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Experimental Evaluation of the Effectiveness of Antishock Therapy for Severe Combined Mechanical and Thermal Injury

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ABSTRACT

BACKGROUND: Modern combat-related surgical trauma is characterized by the combined impact of multiple detrimental factors. This often leads to the simultaneous development of several critical conditions in the body, such as burn disease and crush syndrome. The cross-interaction of these pathogenetic pathways contributes to the development of mutual aggravation syndrome, thereby increasing the likelihood of an unfavorable trauma outcome. Research into the mechanisms of these conditions and the development of pathogenetically justified approaches to correcting these homeostatic disturbances remains highly relevant.

AIM: To evaluate the effectiveness of antishock therapy in a combined experimental model of crush syndrome and burn disease.

METHODS: The study was performed using 360 rats weighing 240–250 g, in which both crush syndrome and deep skin burns were induced. Mortality rates were analyzed depending on the volume and composition of the antishock therapy delivered. Data processing was performed using the standard methods for variation statistics. The alternative hypothesis was accepted at $p < 0.05$.

RESULTS: It was found that in the combined model of crush syndrome and burn disease, the volume of infused fluids should be increased by 40%. Based on this result, the standard Parkland formula was modified to $V = 6{,}5 \times S \times M$. Among the evaluated antishock therapy regimens, the combination of crystalloid and colloid solutions in a 40:60 ratio exhibited the highest efficacy. The mortality rate in this group was 28.9%, which was 2.4 ($p < 0.05$) and 2.6 ($p < 0.05$) times lower than that with 0.9% sodium chloride solution and 10% albumin solution, respectively. The high effectiveness of the substrate-based antihypoxants for the treatment of mechanical and thermal shock was demonstrated. Their administration reduced the mortality rate to 30.4%, which was 2.2 times ($p < 0.05$) lower than the results observed when normal saline was administered. The use of analgesics and antioxidants did not significantly influence the animals' survival rate.

CONCLUSION: The results of this study indicate the potential use of metabolic (substrate-based) antihypoxants in antishock therapy for patients with combined crush syndrome and burn disease. This research direction requires further development and in-depth investigation..

Keywords: skin burns; crush syndrome; shock; intensive care; hypoxia; ischemia; antihypoxants; antioxidants.

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Экспериментальная оценка эффективности противошоковой терапии комбинированного механо-термического поражения тяжелой степени

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АННОТАЦИЯ

Обоснование. Современную боевую хирургическую травму характеризует комбинированное воздействие различных поражающих факторов. Нередко это приводит к одновременному развитию в организме нескольких критических состояний, например, ожоговой болезни, синдрому длительного сдавления и т. д. Перекрестное взаимодействие цепей патогенеза каждого из них способствует возникновению синдрома взаимного отягощения и, как следствие, повышению вероятности неблагоприятного исхода травмы. Актуальность исследований, направленных на изучение механизмов развития данных состояний и разработку возможных патогенетических путей коррекции изменений гомеостаза, не вызывает сомнений.

Цель — оценить эффективность противошоковой терапии при сочетании синдрома длительного сдавления и ожоговой болезни в эксперименте.

Материалы и методы. Исследование выполнено на 360 крысах массой 240–250 г, которым моделировали синдром длительного сдавления тканей и глубокие ожоги кожи. В ходе работы анализировали показатель летальности в зависимости от объема и состава противошоковой терапии. Обработку материалов проводили общепринятыми методами вариационной статистики. Альтернативную гипотезу принимали при $p < 0,05$.

Результаты. Установлено, что при сочетании синдрома длительного сдавления и ожоговой болезни объем вводимых растворов должен быть увеличен на 40%. С учетом этого общепринятая формула Паркланда была модифицирована следующим образом: $V = 6,5 \times S \times M$. При изучении различных схем противошоковой терапии наибольшую эффективность продемонстрировало совместное применение кристаллоидных и коллоидных растворов в соотношении 40 и 60% соответственно. При этом летальность составила 28,9%, что в 2,4 раза ($p < 0,05$) и в 2,6 раза ($p < 0,05$) больше по сравнению с изолированным применением 0,9% раствора натрия хлорида и 10% раствора альбумина. Доказана высокая эффективность субстратных антигипоксантов при лечении механо-термического шока. Их введение позволило снизить показатель летальности до 30,4%, что в 2,2 раза ($p < 0,05$) меньше относительно результатов использования физиологического раствора. У применения анальгетиков и антиоксидантов не было достоверного влияния на выживаемость животных.

