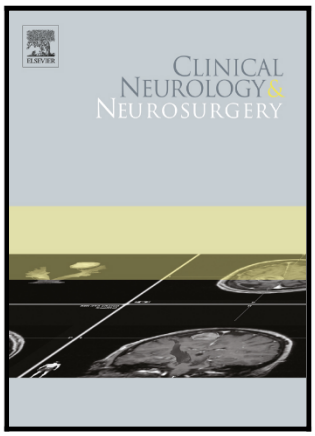
Effect of Cerebrolysin in Severe Traumatic Brain Injury: A multi-center, retrospective cohort study



Lynne Lourdes N. Lucena, Marla Vina A. Briones

PII: S0303-8467(22)00097-X

DOI: https://doi.org/10.1016/j.clineuro.2022.107216

Reference: CLINEU107216

To appear in: *Clinical Neurology and Neurosurgery*

Received date: 8 February 2022
Revised date: 15 March 2022
Accepted date: 18 March 2022

Please cite this article as: Lynne Lourdes N. Lucena and Marla Vina A. Briones, Effect of Cerebrolysin in Severe Traumatic Brain Injury: A multi-center, retrospective cohort study, *Clinical Neurology and Neurosurgery,* (2022) doi:https://doi.org/10.1016/j.clineuro.2022.107216

Effect of Cerebrolysin in Severe Traumatic Brain Injury:

A multi-center, retrospective cohort study

Lynne Lourdes N. Lucena, MD, DPBNS, FPCS, FAFNI[1*], Marla Vina A. Briones, MSc. Epidemiology (Public Health)[2]

[1]Department of Surgery, Bicol Regional Training and Teaching Hospital, Philippines

[2]Independent researcher

*** Correspondence:** Lynne Lourdes N. Lucena lynne.lucena@gmail.com

Abstract

Background: Severe traumatic brain injury (TBI) patients with nonoperative lesions are known to have a poorer prognosis. Recent and ongoing clinical studies have been exploring the utility of Cerebrolysin in improving patient outcomes among TBI patients; however, few studies are available on the effect of Cerebrolysin among nonoperative severe TBI patients.

Objectives: To determine the effects of Cerebrolysin as add-on therapy to the standard medical decompression protocol for nonoperative severe TBI patients.

Methods: The study employed a retrospective cohort design and included 87 severe TBI patients on admission. In addition to the current medical decompression protocol, 42 patients received 30 ml/day Cerebrolysin for 14 days, followed by a subsequent 10 ml/day dosage for another 14 days. The control group included 45 patients who received the standard decompression protocol only. Stata MP version 16 was used for data analysis.

Results: Compared to the control group, a significantly higher proportion of patients who received Cerebrolysin treatment achieved a favourable outcome at Day 21 post-TBI (50% vs. 87%; $p<0.00001$) and GOS ≥4 (18% vs. 39%; $p=0.043$). The mean length of hospital stay was approximately seven days shorter in the Cerebrolysin group (25.61 days vs. 31.92 days; $p<0.00001$), and a significantly lower proportion of Cerebrolysin patients had a LOS ≥30 days (Cerebrolysin: 13%; Control: 51%; $p<0.0001$). No significant group differences were seen in the 28-day mortality rate.

Conclusion: Cerebrolysin is beneficial for severe TBI patients with nonoperative lesions as evidenced by stronger improvement in GCS/GOS and shorter length of hospital stay than standard treatment alone.

Keywords: Cerebrolysin, Severe Traumatic Brain Injury, Glasgow Coma Score, Glasgow Outcome Score, neurorehabilitation

INTRODUCTION

In 2016, about 55 million people worldwide suffered from traumatic brain injury (TBI), most (87%) of which occurred in low- and middle-income countries (LMICs). [1] Fall injuries are the predominant cause of TBI worldwide; however, when stratified by geographical location, road traffic accidents comprise the majority of TBI in most LMICs. [1] With the increasing trend in road traffic injuries in the Philippines [2], the incidence of TBI is also expected to increase.

TBI is also a leading cause of disability, causing 8.1 million years lived with disability in 2016 alone and confers a significant burden on the patients and their family members. [1] The high cost of hospitalization attributed to the extended hospital stay, especially among severe TBI patients, has been elucidated and poses an enormous burden on the healthcare systems, particularly in LMICs.[3] Furthermore, the mortality rate is more than 50% among patients suffering from severe TBI. [4,5]

Proper management of severe TBI patients is, therefore, crucial for a better prognosis. While most severe TBI patients are managed surgically, some patients do not present with operative lesions. Thus, conservative management remains the only option, which aims to prevent secondary injury to the brain by decreasing intracranial pressure and preventing cerebral edema. [6,7] Unfortunately, conservative management of severe TBI patients is associated with higher mortality rates. [6,8]

Cerebrolysin is a peptide preparation that promotes neuroplasticity and neurogenesis in vitro and in vivo; [9–11] thus, was found to be helpful in various neurologic disorders like Alzheimer's disease and stroke.[12–14] Several authors also proposed the use of Cerebrolysin for the management of TBI patients. [15–22] Cerebrolysin helps in preventing secondary injury cascade by controlling oxidative stress, microglial activation, inflammatory process and blood-brain barrier (BBB) dysfunction. [23–28]

However, studies on the effect of Cerebrolysin for nonoperative severe TBI patients remain limited today. Therefore, the current study aims to determine the effect of Cerebrolysin as an add-on therapy to the standard medical decompression protocol in improving clinical outcomes among nonoperative severe TBI patients.

General objective: To determine the effect of Cerebrolysin as add-on therapy to the standard medical decompression protocol in improving clinical outcomes among severe TBI patients admitted in three tertiary hospitals in the Philippines from December 2010 to December 2015.

Specific objectives:

1. To compare the demographic and clinical characteristics of patients between the two groups
2. To compare the proportion of patients with favorable outcomes between the two groups in terms of:

    a. Glasgow Coma Scale (GCS) ≥9
    b. Glasgow Outcome Score (GOS) ≥4
3. To determine the association between Cerebrolysin treatment and favorable outcome in terms of GCS (≥9) and GOS (≥4)
4. To compare the absolute and relative improvement in GCS at Days 7, 14, 21, and 28 between the two groups
5. To compare the GOS at Days 14, 21 and 28 between the two groups.
6. To compare the length of stay between the two groups
7. To compare the 28-day mortality rate between the two groups

MATERIALS AND METHODS

The study employed a retrospective cohort design. Participants were nonoperative severe TBI patients (i.e., GCS 5-7) admitted from December 2010 to December 2015 in three hospitals located in the Bicol Region, Philippines. Patients fulfilling the following inclusion criteria were included in the study: 1) CT scan done within 24 hours of hospital admission, 2) Isolated TBI (AIS of <2 body parts), and 3) ability to speak, read and write prior to the injury. Patients with the following conditions were excluded from the study: 1) spinal cord injury, 2) history of intracranial interventions, ischemic or hemorrhagic stroke, 3) psychiatric disorders or neurodegenerative diseases, 4) history of epileptic seizures, 5) patients under chronic treatment with cortisone, Ca+-channel blockers, antidepressants, antipsychotic drugs, nootropic molecules, and 6) use of concomitant neuroprotective treatment or cholinesterase inhibitors for pre-existing cognitive function deficits.

