Investigation of partial thickness burn injuries with a complex approach: from experimental rat model to meta-analysis of human data

Doctoral (PhD) Thesis

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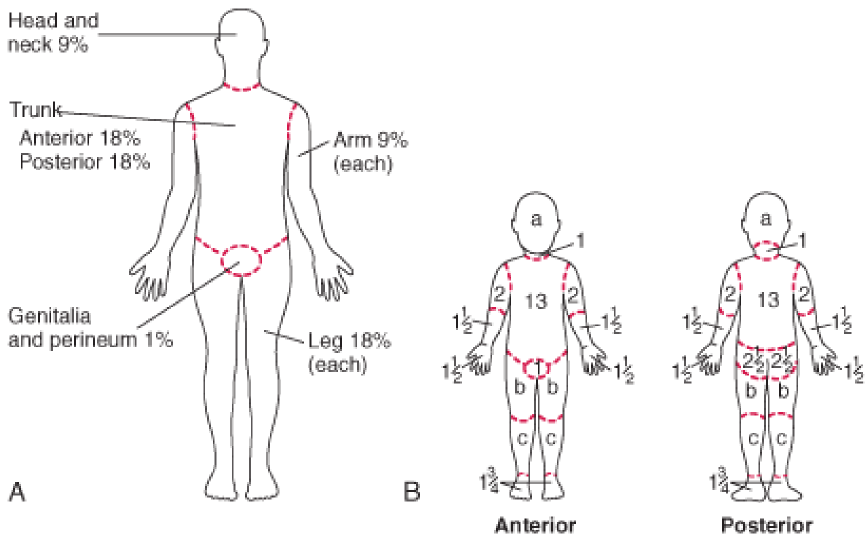
Introduction

A burn injury is a damage to the skin or other organic tissues (e.g., mucosa) commonly caused by heat. However, it can also be due to friction, electricity, radiation, radioactivity, and contact with certain chemicals. The most common causes of burns in the United States are: fire or flame (44%), scalding (33%), hot objects (9%), electricity (4%), chemicals (3%) (American Burn Association, 2016). Scalding is caused by hot liquid or gas, most commonly a hot drink, high-temperature tap water from a bathroom or shower, hot cooking oil, or steam (Gardiner et al., 2009). Scald injuries are most common in children under the age of five (Tintinalli, 2009), and they account for two-thirds of all burn injuries in the United States and Australia (Wolf et al., 2018). Touching hot objects causes 20-30% of burns in children (Wolf et al., 2018). Scalding usually causes superficial and partial-thickness burn injury, but – especially in the case of prolonged contact – it can also cause full-thickness burns (Maguire et al., 2008). In many countries, fireworks are a common cause of burns during holidays (Peden et al., 2008). This risk factor is particularly common among adolescent boys (Peden et al., 2008). Electrical burns are divided into high voltage (1,000 Volts or more) and low voltage (less than 1,000 Volts) injuries (Tintinalli, 2009). In children, electrical burns are most often caused by electrical cords (60%) and electrical outlets (14%) (Wolf et al., 2018). Lightning can also cause electrical burns (Edlich et al., 2005). Chemicals are responsible for 2–11% of burn injuries and nearly 30% of burn-related deaths (Hardwicke et al., 2012). Chemical burns can be caused by more than 25,000 different compounds (Tintinalli, 2009). Most of these are strong bases (55%) or strong acids (26%) (Hardwicke et al., 2012). The most common chemicals include sulfuric acid, sodium hypochlorite and halogenated hydrocarbons (Tintinalli, 2009). Hydrogen fluoride causes particularly deep burns (Makarovsky et al., 2008). Most deaths from chemical burns result from ingestion (Tintinalli, 2009). The majority of burns occur at home (69%) or at work (9%), most of them are accidents, 2% are physical injuries committed by others, and 1-2% are the result of suicide attempts (Peck, 2011). It is also important to highlight other non-accidental burns, because 3-10% of those hospitalized for scald or fire burns are victims of abuse. Other causes include child abuse, personal disputes, spousal violence, elder abuse and business disputes (Peck, 2011). Immersion burns or scalds may indicate child abuse (Maguire et al., 2008). It typically has a sharply defined upper border, and is often symmetrical (Maguire et al., 2008). Other signs strongly suggestive of abuse include a circular burn, absence of spatter marks, burns of uniform depth, and other associated signs of neglect or abuse (Herndon, 2012). In some cultures, such as India, bride burning takes place when the dowry is not considered sufficient by the husband or his family (Jutla et al., 2004; Peden, 2008) or self-immolation in protest (Peck, 2011). In Pakistan, acid burns account for 13% of all intentional burns and are often associated with domestic violence (Blanchard et al., 2016). Smoking is considered a risk factor, but alcohol consumption is not. Burns caused by fire are usually more common in colder climates (Peck, 2011). Risk factors specific to developing countries include cooking over open fires or cooking on the ground (Herndon, 2012), and developmental disabilities in children and chronic diseases in adults (Forjuoh, 2016). Many different elements, including the depth and extent of the burn, the contact time, and the injury's mechanism, influence the burn's severity. Moreover, they are affected by the age and general condition of the injured person, as well as by regional and socioeconomic factors. It can be clearly stated that in countries that are socially and economically more developed, both the extent and time of recovery are much more favorable (American Burn Association, 2016; Brusselaers et al., 2010; Peck, 2021). Recent epidemiological data about the disease demonstrate the topic's importance and relevance. In recent days, 6 million patients per year have sought medical care for burns worldwide. Burn injuries are also quite common in developed countries. For example, in the United Kingdom (total population > 60 million), approximately 250,000 people suffer from burns each year, and 300 of those die because of the injury, which constitutes a severe burden on the healthcare system and economy (American Burn Association, 2016; Peck, 2021). Nearly half a million patients receive medical treatment in the United States (with a population of ~314 million). Unfortunately, over 5,000 of those die from burns among the 1.25 million who suffer from burns every year (American Burn Association, 2016; Peck, 2021). According to a systematic review, the mortality rate of burns ranges from 1.4% to 18% in Europe (Brusselaers et al., 2010). In Hungary, per 100,000 inhabitants, 1-1.5 fatal burns, 11-13 requiring hospital treatment, and 40-50 requiring outpatient treatment occur annually, which can be considered to be low by world standards. As in other developed countries, the two most affected populations are children under 5 years of age and adults over 65 years of age— both groups are overrepresented in burn injuries due to lack of recognition of dangerous situations and limited escape options (Beers, 2004; Csorba, 2005).

About 90% of burns occur in developing countries (Peck, 2011). This is partly due to overpopulation and partly to unsafe cooking methods (Peck, 2011). Almost 60% of fatal burns occur in Southeast Asia, with a rate of ~12:100,000 (Herndon, 2012). In the developed world, adult men die twice as often as women from burns. This is probably because men are more often involved in dangerous and high-risk occupations. However, in many developing countries women are at greater risk of burns than men, often from kitchen accidents or domestic violence. The number of burn deaths among children in developing countries is more than ten times higher than in developed countries (Peck, 2011). Overall, burns are among the fifteen most common causes of death among children (Herndon, 2012).

In clinical practice, burn injuries are often classified based on the size of the affected skin area, which is usually assessed as the percentage of total body surface area (TBSA) (Figure 1). Moreover, it can be characterised based on the depth of the wound, which can be superficial, partial-thickness, or full-thickness burns.



Relative percentage of body surface area (% BSA) affected by growth

	Age				
Body Part	**0 yr**	**1 yr**	**5 yr**	**10 yr**	**15 yr**
a = 1/2 of head	9 1/2	8 1/2	6 1/2	5 1/2	4 1/2
b = 1/2 of 1 thigh	2 3/4	3 1/4	4	4 1/4	4 1/2
c = 1/2 of 1 lower leg	2 1/2	2 1/2	2 3/4	3	3 1/4

Figure 1. *A) TBSA and the rule of nines (for adults). B) Lund-Browder chart (for children) for estimating the extent of burns (Artz & Moncrief, 1969).*

Among the latter classification, superficial burns typically do not require hospital admission and special medical treatment, whereas surgical intervention is always needed for the most severe, full-thickness burns. However, in the case of partial-thickness (earlier called second-degree or II) burns, the subgroups and their therapeutic options are more complex.

Superficial partial-thickness (earlier: II/A or II/1) burns affect the epithelium and penetrate the papillary layer of the skin. They are characterised by moist and red surfaces, fluid-filled blisters, and severe pain upon touching. Deep partial-thickness burns (II/B or II/2) affect the deeper reticular layer of the skin. In such injuries, the skin is usually dry, white or dull red in colour, blisters may also be present, and it is relatively less painful (Jozsa et al., 2017; Markiewicz-Gospodarek et al., 2022; Rowan et al., 2015; Wasiak et al., 2013). While deep partial-thickness burns may require skin grafting and surgery, the superficial forms usually do not need surgical intervention (Figure 2) (Epeneu and Alina, 2015).

