	End date - 31 December 2027

GP20011-P4-36

Study name	Multicenter, double-blind, randomized, controlled, parallel study of the therapeutic equivalence of intramuscular and intravenous forms of administration of the drug Cortexin®, 10 mg, produced by GEROPHARM LLC, Russia, in relation to the degree of functional recovery in patients in the acute period of ischemic stroke
Methods	Multicentre, double-blind, randomised, controlled, parallel study
Participants	974
Interventions	Cortexin 10 mg, produced by GEROPHARM LLC, lyophilisate solution for intramuscular and intravenous administration, 10 mg, no further details provided
Outcomes	No details provided
Starting date	2 December 2021
Contact information	Limited Liability Company "GEROPHARM", 191119, St. Petersburg, St. Petersburg, Zvenigorodskaya st., 9, Russia
Notes	End date: 31 December 2029

IRCT201406169014N36

Study name	The effect of Cerebrolysin versus placebo on improvement of patients with acute ischemic stroke: a double blinded randomized clinical trial
Methods	Interventional, randomised, parallel-group, double-blind (clinical trial)
Participants	122 participants aged 45 to 85 years with ischaemic stroke, referred to the hospital within less than 24 hours after stroke
Interventions	Intervention: Cerebrolysin 10 mL in 100 mL normal saline daily for 7 days added to routine therapy Control: placebo - 100 mL normal saline alone daily for 7 days added to routine therapy
Outcomes	Primary: measuring motor function before intervention and 3 and 7 days after intervention using Canadian Stroke Scale

IRCT201406169014N36 *(Continued)*

	Secondary: measuring motor function 1 month after intervention using modified Rankin Scale and Bartel Index
Starting date	23 July 2013; retrospective registration; no results posted
Contact information	Sajedeh Nazari, Farshchian Hospital, Mirzadeh Eshghi Ave, Hamadan, Iran (Islamic Republic of); +98 81 3264 0021; sajed_nazari@yahoo.com
Notes	Funding: Dr Saeid Bashirian, Vice-chancellor for Research the Technology, Hamadan University of Medical Sciences

ISRCTN54581790 (ESCAS)

Study name	Efficacy and safety of Cerebrolysin in the treatment of aphasia after acute ischemic stroke (ESCAS)
Methods	Exploratory prospective randomised, controlled, double-blind trial
Participants	120 - target samples size, adults, both genders. Participant inclusion criteria: 1. Radiologically (CT or MRI) and clinically confirmed diagnosis of acute ischaemic stroke in the left MCA territory 2. Broca or mixed non-fluent aphasia 3. Inclusion in the study between 3 and 5 days post-stroke 4. Right-handedness 5. Romanian as language of daily use 6. Signed informed consent
Interventions	Treatment group: 30 mL Cerebrolysin/day, diluted with 0.9% saline solution to a total solution of 250 mL, administered by IV infusion and speech therapy (1 h/day), 30 treatment days – 1 to 14, 29 to 42, 57 to 70 Control group: 250 mL 0.9% saline solution administered by IV infusion as procedural placebo and speech therapy for 1 h per day during the study period (30 treatment days) – days 1 to 14, 29 to 42, 57 to 70
Outcomes	Primary outcome measure: Language function assessed by Western Aphasia Battery (Kertesz, 1979) at days 0, 30, 60, 90 Secondary outcome measures: 1. Stroke severity assessed by NIH Stroke Scale (www.nihstrokescale.org) at days 0, 30, 60, 90 2. Functional outcome assessed by Modified Rankin Score (van Swieten et al 1988) at days 0, 30, 60, 90 3. Activities of Daily Living assessed by Barthel Index (Mahoney et al 1965) at days 0, 30, 60, 90
Starting date	15 February 2020 (prospective registration)
Contact information	Contact: Dr Olivia Verisezan Rosu 37 Mircea Eliade Street 400364 Cluj-Napoca Romania Phone: +40740066761 Email: olivia.rosu@ssnn.ro
Notes	Overall trial end date: 31 August 2022

ISRCTN88122184 (CODEC)

Study name	A randomized, placebo-controlled, double-blind trial to asses the effficacy and safety of CEREBROLYSIN in the treatment of Post-Stroke Cognitive Decline (CODEC)
Methods	Randomised, placebo-controlled, double-blind, phase IV study
Participants	290 - target sample size. Adults, both genders. 1. Diagnosis of stroke, ischaemic in origin (TACS or PACS), confirmed by MRI 2. Onset of stroke within 72 h prior to screening 3. NIH Stroke Scale score between 5 and 15 at inpatient admission 4. Pre-stroke mRS of 0 or 1 5. No cognitive impairment prior to stroke with an IQ code score ≤ 3 6. Aged between 40 and 80 years, inclusive 7. Patient is willing and able to comply with the protocol for the duration of the study
Interventions	1. Treatment group: Cerebrolysin solution 30 mL diluted with 0.9% saline solution to 250 mL, administered by IV infusion 2. Placebo group: 250 mL 0.9% saline solution administered by IV infusion
Outcomes	Primary outcome measure: 1. Cognitive function assessed using Stroop Color-Word Test (Stroop, 1935) at 0, 180, 360 days 2. Cognitive function assessed using Trail Making Test Part A (Reitan, 1958) at 0, 180, 360 days 3. Cognitive function assessed using Digit Span Backwards Task (Wechsler adult intelligence scale – third edition) (Wechsler, 1997) at 0, 180, 360 days 4. Cognitive function assessed using Verbal Fluency Test – CFL Version (Benton & Hamsher, 1976) at 0, 180, 360 days 5. Cognitive function assessed using Digit Symbol (Wechsler adult intelligence scale – third edition) (Wechsler, 1997) at 0, 180, 360 days 6. Cognitive function assessed using Rey Auditory Verbal Learning Test (Rey, 1964) at 0, 180, 360 days Secondary outcome measures: 1. Cognitive function assessed using Montreal Cognitive Assessment (MoCA) (Nasreddine, 2005) at 0, 180, 360 days 2. Stroke severity assessed by NIH Stroke Scale (www.nihstrokescale.org/) at 0, 180, 360 days 3. Functional outcome assessed by Modified Rankin Score (van Swieten J et al, 1988) at 0, 180, 360 days 4. Emotional status assessed using Hospital Anxiety and Depression Scale (Zigmond, 1983) at 0, 180, 360 days 5. Functional outcome assessed using EQ-5D-5L (Herdman, 2011) at 0, 180, 360 days
Starting date	20 February 2020 (prospective registration)
Contact information	Contact: Dr Olivia Verisezan Rosu 37 Mircea Eliade Street 400364 Cluj-Napoca Romania Phone: +40744820493 Email: olivia.rosu@ssnn.ro
Notes	Overall trial end date: 01/05/2024

NCT05124353

Study name	Evaluation of the effect of early administration of neuroprotective drug (Cerebrolysin) on the outcome of patients with acute ischemic stroke undergoing endovascular therapy
Methods	Interventional (clinical trial), randomised, parallel assignment, open-label
Participants	100 participants - estimated enrolment Inclusion criteria: acute ischaemic stroke diagnosis; qualification for mechanical thrombectomy, without previous thrombolysis; age > 18
Interventions	Cerebrolysin 30 mL IV administrated in first 6 hours after stroke onset and for 10 days afterwards in Neurology Department or ICU conditions
Outcomes	Primary outcome measures: • Survival (time frame: 6 months) survival rate within first 6 months • NIHSS (time frame: day 1) NIH Stroke Scale • NIHSS (time frame: month 3) NIH Stroke Scale • NIHSS (time frame: month 6) NIH Stroke Scale • Rankin (time frame: day 1) modified Rankin Score • Rankin (time frame: month 3) modified Rankin Score • Rankin (time frame: month 6) modified Rankin Score • Pre mRS (time frame: day 1) modified pre Rankin Score • Pre mRS (time frame: month 3) modified pre Rankin Score • Pre mRS (time frame: month 6) modified pre Rankin Score • IQ code (time frame: month 3) IQ code • IQ code (time frame: month 6) IQ code • Geriatric Depression Scales (time frame: month 3) Geriatric Depression Scales • Geriatric Depression Scales (time frame: month 6) Geriatric Depression Scales • IV (time frame: 10 days) infarct volume of the control CT • Modified treatment in cerebral infarction (mTICI score) (time frame: 1 month) measure the reperfusion grade post thrombectomy - radiological imaging
Starting date	27 April 2021 (retrospective registration)
Contact information	Contact: Klaudyna Kojder Phone: +48692581426 ext +48 Email: klaudynakojder@gmail.com
Notes	Estimated study completion date: 1 April 2023

CT: computed tomography
IV: intravenous
MCA: middle cerebral artery
MRI: magnetic resonance imaging
mRS: modified Rankin Scale
MTICI: modified treatment in cerebral infarction
NIH: National Institutes of Health
NIHSS: National Institutes of Health Stroke Scale
PACS: partial anterior circulation stroke
TACS: total anterior circulation stroke
TLT: thrombolytic therapy

DATA AND ANALYSES

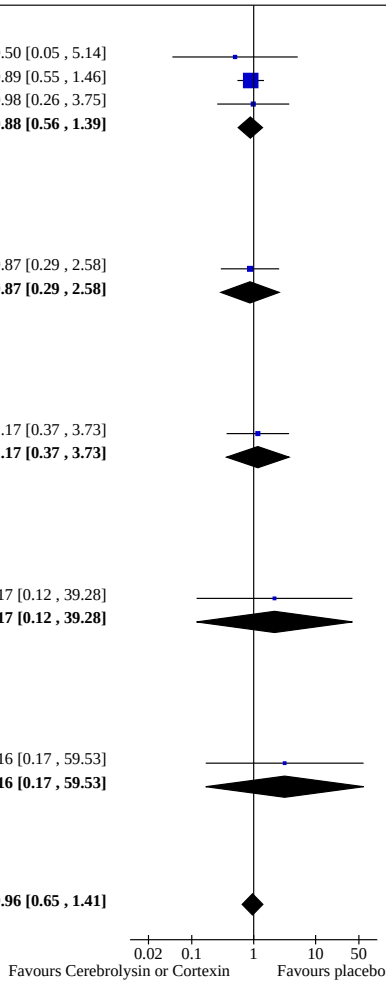
Comparison 1. Cerebrolysin or Cortexin versus placebo