All patients included in the study were under the direct neurosurgical management of the study's primary author. All patients in the study received the standard decompression protocol which utilized osmotherapy with mannitol and hypertonic saline to control ICP and cerebral edema.[29] Cerebrolysin was offered to all patients deemed eligible by the primary author. Cerebrolysin was approved for human use in the Philippines since 2009, and was being administered at the included hospitals as an add-on therapy to standard decompression protocol to nonoperative TBI patients upon patient and/or guardian consent. Those who refused or cannot afford the drug (average cost ~$672) were managed using the standard decompression protocol.

The sample size was computed using G*Power3.1.9.2 software. Parameters were based on previously published literature.[15] Specifying a proportion of favorable outcome equal to 90% using Cerebrolysin + standard decompression protocol and 66% in standard decompression protocol only, and alpha set at 5%, a total of 84 patients—42 for each group—is required to achieve 80% power in detecting a significant difference in proportions. The researcher utilized a total enumeration technique to select study participants.

All 87 patients were included in the study. Forty-two patients received 30 ml/day Cerebrolysin (EVER Neuro Pharma, Austria) for 14 consecutive days, followed by a dosage of 10 ml/day for another 14 days. A heparin lock was inserted in patients discharged before Day 28

so that Cerebrolysin treatment can be continued at home. Forty-five patients who only received the standard medical decompression protocol served as the control group.

Prior to study implementation, the researcher obtained ethical clearance from the BRTTH Institutional Review Board (IRB). Data were collected through a medical chart review from August 1 to 15, 2021. The researcher obtained the baseline data, including age, sex, cause of TBI, TBI diagnosis, and initial GCS score. In addition, the GCS scores at Days 7, 14, 21, and 28 and GOS at Days 14, 21 and 28 were recorded. Likewise, in-hospital mortality was assessed at Day 28.

Data were recorded in an MS Excel by the researcher, and StataMP version16 was used for further processing and analysis. Continuous variables were presented as mean/SD or median/IQR depending on data distribution, while categorical variables were presented as frequency/percentages. Chi square test and Fisher's exact test were used in comparing the proportion of patients who achieved GCS ≥9 and GOS ≥4 between groups. Significant results were further analyzed using simple logistic regression analysis. Repeated Measures ANOVA and Tukey HSD were used to assess the improvement in GCS scores over time. Finally, between-group comparisons of mean GCS, relative and absolute improvement in GCS were compared using an independent t-test while length of stay was compared using Mann Whitney U test. Throughout, p-values ≤0.05 were considered statistically significant.

RESULTS

A total of 87 severe TBI patients were included in the study, of which 42 received Cerebrolysin in addition to the medical management protocol for nonoperative lesions. Table 1 presents the baseline profile of the patients. The majority of the patients in both groups were males, and TBI was predominantly caused by a vehicular accident (2-wheel). No significant difference was observed between the Cerebrolysin and control groups in terms of age, sex, alcohol intoxication, etiology, diagnosis, and mean baseline GCS.

As depicted in Figure 1, the mean GCS of both groups significantly increased over time. The mean GCS score of the Cerebrolysin group was significantly higher than for controls at Day 21 ($p=0.0394$) and Day 28 ($p=0.0002$). Similarly, the proportion of patients who attained a favourable GCS score of 9-15 by Day 21 was significantly higher in the Cerebrolysin group ($p<0.0001$), but was no longer significant at Day 28 ($p=0.115$) as seen in Figure 2. Further analysis revealed that the odds of achieving a GCS ≥9 at Day 21 are about six times higher in the Cerebrolysin group (OR=6.27; $p=0.002$). Table 2 below presents the absolute and relative improvement in GCS between Cerebrolysin and control groups. Compared to baseline, the absolute and relative improvement in GCS at Days 7, 14, 21 and 28 were significantly higher in the Cerebrolysin group than the controls.

A significantly higher percentage of patients treated with Cerebrolysin had a favourable outcome with a GOS score of ≥4, as seen in Figure 3 on Day 21 (Cerebrolysin: 39%; Control 18%; $p=0.043$) and Day 28 (Cerebrolysin: 68%; Control: 21%; $p<0.0001$). A similar trend

towards a better outcome for the active treatment group was also seen at Day 14, with 11% of the Cerebrolysin group compared to 3% of the control group; however, these proportions did not reach statistical significance. Further analysis revealed that the odds of achieving GOS ≥4 at Day 21 are about three times higher in the Cerebrolysin group than controls (OR=2.89; p=0.047).

Among survivors (n=77), the median length of hospital stay (LOS) was significantly shorter in the Cerebrolysin group compared to the control group by about seven days (Cerebrolysin: 25 [IQR: 23-28; mean: 25.61 ± 3.24] vs. Control: 30 [IQR: 25-38; mean: 31.92 ±7.30], p<0.00001). In addition, all patients in the Cerebrolysin group were discharged from the hospital within 33 days compared to 47 days in the control group. Further analysis revealed that the proportion of patients with LOS of ≥30 days was significantly lower in the Cerebrolysin versus the control group (Cerebrolysin: 13% vs. Control: 51%, p<0.0001; Chi-square test).

Mortality at Day 28 did not reveal a significant difference between the study groups, although the rate was descriptively lower for the active treatment group. In the Cerebrolysin group, four patients died versus six patients in the control group (Table 3). All patients died due to pneumonia. In the Cerebrolysin group, two patients died of aspiration pneumonia, one due to hospital-acquired pneumonia (HAP) and one due to sepsis pneumonia. In the control group, five patients died due to HAP and one due to aspiration pneumonia. Almost all mortalities, except for one female patient assigned to the Cerebrolysin group, were males, and the majority was diagnosed with diffuse cerebral edema (DCE) and intracerebral hematoma (ICH).