Заключение. Проведенное исследование свидетельствует о перспективности применения метаболических (субстратных) антигипоксантов при противошоковой терапии у пострадавших с синдромом длительного сдавления и ожоговой болезнью. Для данного направления исследований необходимы дальнейшее развитие и углубленное изучение.

Ключевые слова: ожоги кожи; синдром длительного сдавления; шок; интенсивная терапия; гипоксия; ишемия; антигипоксанты; антиоксиданты.

Как цитировать

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BACKGROUND

Blast injuries were not distinguished as a separate group of combat wounds until the 1980s. However, the era of local armed conflicts has contributed to a change in the qualitative composition of medical losses. Currently, blast injuries are more common than gunshot wounds [1]. Owing to advancements in artillery, missile, and loitering (drones, UAVs) weaponry, the number of blast injury cases accompanied by mechanical and thermal trauma continues to steadily increase. This warrants the medical service of the Ministry of Defense of the Russian Federation to rethink approaches and tactics for providing medical care to military personnel [2].

The use of explosive munitions, including shaped charges, in targeting military personnel, fortifications, and equipment shifts the vector of injuries toward mechanical and thermal damage [3]. Extensive burn injuries to the skin develop into burn disease. The main steps in the pathogenesis of this condition in the first hours after injury are pain syndrome and psychoemotional stress, leading to the active release of vasopressin, oxytocin, and adrenocorticotropin. These hormones contribute to increased acidosis and tissue hypoxia due to an increase in the overall peripheral vascular resistance, blood circulation centralization, and external respiratory function impairment [4]. Hypovolemia plays a major role in disrupting the body's homeostasis in extensive burn injuries. Increased vascular permeability due to the effects of amines, the kinin system, eicosanoids, etc., and changes in oncotic pressure in the microvasculature and interstitium cause active transsudation of fluid from the vasculature into the tissues. The same processes occur in extensive mechanical trauma, including crush syndrome. Prolonged absence of tissue perfusion in damaged areas leads to ischemia and toxic metabolite accumulation, forming a vicious circle of pathogenesis. This warrants early start of antishock therapy, which, if not provided, leads to irreversible tissue damage and death. These conditions are critical.

In an experimental study by Zolotukhina et al. [8], it was demonstrated that even after a 4 cm^2 burn on the withers skin, by the 10th day of observation 80% of rats had died. The combination of the conditions contributes to the development of mutual aggravation syndrome and increases the likelihood of an unfavorable trauma outcome. The primary treatment in this case was early initiation of antishock therapy with large intravenous infusion of crystalloid and colloid solutions. Tissue reperfusion is primarily aimed at decreasing tissue hypoxia. In this regard, there is a number of promising drugs, such as metabolic (substrate) antihypoxants and antioxidants, which, however, are rarely used. Therefore, antishock therapy for burn disease and crush syndrome in combination with these drugs are an important issue in military medicine.

This study aimed to evaluate the effectiveness of antishock therapy in a combined experimental model of crush syndrome and burn disease.

METHODS

The study was conducted on 360 rats weighing 240–250 g at the Department of Pathological Physiology of the S.M. Kirov Military Medical Academy and Research Laboratory of Experimental Surgery of the St. Petersburg State Pediatric Medical University in 2020–2021. The rats were obtained from the Rappolovo laboratory animal vivarium in the Leningrad region and quarantined for 2 weeks. The animals were housed at the institutions' vivarium facilities, meeting the requirements of GOST 33215-2014[1] and GOST 33216-2014.[2] Animals were excluded from the experiment on observation day 7 using the procedure in accordance with section 6.11 of GOST 33215-2014 and the European Commission recommendations on euthanasia of experimental animals.[3] The exclusion was carried out by intravenous infusion of 1 mL of lidocaine under general anesthesia. Biological material was disposed according to the standards for class B waste disposal.