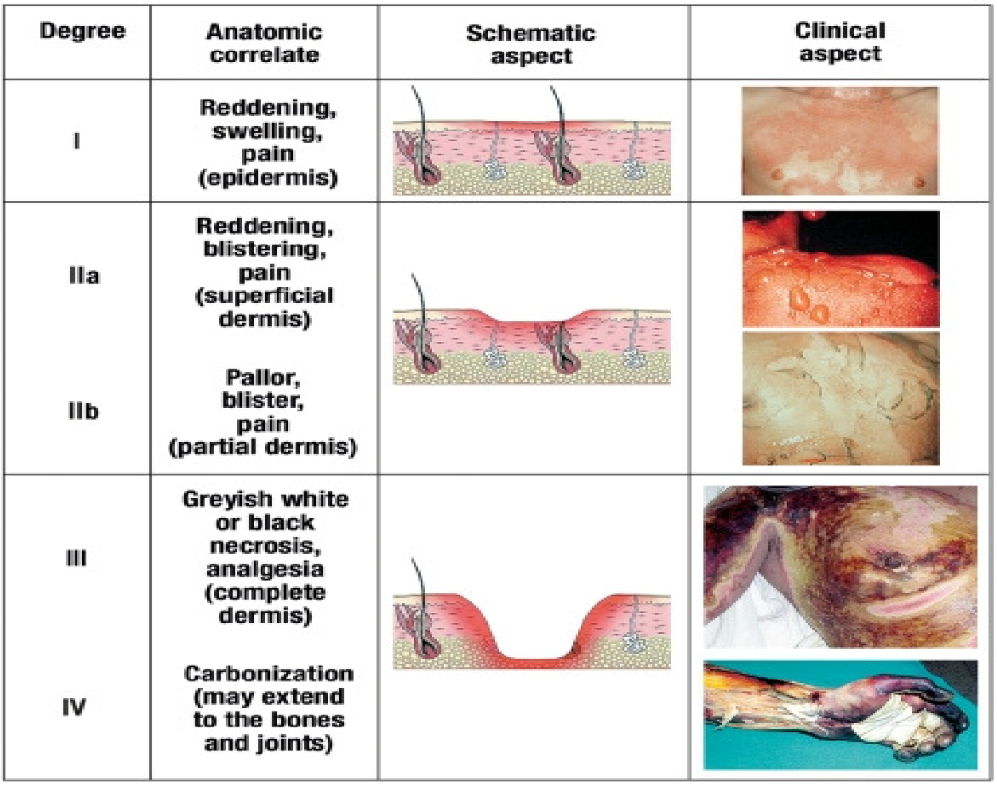


Figure 2. *The degrees of burns (Epeneu and Alina, 2015).*

Among the conservative therapies of the latter form, several topical treatment options are available, including silver-sulfadiazine cream, silver foam dressing, and zinc-hyaluronan-containing gel (Csenkey et al., 2022; Jozsa et al., 2018). Currently, however, there is no gold standard topical treatment for partial-thickness burns. The selection of the actual treatment primarily results from individual experience and institutional habits. Not surprisingly, most of the available treatment options were shown to have certain benefits for burn wound healing. However, a direct comparison of the effects of several dressings at different time points of wound healing has not been performed, partly because of the lack of a reliable and easily reproducible model that could be used for such comparison. The absence of such a preclinical model may hinder the evidence-based selection of the most appropriate treatment.

Besides the beneficial effects on wound healing, when choosing the topical treatment, the need for anaesthesia during dressing changes should also be taken into account. Repeated anaesthesia (e.g., during regular dressing changes of a burn treatment), especially in childhood, can be associated with the impairment of cognitive functions. For example, neonates and infants (less than six months of age) who were anesthetised multiple times developed impaired cognitive functions compared to their peers anesthetised two times or less (Oba et al., 2019). Other psychological factors can make recovery difficult, such as trauma and changing the appearance of the body, which can lead to a distorted self-image and psychological problems. This also shows that post-burn rehabilitation is very complex, involves many specialized fields, and it is a big challenge to achieve the greatest possible physical and mental recovery in the end.

There are no definitive rules or guidelines for prophylactic systemic antibiotics in patients with paediatric burns. Systemic antibiotics are recommended to be reserved for cases where there is clear evidence of infection. However, in 1995 around 60% of UK burn centres had no formal antibiotic use policy and no consensus on antibiotic prophylaxis (Papini et al., 1995). A more recent survey revealed that less than half of UK burn units used standard operating procedures (Lymperopoulos.et al., 2015), while another study found significant variation in the use of guidelines for diagnosing and treating paediatric burn infections (Davies et al., 2017). Inappropriate use of antibiotics in burns can increase the chance of complications and lead to antibiotic resistance, thereby increasing healthcare costs for both patients and the community (Thorpe et al., 2018). Our meta-analysis of published clinical trials aimed to determine whether systemic antibiotic prophylaxis improves outcomes in childhood burns (Csenkey et al., 2019). Similar analyses were performed in adult burn patients and helped to develop guidelines (Avni et al., 2010; Barajas-Nava et al., 2013).

In our work, we used a multiple translational research approach to study the pathomechanisms as well as the clinical relevance of burn injury treatments. First, we aimed to discover a novel preclinical model for the study of treatment options in partial thickness superficial burn injuries (Csenkey et al., 2022). In particular, our goal was to develop an adequate testing model and to compare the effects of four conventional topical treatment methods on superficial partial-thickness burns at three different time points (viz., 5, 10, and 22 days post-injury) of the wound healing. Second, we studied how one of the treatments tested in our animal model performs in human patients (Jozsa et al., 2018). Third, in order to study the appropriateness of the currently used clinical treatments of burns, we investigated the necessity of prophylactic antibiotic treatment in case of superficial partial-thickness burn injury with a meta-analysis (Csenkey et al., 2019).

Aims

In our work, we used a multiple translational research approach to study the pathomechanisms as well as the clinical relevance of burn injury treatments.

1. We aimed to discover a novel preclinical model for the study of treatment options in partial thickness superficial burn injuries. We compared the effects of four conventional topical treatment methods on superficial partial-thickness burns at three different time points of the wound healing.

2. We studied the efficacy of Aquacel Ag foam and Curiosa gel combination in the treatment of superficial partial-thickness pediatric burns.

3. We investigated the necessity of prophylactic antibiotic treatment in case of superficial partial-thickness burn injury with a meta-analysis.

Materials and methods

1. Preclinical experiments

1.1 Animals

In the basic research experiments, we used 90 adult male Wistar rats. The rats were housed in standard plastic cages kept in a room with ambient temperature maintained at ~22°C and humidity at ~35%. The room was on a 12 h light-dark cycle (lights on at 5:00 a.m.). Standard rodent chow and tap water were available ad libitum. At the time of the experiments, the rats weighed 298-466 g. All procedures were conducted under protocols approved by the Institutional Animal Use and Care Committee of the University of Pécs (registration no.: BA02/2000–15/2018, approved on 18 April 2018) and were in accordance with the directives of the National Ethical Council for Animal Research and those of the European Communities Council (86/609/EEC).

1.2 Induction of superficial partial-thickness burn injury

Rats were anaesthetised with the intraperitoneal administration of a ketamine-xylazine cocktail [78 mg/kg (Gedeon Richter Plc.) and 13 mg/kg (Eurovet Animal Health BV), respectively]. Their nape was shaved in a 3×3 cm area; then, the rat was placed on a surgery board.

We used a soldering device (model Industa HF-5100; Stannol Inc.) to induce a burn injury in the centre of the clipped skin area. The standard handpiece of the device was 46 g, and it had a wedge-shaped iron tip with a 4 × 4 mm flat surface on each side (model M-4,2-HF; Stannol Inc.). In previous studies, contact burn wounds were created by heating up the device's tip to 60-200°C and keeping it in direct contact with the skin for 2-60 seconds in different experimental models (for details, see Csenkey et al., 2022). Based on these data, in our experiments, we chose to use 130°C heat and 30 seconds of contact time between the handpiece's tip and the rat's skin. The entire (4 × 4 mm) flat side of the tip of the handpiece was steadily held in direct contact with the skin without applying extra pushing or pulling force to minimise the variability of the impact.

1.3 Treatment groups

We designed four treatment groups: silver-sulfadiazine cream (Dermazin; LEK Pharmaceuticals, Ljubljana, Slovenia); silver foam dressing (Aquacel Ag; ConvaTec Ltd., Deeside, UK); zinc-hyaluronan gel (Curiosa; Gedeon Richter Plc., Budapest, Hungary); and the combination of zinc-hyaluronan gel with a silver foam dressing. In addition, in each group, the burn was covered with a cohesive conforming bandage (Peha-haft; Paul Hartmann AG, Heidenheim, Germany) and a perforated plastic sheet. The latter was needed to prevent the animal from scratching the wound and removing the bandage. Meanwhile, it also maintained the ventilation of the wound.

1.4 Tissue sample collection and histology

The skin tissue samples were collected 5, 10, and 22 days after the induction of the burn injury. For that, the rat was anesthetised the same way as for the burn induction (see above). Then, the bandage was removed, and the entire wound was excised in the centre of a 2 cm × 2 cm tissue sample. The excised tissue samples contained all layers of the skin and part of the underlying muscular layer. After the sample collection, the rat was euthanised with sodium thiopental [400 mg/kg intraperitoneally (Tiobarbital; B. Braun Medical SA, Barcelona, Spain)].