Subgroup analysis was performed by patient age and diagnosis for the study's primary endpoints (Table 4). For patients <40 years old, it has been observed that the percentage of patients achieving GCS of 9-15 and GOS ≥4 at Day 21 were higher in the Cerebrolysin group, while the percentage with GOS≥4 at Day 21 was comparable between the two treatment groups for patients ≥40 years old. In terms of diagnosis, all Cerebrolysin-treated patients diagnosed with cerebral contusion (CC) and diffuse axonal injury (DAI) achieved a GCS of 9-15 at Day 21. Across all diagnoses, patients who received Cerebrolysin showed a higher proportion of a favourable outcome based on the GCS compared to the control group. A GOS ≥4 at Day 21 was only found to be higher in Cerebrolysin patients diagnosed with CC, DAI, and ICH.

DISCUSSION

In our current study, patients in both the Cerebrolysin and control groups received mannitol, hypertonic sodium lactate, or both. Although previous studies reported higher mortality rates among conservatively managed severe TBI patients [6,8], only 11.5% of the patients died in our current study, all because of pneumonia, the most common non-neurological complication of severe TBI. [30] The difference in the mortality estimates in our study is probably due to the variance in the study population—our current study excluded patients with GCS<5 who are known to have an even poorer prognosis. In contrast, the epidemiologic studies included all TBI patients regardless of GCS at hospital presentation. [6,8]

This retrospective study provided evidence that Cerebrolysin is beneficial for severe TBI patients with nonoperative lesions. Although, as expected, control patients also showed a significant increase in GCS scores over time, the improvement in GCS from baseline to Days 7, 21 and 28 were consistently and significantly higher in patients who received Cerebrolysin treatment. As concluded by a recently published meta-analysis of the CAPTAIN trial series, functional and neurological outcomes at Day 10 and 30 favoured Cerebrolysin treatment. [31] Similarly, a previous study demonstrated that Cerebrolysin administration resulted in an improvement in eye-opening and verbal response parameters of the GCS as early as seven days of treatment, followed by improvement in consciousness and cognitive performance, and motor response. [19] These changes could explain the patients' early and good treatment response, particularly in the Cerebrolysin group in the current study with about 50% of patients improving from the initially severe to a mild or moderate TBI at day 14.

According to another previous study, up to 47% of severe TBI patients can achieve a GOS ≥4 at three months by conservative management alone. [32] Our study showed that by the end of 28 days, 68% of severe TBI patients treated with Cerebrolysin already achieved a GOS ≥4 compared to 21% of controls; thus, suggesting faster recovery rates. One meta-analysis also concluded that Cerebrolysin significantly increased GOS, but is only evident in moderate-to-severe TBI cases. [33] A similar study administered Cerebrolysin among moderate-to-severe TBI patients and showed a favourable outcome, defined as GOS 3-5, in 67% of patients at six months. [15] Interestingly, our current study showed that by using the same criteria (GOS of 3-5), 100% of surviving patients already achieved a favourable outcome by 21 days (data not shown). Furthermore, the observation of a shorter length of hospital stay for Cerebrolysin-treated patients in our study also clearly supports the notion of faster recovery rates after treatment with Cerebrolysin as measured by GCS and GOS.

Although the exact mechanism of how Cerebrolysin contributes to a better and faster recovery among TBI patients is not yet fully established, previous research studies have provided convincing explanations. On a molecular and cellular level, prevention of secondary injury entails controlling the excessive formation of nitric oxide and oxidative stress, microglial activation, inflammatory processes, and blood-brain barrier (BBB) dysfunction. [20] Several studies provided evidence on the mechanism of how Cerebrolysin affects the secondary injury cascade, thereby eliciting favourable outcomes in severe TBI patients. By targeting free radical formation, Cerebrolysin combats oxidative stress, as evident by the reduced malondialdehyde (MDA) levels. In vivo and in vitro studies also concluded that Cerebrolysin affects microglial activation and diminishes inflammation, thereby preventing neuronal damage. [23–25] Another recent study also suggested that alterations in dopamine levels result in CNS inflammation, which was also found to be counteracted by Cerebrolysin. [26,27] Furthermore, disruption in BBB can also be attenuated by Cerebrolysin administration. [28] Recently, studies have been proposing the role of brain-derived neurotrophic factor (BDNF) in the prognosis of TBI patients. Serum BDNF was low in TBI patients but could be reversed by Cerebrolysin. [34,35]

In order to gain more information regarding the effects of Cerebrolysin, the current study performed subgroup analyses by patient age and diagnosis. Age is a prognostic factor among TBI patients—the higher the age, the poorer the prognosis. An unfavourable outcome, defined as severe disability or death, was found to be six times more likely among nonoperative TBI patients ≥40 years old. [36] In severe TBI patients ≥40 years old in the current study, 81% achieved a GCS of 9-15 at Day 21 compared to 40% of the control group. In contrast, the proportion showing favourable GOS (≥4) in the ≥40 years subgroup was almost comparable between the two treatment groups, despite the fact that positive effects of Cerebrolysin in elderly TBI patients have been observed in a previous study. [15] This study by Wong et al. has administered a dose of 50 ml of Cerebrolysin, and the outcome was assessed at six months [15], while in the current study a lower dose of 30 ml of Cerebrolysin was applied, and the outcome was only assessed for 21 days. A dose-response effect of Cerebrolysin was recently observed in one animal study [37], thus, increasing the dosage for severe TBI patients could potentially lead to faster and even better recovery rates. In addition, the short duration of follow-up in this study could have contributed to different findings in the said age group. The low sample size of our subgroup analyses prevented us from conducting any significance testing. Therefore, future studies should compare doses of Cerebrolysin which can elicit faster but still safe recovery among severe TBI patients. Longer follow-ups are also needed to document patient progress over time. Since the effect of Cerebrolysin appeared to be different by age group, future studies with larger sample size, powered for age-subgroup analysis should be considered.

The beneficial effects of Cerebrolysin also varied by patient diagnosis. Notably, all Cerebrolysin patients diagnosed with a CC and DAI showed favourable GCS (GCS 9-15) as early as Day 21. In CC patients, raised ICP with edema formation is a common phenomenon, and in patients with high levels of glutamate, a poorer outcome is expected. [38] Studies show that Cerebrolysin exhibits neuroprotection for CC patients based on the following mechanisms: 1) prevention of cytotoxic edema formation, protection, and rescue of neuronal damage induced by glutamate, 3) restoration of cerebral blood flow to higher levels which is reduced by cerebrovascular damage, and 4) attenuation of BBB disruption. [28,38–40] These mechanisms could explain why all CC patients treated with Cerebrolysin in the current study no longer have severe TBI at Day 21, and a higher proportion achieved a GOS ≥4 than controls.