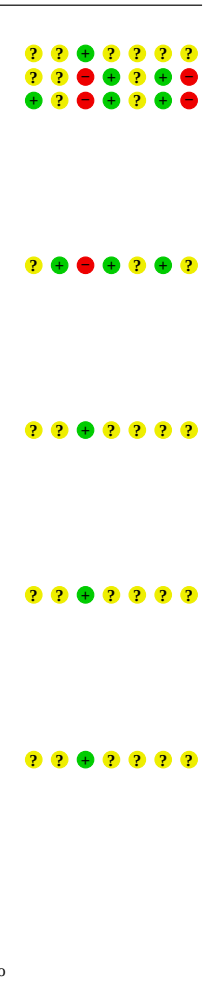
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.1 All-cause death	6	1689	Risk Ratio (M-H, Fixed, 95% CI)	0.96 [0.65, 1.41]
1.1.1 Cerebrolysin dose: 30 mL for 10 days	3	1235	Risk Ratio (M-H, Fixed, 95% CI)	0.88 [0.56, 1.39]
1.1.2 Cerebrolysin dose: 50 mL for 21 days	1	146	Risk Ratio (M-H, Fixed, 95% CI)	0.87 [0.29, 2.58]
1.1.3 Cerebrolysin dose: 10 mL and 50 mL for 10 days	1	36	Risk Ratio (M-H, Fixed, 95% CI)	1.17 [0.37, 3.73]
1.1.4 Cortexin dose: 20 mg for 10 days, 10 days rest, then 20 mg for 10 days	1	168	Risk Ratio (M-H, Fixed, 95% CI)	2.17 [0.12, 39.28]
1.1.5 Cortexin dose: 20 mg for 10 days, 10 days rest, then placebo for 10 days	1	104	Risk Ratio (M-H, Fixed, 95% CI)	3.16 [0.17, 59.53]
1.2 Non-death attrition	6	1689	Risk Ratio (M-H, Random, 95% CI)	0.72 [0.38, 1.39]
1.2.1 Cerebrolysin dose: 30 mL for 10 days	3	1235	Risk Ratio (M-H, Random, 95% CI)	0.87 [0.50, 1.52]
1.2.2 Cerebrolysin dose: 50 mL for 21 days	1	146	Risk Ratio (M-H, Random, 95% CI)	0.26 [0.08, 0.91]
1.2.3 Cerebrolysin dose: 10 mL and 50 mL for 10 days	1	36	Risk Ratio (M-H, Random, 95% CI)	Not estimable
1.2.4 Cortexin dose: 20 mg for 10 days, 10 days rest, then 20 mg for 10 days	1	168	Risk Ratio (M-H, Random, 95% CI)	Not estimable
1.2.5 Cortexin dose: 20 mg for 10 days, 10 days rest, then placebo for 10 days	1	104	Risk Ratio (M-H, Random, 95% CI)	Not estimable
1.3 Total number of people with SAEs	3	1335	Risk Ratio (M-H, Fixed, 95% CI)	1.16 [0.81, 1.66]
1.3.1 Cerebrolysin dose: 30 mL for 10 days	2	1189	Risk Ratio (M-H, Fixed, 95% CI)	1.23 [0.83, 1.81]
1.3.2 Cerebrolysin dose: 50 mL for 21 days	1	146	Risk Ratio (M-H, Fixed, 95% CI)	0.75 [0.26, 2.12]
1.4 Total number of people with fatal SAEs	3	1335	Risk Ratio (M-H, Fixed, 95% CI)	0.90 [0.59, 1.38]
1.4.1 Cerebrolysin dose: 30 mL for 10 days	2	1189	Risk Ratio (M-H, Fixed, 95% CI)	0.90 [0.57, 1.44]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.4.2 Cerebrolysin dose: 50 mL for 21 days	1	146	Risk Ratio (M-H, Fixed, 95% CI)	0.87 [0.29, 2.58]
1.5 Total number of people with non-fatal SAEs	3	1335	Risk Ratio (M-H, Fixed, 95% CI)	2.39 [1.10, 5.23]
1.5.1 Cerebrolysin dose: 30 mL for 10 days	2	1189	Risk Ratio (M-H, Fixed, 95% CI)	2.87 [1.24, 6.69]
1.5.2 Cerebrolysin dose: 50 mL for 21 days	1	146	Risk Ratio (M-H, Fixed, 95% CI)	0.29 [0.01, 7.03]
1.6 Total number of people with adverse events	4	1607	Risk Ratio (M-H, Fixed, 95% CI)	1.03 [0.92, 1.14]
1.6.1 Cerebrolysin dose: 30 mL for 10 days	2	1189	Risk Ratio (M-H, Fixed, 95% CI)	1.00 [0.90, 1.11]
1.6.2 Cerebrolysin dose 50 mL for 21 days	1	146	Risk Ratio (M-H, Fixed, 95% CI)	1.62 [0.69, 3.82]
1.6.3 Cortexin dose: 20 mg for 10 days, 10 days rest, then 20 mg for 10 days	1	168	Risk Ratio (M-H, Fixed, 95% CI)	1.57 [0.50, 4.96]
1.6.4 Cortexin dose: 20 mg for 10 days, 10 days rest, then placebo for 10 days	1	104	Risk Ratio (M-H, Fixed, 95% CI)	1.22 [0.42, 3.55]

Analysis 1.1. Comparison 1: Cerebrolysin or Cortexin versus placebo, Outcome 1: All-cause death

Study or Subgroup	Cerebrolysin or Cortexin Events	Total	Placebo Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.1.1 Cerebrolysin dose: 30 mL for 10 days						
Amiri Nikpour 2014	1	23	2	23	4.0%	0.50 [0.05 , 5.14]
CASTA 2012	28	529	32	541	63.8%	0.89 [0.55 , 1.46]
CERE-LYSE-1 2012	4	60	4	59	8.1%	0.98 [0.26 , 3.75]
Subtotal (95% CI)		**612**		**623**	**76.0%**	**0.88 [0.56 , 1.39]**
Total events:	33		38			
Heterogeneity: Chi² = 0.26, df = 2 (P = 0.88); I² = 0%						
Test for overall effect: Z = 0.54 (P = 0.59)						
1.1.2 Cerebrolysin dose: 50 mL for 21 days						
Ladurner 2005	6	78	6	68	12.9%	0.87 [0.29 , 2.58]
Subtotal (95% CI)		**78**		**68**	**12.9%**	**0.87 [0.29 , 2.58]**
Total events:	6		6			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.25 (P = 0.80)						
1.1.3 Cerebrolysin dose: 10 mL and 50 mL for 10 days						
Skvortsova 2004	7	24	3	12	8.1%	1.17 [0.37 , 3.73]
Subtotal (95% CI)		**24**		**12**	**8.1%**	**1.17 [0.37 , 3.73]**
Total events:	7		3			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.26 (P = 0.79)						
1.1.4 Cortexin dose: 20 mg for 10 days, 10 days rest, then 20 mg for 10 days						
Cortexin-Shamalov 2014	4	136	0	32	1.6%	2.17 [0.12 , 39.28]
Subtotal (95% CI)		**136**		**32**	**1.6%**	**2.17 [0.12 , 39.28]**
Total events:	4		0			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.52 (P = 0.60)						
1.1.5 Cortexin dose: 20 mg for 10 days, 10 days rest, then placebo for 10 days						
Cortexin-Shamalov 2014	3	72	0	32	1.4%	3.16 [0.17 , 59.53]
Subtotal (95% CI)		**72**		**32**	**1.4%**	**3.16 [0.17 , 59.53]**
Total events:	3		0			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.77 (P = 0.44)						
Total (95% CI)		**922**		**767**	**100.0%**	**0.96 [0.65 , 1.41]**
Total events:	53		47			
Heterogeneity: Chi² = 1.46, df = 6 (P = 0.96); I² = 0%						
Test for overall effect: Z = 0.22 (P = 0.82)						
Test for subgroup differences: Chi² = 1.20, df = 4 (P = 0.88), I² = 0%						

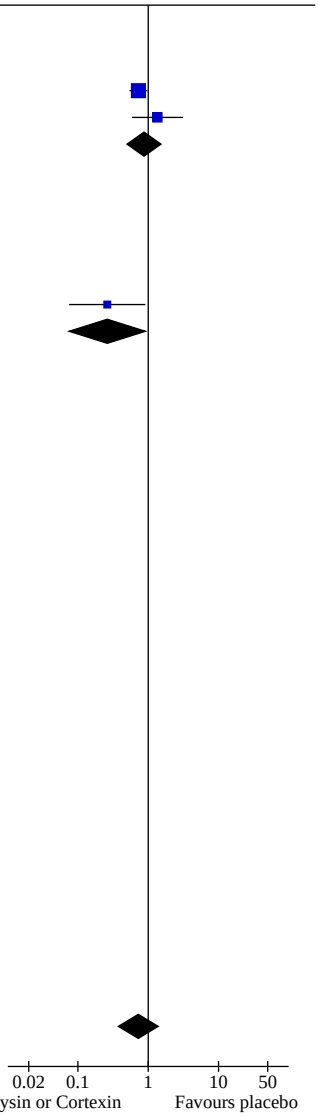




Risk of bias legend
(A) Allocation concealment
(B) Sequence generation
(C) Incomplete outcome data
(D) Blinding of outcome assessors
(E) Selective outcome reporting
(F) Blinding of participants and personnel
(G) Other sources of bias

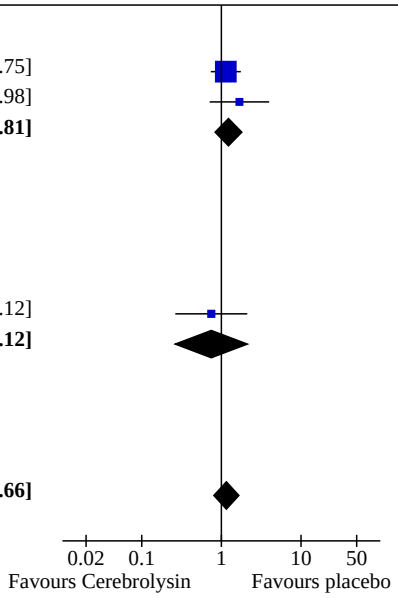
Analysis 1.2. Comparison 1: Cerebrolysin or Cortexin versus placebo, Outcome 2: Non-death attrition

Study or Subgroup	Cerebrolysin or Cortexin Events	Total	Placebo Events	Total	Weight	Risk Ratio M-H, Random, 95% CI
1.2.1 Cerebrolysin dose: 30 mL for 10 days						
Amiri Nikpour 2014	0	23	0	23		Not estimable
CASTA 2012	66	529	93	541	51.8%	0.73 [0.54 , 0.97]
CERE-LYSE-1 2012	11	60	8	59	29.6%	1.35 [0.59 , 3.12]
Subtotal (95% CI)		**612**		**623**	**81.5%**	**0.87 [0.50 , 1.52]**
Total events:	77		101			
Heterogeneity: Tau² = 0.09; Chi² = 1.89, df = 1 (P = 0.17); I² = 47%						
Test for overall effect: Z = 0.49 (P = 0.63)						
1.2.2 Cerebrolysin dose: 50 mL for 21 days						
Ladurner 2005	3	78	10	68	18.5%	0.26 [0.08 , 0.91]
Subtotal (95% CI)		**78**		**68**	**18.5%**	**0.26 [0.08 , 0.91]**
Total events:	3		10			
Heterogeneity: Not applicable						
Test for overall effect: Z = 2.11 (P = 0.04)						
1.2.3 Cerebrolysin dose: 10 mL and 50 mL for 10 days						
Skvortsova 2004	0	24	0	12		Not estimable
Subtotal (95% CI)		**24**		**12**		**Not estimable**
Total events:	0		0			
Heterogeneity: Not applicable						
Test for overall effect: Not applicable						
1.2.4 Cortexin dose: 20 mg for 10 days, 10 days rest, then 20 mg for 10 days						
Cortexin-Shamalov 2014	0	136	0	32		Not estimable
Subtotal (95% CI)		**136**		**32**		**Not estimable**
Total events:	0		0			
Heterogeneity: Not applicable						
Test for overall effect: Not applicable						
1.2.5 Cortexin dose: 20 mg for 10 days, 10 days rest, then placebo for 10 days						
Cortexin-Shamalov 2014	0	72	0	32		Not estimable
Subtotal (95% CI)		**72**		**32**		**Not estimable**
Total events:	0		0			
Heterogeneity: Not applicable						
Test for overall effect: Not applicable						
Total (95% CI)		**922**		**767**	**100.0%**	**0.72 [0.38 , 1.39]**
Total events:	80		111			
Heterogeneity: Tau² = 0.19; Chi² = 4.69, df = 2 (P = 0.10); I² = 57%						
Test for overall effect: Z = 0.98 (P = 0.33)						
Test for subgroup differences: Chi² = 2.98, df = 1 (P = 0.08), I² = 66.4%						



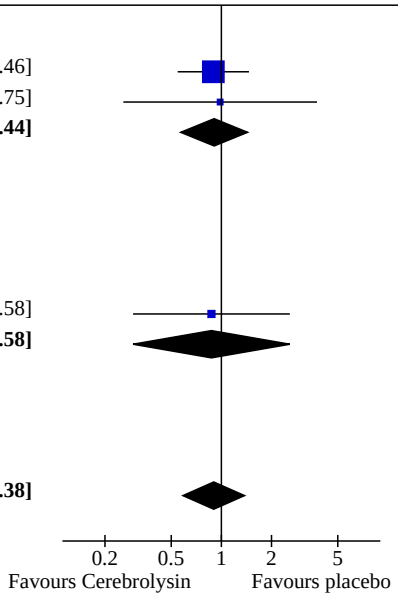
Analysis 1.3. Comparison 1: Cerebrolysin or Cortexin versus placebo, Outcome 3: Total number of people with SAEs

Study or Subgroup	Cerebrolysin Events	Cerebrolysin Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.3.1 Cerebrolysin dose: 30 mL for 10 days						
CASTA 2012	40	529	36	541	71.0%	1.14 [0.74 , 1.75]
CERE-LYSE-1 2012	12	60	7	59	14.1%	1.69 [0.71 , 3.98]
Subtotal (95% CI)		**589**		**600**	**85.1%**	**1.23 [0.83 , 1.81]**
Total events:	52		43			
Heterogeneity: Chi² = 0.64, df = 1 (P = 0.42); I² = 0%						
Test for overall effect: Z = 1.04 (P = 0.30)						
1.3.2 Cerebrolysin dose: 50 mL for 21 days						
Ladurner 2005	6	78	7	68	14.9%	0.75 [0.26 , 2.12]
Subtotal (95% CI)		**78**		**68**	**14.9%**	**0.75 [0.26 , 2.12]**
Total events:	6		7			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.55 (P = 0.58)						
Total (95% CI)		**667**		**668**	**100.0%**	**1.16 [0.81 , 1.66]**
Total events:	58		50			
Heterogeneity: Chi² = 1.42, df = 2 (P = 0.49); I² = 0%						
Test for overall effect: Z = 0.79 (P = 0.43)						
Test for subgroup differences: Chi² = 0.77, df = 1 (P = 0.38), I² = 0%						