For histology, the collected tissue samples were placed in a 10% formalin solution. Two days later, the biopsy was serially sectioned and submitted entirely for histopathological examination

(5-8 slices). The tissue samples were dehydrated in a graded series of ethanol solutions, embedded in paraffin, and cut into approximately 3-μm sections. The hematoxylin and eosin staining was performed according to the routine procedure by a Leica ST 4040 linear automatic stainer (Leica Microsystems GmbH, Wetzlar, Germany). Histological changes were evaluated under a light microscope (DM500; Leica Microsystems GmbH, Wetzlar, Germany) by an expert histologist who was blinded to the treatment of the rat.

Complex histological evaluations included the assessment of the degree of re-epithelialisation and final wound contraction. On day 5, the ratio of the unhealed burned surface was calculated as the percentage of the not epithelialised distance compared to the total length of the wound (for an example, see Figure 3).

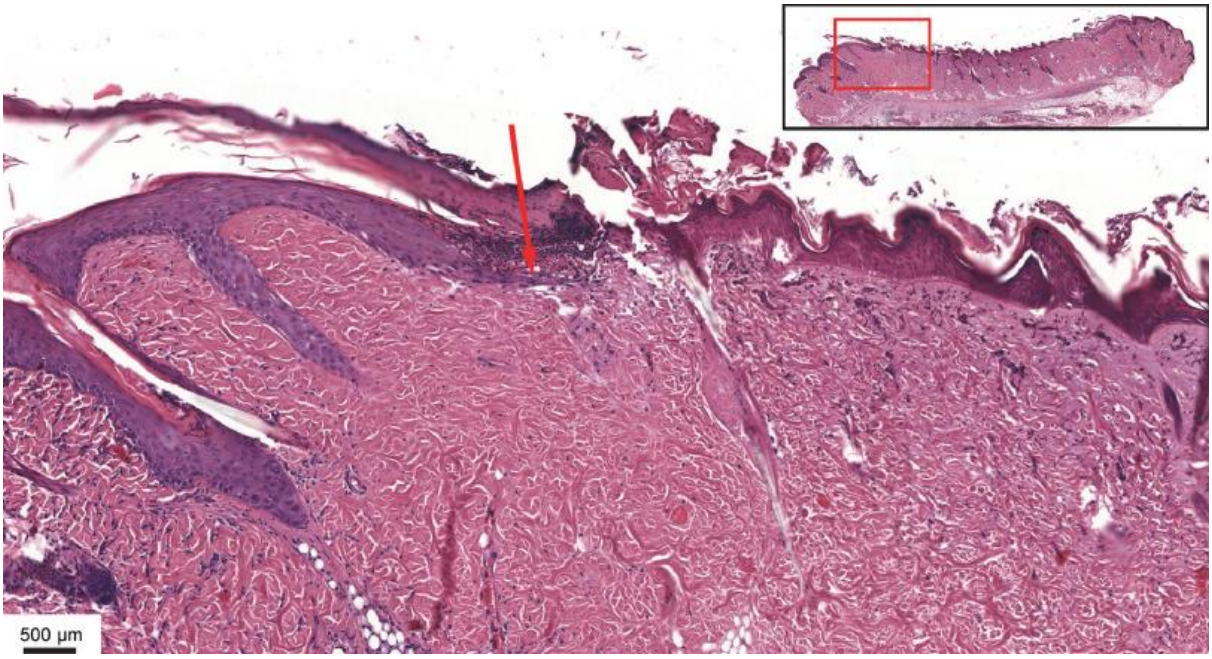


Figure 3. *Representative photo of the excised skin sample on day five post-burn injury. The extent of the re-epithelialisation was assessed by measuring the distance from the edge of the wound to the furthest newly formed keratinocyte (arrow). The insert on the top right shows the whole tissue section with a red box indicating the magnified area for orientation purposes (Csenkey et al., 2022).*

On day 10, the re-epithelialisation of the wound was evaluated by a 3-score system (0: no re-epithelialisation; 1: partial re-epithelialisation; and 2: complete re-epithelialisation that is multiple epithelial layers over the entire length of the wound) (Figure 4).

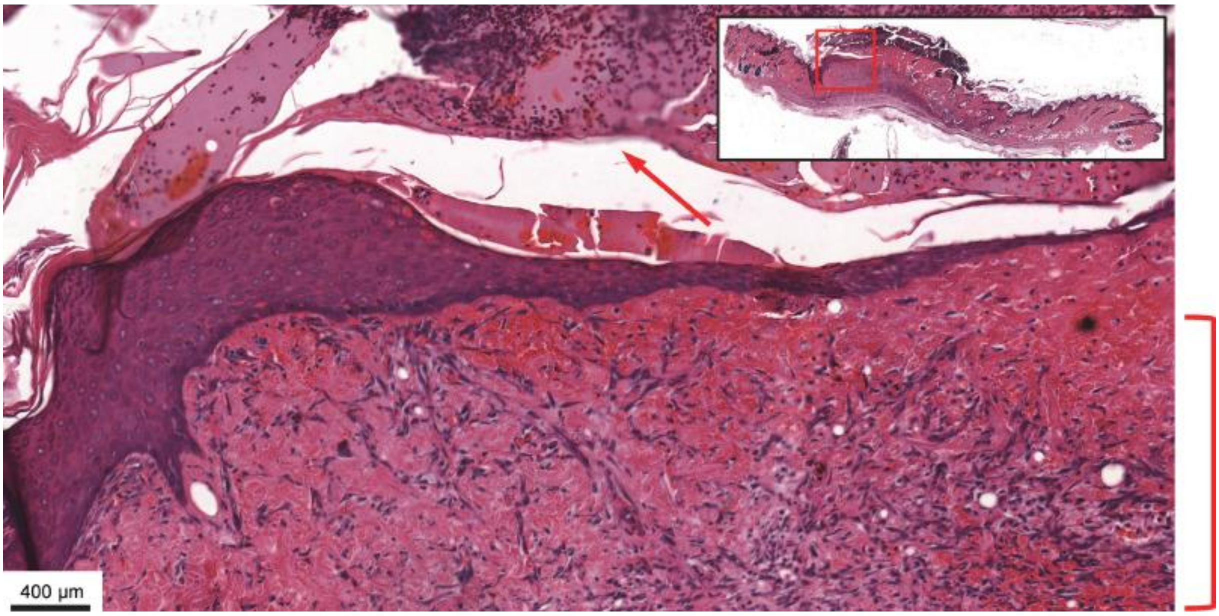


Figure 4. *Representative photomicrograph of the partially re-epithelialised skin tissue on day ten post-burn injury. Loose granulation tissue (indicated by the red bracket) is present beneath the re-epithelialised surface, which contains newly-forming capillaries and plump fibroblasts. Scab is attached to the re-epithelialised surface from the top (arrow). For orientation purposes, the insert on the top right shows the whole tissue section with a red box indicating the magnified area (Csenkey et al., 2022).*

Finally, on day 22, the scar thickness was measured as the distance between the basal epidermal cells and the lowest cell layer of the dermis (Figure 5).

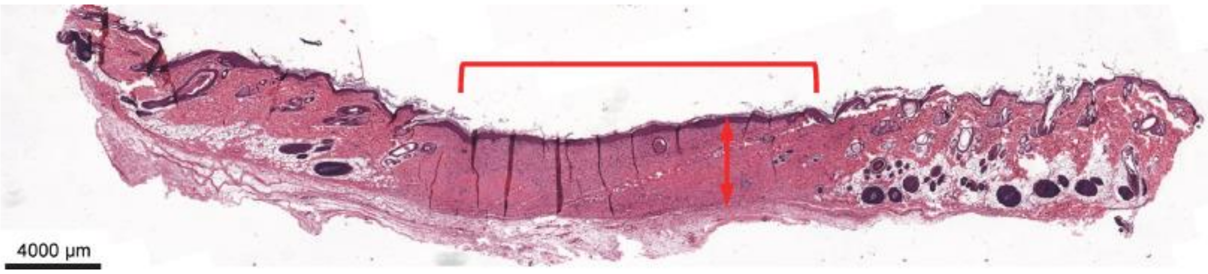


Figure 5. *Representative photomicrograph of the completely healed skin tissue on day 22 post-burn injury. A slightly more cellular dermis and a lack of adnexal structures are present in the centre of the skin section (indicated by the red bracket) as remnants of the previous burn. The scar thickness of the burn wound is shown by the arrow (Csenkey et al., 2022).*

1.5 Statistical analysis

Data on unhealed wound percentage, scores of re-epithelialisation, and scar thickness were compared by one-way ANOVA, followed by Fisher's LSD post hoc test, as appropriate. For statistical analyses, the Sigmaplot 11.0 (Systat Software, San Jose, CA, USA) software was used. The significance level was set at $p < 0.05$. All data are reported as mean ± SEM.