The effect of Cerebrolysin on DAI has been explored in two human studies. Surprisingly, the improvement in patient outcome was more significant in controls than Cerebrolysin patients in one study that administered 10 ml of Cerebrolysin. [41] In a study done in China, a significant improvement in GCS was evident within two weeks of Cerebrolysin treatment among DAI patients, compared to controls. [42] Secondary injuries are common in DAI patients, leading to poorer patient prognosis. [43] The secondary injury starts with the activation of caspase-3 and neuronal damage caused by the accumulation of calpain. [44] Therefore, the calpain-inhibitory effect of Cerebrolysin may explain the beneficial effect of Cerebrolysin in DAI patients in the current study. [20]

Patients with other clinical diagnoses (DCE, ICH, SDH) also showed favourable responses in GCS. In contrast, Cerebrolysin failed to show additional benefit in terms of GOS among DCE and SDH patients. However, the sample size of patients for each subgroup was too low to conduct significance testing and to provide a definite conclusion on the benefit of Cerebrolysin. Additional studies with a higher sample size for each specific subpopulation are therefore warranted.

Several limitations were noted for this study. First, the beneficial effect of Cerebrolysin was only tested for nonoperative patients who completed the recommended dosage. Patients with poorer prognosis (e.g., GCS 3-4) failed to complete the treatment, thus, were excluded in this research. The results of the study are, therefore, limited to GCS 5-7 patients. Furthermore, the results of the study may be different for those who are candidates for surgery since only patients with nonoperative lesions were included. Second, the outcome measures—GCS, GOS and mortality—were only measured up to Day 28 of treatment. Due to the retrospective nature of this research, follow-up data of discharged patients beyond 28 days is not available. Previous studies with long-term follow-ups have suggested that the benefit of Cerebrolysin on functional and cognitive recovery persists for several months. [16,17,45–47] In the recently published meta-analysis, functional and neuropsychological outcomes favour Cerebrolysin up to Day 90 of follow-up. Third, even after hospital discharge, TBI patients are faced with a lengthy recovery period and the possibility of adverse sequelae. [48,49] Thus, in patients who suffered TBI requiring rehabilitation, treatment with Cerebrolysin could lead to higher and faster recovery rates. [20] Future studies should, therefore, explore this possibility. Fourth, information bias is likely to have occurred since patients were not blinded to the treatment they received. Furthermore, the primary author served as the attending physician of these patients during the time of admission, thus, was also responsible in assessing the patients' clinical outcomes. Randomized controlled trials are warranted to provide a higher level of evidence on the efficacy of Cerebrolysin. Finally, the cost of treatment could have served as a barrier in availing Cerebrolysin. The characteristics of patients who refused the treatment due to financial concerns may be different from those who consented; however, due to the retrospective nature of this research, the authors cannot identify those who refused treatment due to cost from those who refused due to other reasons. Nevertheless, the shorter length of stay and faster recovery rates could have been beneficial to the patients; unfortunately, cost analysis was not performed due to the unavailability of billing records. Hence, future studies should explore the cost-effectiveness of this add-on therapy.

CONCLUSION

Cerebrolysin treatment is beneficial for severe traumatic brain injury patients with nonoperative lesions. Patients showed faster recovery rates as evidenced by more significant improvement in GCS/ GOS and lower duration of hospital stay than standard treatment alone. In addition, all Cerebrolysin-treated patients presenting with a cerebral contusion and diffuse axonal injury showed favourable GCS at Day 21. Therefore, future studies should explore the long-term outcomes and cost-effectiveness of Cerebrolysin treatment while considering patient diagnosis among conservatively managed severe TBI patients.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

LL: Conceptualization, investigation, writing- review & editing; MB: Methodology, formal analysis, writing-original draft

Funding

No funding or grant has been obtained from any agency for this study.

Acknowledgments

We thank the following institutions for allowing us to conduct this research: Bicol Regional Training and Teaching Hospital (BRTTH), Bicol Medical Center (BMC), and Universidad de Santa Isabel-Mother Seton Hospital (USI-MSH).

References

1. James SL, Theadom A, Ellenbogen RG, et al. Global, regional, and national burden of traumatic brain injury and spinal cord injury, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol*. 2019;18(1):56-87. doi:10.1016/S1474-4422(18)30415-0

2. Rey A. IN CHARTS: How deadly are Metro Manila roads? *Rappler.com*. www.rappler.com/newsbreak/in-depth/199896-metro-manila-road-crash-2017. Published April 11, 2018.

3. van Dijck JTJM, Dijkman MD, Ophuis RH, de Ruiter GCW, Peul WC, Polinder S. In-hospital costs after severe traumatic brain injury: A systematic review and quality assessment. *PLoS One*. 2019;14(5). doi:10.1371/journal.pone.0216743 Free PMC article

4. Majdan M, Mauritz W, Wilbacher I, et al. Traumatic brain injuries caused by traffic accidents in five European countries: Outcome and public health consequences. *Eur J Public Health*. 2013;23(4):682-687. doi:10.1093/eurpub/cks074

5. Adeleye AO, Ogun MI. Clinical epidemiology of head injury from road-traffic trauma in a developing country in the current era. *Front Neurol*. 2017;8(DEC):695. doi:10.3389/fneur.2017.00695

6. Agrawal D, Ahmed S, Khan S, Gupta D, Sinha S, Satyarthee G. Outcome in 2068 patients of head injury: Experience at a level 1 trauma centre in India. *Asian J Neurosurg*. 2016;11(2):143. doi:10.4103/1793-5482.145081

7. Boone M, Oren-Grinberg A, Robinson T, Chen C, Kasper E. Mannitol or hypertonic saline in the setting of traumatic brain injury: What have we learned? *Surg Neurol Int*. 2015;6(1):177. doi:10.4103/2152-7806.170248

8. Feliciano CE, De Jesus O. Conservative management outcomes of traumatic acute subdural hematomas. *P R Health Sci J*. 2008;27(3):220-223.

9. Satou T, Itoh T, Tamai Y, Ohde H, Anderson AJ, Hashimoto S. Neurotrophic effects of FPF-1070 (Cerebrolysin®) on cultured neurons from chicken embryo dorsal root ganglia, ciliary ganglia, and sympathetic trunks. *J Neural Transm*. 2000;107(11):1253-1262. doi:10.1007/s007020070015

10. Akai F, Hiruma S, Sato T, et al. Neurotrophic factor-like effect of FPF1070 on septal cholinergic neurons after transections of fimbria-fornix in the rat brain. *Histol Histopathol*. 1992;7(2):213-221.