The animals were randomized based on body weight. Depending on assigned tasks, the animals were divided into 28 equal groups. The first 6 groups, each consisting of 11 rats, were used to assess the impact of burn injury area (10, 20, 30, 40, 50, and 60 cm^2) on mortality. The next 9 groups, each consisting of 11 rats, were used to assess the impact of the volume of 0.9% sodium chloride infusion (0, 5, 10, 15, 20, 25, 30, 35, and 40 mL) on mortality. The third set of 13 groups, each consisting of 15 rats, was used to evaluate the effectiveness of intensive therapy (0 [control group, no treatment]); 0.9% sodium chloride (NaCl) solution; 0.9% NaCl solution + 10% albumin solution (80:20); 0.9% NaCl solution + 10% albumin solution (60:40); 0.9% NaCl solution + 10% albumin solution (40:60); 0.9% NaCl solution + 10% albumin solution (20:80); 10% albumin solution; Remaxol®; Mafusol®; polyoxyfumarine solution; 0.9% NaCl solution+superoxide dismutase

[1] *Guide to the Care and Maintenance of Laboratory Animals.* Moscow: Standartinform, 2016, 20.

[2] *Guide to the Care and Maintenance of Laboratory Animals. Rules for the care and maintenance of laboratory rodents and rabbits.* Moscow: Standartinform, 2016, 17.

[3] Directive 2010/63/EU of the European Parliament and of the Council *On the Protection of Animals Used for Scientific Purposes* dated September 22, 2010 // ***Official Journal of the European Union***. L 276/33 dated October 20, 2010.

Fig. 1. Skin of rats after reproducing a third-degree burn.

solution; 0.9% NaCl solution + 2% xylazine solution; and polyoxyfumarine solution + superoxide dismutase solution + 2% xylazine solution) in the treatment of mechanical and thermal shock.

Crush syndrome was modeled under general anesthesia by applying pressure to the soft tissues of the thigh over a 5 cm^2 area using metal clamps with a force of 8–10 kg/cm^2 for 4 h [10]. Then, using our original method,[4] third-degree deep skin burns were reproduced on 10, 20, 30, 40, 50, and 60 cm^2 areas. Before modeling a burn wound, the hair on the animal's back was removed using sodium sulfide solution heated to 36 °C. Furthermore, an asbestos stencil was applied to the selected area, the surface area of which was calculated using the Lee formula [11]:

$$S = 12.54 \times M \times 0.66,$$

where S is the body surface area (cm^2) and M is the body weight (kg).

Burn was inflicted by light radiation of a “KDB-22” lamp (with a power of 500 W), positioned 2.5 cm away from the animal's skin for 20 s at 85 °C–90 °C. The lamp was controlled using the thermocouple sensor of the multimeter. The depth of the lesion was visually assessed by evaluating the vascular response. Upon external examination, a whitish color of the dermis was noted in the burn area, with pronounced tissue swelling and a clear border with intact skin. The vascular response was assessed by applying pressure to the injured area until a “white spot” appeared. This phenomenon reflects microvasculature spasm. In superficial lesions, exposure cessation leads to arteriole and capillary dilation, rapid microcirculation restoration, and arterial hyperemia. Slowing of microvasculature filling, manifested by the prolonged retention of a “white spot,” is characteristic of a borderline skin burn. The absence of vascular response indicates a deep lesion.

Prior to injury modeling, general anesthesia was performed through intramuscular injection of 2% xylazine solution in the thigh at 0.1 mL per 100 g of body weight. Anesthesia was maintained by ether vapor inhalation. Antishock therapy involved infusing crystalloid and colloid solutions, antihypoxants, and antioxidants. The needle was intraperitoneally inserted through parenteral access in the thigh. The volume of solutions was calculated using the Parkland formula [12]:

$$V = 4 \times \mathrm{M} \times \%,$$

where V is the volume of infusion (mL), M is the body weight of the animal (kg); and % is the burn area (absolute units).

During the observation, mortality (%) was evaluated in consideration of the antishock therapy. The study lasted for 7 days.