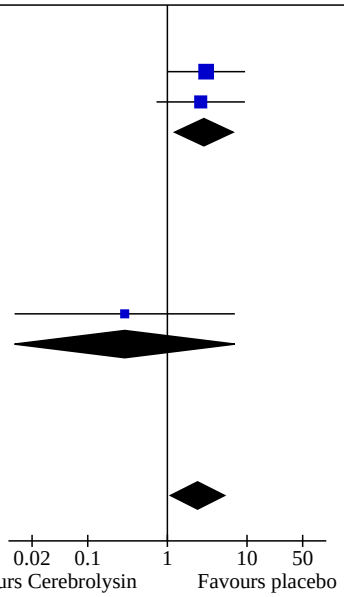
Analysis 1.4. Comparison 1: Cerebrolysin or Cortexin versus placebo, Outcome 4: Total number of people with fatal SAEs

Study or Subgroup	Cerebrolysin Events	Cerebrolysin Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.4.1 Cerebrolysin dose: 30 mL for 10 days						
CASTA 2012	28	529	32	541	75.2%	0.89 [0.55 , 1.46]
CERE-LYSE-1 2012	4	60	4	59	9.6%	0.98 [0.26 , 3.75]
Subtotal (95% CI)		**589**		**600**	**84.8%**	**0.90 [0.57 , 1.44]**
Total events:	32		36			
Heterogeneity: Chi² = 0.02, df = 1 (P = 0.90); I² = 0%						
Test for overall effect: Z = 0.42 (P = 0.67)						
1.4.2 Cerebrolysin dose: 50 mL for 21 days						
Ladurner 2005	6	78	6	68	15.2%	0.87 [0.29 , 2.58]
Subtotal (95% CI)		**78**		**68**	**15.2%**	**0.87 [0.29 , 2.58]**
Total events:	6		6			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.25 (P = 0.80)						
Total (95% CI)		**667**		**668**	**100.0%**	**0.90 [0.59 , 1.38]**
Total events:	38		42			
Heterogeneity: Chi² = 0.02, df = 2 (P = 0.99); I² = 0%						
Test for overall effect: Z = 0.49 (P = 0.63)						
Test for subgroup differences: Chi² = 0.00, df = 1 (P = 0.95), I² = 0%						

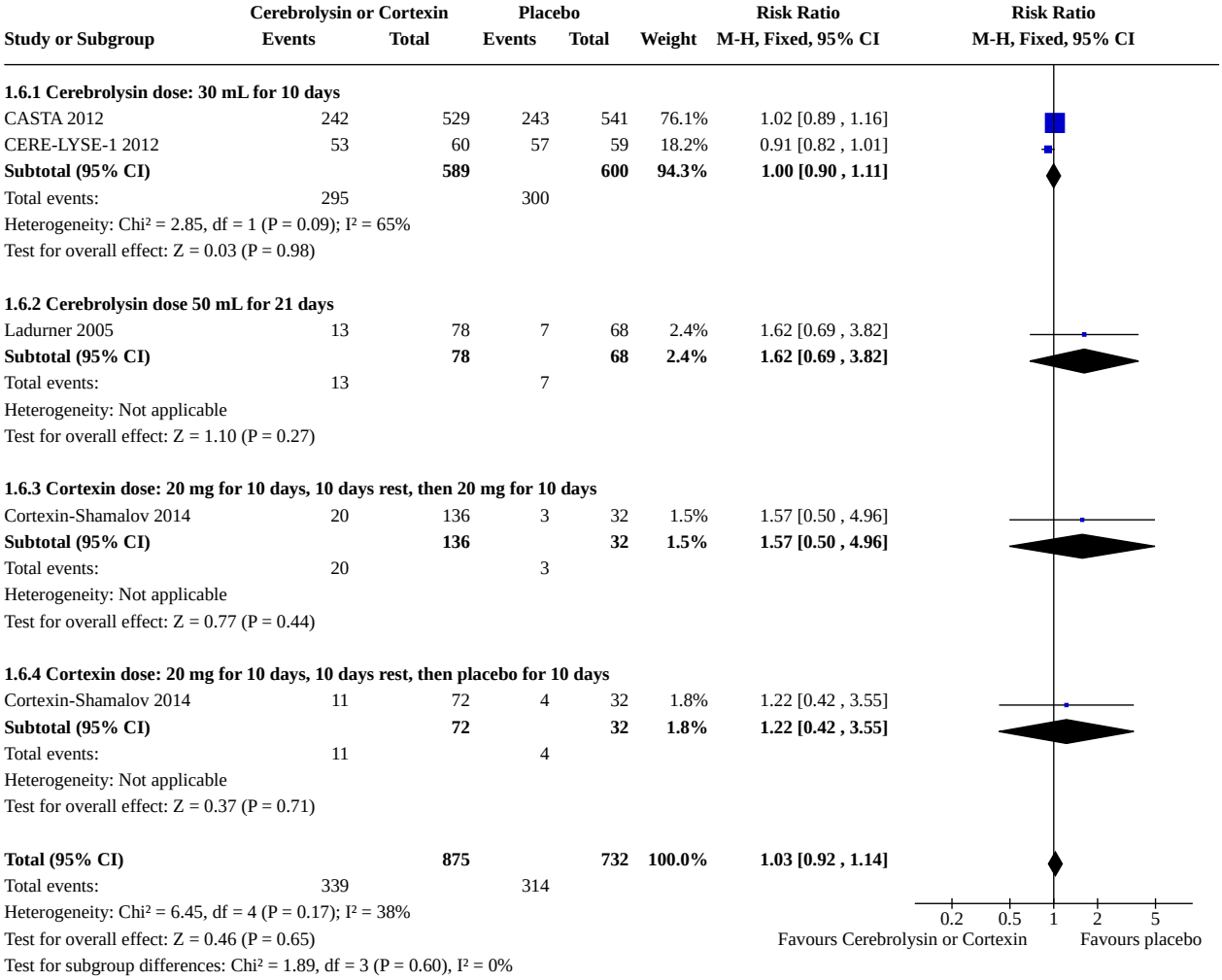


Analysis 1.5. Comparison 1: Cerebrolysin or Cortexin versus placebo, Outcome 5: Total number of people with non-fatal SAEs

Study or Subgroup	Cerebrolysin Events	Cerebrolysin Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.5.1 Cerebrolysin dose: 30 mL for 10 days						
CASTA 2012	12	529	4	541	46.1%	3.07 [1.00 , 9.45]
CERE-LYSE-1 2012	8	60	3	59	35.3%	2.62 [0.73 , 9.41]
Subtotal (95% CI)		**589**		**600**	**81.3%**	**2.87 [1.24 , 6.69]**
Total events:	20		7			
Heterogeneity: Chi² = 0.03, df = 1 (P = 0.86); I² = 0%						
Test for overall effect: Z = 2.45 (P = 0.01)						
1.5.2 Cerebrolysin dose: 50 mL for 21 days						
Ladurner 2005	0	78	1	68	18.7%	0.29 [0.01 , 7.03]
Subtotal (95% CI)		**78**		**68**	**18.7%**	**0.29 [0.01 , 7.03]**
Total events:	0		1			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.76 (P = 0.45)						
Total (95% CI)		**667**		**668**	**100.0%**	**2.39 [1.10 , 5.23]**
Total events:	20		8			
Heterogeneity: Chi² = 1.89, df = 2 (P = 0.39); I² = 0%						
Test for overall effect: Z = 2.19 (P = 0.03)						
Test for subgroup differences: Chi² = 1.86, df = 1 (P = 0.17), I² = 46.1%						



Analysis 1.6. Comparison 1: Cerebrolysin or Cortexin versus placebo, Outcome 6: Total number of people with adverse events

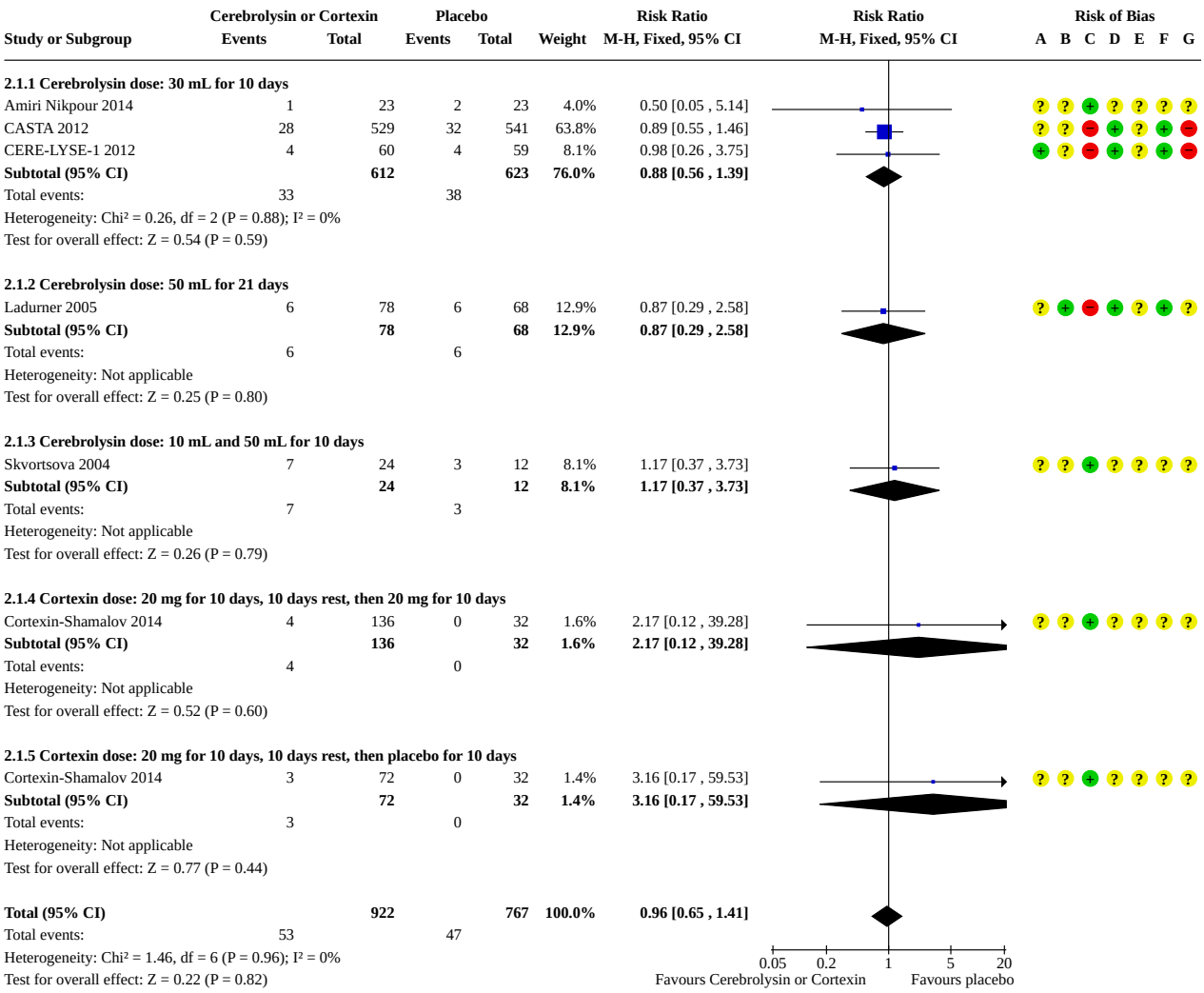


Comparison 2. Sensitivity analyses: Cerebrolysin or Cortexin versus placebo

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.1 All-cause death. Sensitivity 1. Best-case	6	1689	Risk Ratio (M-H, Fixed, 95% CI)	0.96 [0.65, 1.41]
2.1.1 Cerebrolysin dose: 30 mL for 10 days	3	1235	Risk Ratio (M-H, Fixed, 95% CI)	0.88 [0.56, 1.39]
2.1.2 Cerebrolysin dose: 50 mL for 21 days	1	146	Risk Ratio (M-H, Fixed, 95% CI)	0.87 [0.29, 2.58]
2.1.3 Cerebrolysin dose: 10 mL and 50 mL for 10 days	1	36	Risk Ratio (M-H, Fixed, 95% CI)	1.17 [0.37, 3.73]
2.1.4 Cortexin dose: 20 mg for 10 days, 10 days rest, then 20 mg for 10 days	1	168	Risk Ratio (M-H, Fixed, 95% CI)	2.17 [0.12, 39.28]