2. Investigations in human patients

Between 1 January 2014 and 31 January 2017, a prospective clinical study was performed at the Surgical Division, Department of Paediatrics, Medical School, University of Pécs, Hungary. Thirty-seven children with superficial and mixed-type of second-degree hand burns were included in the study in whom the burning injury was treated with Zn-hyaluronic gel combined with Aquacel Ag foam. In nearly 90% of the cases, burn depth was undoubtedly superficial. Aquacel Ag foam dressing with Zn-hyaluronic gel was applied primarily after wound cleaning and blister removal, which included the removal of the vesicles and blisters (i.e., bullectomy, which is not equivalent to necrectomy) but not the burned epidermis. In cases when the burn depth was not clearly assessable (II/1 or II/2) by the primary surgeon, silver nitrate solution was used for 24 hours. On the following day, the burn depth was reassessed by a burn specialist (consultant). At primary treatment, wound cleaning and blister removal were carried out under sedation or general anaesthesia. When the burn was superficial (II/A), the above-described conservative therapy (Aquacel Ag foam dressing with Zn-hyaluronic gel) was applied. In cases when the burn depth was II/B degree (or more profound), the patients were excluded from the study. In patients treated with the combination of a silver foam dressing with Zn-hyaluronic gel, the dressing was checked on the second day and removed on the sixth or the seventh day. However, clinical application of the dressing combination has been accepted and permitted in 2010 by our Hungarian Paediatric Surgery Committee medical board. Possible benefits and complications, along with other treatment options, were explained to the parents of each child (Jozsa et al., 2018).

3. Meta-analysis

3.1 Search strategy

Our search strategy for the meta-analysis is described in detail in the published paper (Csenkey et al., 2019). In brief, we followed the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols. The question of our analysis was formulated with the Participants, Intervention, Comparison, Outcome (PICO) model: in children with burn injuries, we aimed to assess the effect of systemic antibiotic prophylaxis on infectious complications. Our meta-analysis was registered with PROSPERO (registration number: CRD42018102498).

We searched the Cochrane Library, EMBASE, and PubMed databases for eligible papers from inception to August 2019 with the following search key: "(antibiotic* OR antimicrobial*) AND (prophylaxis OR prophylactic) AND (burn* OR scald OR flame) AND (paediatric* OR child*)". We filtered the hits for human studies. The search was conducted separately by myself and a coauthor, and we also assessed study eligibility and extracted data from the selected studies independently. Disagreements were resolved by consensus, if needed, with the help of the supervisors. As a specific example for the search, in the EMBASE database, which identified the highest number of articles, the term "(antibiotic* OR antimicrobial*) AND (prophylaxis OR prophylactic) AND (burn* OR scald OR flame) AND (paediatric* OR child*)" was entered and retrieved 230 records, which decreased to 213 studies after the "humans" filter was selected.

3.2 Study selection and data extraction

After screening the titles and abstracts of the identified articles, the full texts of potentially eligible papers were obtained. We included studies which compared event rates of systemic and local complications of burns between children receiving and not receiving systemic antibiotic prophylaxis. Antibiotic prophylaxis was defined as systemic antibacterial drug administration to patients without confirmed infection and systemic inflammatory signs. Wound infection was considered a local complication, while systemic complications included sepsis and suspected toxic shock syndrome. From the included studies, we extracted the country of origin, patient population characteristics (sample size, age, TBSA), and complication events in the different treatment groups (i.e., with or without systemic antibiotic prophylaxis) of children with burn injuries. The quality of the studies included in the meta-analysis was evaluated by myself and another coauthor with the Cochrane Risk of Bias Tool for Randomized Controlled studies and by the Newcastle-Ottawa Scale, as appropriate.

3.3 Statistical analysis

The statistical analysis was performed according to the standard methods of meta-analysis. Patients were grouped as either treated with systemic antibiotic prophylaxis or not. Pooled odds ratio (OR) with 95% confidence intervals (CI) for infectious complications in paediatric patients with burn injuries were calculated for the dichotomous outcomes. In all forest plots, we applied the random-effect model with DerSimonian-Laird estimation. The OR was calculated by dividing the ratio of events to no events in the antibiotic-treated group with the same ratio in the group without systemic antibiotic prophylaxis. Statistical heterogeneity was determined by the I^2 statistical test ($p < 0.1$ indicating significant heterogeneity), while publication bias was assessed by the visual inspection of funnel plots, as described elsewhere (Olah et al., 2018; Rumbus et al., 2017). Heterogeneity in clinical outcomes was explored by creating different subgroups (age, income, TBSA, type of complication). Sensitivity analysis (i.e., iteratively removing one study from the analyses and recalculating the OR to investigate the impact of each individual study on the summary estimate) showed no difference in the final pooled results. The analyses were performed using the Stata 11 SE software (StataCorp LLC, College Station, TX, USA).

Results

1. Animal experiment

1.1 Percentage of the non-epithelialised wound area on day five post-burn injury

Five days after the induction of the burn injury, the ratio of the not epithelialised wound surface to the whole wound diameter, as well as the depth of the burn, was analysed histologically. In all rats, we confirmed that the applied method for burn induction resulted in a superficial partial-thickness burn injury (for a representative photo, see Figure 6).

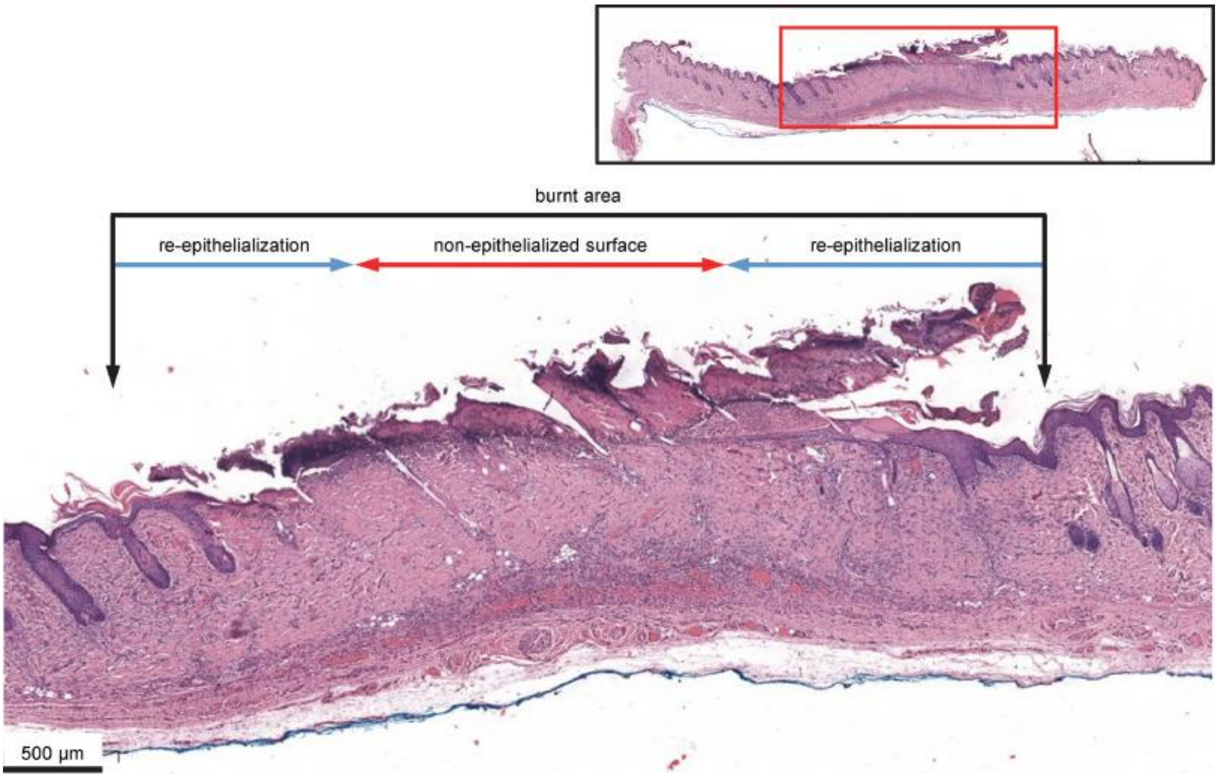


Figure 6. *The ratio of the not epithelialised surface and the burn wound diameter was calculated to assess the healing of the burn on day five post-injury. The diameter of the entire burn wound, as well as the epithelial invasion from both sides and the not epithelialised surface, are marked with black, blue, and red arrows, respectively. For orientation purposes, the insert on the top right shows the whole tissue section with a red box indicating the magnified area (Csenkey et al., 2022).*

We also compared the effects of the four treatments used: silver-sulfadiazine cream, silver foam dressing, zinc-hyaluronan gel, and the combination of zinc-hyaluronan gel with a silver foam dressing. We found that the treatment had a significant effect on wound healing ($p = 0.08$) based on the percentage of the not epithelialised surface to the whole diameter of the burn on day 5 (for an example, see Figure 6). The post hoc analysis revealed that the zinc-hyaluronan gel and the combination treatment resulted in a significantly smaller ratio of the not epithelialised area (29 ± 10% and 28 ± 13%, respectively) than the silver-sulfadiazine cream (69 ± 4%; $p < 0.01$ compared to both) (Figure 7).