11. Zhang Y, Chopp M, Zhang ZG, et al. Cerebrolysin Reduces Astrogliosis and Axonal Injury and Enhances Neurogenesis in Rats After Closed Head Injury. *Neurorehabil Neural Repair*. 2019;33(1):15-26. doi:10.1177/1545968318809916

12. Rockenstein E, Mante M, Adame A, et al. Effects of Cerebrolysin^{TM} on amyloid-β deposition in a transgenic model of Alzheimer's disease. *J Neural Transm Suppl*. 2002;113(62):327-336. doi:10.1007/978-3-7091-6139-5_31

13. Heiss WD, Brainin M, Bornstein NM, Tuomilehto J, Hong Z. Cerebrolysin in patients with acute ischemic stroke in Asia: Results of a double-blind, placebo-controlled randomized trial. *Stroke*. 2012;43(3):630-636. doi:10.1161/STROKEAHA.111.628537

14. Wang Z, Shi L, Xu S, Zhang J. Cerebrolysin for functional recovery in patients with acute ischemic stroke: A meta-analysis of randomized controlled trials. *Drug Des Devel Ther*. 2017;11:1273-1282. doi:10.2147/DDDT.S124273

15. Wong GKC, Zhu XL, Poon WS, Zhu XL, Poon WS. Beneficial effect of cerebrolysin on moderate and severe head injury patients: result of a cohort study. *Acta Neurochir Suppl*. 2005;95(March 2001):59-60. doi:10.1007/3-211-32318-x_13

16. Álvarez XA, Sampedro C, Figueroa J, et al. Reductions in qEEG slowing over 1 year and after treatment with Cerebrolysin in patients with moderate-severe traumatic brain injury. *J Neural Transm*. 2008;115(5):683-692. doi:10.1007/s00702-008-0024-9

17. Chen C-C, Wei S-T, Tsaia S-C, Chen X-X, Cho D-Y. Cerebrolysin enhances cognitive recovery of mild traumatic brain injury patients: double-blind, placebo-controlled, randomized study. *Br J Neurosurg*. 2013;27(6):803-807. doi:10.3109/02688697.2013.793287

18. Álvarez XA, Sampedro C, Pérez P, et al. Positive effects of Cerebrolysin on electroencephalogram slowing, cognition and clinical outcome in patients with postacute traumatic brain injury: An exploratory study. *Int Clin Psychopharmacol*. 2003;18(5):271-278. doi:10.1097/00004850-200309000-00003

19. Konig P, Waanders R, Witzmann A, et al. Cerebrolysin in TBI - A pilot study of a neurotropic and neurogenic agent in the treatment of acute traumatic brain injury. *J fur Neurol Neurochir und Psychiatr*. 2006;7:12-20.

20. Onose G, Mureşanu DF, Ciurea A V, et al. Neuroprotective and consequent neurorehabilitative clinical outcomes, in patients treated with the pleiotropic drug cerebrolysin. *J Med Life*. 2009;2(4):350-360.

21. Muresanu DF, Florian S, Hömberg V, et al. Efficacy and safety of cerebrolysin in neurorecovery after moderate-severe traumatic brain injury: results from the CAPTAIN II trial. *Neurol Sci*. Published online 2020. doi:10.1007/s10072-019-04181-y

22. Poon W, Matula C, Vos PE, et al. Safety and efficacy of Cerebrolysin in acute brain injury and neurorecovery: CAPTAIN I—a randomized, placebo-controlled, double-blind, Asian-Pacific trial. *Neurol Sci*. 2020;41(2):281-293. doi:10.1007/s10072-019-04053-5

23. Alvarez X, Lombardi V, Fernandez-Novoa L, et al. Cerebrolysin reduces microglial activation in vivo and in vitro: a potential mechanism of neuroprotection. *J Neural Transm Suppl*. 2000;59:281-292. doi:10.1007/978-3-7091-6781-6_30

24. Malashenkova IK, Krynskiy SA, Hailov NA, et al. Anti-inflammatory effects of neurotrophic therapy (a pilot study). *Zhurnal Nevrol i psikhiatrii im SS Korsakova*. 2018;118(5):39. doi:10.17116/jnevro20181185139

25. Mahmoudi J, Mohaddes G, Erfani M, et al. Cerebrolysin attenuates hyperalgesia, photophobia, and neuroinflammation in a nitroglycerin-induced migraine model in rats. *Brain Res Bull*. 2018;140:197-204. doi:10.1016/j.brainresbull.2018.05.008

26. Chen Y-H, Huang EY-K, Kuo T-T, Miller J, Chiang Y-H, Hoffer BJ. Impact of Traumatic Brain Injury on Dopaminergic Transmission. *Cell Transplant*. 2017;26(7):1156-1168. doi:10.1177/0963689717714105

27. Calderón Guzmán D, Brizuela NO, Herrera MO, et al. Effect of cerebrolysin on dopaminergic neurodegeneration of rat with oxidative stress induced by 3-nitropropionic acid. *Acta Pharm*. 2016;66(3):443-448. doi:10.1515/acph-2016-0027

28. Sharma HS, Zimmermann-Meinzingen S, Johanson CE. Cerebrolysin reduces blood-cerebrospinal fluid barrier permeability change, brain pathology, and functional deficits following traumatic brain injury in the rat. *Ann N Y Acad Sci*. 2010;1199(1):125-137. doi:10.1111/j.1749-6632.2009.05329.x

29. Carney N, Totten AM, Reilly CO, et al. *Guidelines for the Management of Severe Traumatic Brain Injury 4th Edition*.; 2016. doi:10.1227/NEU.0000000000001432

30. Lim HB, Smith M. Systemic complications after head injury: A clinical review. *Anaesthesia*. 2007;62(5):474-482. doi:10.1111/j.1365-2044.2007.04998.x

31. Vester J, Buzoianu A, Florian S, et al. Cerebrolysin after moderate to severe traumatic brain injury: prospective meta-analysis of the CAPTAIN trial series. *Neurol Sci*. Published online 2021. doi:10.1007/sl0072-020-04974-6

32. An L, Han X, Li H, et al. Effects and mechanism of cerebroprotein hydrolysate on learning and memory ability in mice. *Genet Mol Res*. 2016;15(3). doi:10.4238/gmr.15038804

33. Ghaffarpasand F, Torabi S, Rasti A, et al. Effects of cerebrolysin on functional outcome of patients with traumatic brain injury: a systematic review and meta-analysis. *Neuropsychiatr Dis Treat*. 2019;15:127-135. doi:10.2147/NDT.S186865

34. Dharmajaya R. Determinants of Glasgow outcome scale in patients with severe traumatic brain injury for better quality of life To. *IOP Conf Ser Earth Env Sci*. 2018;125.