Statistical analysis of obtained data was performed using Microsoft Excel and SPSS Statistics 17.0 software. The work with databases was conducted using generally accepted methods of variation statistics in three steps: research model development, plan drafting and implementation, and material collection. Quantitative parameters were evaluated using the nonparametric Mann–Whitney test (pairwise comparison) and Kruskal–Wallis test to eliminate the problem of multiple pairwise comparisons. Frequency indicators were assessed using Pearson's criterion (χ^2-test). The alternative hypothesis was accepted at p <0.05.

RESULTS

Figure 2 presents the study of rat mortality during tissue reperfusion after modeling prolonged crush syndrome and inflicting deep burns without administering antishock therapy.

The critical burn area in rats was 30 cm^2. In this group, all rats had an average lifespan of 64.3±10.7 h. Moreover, an increase in the burn area of the skin to ≥60 cm^2 was

[4] Rationalization Proposal No. 14287/1 dated January 19, 2016. *Methodology for Reproducing Third-Degree Skin Burns in an Experiment* / E.V. Zinoviev. S.M. Kirov Military Medical Academy. 2016.

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characterized by 100% mortality. These values did not change despite infusion therapy, whereas the administration of a 0.9% sodium chloride solution, in the amount calculated using the Parkland formula, in rats with a burn area of 30 cm^2 increased their survival by 19%. Thus, the model of 30 cm^2 deep skin burns was chosen for further study of the effectiveness of the proposed antishock therapy.

Figure 3 shows the effectiveness of 0.9% sodium chloride infusions of various volumes as antishock therapy.

Data presented in Fig. 3 indicate that antishock therapy with a volume of 25 mL of 0.9% sodium chloride solution contributed to achieving minimal mortality (68.2%) during the tissue reperfusion period after modeling crush syndrome and inflicting deep skin burns over an area of 30 cm^2. After decreasing the infusion volume to 20 mL and increasing the infusion volume to 30 mL, the mortality rate was 76.7% and 82.1%, respectively. Thus, the highest survival rate was noted when calculating the volume of 0.9% sodium chloride solution using the modified Parkland formula (mL) V=6.5×M×%, which is 39% higher than its classical variant.

Figure 4 presents mortality during tissue reperfusion after modeling crush syndrome and inflicting a 30 cm^2 deep skin burn, considering antishock therapy including 0.9% sodium chloride solution and 10% albumin solution in various volumes.

According to the study results presented in Fig. 4, the infusion of 0.9% NaCl solution combined with 10% albumin solution in a 40:60 ratio, in a volume calculated using the Parkland formula + 40% of the calculated volume, achieves the lowest mortality rate (28.9%) in small laboratory animals. Isolated administration of saline or 10% albumin solution led to an increase in the frequency of fatal outcomes among rats by 2.4 times ($p < 0.05$) and 2.6 times ($p < 0.05$), respectively.

Notably, the administration of substrate antihypoxants contributed to the increased survival of experimental animals. Polyoxyfumarine showed the greatest efficacy among these drugs. Drug infusion in animals with combined lesions led to a 2.2-fold decrease in mortality (p <0.05) compared with isolated saline infusion. When Mafusol® and Remaxol® were administered, the mortality rate was 47.6% and 50.2%, respectively, which was 1.4 times (p <0.05) and 1.2 times (p <0.05) lower compared with isolated saline infusion (68.2%).

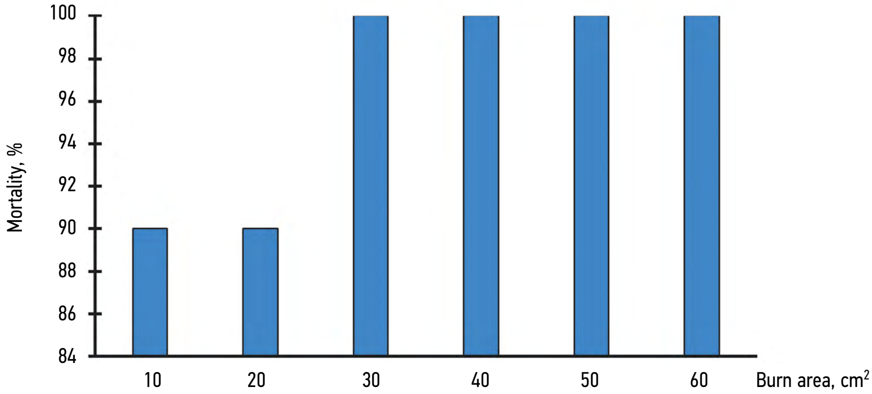

Fig. 2. Rat mortality during tissue reperfusion after modeling crush syndrome and inflicting a skin burn, with consideration of the area of deep lesions.