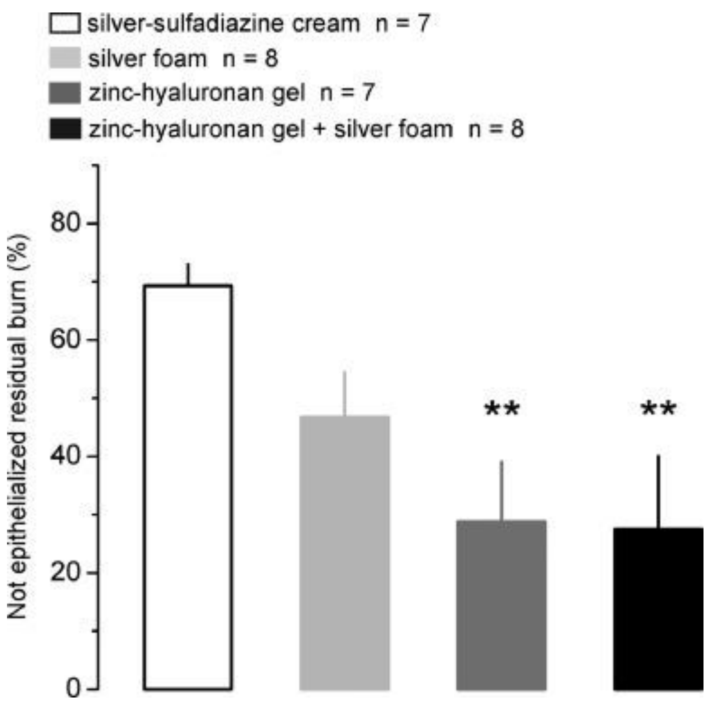


Figure 7. *The percentage of the open (not epithelialised) surface compared to the whole diameter of the burn wound five days after the induction of the burn injury in rats (treatment groups and the number of animals are indicated). **p < 0.01 vs silver-sulfadiazine cream as determined by one-way ANOVA followed by Fisher LSD test. Data are presented as mean ± SE (Csenkey et al., 2022).*

1.2 Extent of wound re-epithelialisation on day ten post-burn injury

Ten days after the induction of the burn injury, the whole wound diameter was somewhat epithelialised in most of the rats (in specific, in 20 of the 28 animals); thus, we used a quite simple scoring system to assess the healing of the wound: when at least some part of the wound was not re-epithelialised, it scored 0; when the entire wound was re-epithelialised but only partially (i.e., in a single layer), it scored 1; when the wound was entirely closed in multiple layers, it scored 2.

The effect of the treatment was significant on the re-epithelialisation score ($p < 0.001$). We found that the extent of re-epithelialisation was the lowest (0.2 ± 0.2) in the silver-sulfadiazine cream group. At the same time, in the case of the other three treatments, it was significantly higher with scores of 1.0 ± 0.2 for silver foam ($p = 0.008$), 1.0 ± 0.4 for zinc-hyaluronan ($p = 0.012$), and 2.0 ± 0.0 for the combination treatment ($p < 0.001$) (Figure 8). Notably, the combination treatment resulted in the maximal score of 2 in every rat, which was higher than the scores in the other treatment groups ($p < 0.001$ vs silver foam and $p = 0.002$ vs zinc-hyaluronan).

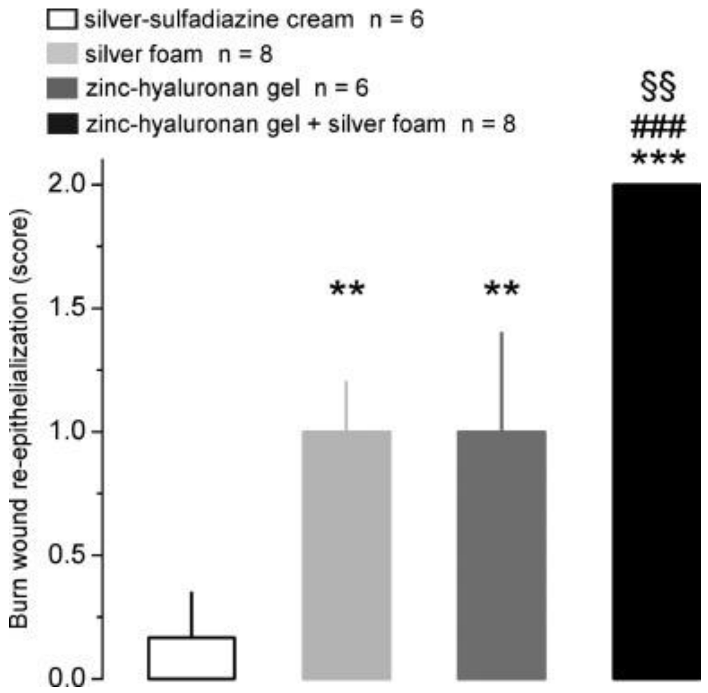


Figure 8. *The score for the re-epithelialisation of the burn wound ten days after the induction of the burn injury in rats (treatment groups and the number of animals are indicated). 0: no re-epithelialisation; 1: partial re-epithelialisation; and 2: complete re-epithelialisation in multiple layers over the entire wound length. **p < 0.01 and ***p < 0.001 vs silver-sulfadiazine cream; ###p < 0.001 vs silver foam dressing; §§p < 0.01 vs zinc-hyaluronan gel as determined by one-way ANOVA followed with Fisher LSD test. Data are presented as mean ± SE (Csenkey et al., 2022).*

1.3 Scar thickness of the wound on day 22 post-burn injury

By day 22 post-burn injury, the wounds were fully re-epithelialised in all rats. In order to further evaluate the healing process, we analysed whether there was a difference in the scar thickness among the treatment groups since an increased scar thickness can be an indicator of hypertrophic scarring, which remains a core challenge following burn injury (Finnerty et al., 2016).

We found that the scar thickness was the smallest in the combination treatment group (560 ± 42 µm), which was significantly less than in the silver-sulfadiazine cream group (712 ± 38 µm; p = 0.024) (Figure 9).

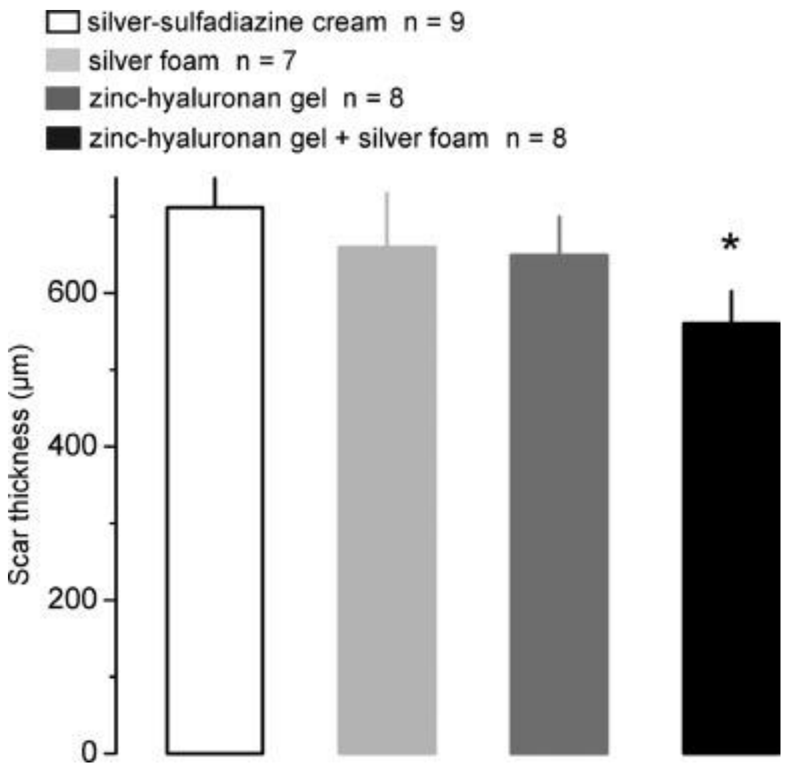


Figure 9. *The scar thickness of the burn wound was evaluated 22 days after the induction of the burn injury in rats (treatment groups and the number of animals are indicated). The star sign (*) indicates p < 0.05 compared to silver-sulfadiazine cream as determined by one-way ANOVA followed by the Fisher LSD test. Data are presented as mean ± SE (Csenkey et al., 2022).*

For macroscopic visualisation of the burned skin, digital photographs of the burn wound in randomly selected rats at the beginning and the end of the experiment are shown in Figure 10.