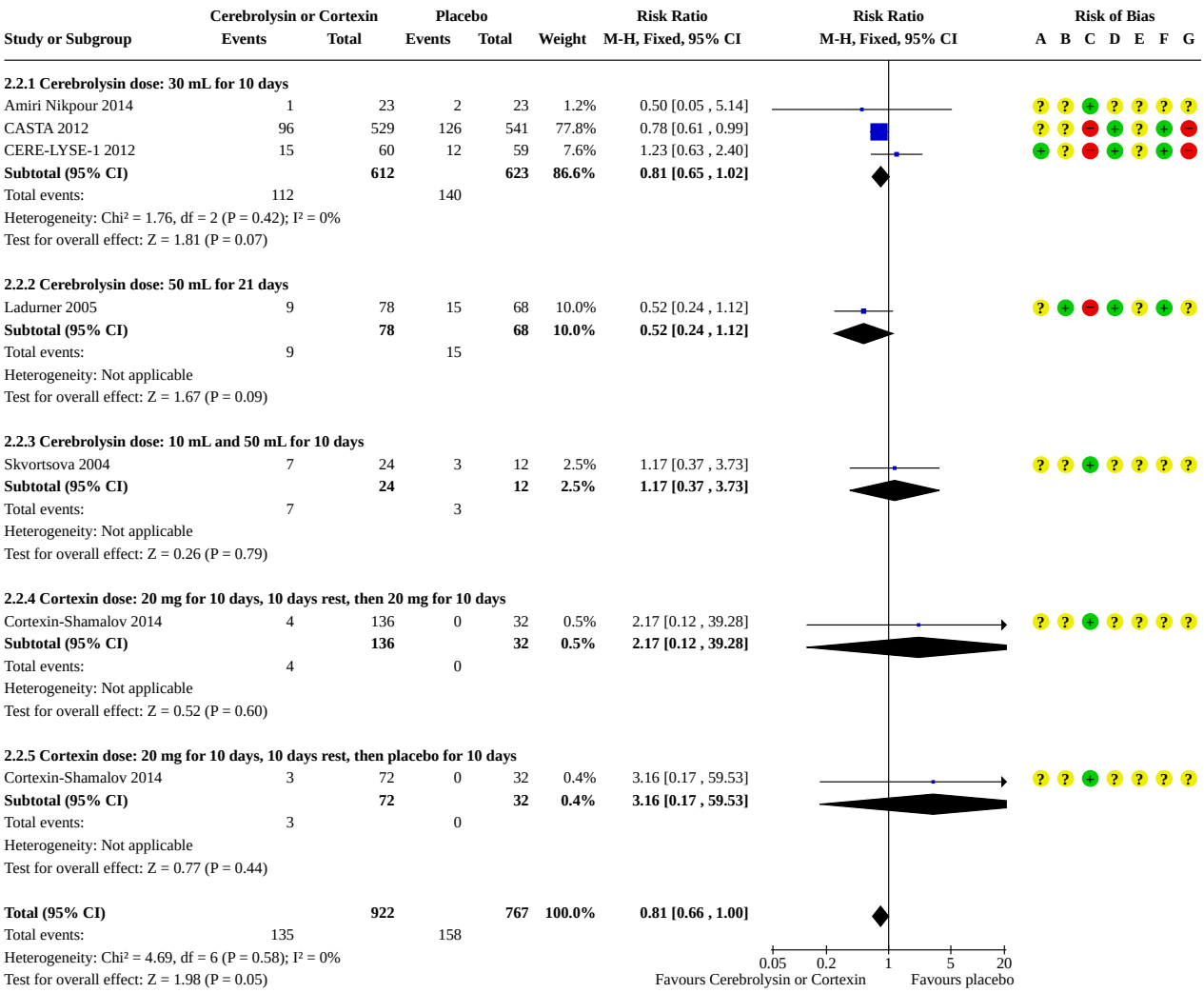
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.1.5 Cortexin dose: 20 mg for 10 days, 10 days rest, then placebo for 10 days	1	104	Risk Ratio (M-H, Fixed, 95% CI)	3.16 [0.17, 59.53]
2.2 All-cause death. Sensitivity 2. Worst-case	6	1689	Risk Ratio (M-H, Fixed, 95% CI)	0.81 [0.66, 1.00]
2.2.1 Cerebrolysin dose: 30 mL for 10 days	3	1235	Risk Ratio (M-H, Fixed, 95% CI)	0.81 [0.65, 1.02]
2.2.2 Cerebrolysin dose: 50 mL for 21 days	1	146	Risk Ratio (M-H, Fixed, 95% CI)	0.52 [0.24, 1.12]
2.2.3 Cerebrolysin dose: 10 mL and 50 mL for 10 days	1	36	Risk Ratio (M-H, Fixed, 95% CI)	1.17 [0.37, 3.73]
2.2.4 Cortexin dose: 20 mg for 10 days, 10 days rest, then 20 mg for 10 days	1	168	Risk Ratio (M-H, Fixed, 95% CI)	2.17 [0.12, 39.28]
2.2.5 Cortexin dose: 20 mg for 10 days, 10 days rest, then placebo for 10 days	1	104	Risk Ratio (M-H, Fixed, 95% CI)	3.16 [0.17, 59.53]
2.3 All-cause death. Sensitivity 3. Complete case (missing data excluded)	6	1496	Risk Ratio (M-H, Fixed, 95% CI)	0.92 [0.63, 1.35]
2.3.1 Cerebrolysin dose: 30 mL for 10 days	3	1054	Risk Ratio (M-H, Fixed, 95% CI)	0.85 [0.54, 1.33]
2.3.2 Cerebrolysin dose: 50 mL for 21 days	1	134	Risk Ratio (M-H, Fixed, 95% CI)	0.79 [0.27, 2.31]
2.3.3 Cerebrolysin dose: 10 mL and 50 mL for 10 days	1	36	Risk Ratio (M-H, Fixed, 95% CI)	1.17 [0.37, 3.73]
2.3.4 Cortexin dose: 20 mg for 10 days, 10 days rest, then 20 mg for 10 days	1	168	Risk Ratio (M-H, Fixed, 95% CI)	2.17 [0.12, 39.28]
2.3.5 Cortexin dose: 20 mg for 10 days, 10 days rest, then placebo for 10 days	1	104	Risk Ratio (M-H, Fixed, 95% CI)	3.16 [0.17, 59.53]
2.4 All-cause death. Sensitivity 4. Risk of bias	6	1689	Risk Ratio (M-H, Fixed, 95% CI)	0.96 [0.65, 1.41]
2.4.1 Low and unclear risk of bias	3	354	Risk Ratio (M-H, Fixed, 95% CI)	1.28 [0.51, 3.21]
2.4.2 High risk of bias for incomplete outcome data	3	1335	Risk Ratio (M-H, Fixed, 95% CI)	0.90 [0.59, 1.38]

Analysis 2.1. Comparison 2: Sensitivity analyses: Cerebrolysin or Cortexin versus placebo, Outcome 1: All-cause death. Sensitivity 1. Best-case



Risk of bias legend
(A) Allocation concealment
(B) Sequence generation
(C) Incomplete outcome data
(D) Blinding of outcome assessors
(E) Selective outcome reporting
(F) Blinding of participants and personnel
(G) Other sources of bias

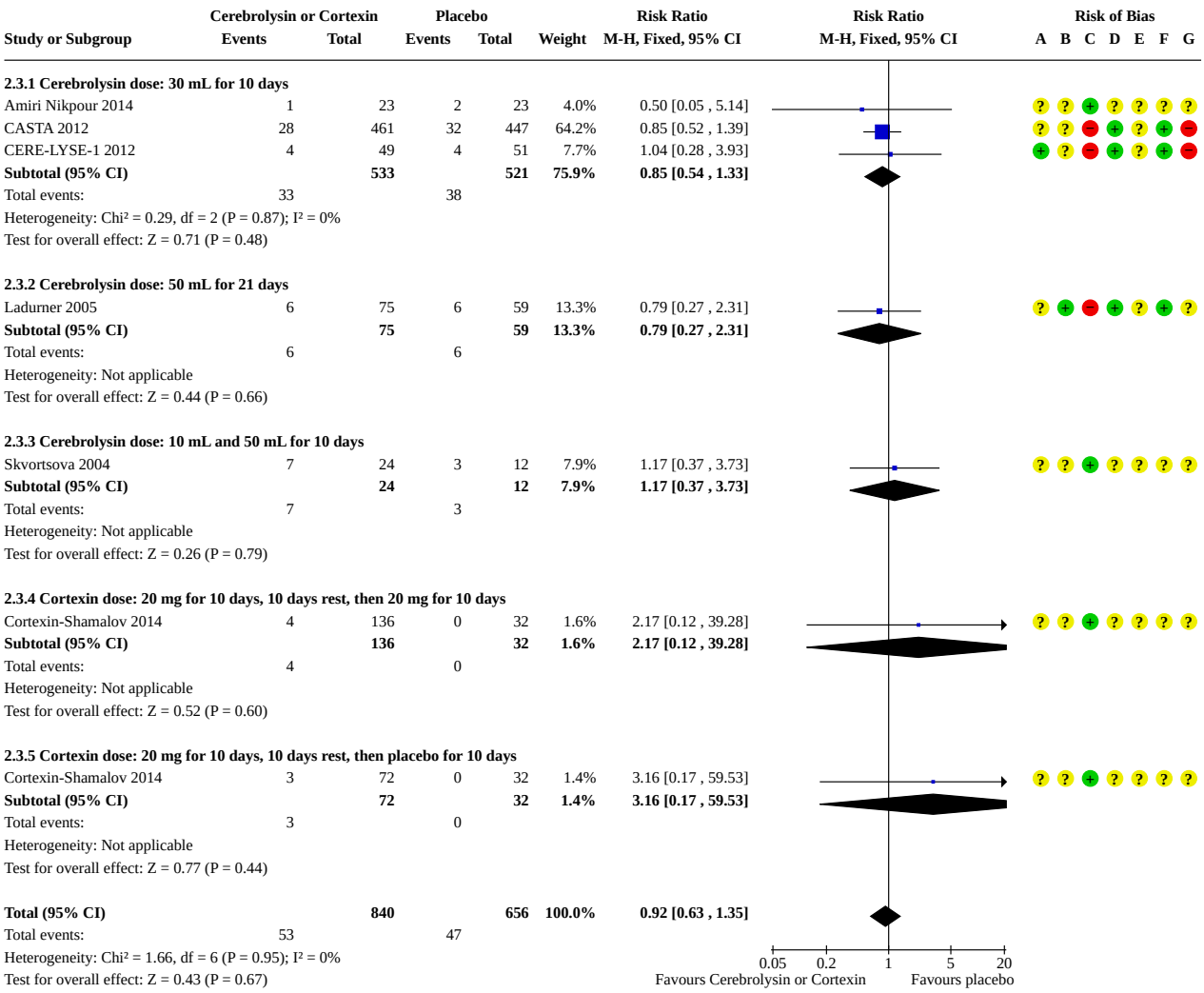
Analysis 2.2. Comparison 2: Sensitivity analyses: Cerebrolysin or Cortexin versus placebo, Outcome 2: All-cause death. Sensitivity 2. Worst-case



Study or Subgroup	Cerebrolysin or Cortexin Events	Total	Placebo Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI	Risk of Bias A B C D E F G
2.2.1 Cerebrolysin dose: 30 mL for 10 days							
Amiri Nikpour 2014	1	23	2	23	1.2%	0.50 [0.05 , 5.14]	? ? + ? ? ? ?
CASTA 2012	96	529	126	541	77.8%	0.78 [0.61 , 0.99]	? ? − + ? + −
CERE-LYSE-1 2012	15	60	12	59	7.6%	1.23 [0.63 , 2.40]	+ ? − + ? + −
Subtotal (95% CI)		**612**		**623**	**86.6%**	**0.81 [0.65 , 1.02]**	
Total events:	112		140				
Heterogeneity: Chi² = 1.76, df = 2 (P = 0.42); I² = 0%							
Test for overall effect: Z = 1.81 (P = 0.07)							
2.2.2 Cerebrolysin dose: 50 mL for 21 days							
Ladurner 2005	9	78	15	68	10.0%	0.52 [0.24 , 1.12]	? + − + ? + ?
Subtotal (95% CI)		**78**		**68**	**10.0%**	**0.52 [0.24 , 1.12]**	
Total events:	9		15				
Heterogeneity: Not applicable							
Test for overall effect: Z = 1.67 (P = 0.09)							
2.2.3 Cerebrolysin dose: 10 mL and 50 mL for 10 days							
Skvortsova 2004	7	24	3	12	2.5%	1.17 [0.37 , 3.73]	? ? + ? ? ? ?
Subtotal (95% CI)		**24**		**12**	**2.5%**	**1.17 [0.37 , 3.73]**	
Total events:	7		3				
Heterogeneity: Not applicable							
Test for overall effect: Z = 0.26 (P = 0.79)							
2.2.4 Cortexin dose: 20 mg for 10 days, 10 days rest, then 20 mg for 10 days							
Cortexin-Shamalov 2014	4	136	0	32	0.5%	2.17 [0.12 , 39.28]	? ? + ? ? ? ?
Subtotal (95% CI)		**136**		**32**	**0.5%**	**2.17 [0.12 , 39.28]**	
Total events:	4		0				
Heterogeneity: Not applicable							
Test for overall effect: Z = 0.52 (P = 0.60)							
2.2.5 Cortexin dose: 20 mg for 10 days, 10 days rest, then placebo for 10 days							
Cortexin-Shamalov 2014	3	72	0	32	0.4%	3.16 [0.17 , 59.53]	? ? + ? ? ? ?
Subtotal (95% CI)		**72**		**32**	**0.4%**	**3.16 [0.17 , 59.53]**	
Total events:	3		0				
Heterogeneity: Not applicable							
Test for overall effect: Z = 0.77 (P = 0.44)							
Total (95% CI)		**922**		**767**	**100.0%**	**0.81 [0.66 , 1.00]**	
Total events:	135		158				
Heterogeneity: Chi² = 4.69, df = 6 (P = 0.58); I² = 0%							
Test for overall effect: Z = 1.98 (P = 0.05)							
Test for subgroup differences: Chi² = 2.92, df = 4 (P = 0.57), I² = 0%							