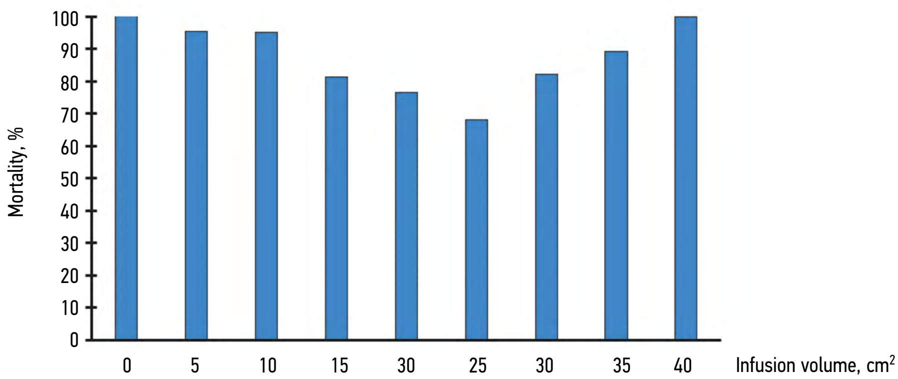

Fig. 3. Rat mortality during tissue reperfusion after modeling crush syndrome and inflicting a 30 cm2 skin burn, with consideration of the volume of 0.9% sodium chloride infusion.

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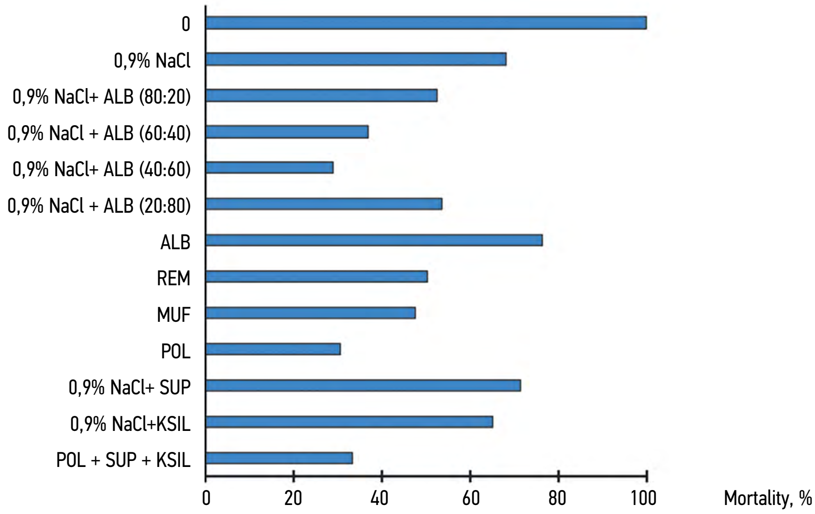

Fig. 4. Rat mortality during tissue reperfusion after modeling crush syndrome and inflicting a 30 cm2 skin burn, with consideration of infusion therapy calculated according to the Parkland formula + 40% of the calculated volume: 0, control group (no treatment); NaCl, 0.9% sodium chloride solution; ALB, 10% albumin solution; REM, Remaxol®; MUF, Mafusol®; POL, polyoxifumarine solution; SUP, superoxide dismutase solution; KSIL, 2% xylazine solution.

The effectiveness of using 0.9% NaCl solution and superoxide dismutase solution was comparable to the isolated saline infusion, with 71.3% and 68.2% mortality rate, respectively. No significant differences were noted in survival rates between the 0.9% NaCl solution + xylazine solution and 0.9% NaCl solution groups.

Thus, the most effective treatment regimens for animals with burn shock and crush syndrome are 0.9% NaCl solution + 10% albumin solution (40:60); polyoxyfumarine solution; and combined polyoxyfumarine, superoxide dismutase, and xylazine solution. The mortality rates were 28.9%, 30.4%, and 33.2%, respectively.