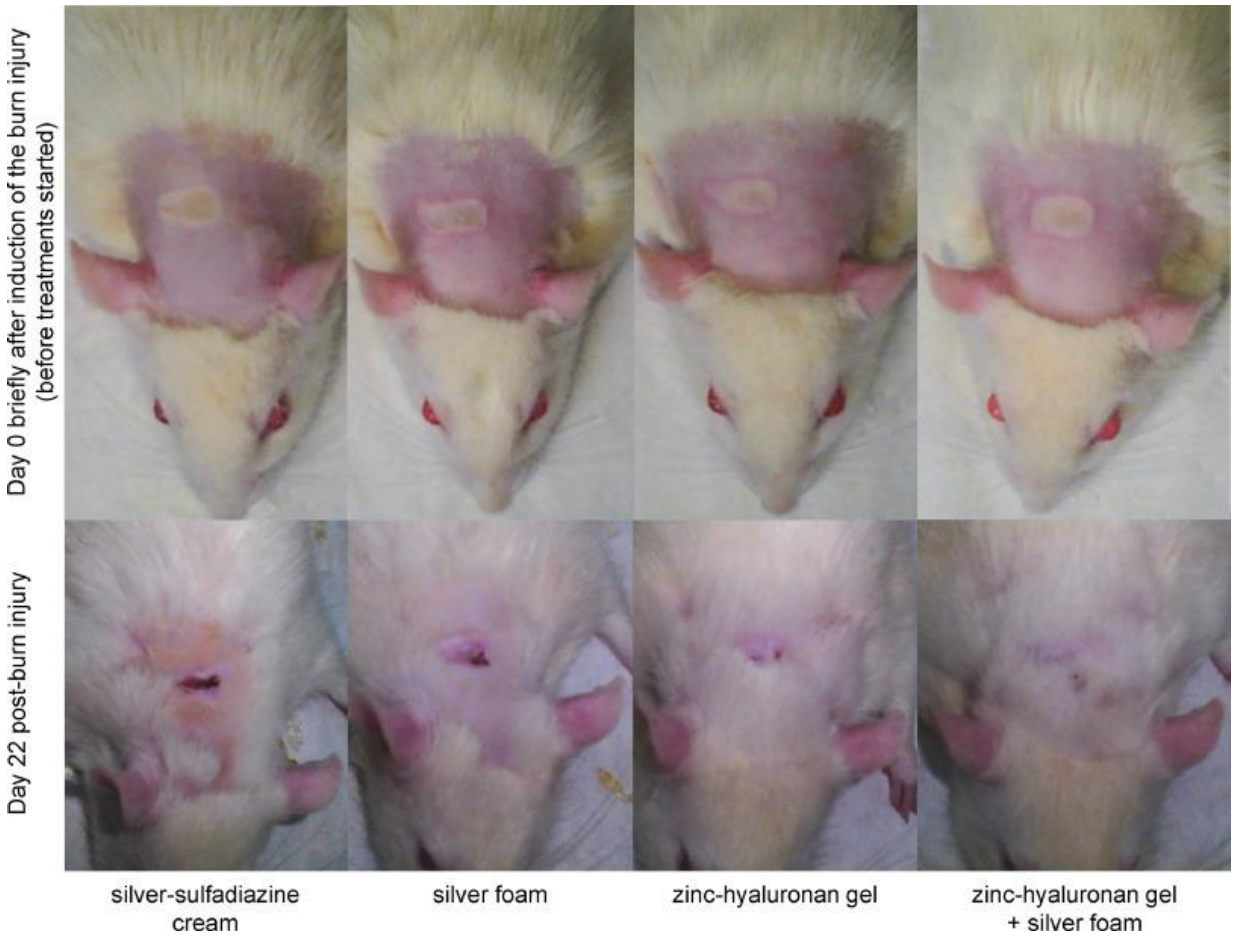


Figure 10. *Digital photographs of four randomly selected rats on day 0 briefly after the induction of the burn wound before any of the treatments were applied (top row) and a picture of one randomly selected rat from each treatment group at the end of the experiment on day 22 post-burn injury (bottom row) (Csenkey et al., 2022).*

2. Human study

Since we showed that the zinc-hyaluronan treatment is quite beneficial in our newly developed preclinical model (see above), we wanted to know whether its benefits can also be found in human patients. For that reason, we conducted a small, single-centre clinical trial (Jozsa et al., 2018). Most of the studied children were younger than five years old. With regards to gender distribution, out of the 37 injured children, 27 were boys, and 10 were girls. This gender ratio is similar to international and European incidence rates, namely that boys (73%) are more likely to be exposed to burn injury (Figure 11) (Jozsa et al., 2018).

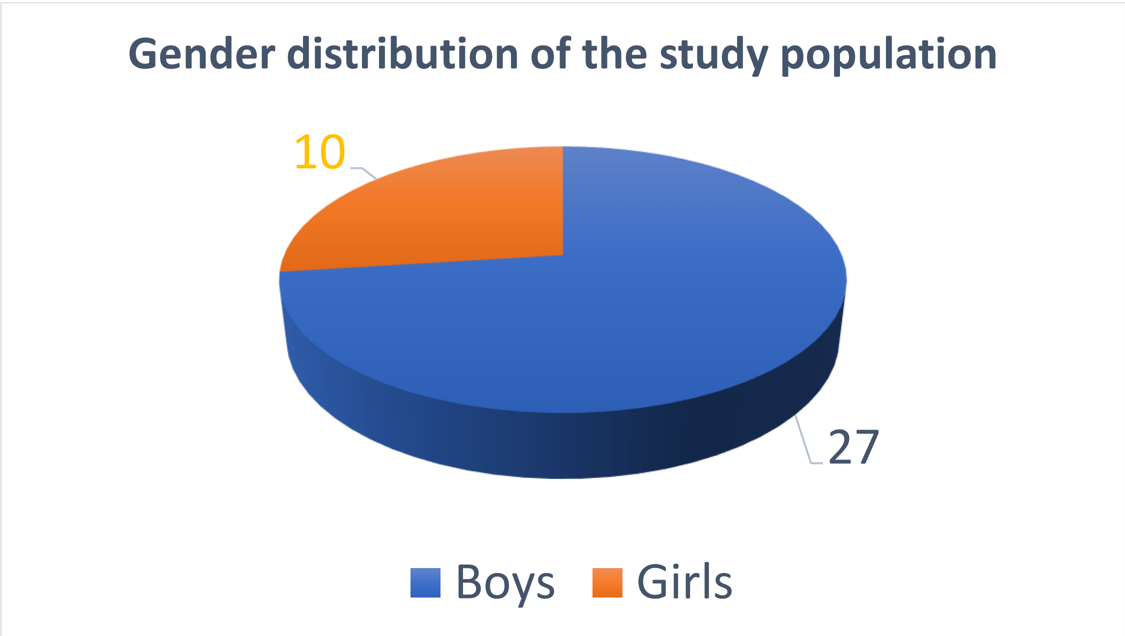


Figure 11. *Gender distribution of the study population (Jozsa et al., 2018).*

Concerning the causes of the hand burns, touching a heater or a stove with the palm of the hand was the most common (16/37 pts), while injuries caused by household equipment (12/37 pts) and hot water (8/37 pts) were also frequent. Only one child in the sample had injuries caused by electricity (Figure 12) (Jozsa et al., 2018).

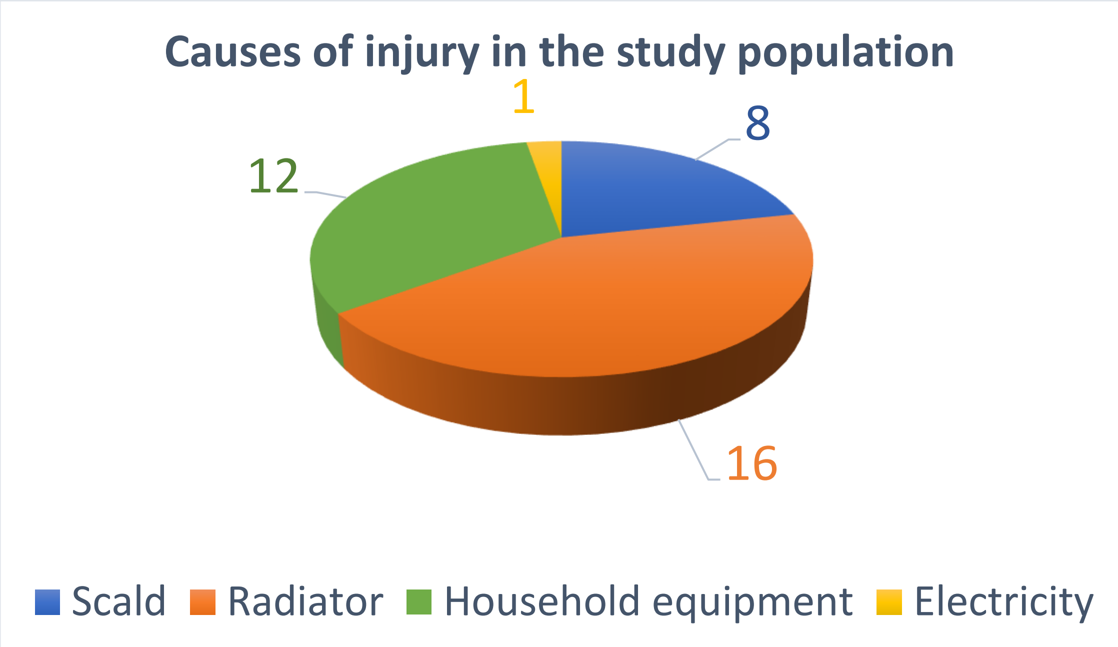


Figure 12. *Causes of injury in the study population (Jozsa et al., 2018).*

No wound infection was diagnosed in patients treated with the Zn-hyaluronan gel combined with Aquacel Ag foam dressing, corresponding to an infection ratio of 0/37. The bandages were kept in place and usually uncontaminated for the duration of the treatment. In general, epithelialisation of the burned area was observed 6 to 7 days after primary treatment (Figure 13), which corresponds well with the results from other methods of dressing for this type of burn. The same combined treatment resulted in similarly improved outcomes when we applied it to partial-thickness burn injuries of body parts other than the hands in children (Jozsa et al., 2018).

Figure 13. *Superficial second-degree burn injury of the right hand before (A) and after the removal of bullae (B). On the seventh day after initiation of the treatment, the burn injury is healed (C). The cosmetic result was good one month after the injury (D) (Jozsa et al., 2018).*

3. Meta-analysis

In our third approach to studying the importance of burn injuries and their treatments, we conducted a meta-analysis to evaluate the necessity of prophylactic systemic antibiotic treatment in children with burns (Csenkey et al., 2019). The search strategy is described in Methods. The flowchart of the search is shown in Figure 14.