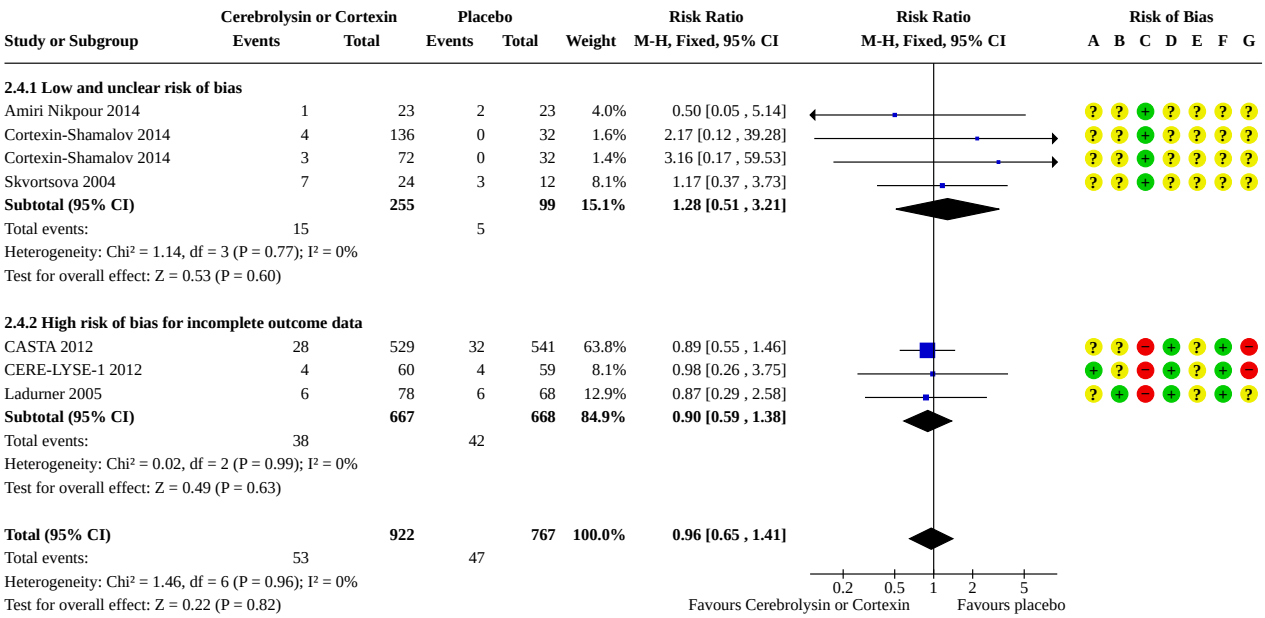
Risk of bias legend
(A) Allocation concealment
(B) Sequence generation
(C) Incomplete outcome data
(D) Blinding of outcome assessors
(E) Selective outcome reporting
(F) Blinding of participants and personnel
(G) Other sources of bias

Analysis 2.3. Comparison 2: Sensitivity analyses: Cerebrolysin or Cortexin versus placebo, Outcome 3: All-cause death. Sensitivity 3. Complete case (missing data excluded)



Risk of bias legend
(A) Allocation concealment
(B) Sequence generation
(C) Incomplete outcome data
(D) Blinding of outcome assessors
(E) Selective outcome reporting
(F) Blinding of participants and personnel
(G) Other sources of bias

Analysis 2.4. Comparison 2: Sensitivity analyses: Cerebrolysin or Cortexin versus placebo, Outcome 4: All-cause death. Sensitivity 4. Risk of bias



Risk of bias legend
(A) Allocation concealment
(B) Sequence generation
(C) Incomplete outcome data
(D) Blinding of outcome assessors
(E) Selective outcome reporting
(F) Blinding of participants and personnel
(G) Other sources of bias

ADDITIONAL TABLES

Table 1. Outcome all-cause death and sensitivity analyses

Analysis	Participants	Nominator	Denominator
Primary analysis (Intention-to-treat, best-case) Sensitivity analysis 1 - best-case	Lost to follow-up	Excluded[a]	Included
Sensitivity analysis 2 - worst-case[b]	Lost to follow-up	Included as deaths	Included
Observed case analysis (as per trial authors)	Lost to follow-up	Excluded	Excluded

[a]"Excluded" means removed from the calculation.
[b]To re-classify missing participants (missing data, including losses to follow-up) as treatment failures. For negative outcomes (death) this represents a true worst-case scenario.

Table 2. Ongoing studies

	Number of

Table 2. Ongoing studies *(Continued)*

Records identified through database searching (ClinicalTrials.gov and WHO ICTRP)	40
Records identified through other sources, namely Russian trial registry (GRLS)	11
Duplicates removed	1
Records screened	50
Records excluded as irrelevant	33
Trial records assessed for eligibility	17
Trial records excluded	11
Studies included in the list of ongoing studies	6
Studies included in the list of ongoing studies in previous version of review	2
Total number of studies included in the list of ongoing studies	8

Table 3. NIHSS score at admission

Study	Baseline score at admission
Amiri Nikpour 2014	Cerebrolysin - 14 (13 to 15) Placebo - 14 (12 to 16) *Median (IQR)*
CASTA 2012	Cerebrolysin - 9 (6 to 33) Placebo - 9 (6 to 26) *Median (range)*
CERE-LYSE-1 2012	Cerebrolysin 12.3 (5.39) Placebo 11.0 (5.44) *Mean (SD)*
Ladurner 2005	No info on NIHSS, the Canadian Neurological Scale (CNS) and the Glasgow Coma Scale (GCS) were measured Cerebrolysin - CNS 6.88 (0.09); GCS 14.1 (0.20) Placebo - CNS 6.68 (0.14); 14.4 (0.16) *Mean (SEM)*
Skvortsova 2004	Cerebrolysin 11.2 ± 4.7 Placebo 12.2 ± 2.8 *Mean (±)*
Cortexin-Shamalov 2014	Cortexin + Cortexin 7.03 (3.63); 6.0

Table 3. NIHSS score at admission *(Continued)*

	Cortexin + placebo 7.68 (4.94); 6.0 Placebo + placebo 7.94 (4.58); 6.0 *Mean (SD); median*
Xue 2016	Cerebrolysin 10.60 (4.74) Placebo 10.20 (3.72) *Mean (SD)*

IQR: interquartile range
NIHSS: National Institutes of Health Stroke Scale
SD: standard deviation
SEM: standard error of the mean

Table 4. Loss to follow-up (attrition, missing data)

Study	Number of randomised participants	Number lost to follow-up (%)	Number lost to follow-up Cerebrolysin/Cortexin	Number analysed by authors **Cerebrolysin/Cortexin** (denominator observed case)	Number lost to follow-up Placebo	Number analysed by authors **Placebo** (denominator observed case)
Amiri Nikpour 2014	46	0 (0)*	0 (0)	23	0 (0)	23
CASTA 2012	1070	162 (15)	66 + 2 (premature discontinuation) = 68	461	93 + 1 (no treatment) = 94	447
CERE-LYSE-1 2012	119	19 (16)	11	49	8	51
Ladurner 2005	146	12 (8)	3	75	9	59
Skvortsova 2004	36	0 (0)*	0 (0)	24	0 (0)	12
Cortexin-Shamalov 2014	272	0 (0)*	0 (0)	208	0 (0)	64
Xue 2016	84	24 (29)	n/a	n/a	n/a	n/a

*Number lost to follow-up not stated; we assumed the value to be '0'.

APPENDICES

Appendix 1. CENTRAL (the Cochrane Library) search strategy

ID SearchHits
#1 [mh ^"cerebrovascular disorders"] or [mh ^"basal ganglia cerebrovascular disease"] or [mh "brain ischemia"] or [mh ^"carotid artery diseases"] or [mh ^"carotid artery thrombosis"] or [mh ^"carotid artery, internal, dissection"] or [mh ^"stroke, lacunar"] or [mh ^"intracranial arterial diseases"] or [mh ^"cerebral arterial diseases"] or [mh ^"infarction, anterior cerebral artery"] or [mh ^"infarction, middle cerebral artery"] or [mh ^"infarction, posterior cerebral artery"] or [mh "intracranial embolism and thrombosis"] or [mh ^stroke] or [mh "brain infarction"] or [mh ^"vertebral artery dissection"]

#2 ((brain or cerebr* or cerebell* or vertebrobasil* or hemispher* or intracran* or intracerebral or infratentorial or supratentorial or middle cerebr* or mca* or anterior circulation) near/5 (isch*emi* or infarct* or thrombo* or emboli* or occlus* or hypoxi*)):ti,ab,kw
#3 (isch*emi* near/6 (stroke* or apoplex* or cerebral vasc* or cerebrovasc* or cva or attack*)):ti,ab,kw
#4 #1 or #2 or #3
#5 (cerebrolysin* or CERE or "FPF-1070" or FPF1070 or "FPF 1070" or "FPF 10-70"):ti,ab,kw
#6 #4 and #5

Appendix 2. MEDLINE (Ovid) search strategy

1. cerebrovascular disorders/ or basal ganglia cerebrovascular disease/ or exp brain ischemia/ or carotid artery diseases/ or carotid artery thrombosis/ or carotid artery, internal, dissection/ or stroke, lacunar/ or intracranial arterial diseases/ or cerebral arterial diseases/ or infarction, anterior cerebral artery/ or infarction, middle cerebral artery/ or infarction, posterior cerebral artery/ or exp "intracranial embolism and thrombosis"/ or stroke/ or exp brain infarction/ or vertebral artery dissection/

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

3. (isch?emi$ adj6 (stroke$ or apoplex$ or cerebral vasc$ or cerebrovasc$ or cva or attack$)).tw.

4. 1 or 2 or 3

5. (cerebrolysin$ or CERE or FPF-1070 or FPF1070 or FPF 1070 or FPF 10-70).tw.

6. 4 and 5

7. exp animals/ not humans.sh.

8. 6 not 7

Appendix 3. Embase (Ovid) search strategy

1. cerebrovascular disease/ or brain infarction/ or brain stem infarction/ or cerebellum infarction/ or exp brain ischemia/ or carotid artery disease/ or exp carotid artery obstruction/ or cerebral artery disease/ or exp cerebrovascular accident/ or exp occlusive cerebrovascular disease/ or stroke patient/
2. ((brain or cerebr$ or cerebell$ or vertebrobasil$ or hemispher$ or intracran$ or intracerebral or infratentorial or supratentorial or middle cerebr$ or mca$ or anterior circulation) adj5 (isch?emi$ or infarct$ or thrombo$ or emboli$ or occlus$ or hypoxi$)).tw.
3. (isch?emi$ adj6 (stroke$ or apoplex$ or cerebral vasc$ or cerebrovasc$ or cva or attack$)).tw.
4. 1 or 2 or 3
5. cerebrolysin/
6. (cerebrolysin$ or CERE or FPF-1070 or FPF1070 or FPF 1070 or FPF 10-70).tw.
7. 5 or 6
8. 4 and 7
9. (exp animals/ or exp invertebrate/ or animal experiment/ or animal model/ or animal tissue/ or animal cell/ or nonhuman/) not (human/ or normal human/ or human cell/)
10. 8 not 9

Appendix 4. Web of Science Core Collection search strategy

#1. TOPIC: (stroke* or apoplex* or cerebral vasc* or cerebrovasc* or cva)
#2. TOPIC: (cerebrolysin*)
#3. #2 AND #1

Appendix 5. LILACS search strategy

cerebrolysin or CERE or FPF-1070 or FPF1070 or cortexin or CORT or N-PEP-12F

Appendix 6. OpenGrey search strategy

cerebrolysin or CERE or FPF-1070 or FPF1070 or cortexin or CORT or N-PEP-12F

Appendix 7. Russian databases search strategy

#1. инсульт or цереброваск* or церебральн* or цвб*

#2. церебролизин or ЦЕРЕ or кортексин or КОРТ

#3. #1 and #2

Appendix 8. US National Institutes of Health Ongoing Trials Register ClinicalTrials.gov

Cerebrolysin AND (ischaemic stroke OR brain infarction OR brain ischemia OR carotid artery obstruction OR cerebral ischemia) [DISEASE]

Appendix 9. World Health Organization International Clinical Trials Registry Platform

Trial search

Basic search: cerebrolysin
Phases are: ALL

Appendix 10. Retraction Watch Database

Retraction Watch

Retraction Watch Search database

Basic search: cerebrolysin

F E E D B A C K

Response from authors of the Bornstein (2018) meta-analysis, 29 July 2020

Summary

Dear colleagues

As the first author of a meta-analysis that is very much in the highlight of this review (Bornstein 2018), I feel it is appropriate to offer Cochrane readership the opportunity to understand our point of view on serious matters that are being raised by Ziganshina et al (2020), on behalf of the authors of our manuscript.