CONCLUSION

The outcome of severe mechanical and burn shock directly correlates with the volume and composition of infusion therapy. The infusion volume calculated using the generally accepted Parkland method is insufficient and requires at least a 40% increase. The modified Parkland formula was modified as follows: $V = 6.5 \times M \times \%$. This allowed for achieving a minimal animal mortality of 68.2%, which is 31.8% lower ($p < 0.05$) compared with the control group. Antishock therapy is an important regimen. It should consist of crystalloid and colloid solutions in a 40%–60% ratio. This allows a significant decrease in the frequency of fatal outcomes in laboratory animals to 28.2%. With isolated administration of the abovementioned infusion regimens, 7 out of 10 rats died.

Study data justify the addition of metabolic (substrate) drugs with antihypoxic effects in antishock therapy. The introduction of polyoxyfumarine solution during tissue reperfusion decreases the mortality of rats by almost two times (30.4%). Moreover, no positive effect was noted in the use of antioxidant drugs, with the mortality rate of 71.3%, which is 3.1% higher ($p > 0.05$) compared to the isolated infusion of an equivalent volume of saline. Furthermore, it was noted that the additional administration of analgesics had no effect on the mortality rate of rats. In this case, mortality was comparable to that seen with 0.9% NaCl infusion: 65.1% and 68.2% ($p > 0.05$), respectively.

The cross-interaction of the pathogenetic mechanisms of extensive burn injuries and crush syndrome significantly affects trauma outcome, with hypoxia and tissue ischemia playing the central role. The study demonstrates the potential of using metabolic (substrate) antihypoxants in treating patients with mechanical and thermal shock. However, the existing theoretical basis is insufficient to justify the inclusion of these drugs in antishock therapy. This requires further development and in-depth investigation.

ADDITIONAL INFORMATION

Authors' contribution: *V.N. Tsygan*: development of a general concept, research design, writing an article; *E.V. Ivchenko*: research design, making final edits; *N.K. Sokolov*: collection and processing of materials, data analysis, writing an article; *E.V. Zinoviev*: development of a general concept, research design, writing an article. The authors have approved the version for publication and have also agreed to be responsible for all aspects of the work, ensuring that issues relating to the accuracy and integrity of any part of it are properly considered and addressed.

Ethics approval. The study was approved by the local Ethical Committee of the Kirov Military Medical Academy (Protocol No. № 221 from 23.04.2019).

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Statement of originality. The authors did not use previously published information (text, illustrations, data) to create this paper.

Data availability statement. All the data obtained in this study is available in the article.

Generative AI. Generative AI technologies were not used for this article creation.

Provenance and peer review. This work was submitted to the journal on its own initiative and reviewed according to the usual procedure. Two internal reviewers participated in the review.

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. В.Н. Цыган — разработка общей концепции, дизайн исследования, написание статьи; Е.В. Ивченко — дизайн исследования, внесение окончательной правки; Н.К. Соколов — сбор и обработка материалов, анализ данных, написание статьи; Е.В. Зиновьев — разработка общей концепции, дизайн исследования, написание статьи. Авторы одобрили версию для публикации, а также согласились нести ответственность за все аспекты работы, гарантируя надлежащее рассмотрение и решение вопросов, связанных с точностью и добросовестностью любой ее части.

Этическая экспертиза. Проведение исследования одобрено локальным этическим комитетом Военно-медицинской академии им. С.М. Кирова (протокол № 221 от 23.04.2019).

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

Раскрытие интересов. Авторы заявляют об отсутствии отношений, деятельности и интересов за последние три года, связанных с третьими лицами (коммерческими и некоммерческими), интересы которых могут быть затронуты содержанием статьи.

Оригинальность. При создании настоящей работы авторы не использовали ранее опубликованные сведения (текст, иллюстрации, данные).

Доступ к данным. Все данные, полученные в настоящем исследовании, доступны в статье.

Генеративный искусственный интеллект. При создании настоящей статьи технологии генеративного искусственного интеллекта не использовали.

Рассмотрение и рецензирование. Настоящая работа подана в журнал в инициативном порядке и рассмотрена по обычной процедуре. В рецензировании участвовали два внутренних рецензента.

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