35. Karakulova Y V., Selyanina N V. Monitoring of neurotrophic factors and cognitive function in patients with traumatic brain injury. *Zhurnal Nevrol i Psihiatr Im SS Korsakova*. 2017;117(10):34-37. doi:10.17116/jnevro201711710134-37

36. Dhandapani S, Manju D, Sharma B, Mahapatra A. Prognostic significance of age in traumatic brain injury. *J Neurosci Rural Pract*. 2012;3(2):131. doi:10.4103/0976-3147.98208

37. Zhang Y, Chopp M, Gang Zhang Z, et al. Prospective, randomized, blinded, and placebo-controlled study of Cerebrolysin dose-response effects on long-term functional outcomes in a rat model of mild traumatic brain injury. *J Neurosurg*. Published online 2018:1-10. doi:10.3171/2017.6.JNS171007

38. Ragaisis V. [Brain contusion: morphology, pathogenesis and treatment]. *Medicina (Kaunas)*. 2002;38(3):243-249; quiz 354.

39. Schwab M, Bauer R, Zwiener U. Physiological effects and brain protection by hypothermia and Cerebrolysin after moderate forebrain ischemia in rats. *Exp Toxicol Pathol*. 1997;49(1-2):105-116. doi:10.1016/S0940-2993(97)80078-4

40. Schauer E, Wronski R, Patockova J, et al. Neuroprotection of Cerebrolysin in tissue culture models of brain ischemia: Post lesion application indicates a wide therapeutic window. *J Neural Transm*. 2006;113(7):855-868. doi:10.1007/s00702-005-0384-3

41. Asghari M, Meshkini A, Salehpoor F, Aghazadeh J. Investigation of the effect of cerebrolysin on patients with head trauma and diffuse axonal injury. *Int J Curr Res Acad Rev*. 2014;2(8):62-69.

42. Wang J, Liu C, Xiong Y, Wuang L. Clinical study on cerebroprotein hydrolysate Injection for diffuse axonal injury. *Acta Acad Med Zunyi*. Published online 2006. http://en.cnki.com.cn/Article_en/CJFDTotal-ZYYB200603023.htm

43. Mesfin FB, Dulebohn SC. *Diffuse Axonal Injury (DAI)*. StatPearls Publishing; 2018.

44. Ma J, Zhang K, Wang Z, Chen G. Progress of research on diffuse axonal injury after traumatic brain injury. *Neural Plast*. 2016;2016. doi:10.1155/2016/9746313

45. Masliah E, Díez-Tejedor E. The pharmacology of neurotrophic treatment with cerebrolysin: Brain protection and repair to counteract pathologies of acute and chronic neurological disorders. *Drugs of Today*. 2012;48(SUPPL. A):3-24. doi:10.1358/dot.2012.48(Suppl.A).1739716

46. Khalili H, Niakan A, Ghaffarpasand F. Effects of cerebrolysin on functional recovery in patients with severe disability after traumatic brain injury: A historical cohort study. *Clin Neurol Neurosurg*. 2017;152:34-38. doi:10.1016/j.clineuro.2016.11.011

47. Talypov AE, Myatchin MY, Kuksova NS, Kordonsky AY. Cerebrolysin in the treatment of brain injuries of moderate severity. *Zhurnal Nevrol i Psihiatr Im SS Korsakova*. 2014;2014(11):98-106.

48. Fleminger S, Ponsford J. Long term outcome after traumatic brain injury. *BMJ*. 2005;331(7530):1419. doi:10.1136/bmj.331.7530.1419

49. Stocchetti N, Zanier ER. Chronic impact of traumatic brain injury on outcome and quality of life: a narrative review. *Crit Care*. 2016;20(1):148. doi:10.1186/s13054-016-1318-1