1. Related citation

"The most recent meta-analysis published in Bornstein 2018 included a lengthy list of authors who had potential conflicts of interest due to their involvement with EVER Neuro Pharma, the manufacturer of Cerebrolysin. All of the studies included in the Bornstein 2018 meta-analysis were supported either totally or partially by EVER Neuro Pharma, or did not provide any information on funding or disclosure."

Our commentary

• We kindly ask the authors of this review to consider revisiting these statements, as they imply that all authors of our cited meta-analysis have had financial involvement with EVER Neuro Pharma, and that all studies included in the meta-analysis have received support from this company. None of the above are true.

• None of the authors of the review group received any honoraria for their participation in this meta-analysis. Three authors were coordinating investigators of included double-blind randomized controlled trials. Since the meta-analysis was based on Individual Patient Data (IPD) of these studies, which is regarded as the 'gold standard' for the meta-analytic approach [1], collaboration is natural for obtaining proper access to data. We refer to the appreciation of the Cochrane Collaboration Methods Group on IPD meta-analyses [2]: "IPD meta-analyses can improve the quality of the data and the type of analyses that can be done and produce more reliable results. For this reason, they are considered to be a 'gold standard' of systematic questions, which might not have been obtained from summary data." Similar acknowledgment is provided in the Cochrane Handbook for Systematic Reviews of Interventions [3]: "The IPD approach can bring substantial improvements to the quality of data available and offset inadequate reporting of individual studies. Risk of bias can be assessed more thoroughly and IPD enables more detailed and flexible analysis than is possible in systematic reviews of aggregate data." We consider it problematic to conduct research in any field without collaborating with individuals with hands-on experience with the topic at hand.

• For the methodological part of this large-scale review, two internationally renowned biostatisticians with great methodological experience were included in the review group. Prof. Johannes C. Vester, President of the World Academy for Multidisciplinary Neurotraumatology and a highly experienced methodologist, is requested for more than three decades by multiple international organizations and regulatory authorities. Dr. Volker W. Rahlfs, the founder of IDV, the oldest German biometric institution (1967), chairman of the IDV Methodology Group, author of more than 150 methodological publications, Certificate 'Biometry in Medicine', Member of the Royal Statistical Society, routinely provides consultancy for numerous regulatory and academic institutions.

• Professors Volker Hömberg (Secretary General of the World Federation for Neurorehabilitation, Dafin Muresanu (President of the European Federation of Neurorehabilitaiton), and myself, are highly committed clinicians and research scientists, dedicating their lifework to progress in stroke and neurorehabilitation.

• Regarding industry involvement in clinical trials, we provide the example of the study with the strongest effect size of all included trials, performed by Xue et al. (2016). As per manuscript acknowledgments, this study was supported by the Shanghai Jiao Tong University Affiliated Sixth People's Hospital (grant nos. 1462 and 1583) and the Shanghai Science and Technology Council (grant no. 13411951401),

with no contribution at all from EVER Neuro Pharma. The same applies to the study performed by Amiri-Nikpour (2014) - the study was supported by a grant from the Urmia University of Medical Sciences.

We, therefore, feel that inaccurate assertions by Ziganshina et al bring unjust prejudice to the image and impact of our research group.

References

1. Thomas D, Radji A, Benedetti, A - Systematic review of methods for individual patient data meta-analysis with binary outcomes. BMC Med Res Methodol. 2014;14:79.

2. https://methods.cochrane.org/ipdma/about-ipd-meta-analyses

3. Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). Cochrane Handbook for Systematic Reviews of Interventions version 6.0 (updated July 2019). Cochrane, 2019. Available from www.training.cochrane.org/handbook.

2. Related citation

"In addition to contrasting with our results, the meta-analysis of Bornstein 2018 is in contrast to another recent meta-analysis that showed lack of benefit from Cerebrolysin treatment for ischaemic stroke compared to placebo for functional recovery at day 90 (Wang 2017). Of the six studies included in Wang 2017, four overlapped with those included in this Cochrane Review (Amiri Nikpour 2014; CASTA 2012; CERE-LYSE-1 2012; Ladurner 2005). Wang 2017 also included studies that we have excluded owing to dealing with different research questions and not meeting our eligibility criteria (CARS study - Chang 2016; Muresanu 2016a)."

Our commentary

• This paragraph is an example of a double standard logical fallacy, namely that both similarity (i.e. four overlapped studies) and difference (i.e. excluded studies) are used to offend the meta-analysis (Bornstein, 2018). What Ziganshina et al. do not mention is that in reality, neither comparison is appropriate, owing to totally different approaches in terms of research questions, outcomes selection, and statistical analysis.

• To note that the meta-analyses of Ziganshina (2020) evaluate beneficial effects using mortality as the primary outcome. The overall death rate in the included studies was 6%, thus any group differences are hardly expected, except with very large sample sizes. The status of the 94% survivors is completely overlooked in this review. On the contrary, our meta-analysis focused predominantly on neurological function, thus, addressing especially beneficial effects for survivors. Two completely different approaches, which cannot be used as a scientific rationale against our meta-analysis.

• In our work, we provide clear references to support our methodological choices. For the sake of diversity of opinion, and increased objectiveness that would be appropriate for an organization such as the Cochrane collaboration, we invite authors to also reference positive articles that have been published on the topic, such as this systematic review developed by an independent group of researchers from Australia, Canada, and Sweden: https://www.medicaljournals.se/jrm/content/html/10.2340/16501977-2536.

We suggest that the implications and interpretation of the above-mentioned statements are clarified, in order to enhance the quality of this material.

Conclusion

The review's comments related to applied methodology will be addressed separately by the review group. Arguments presented by Ziganshina et al. (2020) against our meta-analysis build a conspiratorial narrative that includes severe allegations of scientific misconduct. We hope for constructive dialogue of objective scientific matters. On subjectively approached issues, such as those related to conflict of interest, we expect authors to nuance the manuscript's language, or to remove inappropriate passages that are not based on any evidence.

Reply

Dear Professor Bornstein

Thank you for your interest in our Cochrane Review 'Cerebrolysin for acute ischaemic stroke'.

There is no 'Disclosure' statement in the published paper Bornstein 2018, yet all the authors are known to be involved with or have been involved with EVER Neuro Pharma, the manufacturer of Cerebrolysin.

Here is the summary, which we prepared from published studies on Cerebrolysin in stroke. For direct citations and specifics of overlapping trials, please see the Characteristics of included studies table of our Cochrane Review:

- **Dr Bornstein** has been a consultant for EVER Neuro Pharma and has received honoraria for this activity. He was also active in the speaker's bureau of EVER Neuro Pharma;
- **Alla Guekht** is a principal investigator of the CARS2 trial. Reports receipt of grants/research support from EVER Neuro Pharma;
- **Johannes C.** Vester has been a senior biometric consultant of IDV (Advisory Board for EVER Neuro Pharma);

- **Wolf Dieter Heiss** has served on the Advisory Board and Speakers bureau for EVER Neuro Pharma;
- **Eugene Gusev** was a CARS 2 investigator;
- **Volker Homberg** has been a member of the CAPTAIN trial scientific advisory board;
- **Volker Rahlfs** has been an employee of IDV. Consultant for EVER Neuro Pharma and has received honoraria for this activity
- **Ovidiu Bajenaru** was a principal investigator of the CARS trial. Reports a receipt of grants/research support from EVER Neuro Pharma;
- **Bogdan Popescu** was a principal investigator of the CARS trial; worked for Ebewe/Ever Neuro Pharma-clinical studies 2008–2012, received Ebewe/Ever Neuropharma–speaker fees 2008–2014;
- **Dafin Fior Muresanu** was a coordinating investigator of the Cerebrolysin and Recovery After Stroke (CARS) trial and a member of the Cerebrolysin Asian Pacific Trial in Acute Brain Injury and Neurorecovery (CAPTAIN) trial scientific advisory board. Reports receipt of grants/research supports from EVER Neuro Pharma.

To clearer reflect this we have amended the description in the review to read:

“The most recent meta-analysis published in Bornstein 2018 included a lengthy list of authors who have previously declared conflicts of interest relating to EVER Neuro Pharma, the manufacturer of Cerebrolysin. All of the studies included in the Bornstein 2018 meta-analysis were supported either totally or partially by EVER Neuro Pharma, or did not provide any information on funding or disclosure. For specifics of the six overlapping trials, please see Characteristics of included studies.”

In the citation you look at, we say at the very end: “ ... or did not provide any information on funding or disclosure."

Xue 2016: no disclosure or conflict of interest statement.

Amiri-Nikpour 2014: we judged the information on funding to be unclear. There was no information on sources of study drug or placebo. Added to the unavailability of the study protocol, we judged this information to be missing.

We support all our judgements to make the reviewing process fully transparent as per MECIR standards R52-55 (mandatory).

We would like to draw attention to a concern about the reporting of the Bornstein 2018 paper and potential problems with study protocol registration:

The meta-analysis does not have either a protocol, or an official registration. Instead the paper contains the section 2. ***Protocol and registration,*** reading:

"This meta-analysis follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses [3]. The nonparametric approach and the method of synthesis were operationalized under blinded conditions in the final statistical analysis plan of study CARS-2 (2014). **A separate review protocol has not been prepared for this meta-analysis** and the meta-analysis has not been included in any study registry since the objective of this meta-analysis was to verify the findings of the previously published meta-analysis on early neurological benefit (CARS-1, CARS-2) [2], using identical methodology."

In addition to this, despite the statement in the Eligibility criteria of the Bornstein 2018 paper: "Eligible studies published as abstract only were not included in this meta-analysis", the authors included in their meta-analysis an abstract, Guekht 2015a, listed under Excluded studies in this Cochrane review.

Thus, we provide here clear explanations to our statements and contend that our statements and judgements are accurate and bring light to the image and impact of Cerebrolysin author team.

Contributors

Commentary submitted by Natan Bornstein, Professor of Neurology, Shaare Zedek Medical Center, Jerusalem, Israel

Response submitted by the authors of this Cochrane Review

Methodological commentary from authors of the Bornstein (2018) meta-analysis, 29 July 2020

Summary

Dear corresponding author

I hereby submit a commentary to the 'Cerebrolysin for acute ischaemic stroke' Cochrane Review.

Topic: Utilization of LOCF in the Bornstein (2018) meta-analysis

Related citation from Ziganshina (2020)

“We would like to reiterate here that there is an inherent bias in the 'last observation carried forward' method, and its use is deprecated (Lachin 2016; Molnar 2008; Salim 2008), as we mentioned in Risk of bias in included studies.”

Commentary

• Ziganshina 2020 cites Lachin 20161 as reference for the LOCF criticism of Bornstein 2018, overlooking the critical warning by Lachin 2016 regarding 'last observation carried forward' (LOCF) method refers explicitly to a substantial fraction of missing data: "Regulatory agencies and journal editors (and reviewers) should be critical of any study with a substantial fraction of missing data" (Lachin 2016) [1]

• The rate of missing NIHSS values as compared to randomized subjects was below 10% in eight out of nine trials, thus well fulfilling the criteria for class I studies (American Academy of Neurology 2018 benchmark for class I studies [2]: <20%; to note: the 20% cutoff was suggested by David Sackett, OC, FRSC - a pioneer of EBM [3]).

• In three studies, anyhow, only observed cases (OC) data were available for NIHSS evaluation[4]; the missing rates were well comparable between Cerebrolysin and placebo (7/67 vs. 8/66).

• For two[5] of the studies included in the meta-analysis, sensitivity analysis comparing LOCF vs. observed case analysis (OC) was available on the associated primary efficacy criterion. It was shown that there was no indication for bias, results did well agree: "The OC result is well supporting the LOCF analysis (MWOC 0.62 with POC < 0.0001 vs. MWLOCF 0.62 with PLOCF < 0.0001)"[6].

• Thus, all in all, and in particular with respect to the very low dropout rate and a very low P-value in the primary meta-analysis (<0.0001), we do not see a rationale for rating down the level of evidence due to the non-substantial fraction of LOCF imputations.