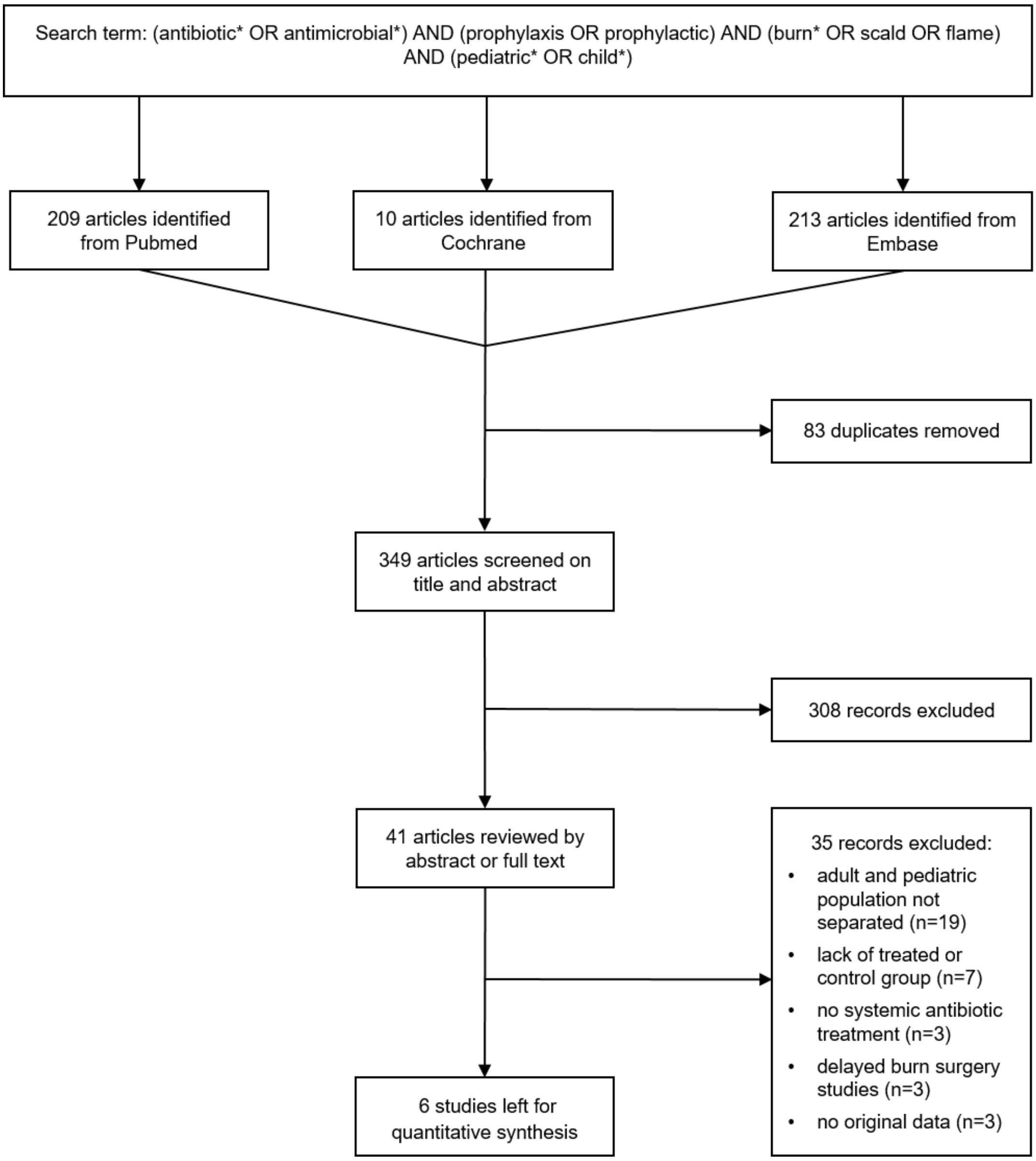


Figure 14. *Flow chart of study selection and inclusion (Csenkey et al., 2019).*

3.1 Study selection

Until August 2019, the electronic literature search identified 432 human studies from three databases: Cochrane Library, EMBASE, and PubMed altogether. After removing duplicates, 349 articles remained, which were screened on title and abstract for inclusion criteria. In addition, full texts of 41 articles were reviewed, and, in the end, six publications were found eligible for statistical analysis (Chahed et al., 2014; Ergun et al., 2004; Mulgrew et al., 2014;

Rashid et al., 2005; Rosanova et al., 2013; Sheridan et al., 2001), which included data from a total of 1,735 patients.

3.2 Effects of systemic antibiotic prophylaxis on local and systemic infectious complications in children with burn injuries

First, we analysed whether systemic antibiotic prophylaxis has an effect on the OR for infectious complications either locally or systemically. Studies which separately reported the event rates of local (Ergun et al., 2004; Sheridan et al., 2001) or systemic complications (Ergun et al., 2004; Mulgrew et al., 2014; Rashid et al., 2005) were included in the forest plot (Figure 15). Prophylactic administration of systemic antibiotics did not cause a significant change in the odds of systemic infections (OR = 0.74; 95% CI, 0.38, 1.45). With regards to local complications, the use of antibiotics did not have a significant effect in the two included studies, but the averaged result (OR = 0.99; 95% CI, 0.40, 2.47) should be considered carefully because of the low number of studies in this subgroup. The odds of all (local and systemic together) infectious complications was also not significantly different between the antibiotic-treated and non-treated groups (OR = 0.82; 95% CI, 0.48, 1.40) (Figure 15).

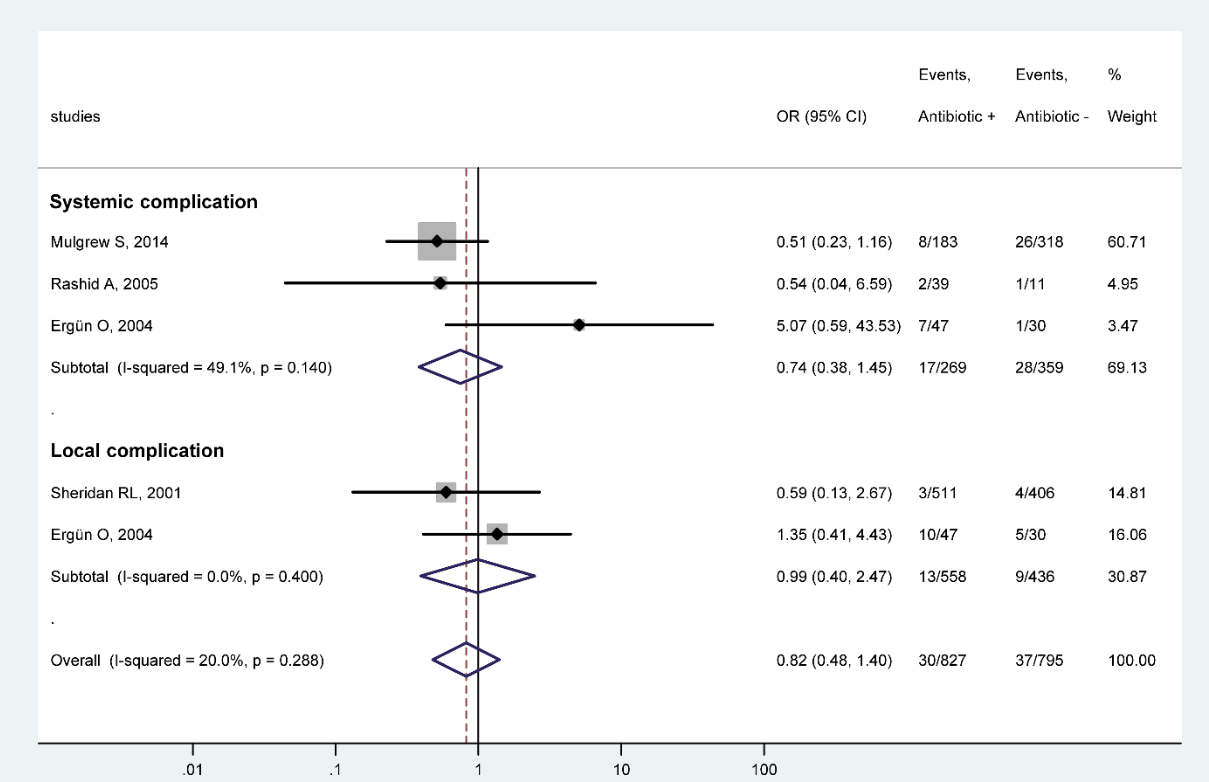


Figure 15. *Forest plot of the odds ratios (ORs) for subgroups of systemic and local infectious complications in children with burn injuries who received systemic antibiotic prophylaxis compared to those who did not. Here, and in Figures 16-18, black circles represent the OR for each study; the horizontal arms of the circles indicate the corresponding 95% confidence intervals (CI) for the OR. The size of the grey square indicates the study's sample size: the more extensive the square, the larger the sample size, and thus the relative weight of the study. The rhombi represent the average OR calculated from the ORs of the individual studies in each subgroup (top and middle) and all studies (bottom). The horizontal diagonals of the rhombi represent the 95% CI of the average ORs. The vertical dashed line is determined by the vertical diagonal of the bottom rhombus and indicates the value of the average OR of all studies in the forest plot. An OR lesser than 1 indicates that systemic antibiotic prophylaxis decreased the chance of infectious complications. In contrast, an OR higher than 1 indicates an increased chance of infections in antibiotic-treated children (Csenkey et al., 2019).*

3.3 Odds for infections in different subgroups of burnt children treated or not treated with systemic antibiotic prophylaxis

We also divided the studies into different subgroups according to the known risk factors of the outcome of burns when enough data were available. Unlike in the first forest plot (Figure 15), where systemic and local complications were distinguished from each other, in the remaining part of our meta-analysis, we considered all (i.e., both local and systemic) complications together as the outcome.