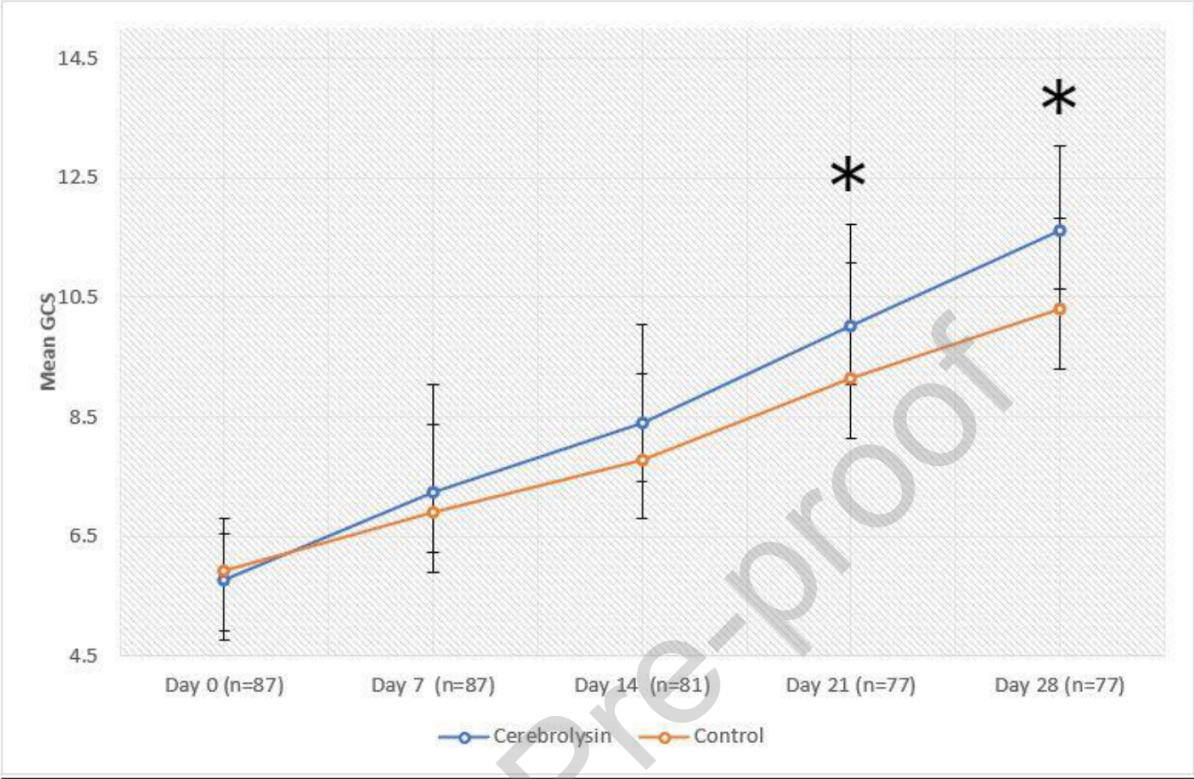


Figure 1. Mean Glasgow Coma Scale (GCS) over time by group. *The sample sizes for each time point corresponds to the total number of patients with GCS score for both groups. Error bars represent standard deviation; mean difference in GCS between Cerobrolysin and control groups at each time period were analysed using independent t-test; statistical significance is indicated by an asterisk where p≤0.05*

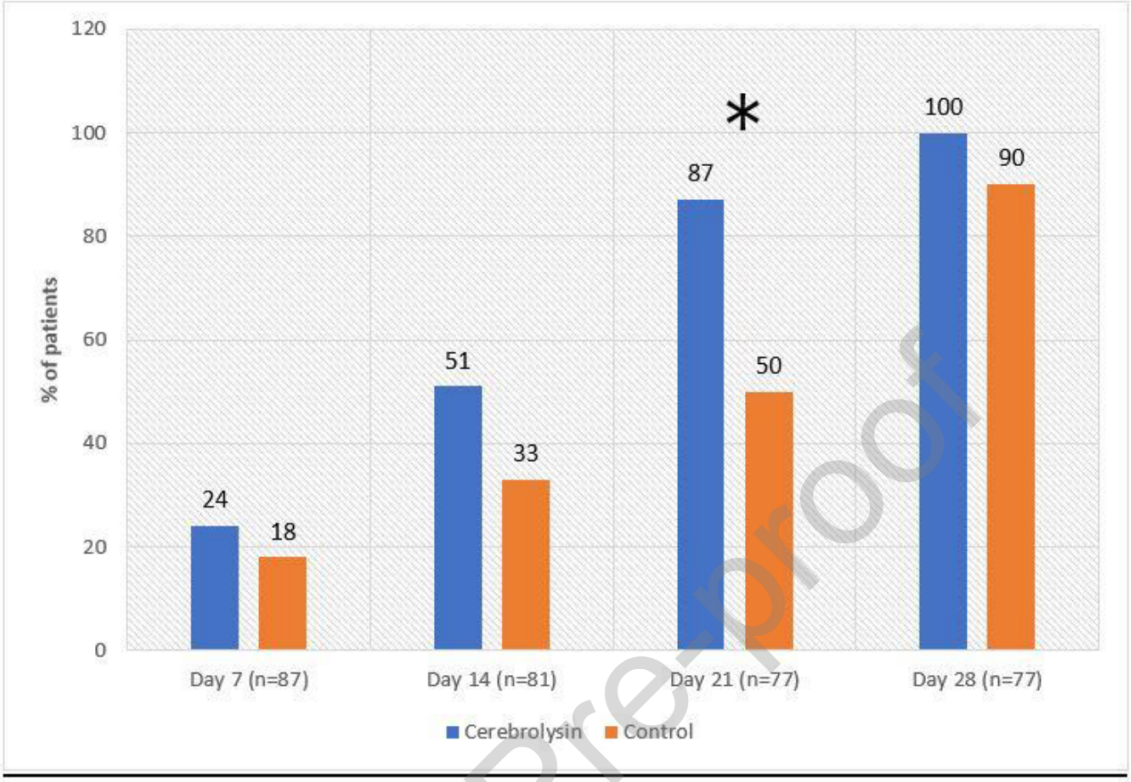


Figure 2. Proportion of patients that improved from a severe to a mild to moderate TBI (GCS 9-15) over time. *The sample sizes for each time point corresponds to the total number of patients with GCS score for both groups; values above the bar graphs represents the percentage of patients for each group with GCS 9-15; the difference in proportions between Cerebrolysin and Control groups at each time period were analysed using Chi Square test for Days 7,14 and 21 and Fisher's exact test for Day 28; statistical significance is indicated by an asterisk where $p \leq 0.05$*

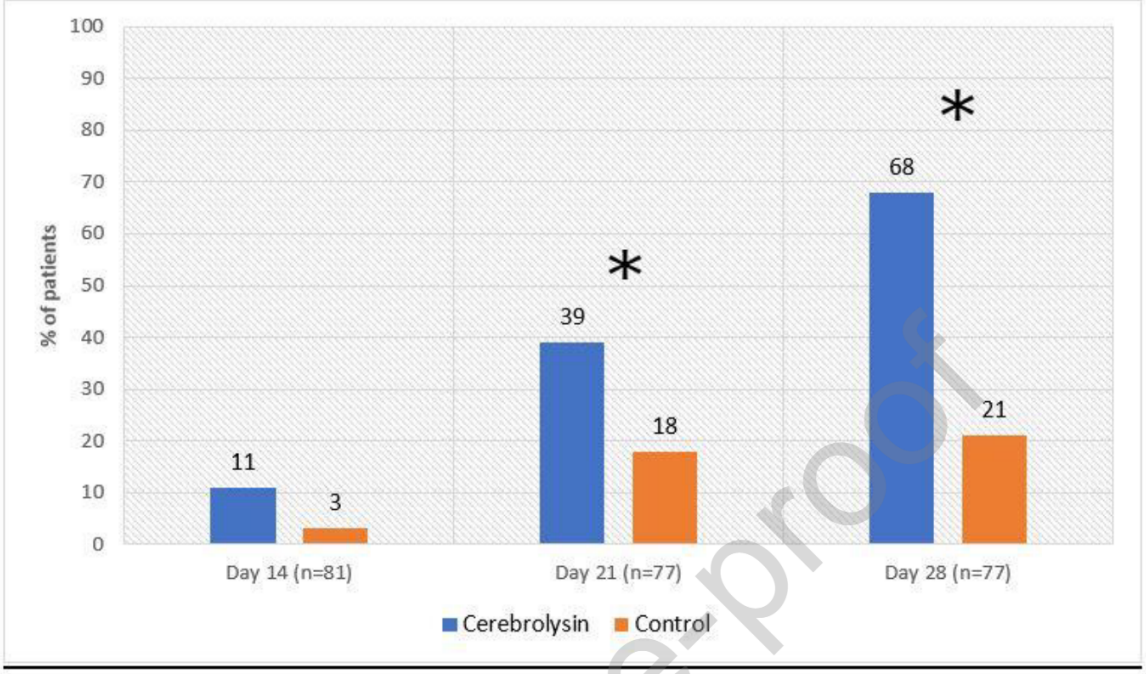


Figure 3. Proportion of patients with GOS ≥4 between groups. At Days 14, 21 and 28. *The sample sizes for each time point corresponds to the total number of patients with GOS score for both groups; values above the bar graphs represents the percentage of patients for each group with GOS ≥4; the difference in proportions between Cerebrolysin and Control groups at each time period were analysed using Fisher's exact test for Day 14 and Chi Square test for Day 21 and 28; statistical significance is indicated by an asterisk where p≤0.05*