References

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3. Sackett, DL, Rosenberg WMC, Muir Gray JA, Haynes RB, Richardson WS. Evidence-based medicine. BMJ 1996;312:71

4. Skvortsova 2003, Shamalov 2010, Amiri-Nikpour 2014

5. CARS-1, CARS-2

6. Safety and efficacy of Cerebrolysin in motor function recovery after stroke: a meta-analysis oft he CARS trials. Neurol Sci 2017. DOI 10.1007/s10072-017-3037-z

Topic: Utilization of t-test vs. Wilcoxon Test in the Bornstein (2018) meta-analysis

Related citation from Ziganshina (2020)

"They state that it is the preferred analysis method if the outcome variables are not continuous or might have skewed distributions or outliers. The authors do not examine the distribution of the populations."

Commentary

• Non-normality is not an assumption for the Wilcoxon-test, it is the opposite: normal distribution is an assumption for the t-test (as well as homogeneity of variances).

• Assumptions have to be checked for validity of the t-test, not for the Wilcoxon test (which has a minimum of assumptions). To make a pre-test on normality and then switch to the Wilcoxon test in case of non-normality is not a recommended approach since the multiple level alpha is not preserved. The more, in the case of small sample sizes such pre-tests are highly underpowered.

• Besides, statistically significant non-normality of the distributions was formally demonstrated and reported as per manuscript of the included study CARS-1 (Shapiro-Wilk test of normality, P = 0.0137).

• See also LaVange 2005 [1] (2011-2017 Director of the Office of Biostatistics, FDA): "methods with essentially no assumptions external to the study design are ideal. Nonparametric methods in general require minimal assumptions. In a regulator setting, the failure to meet assumption may cast doubt on the study results, even if the findings a are robust to that failure. Thus minimizing assumptions is a recommended approach."

• Leading biostatisticians note, that rating scales or composite index values are by design ordinal scales and should be only evaluated using Wilcoxon test (see, e.g., Munzel 1998 [2]: "Hence, when analyzing data from … rating scales, statistics that are based on differences and means of scores are not appropriate").

• The NIHSS outcome variable is not continuous (interval/ratio), it is an ordinal rating scale. Thus, also in this respect the Wilcoxon-Mann-Whitney test is the recommended approach, not the t-test as recommended by Ziganshina 2020.

References

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2. Munzel U, Bandelow B. The use of parametric vs. nonparametric tests in the statistical evaluation of rating scales. Pharmacopsychiary 1998;31:222-4.

Related citation from Ziganshina (2020)

"Given the size of the populations under study here in the meta-analysis, a t-test would be preferable."

Commentary

• This statement is highly misleading. For the inappropriateness of the t-test see the previous comment. Else, it is a common misunderstanding that violation of assumptions can be neglected with higher sample sizes. See, e.g. LaVange (as cited above): “In a regulatory setting, the failure to meet assumption may cast doubt on the study results, even if the findings a are robust to that failure. Thus, minimizing assumptions is a recommended approach.”

• The size of the nine individual studies, on which the statistical test is applied, goes down to 16 vs 17 patients (MRI-1). The majority of the studies has sample sizes below 50 per group. Thus, the t-test with its various assumptions is not preferable “given the size of the populations under study”.

• In a particular meta-analysis, there can be only one common effect size. Thus, even if one of the studies would verifiably meet the assumptions of the t-test, the Wilcoxon test is still the preferable method for the ensemble of the trials.

Related citation from Ziganshina (2020)

"It is well known that the Wilcoxon test is more powerful than a t-test under certain conditions, but it can also yield a significant result when the t-test does not (Lumley 2002)."

Commentary

• This statement is correct only for special non-normal distributions, where anyhow the t-test is not appropriate. However, under the assumption of a normal distribution, the Wilcoxon test is not “more powerful” than a t-test - the opposite is true! The asymptotic relative efficiency (A.R.E.) of a Wilcoxon test is 3/π, which means that the Wilcoxon test has a power of 0.96 as compared to the t-Test [1,2]. If there is no normal distribution, then the Wilcoxon test is anyhow the appropriate approach and preferable to the t-test!

References

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Topic: Alleged misuse of Mann-Whitney benchmarks and interpretation in the Bornstein (2018) meta-analysis

Related citation from Ziganshina (2020)

"The following benchmark values hold for the test group under fairly general conditions: 0.50 = equality, 0.56 = small superiority, 0.64 = medium-sized/relevant superiority, 0.71 = large superiority". Whilst the Mann-Whitney statistic of 0.5 represents complete overlap of the data, and values of 0 and 1 represent complete non-overlap one way or the other, these benchmarks are arbitrary, and the authors do not define the terms "small superiority", "medium-sized superiority", or "large superiority". The use of the word 'superiority' shows prejudice: 'difference' would be more neutral and more accurate."

Commentary

• Neither the above statement nor the citation is correct. The correct citation of the Mann-Whitney benchmarks provided in Bornstein 2018 includes inferiority: “The traditional benchmarks for the MW effect size measure are [23, 24]: 0.29 = large inferiority, 0.36 = medium inferiority, 0.44 = small inferiority, 0.50 = equality, 0.56 = small superiority, 0.64 = medium superiority, 0.71 = large superiority.“

• The cited benchmarks are by no means “arbitrary”:

o Bornstein 2018 provides the key references for the benchmarks (Cohen 1988, Colditz 1988) [1,2].

o Under the assumption of a normal distribution the benchmarks can directly be converted to the standardized mean difference (SMD) and its associate benchmarks.

o A comprehensive overview of the transformation pathways of the Mann-Whitney statistic to other well-known effect size measures including the associated conversion formulas is provided by Rahlfs 2019 (Effect size measures and their benchmark values for quantifying benefit or risk of medicinal products) [3].

• The Mann-Whitney effect size (MW) has been shown by many authors to be a gold standard for ordinal/rating scales, see, e.g., Munzel 1998 (see citation above). The use of the MW measure for obtaining a good measure of relevance in clinical research has been recommended for many years. We cite Brunner and Munzel [4], 2002, Colditz et al.2, 1988, Munzel and Hauschke [5], 2003, Newcombe [6], 2006, Wei and Lachin [7], 1984, and Wolfe and Hogg [8], 1971, among others.

• The importance of the Mann-Whitney statistics for clinical research may be further highlighted by the fact that the leading biometric journal Statistics in Medicine dedicated a whole volume to the Mann-Whitney Statistic on the occasion of the 25th Anniversary of the journal (d'Agostino, Campell, M., Greenhouse, J., (ed.) 2006, The Mann-Whitney statistic: continuous use and discovery) [9]. For the use of the Mann-Whitney approach in ordinal data analysis see also Rothmann 2012 [10] (Mark Rothmann, Director, Division of Biostatistics II, FDA).

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Conclusion

The conclusions of Ziganshina et al (2020) on Bornstein (2018) are not valid since they are based on incorrect or incomplete biometric statements and citations. We consider it mandatory to correct or remove the commented passages.

Reply

Dear Dr Rahlfs

Thank you for your interest in our Cochrane Review 'Cerebrolysin for acute ischaemic stroke'.

Re: Topic: Utilization of LOCF in the Bornstein (2018) meta-analysis

In the Discussion section (subsection 'Agreements and disagreements with other studies or reviews'/'Use of statistical instruments') of our Cochrane review, from which you extracted our sentence, we do not rate levels of evidence in the Bornstein 2018 paper, we just very briefly describe this work and comment on the appropriateness of the use of LOCF method. In this discussion we do not go into individual trial appraisal. For this, please refer to the relevant sections of our Cochrane Review.

However, answering your query, we would like to present the full citation from Lachin 2016 and draw your attention to the last part of it, unfortunately omitted from your citation:

"Regulatory agencies and journal editors (and reviewers) should be critical of any study with a substantial fraction of missing data, **and should be highly skeptical of the veracity of any results and pursuant claims based on LOCF analyses**."

Furthermore, Lachin 2016 concludes:

“In summary, the well-known statistical properties of the mixture of two distributions are employed to demonstrate that LOCF analyses can introduce a positive or negative bias that can grossly inflate or deflate, respectively, the probability of a statistically significant test result under either the null or alternative hypothesis. Accordingly, without exception, all analyses using LOCF are suspect and should be dismissed. Statistically, last observation carried forward is specious (def: appearing to be true but actually false).”

Therefore, we confirm that all we said in our Cochrane review on the use of LOCF method was referenced to authoritative sources.

Re: Topic: Alleged misuse of Mann-Whitney benchmarks and interpretation in the Bornstein (2018) meta-analysis

These topics and comments deal with two paragraphs of the Discussion section ('Agreements and disagreements with other studies or reviews'/'Use of statistical instruments'). Separate sentences, taken out of context, lose coherence and should be read and comprehended together. Further we provide these two paragraphs in full:

“The authors of the Bornstein 2018 meta-analysis, and those of several of the studies with involvement of the same author team members, used the Wilcoxon-Mann-Whitney test. They state that it is the preferred analysis method if the outcome variables are not continuous or might have skewed distributions or outliers. The authors do not report the distribution of the populations. Given the sizes of the populations under study here in the meta-analysis, a t-test should be preferable. It is well known that the Wilcoxon test is more powerful than a t-test under certain conditions, but it can also yield a significant result when the t-test does not (Lumley 2002). The authors of the meta-analysis state: **"The effect size measure associated with the Wilcoxon-Mann-Whitney test is the Mann Whitney** statistic (MW). It defines the probability that a randomly selected patient of the treatment group is better off than a randomly chosen patient from the reference group. The following **benchmark** values hold for the test group under fairly general conditions: **0.50 = equality, 0.56 = small superiority, 0.64 = medium-sized/relevant superiority, 0.71 = large superiority".**

Whilst the Mann-Whitney statistic of 0.5 represents complete overlap of the data, and values of 0 and 1 represent complete non-overlap one way or the other, these benchmarks are arbitrary, and the authors do not define the terms "small superiority", "medium-sized superiority", or "large superiority". The use of the word 'superiority' suggests a degree of prejudice: 'difference' would be more neutral and more accurate.”

Here we refer to the author team both of the Bornstein 2018 meta-analysis and of several of the included studies through the entire section.

We apologise that we did not cite the CASTA 2012 trial report, which we had intended. We lost the citation in the review drafting process.

This part of the paragraph should read:

The author team members state in (CASTA 2012):

"The effect size measure associated with the Wilcoxon-Mann-Whitney test is the Mann Whitney statistic (MW). It defines the probability that a randomly selected patient of the treatment group is better off than a randomly chosen patient from the reference group. The following benchmark values hold for the test group under fairly general conditions: 0.50 = equality, 0.56 = small superiority, 0.64 = medium-sized/relevant superiority, 0.71 = large superiority". This approach is also used in the Bornstein 2018 meta-analysis.”

However, we confirm here that despite the misplaced citation the use of the word 'superiority' indeed shows prejudice: 'difference' would be more neutral and more accurate, including from the statistical point of view.

Here, we will not go into discussion of validity of t-test versus Wilcoxon-Mann-Whitney test or vice versa, we think that the following citation from Lumley 2002 speaks for itself:

“The Wilcoxon test is widely known to be more powerful than the t-test when the distribution of data in the two groups has long tails and has the same shape in each group but has been shifted in location. Conversely, it is less powerful than the t-test when the groups differ in the number and magnitude of extreme outlying distributions, as recognized in EPA guidelines for testing for environmental contamination in soil (33). Although its power relative to other tests depends on the details of the null and alternative hypotheses, the Wilcoxon test always has the disadvantage that it does not test for equality in any easily described summary of the data. This is illustrated by the analysis of Rascati et al. (21) in comparing overall medical costs for asthmatics prescribed steroids compared with other treatments. Although the mean cost was lower in the steroid group, a Wilcoxon test reported significantly higher costs for that group. A related disadvantage is that it is not easy to construct confidence intervals that correspond to the Wilcoxon test.

The t-test and least-squares linear regression do not require any assumption of Normal distribution in sufficiently large samples. Previous simulations studies show that “sufficiently large” is often under 100, and even for our extremely nonNormal medical cost data it is less than 500. This means that in public health research, where samples are often substantially larger than this, the t-test and the linear model are useful default tools for analyzing differences and trends in many types of data, not just those with Normal distributions. Formal statistical tests for Normality are especially undesirable as they will have low power in the small samples where the distribution matters and high power only in large samples where the distribution is unimportant.”

Therefore, we believe that all our statements and citations are valid.

We have introduced one more reference to the CASTA 2012 trial report published in *Stroke*, to make it explicitly clear what is the source of the citation coming from the same author team members.

Contributors

Commentary submitted by Volker Rahlfs, Chairman of the IDV Methodology Group, Methodology Group, IDV Data Analysis and Study Planning.

Response submitted by the authors of this Cochrane Review.

Issues with selection bias in Ziganshina (2020), 29 July 2020

Summary

Dear corresponding author

In this message, I want to express my perspective as researcher involved in clinical trials excluded by this review, as well as coordinator of ongoing, similar level review initiatives on the same topic. My first inquiry is related to research question selection. Authors state they "compared Cerebrolysin added to standard treatment against either placebo or no treatment added to standard treatment, while acknowledging that standard treatment is not defined precisely and differs between studies".