Table 1. Demographic and clinical characteristics of patients (n=87)

	CEREBROLYSIN (n=42) n(%)	**CONTROL (n=45) n(%)**	**P VALUE**
Age (in years), mean	34.14 ± 15.81	32.80 ± 13.64	0.6719
<18 years old	3 (7)	3 (7)	1.000
≥18 years old	39 (93)	42 (93)	
Sex			
Male	33 (79)	40 (89)	0.247
Female	9 (21)	5 (11)	
Alcohol intoxication			
Positive	20 (48)	21 (47)	1.000
Negative	22 (52)	24 (53)	
Etiology of TBI			
Fall	5 (12)	0	0.163
Mauling	2 (5)	4 (9)	
Vehicular accident (Pedestrian)	5 (12)	5 (11)	

Vehicular accident (2-wheel)	24 (57)	31 (69)	
Vehicular accident (4-wheel)	6 (14)	5 (11)	
Diagnosis			
Intracerebral hematoma (ICH)	14 (33)	13 (29)	0.833
Cerebral contusion (CC)	7 (17)	7 (16)	
Epidural hematoma (EDH)	2 (5)	1 (2)	
Subdural hematoma (SDH)	5 (12)	11 (24)	
Subarachnoid hemorrhage (SAH)	3 (7)	4 (9)	
Diffuse cerebral edema (DCE)	6 (14)	5 (11)	
Diffuse axonal injury (DAI)	5 (12)	4 (9)	
GCS at baseline, mean	5.76 ± 0.79	5.93 ± 0.86	0.3379

Table 2. Improvement in GCS scores over time (n=87)

PERIOD	n	CEREBROLYSIN Mean ± SD	CONTROL Mean ± SD	P VALUE
Absolute improvement from baseline				
Day 7 - Baseline	87	1.48 ± 1.33	0.98 ± 0.97	0.0475*
Day 14 - Baseline	81	2.64 ± 1.22	1.83 ± 0.96	0.0014*
Day 21 - Baseline	77	4.24 ± 1.26	3.15 ± 1.39	0.0006*
Day 28 – Baseline	77	5.84 ± 1.08	4.31 ± 1.08	<0.00001*
Relative improvement from baseline (%)				
Day 7 - Baseline	87	24.75 ± 21.58	16.11 ± 15.54	0.0340*
Day 14 - Baseline	81	45.90 ± 21.07	30.90 ± 15.61	0.0005*
Day 21 - Baseline	77	73.72 ± 21.29	52.11 ± 20.12	<0.00001*
Day 28 – Baseline	77	102.61 ± 23.36	72.72 ± 18.82	<0.00001*

Table 3. Characteristics of patients who died during the study period (n=10)

Patient	Group	Age/ Sex	Diagnosis	Day of death post TBI	Reason for death
TZ	Cerebrolysin	20/F	DCE	15	Aspiration pneumonia
RT	Cerebrolysin	28/M	ICH	10	Sepsis pneumonia
JJ	Cerebrolysin	17/M	DCE	7	Aspiration pneumonia

SO	Cerebrolysin	65/M	CC	8	Hospital-acquired pneumonia
RR	Control	21/M	CC	18	Hospital-acquired pneumonia
MDC	Control	60/M	DCE	8	Hospital-acquired pneumonia
CP	Control	27/M	DCE	10	Aspiration pneumonia
SP	Control	29/M	SDH	15	Hospital-acquired pneumonia
RV	Control	60/M	CC	9	Hospital-acquired pneumonia
OR	Control	32/M	ICH	21	Hospital-acquired pneumonia

DCE-Diffuse cerebral edema; ICH-Intracerebral hematoma; CC-Cerebral contusion; SDH-Subdural hematoma

Table 4. Proportion of patients achieving a GCS of 9-15 and GOS≥4 at Day 21 by age category and diagnosis

	GCS 9-15 AT DAY 21		GOS ≥4 AT DAY 21	
	CEREBROLYSIN n(%)	**CONTROL n(%)**	**CEREBROLYSIN n(%)**	**CONTROL n(%)**
AGE				
< 40 years old	*(n=27)*	*(n=29)*	*(n=27)*	*(n=29)*
	24 (89)	16 (66)	12 (44)	4 (14)
≥40 years old	*(n=11)*	*(n=10)*	*(n=11)*	*(n=9)*
	9 (81)	4 (40)	3 (27)	3 (33)
DIAGNOSIS				
CC	*(n=6)*	*(n=5)*	*(n=6)*	*(n=4)*
	6 (100)	2 (40)	4 (67)	1 (25)
DAI	*(n=5)*	*(n=4)*	*(n=5)*	*(n=4)*
	5 (100)	2 (50)	2 (40)	0
DCE	*(n=4)*	*(n=3)*	*(n=4)*	*(n=3)*
	3 (75)	2 (67)	1 (25)	1 (33)
ICH	*(n=13)*	*(n=12)*	*(n=13)*	*(n=12)*
	12 (92)	6 (50)	5 (38)	1 (8)
SDH	*(n=5)*	*(n=10)*	*(n=5)*	*(n=10)*
	4 (80)	5 (50)	1 (20)	2 (20)

Values presented are frequency and percentages of patients who achieved a GCS of 9-15 and GOS ≥4 at Day21 for each subgroup

CRediT authorship contribution statement

LL: Conceptualization, investigation, writing- review & editing; MB: Methodology, formal analysis, writing-original draft

Highlights

- Cerebrolysin is beneficial for severe TBI patients with nonoperative lesions
- Cerebrolysin led to faster recovery than standard treatment alone
- Cerebrolysin also showed shorter duration of hospital stay than standard treatment