The review lists among exclusion criteria (ineligible research question) the CARS trial, the study of Stan (2017), as well as other studies we believe contribute to describing the effect of Cerebrolysin in the acute ischemic stroke population. The research question of the excluded CARS study is similar to the research question of included studies (randomized, placebo-controlled, double-blind multicenter trial to investigate the effects of Cerebrolysin after acute ischemic stroke). While the primary criterion was improved motor function in the upper extremity (ARAT), other common stroke outcomes as NIHSS or mRS, were available. It may be regarded as critical and prone to selection bias that our study (Muresanu 2016a), a class I randomized, placebo-controlled, double-blind multicenter trial, well demonstrating statistically significant results on the primary efficacy criterion ARAT ($P < 0.0001$), as well on the NIHSS ($P < 0.0000$) and other stroke outcomes, was excluded from the review Ziganshina (2020). For evaluation of potential selection bias, we suggest to provide further details of exclusion for all trials.

On the same topic of standard treatment definition, in addition to important above-mentioned issues with study inclusion (selection bias), a crucial distinction must be made based on whether patients benefit or not from neurorehabilitation programs, when conducting broad-goal meta-analyses. Some other clinical studies were excluded due to “ineligible question: neurorehabilitation”. We kindly ask for rationale for this exclusion criterion. As rehabilitation regimens are widely accepted as effective post-stroke interventions, we cannot say that we are comparing the same intervention in the standalone (Cerebrolysin) vs. add-on treatment (Cerebrolysin + neurorehabilitation) paradigms. In this case, the agent’s multimodal mechanism of action further expands discrepancies between these approaches beyond the added effect of physical therapy, as the intervention work both on its own to mitigate brain damage (i.e. neuroprotection for apoptosis/inflammation), but also to pharmacologically support existing efforts, enhancing neurorecovery. Therefore, differentiation of existing literature based these criteria should at least be attempted, to ensure both internal and exteral validity of the review. Neurorehabilitation is more and more the standard of care and it is incomprehensible why beneficial effects of a pharmacological treatment should be discarded due to the additional presence of neurorehabilitation. In contrary, neurorehabilitation might open the pathway for pharmacological mode of action. Rather studies without standard neurorehabilitation could be excluded or be part of a separate review question. We see here a major limitation of the overall review conclusions. The associated limitation should at least be very clearly expressed.

In addition, one would inquire what is the rationale for restricting the initiation of treatment in the first 48 hours after stroke onset? Since this is also an exclusion criterion for the positive CARS trial, I feel this important component was left completely unreferenced in the manuscript. While the timing window for treatment initiation only partly exceeds the chosen selection benchmark (benchmark Ziganshina 2020: 48h, excluded study Muresanu 2016a: treatment initiation 24h to 72 hours after stroke onset), the exclusion of a highly positive study, based on an arbitrarily window needs to be explicitly mentioned as a limitation related to risk of selection bias. We suggest to consider an acute phase initiation window (within a week of stroke onset), based on professor Julie Bernhardt’s paper "Agreed definitions and a shared vision for new standards in stroke recovery research: The Stroke Recovery and Rehabilitation Roundtable taskforce".

The general impression upon reading this update is that your team went above and beyond to explore comparators, as well as a selection of absent indicators, such as quality of life, while excluding a wealth of information from studies that were not eligible for your research question. Since there a quite a few papers in this situation, I would argue that at least a subgroup/sensitivity analysis should address existing evidence.

Conclusion

Given the all the large number of restrictions this review has applied – (1) unreasonably restricted initiation window, (2) harsh exclusion of trials with slightly difference explicit, but identical implicit research questions, (3) decision not to analyze a wide range of outcome scales and existing evidence – I feel that the review’s summary translation of findings into lay language does not do justice to published literature regarding Cerebrolysin’s potential to improve outcome after acute ischemic stroke. The paper draws broad, overarching conclusions about the agent, but does not analyze all available information, nor does it suggest that an expanded approach is warranted.

Reply

Dear Dr Dafin Muresanu

Thank you for your interest in our Cochrane Review 'Cerebrolysin for acute ischaemic stroke'.

We would like to assure you that since 2008, when the protocol for this Cochrane Review had been first published, the understanding in the academic community of 'acute stroke' has not changed, nor has our eligibility criteria for participants with acute stroke:

“People with acute ischemic stroke, irrespective of age, gender, or social status, whose symptom onset was less than 48 hours previously.” (citing the Protocol of 2008, Types of participants).

“Study medication must have been started within 48 hours of stroke onset and must have been continued for at least two weeks.” (citing the Protocol of 2008, Types of interventions).

These remain the same through the last 12 years and six versions (one protocol, the first published review, and four subsequent updates):

“People with acute ischaemic stroke, irrespective of age, sex, or social status, whose symptom onset was less than 48 hours previously.” (citing the Review, latest version 2020, Types of participants).

“Study medication must have been started within 48 hours of onset of stroke and continued for any period of time.” (citing the Review, latest version 2020, Types of interventions).

The Cochrane review title is: **Cerebrolysin for acute ischaemic stroke**

The review objective is: “To assess the benefits and harms of Cerebrolysin for treating acute ischaemic stroke.”

The title of the paper Muresanu 2016a is: **Cerebrolysin and Recovery After Stroke (CARS): a randomized, placebo-controlled, double-blind, multicenter trial**

The Muresanu 2016a objective: “The purpose of this Cerebrolysin and Recovery After Stroke (CARS) trial was to analyze the efficacy and safety of Cerebrolysin during recovery after stroke.”

The Muresanu 2016a intervention is described as follows:

“The study medication was administered once daily for 21 days as an intravenous infusion for 20 minutes, beginning at 24 to 72 hours after stroke onset. In previous studies, drug dosages from 10 to 50 mL per day were used, and the treatment periods ranged from 10 to 30 days, with once-daily infusions of Cerebrolysin.15–28,31Each patient included in our study participated in an accompanying standardized rehabilitation program for 21 days, beginning within 48 to 72 hours after stroke onset (5 d/wk for 2h/d). This pro-gram included massages and passive and active movements of the upper and lower limbs.”

Hence our reason for exclusion of this trial still stands: **Ineligible question and ineligible timing of cerebrolysin initiation after stroke onset**

This is in full compliance with Cochrane's mandatory MECIR standards on eligibility criteria, which are described: “Predefined, unambiguous eligibility criteria are a fundamental prerequisite for a systematic review”.

MECIR stanard C2: Predefining objectives

“Define in advance the objectives of the review, including participants, interventions, comparators and outcomes (PICO).”

Thus, we regret to inform you that the study Muresanu 2016a, reported in two publications:

- Muresanu D, Heiss WD, Bajenaru O, Popescu CD, Vester J, Guekht A. Cerebrolysin and recovery after stroke (CARS): a randomized, placebo-controlled, double-blind, multicenter, phase II clinical study. *International Journal of Stroke* 2015;10 Suppl 2:92.
- Muresanu DF, Heiss WD, Hoemberg V, Bajenaru O, Popescu CD, Vester JC, et al. Cerebrolysin and recovery after stroke (CARS): a randomized, placebo-controlled, double-blind, multicenter trial. *Stroke* 2016;47(1):151-9.

is indeed truly ineligible for this Cochrane review: Cerebrolysin for acute ischaemic stroke

Contributors

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Response submitted by the authors of this Cochrane Review

WHAT'S NEW

Date	Event	Description
11 October 2023	New citation required and conclusions have changed	We identified one new study on a Cerebrolysin-like agent, Cortexin, which we included for the outcome 'all-cause death'. The review now has seven included studies involving 1773 participants. We updated the text, risk of bias tables, analyses, summary of findings table, and conclusions. The conclusions now include Cerebrolysin-like agents in addition to Cerebrolysin.
11 October 2023	New search has been performed	Searches and PRISMA diagram updated. Background section revised and updated, and new references added.

HISTORY

Protocol first published: Issue 2, 2008
Review first published: Issue 4, 2010

Date	Event	Description
8 September 2020	New citation required and conclusions have changed	In response to feedback we added a reference to CASTA 2012 for a citation in the 'Discussion', 'Agreements and disagreements with other studies or reviews', and 'Use of statistical instruments' sections of the review, and edited the first sentence of the subsection 'Commercial influences or risks of sponsored science'.
13 November 2019	New search has been performed	Searches updated. Background section revised and updated, and new references added. Searches updated and PRISMA diagram updated. We identified one new study; the review now has seven included studies involving 1601 participants. We edited and updated the text, risk of bias tables, and summary of findings table.
13 November 2019	New citation required but conclusions have not changed	The conclusions have not changed. New author added.
11 April 2017	Amended	In response to feedback, we refined the outcome serious adverse events (SAEs) and replaced it with: total number of people with SAEs; total number of people with fatal SAEs; and total number of people with non-fatal SAEs.
11 April 2017	New citation required and conclusions have changed	Conclusions changed.
27 May 2016	New citation required and conclusions have changed	The conclusions of the review have changed.
27 May 2016	New search has been performed	We refined the inclusion criteria to allow the inclusion of trials where the length of Cerebrolysin use was not restricted to 14 days (any length of use). We performed a new search and included five new studies. The review now has six included studies involving 1501 participants. Ludivine Vernay joined the author team. We used Covidence for managing records, papers, and tri-

Date	Event	Description
		als, to extract data and assess risk of bias, and to resolve conflicting opinions of the authors. We refined the conclusions.
27 January 2015	New citation required but conclusions have not changed	We performed a new search. The conclusions have not changed.
15 July 2008	Amended	Converted to new review format.

CONTRIBUTIONS OF AUTHORS

Liliya-Eugenevna Ziganshina (LEZ) prepared the protocol, was the author of the original review, and was responsible for this update jointly with the other co-authors; involved in the conception of this review update; assessed citations, abstracts, and full texts of trial reports for eligibility; extracted data, assessed risk of bias, and managed the references; drafted the updated sections of the review text.

Tatyana R Abakumova (TRA) was responsible for this update jointly with the other co-authors; involved in the conception of this review update; performed literature searches of the Russian language studies; assessed citations, abstracts, and full texts of trial reports for eligibility.

Dilyara Nurkhametova (DN) was responsible for this update jointly with the other co-authors; involved in the conception of this review update; assessed citations, abstracts, and full texts of trial reports for eligibility; extracted data, assessed risk of bias, and managed the references; drafted the updated sections of the review text.

Kristina Ivanchenko (KI) was responsible for this update jointly with other co-authors; involved in the conception of this review update; performed literature searches of the Russian language studies; assessed citations, abstracts, and full texts of trial reports for eligibility; extracted data, assessed risk of bias, and managed the references; drafted the updated sections of the review text.

DECLARATIONS OF INTEREST

LEZ: is a Cochrane director (Cochrane Russia). She was not involved in the editorial process.

TRA: none known.

DN: none known.

KI: none known.

SOURCES OF SUPPORT

Internal sources

- Cochrane Stroke, UK

  Editorial support and advice
- Liverpool School of Tropical Medicine, UK

  Mentoring support at the initiation stage of the title registration

External sources

- None, Other

  None

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

2010, Issue 4 (first review version): we followed the Cochrane protocol precisely (Ziganshina 2010a).

2015, Issue 6 (second review version): we did not incorporate changes to the structure of the previously published version of the review. We updated searches, followed the protocol precisely, and confirmed the conclusions (Ziganshina 2015).

2016, Issue 11 (third review version): we changed the inclusion criteria to allow varying durations of Cerebrolysin use and included a total of six studies with one comparison: Cerebrolysin versus placebo for acute ischaemic stroke. We restructured the outcomes: all-cause death became the primary outcome, with the remaining outcomes listed as secondary outcomes. We changed the wording of "total number of adverse events" to "total number of people with adverse events". Ludivine Verney joined the team as a co-author (Ziganshina 2016).

2017, Issue 4 (fourth review version): we refined the outcome serious adverse events (SAEs), replacing it with the following three outcomes: total number of people with SAEs; total number of people with fatal SAEs; and total number of people with non-fatal SAEs (Ziganshina 2017).

2020 (fifth review version): we refined the eligibility criteria (types of participants) for future updates. In the current update we added two new secondary outcomes: non-death attrition and cause of death.

2023 (sixth review version): we did not change the eligibility criteria from the last update and did not add any new outcomes. In the current update we added one new trial testing a Cerebrolysin-like agent, Cortexin.

INDEX TERMS

Medical Subject Headings (MeSH)

Acute Disease; Amino Acids [adverse effects] [*therapeutic use]; Bias; Brain Ischemia [complications]; Cause of Death; Neuroprotective Agents [adverse effects] [*therapeutic use]; Patient Dropouts [statistics & numerical data]; Randomized Controlled Trials as Topic; Stroke [*drug therapy] [etiology] [mortality]

MeSH check words

Humans