We merged the local and systemic complications because it allowed us to include two studies in the analysis in which the separate event rates of local and systemic complications were not reported. Further justifying the merging of local and systemic complications, we did not find a significant difference in the OR between systemic and local complications (Figure 15). Based on the age range of the patient populations, the studies were divided into two subgroups: limited to children only, viz., under ten years of age, or also including adolescents up to the age of 16 years. Systemic antibiotic prophylaxis did not change the odds of complications in either of the age groups (Figure 16).

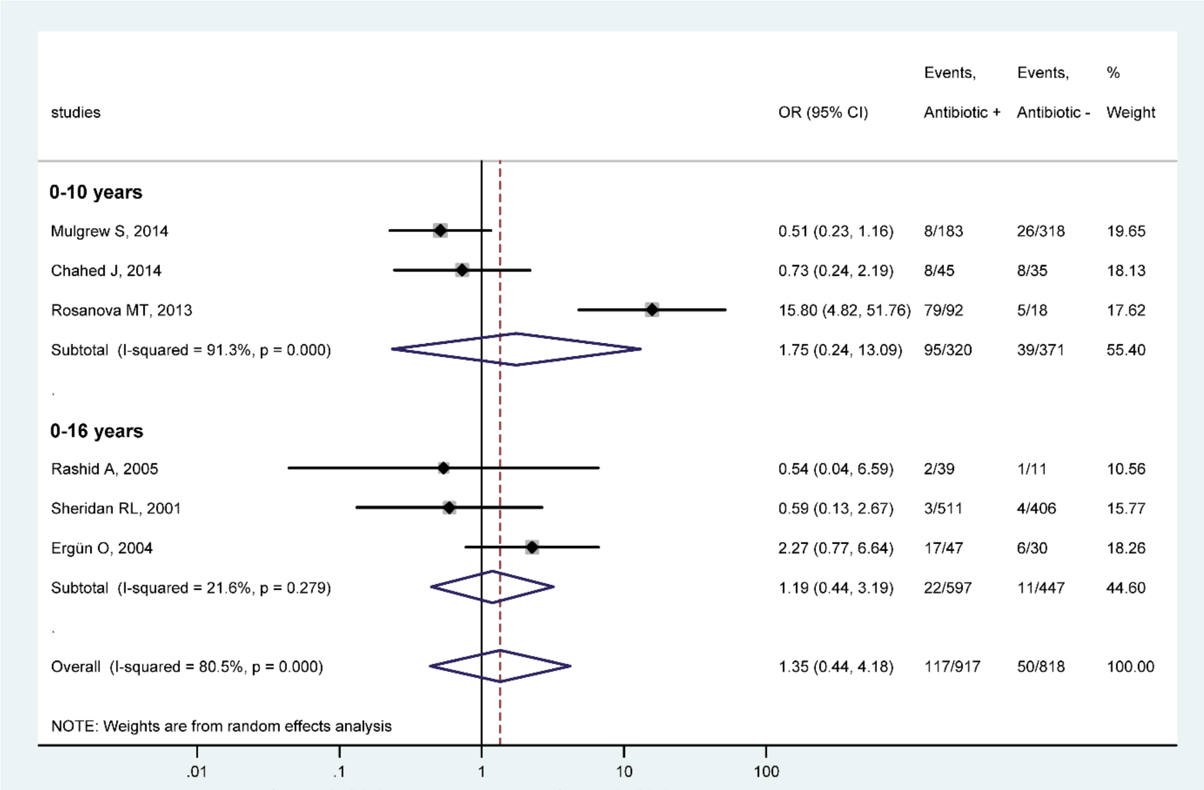


Figure 16. *Forest plot of the odds ratios (ORs) for all infectious complications in children of 0–10 years (top) and 0–16 years (bottom) with burn injuries who received versus those who did not receive systemic antibiotic prophylaxis (Csenkey et al., 2019).*

The OR in the younger (children only) group was 1.75 (95% CI, 0.24, 13.09), while in the older group, which also included adolescents, it was 1.19 (95% CI, 0.44, 3.19). Antibiotic prophylaxis did not have any effect on the chance of infections when all six studies in the forest plot were combined (OR = 1.35, 95% CI, 0.44, 4.18) (Figure 16).

Based on the mean TBSA affected by the burns, the studies were grouped as less than 20% and more than 20% of injured TBSA. We did not find a significant effect of systemic antibiotic prophylaxis on the odds of infectious complications in the subgroup with less than 20% affected TBSA (OR = 0.84, 95% CI, 0.37, 1.91) (Figure 17).

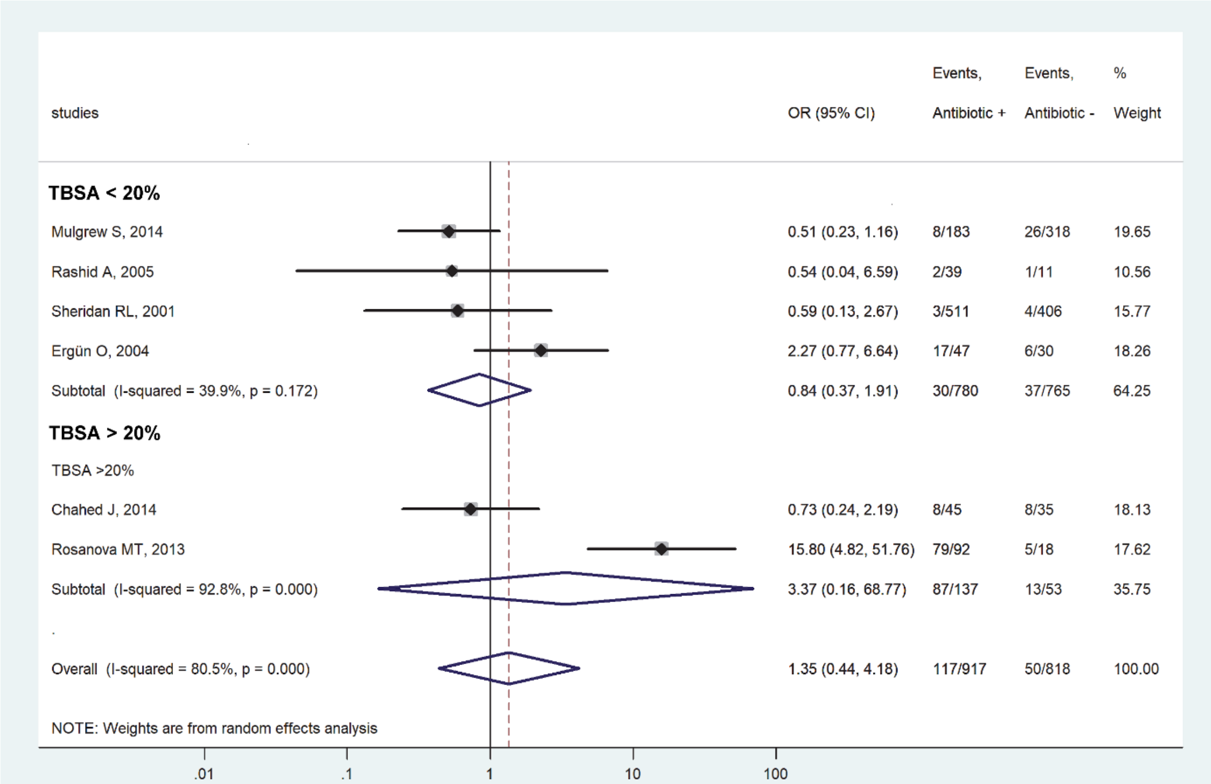


Figure 17. *Forest plot of the odds ratios (ORs) for all infectious complications in paediatric patients with burn injuries who received versus those who did not receive systemic antibiotic prophylaxis in subgroups of less than 20% (top) and more than 20% (bottom) mean extent of the injury as related to the total body surface area (TBSA) (Csenkey et al., 2019).*

Though the OR was also not significant in the subgroup with more than 20% of injured TBSA, this group included only two studies, which is not sufficient for proper meta-analysis; thus, the merged results should be taken with scrutiny. Regarding the economic status of the country of the studies, the studies were divided into middle-income and high-income subgroups based on the categorisation of the countries in the World Bank Data. Our analysis showed no significant effect of antibiotic prophylaxis on the chance of infections in either of the subgroups. The OR in the high-income subgroup was 1.35 (95% CI, 0.21, 8.77) (Figure 18). The use of antibiotics was also without an effect in either of the two studies in middle-income countries (Figure 18). However, caution is needed regarding their averaged OR due to this subgroup's low number of studies.

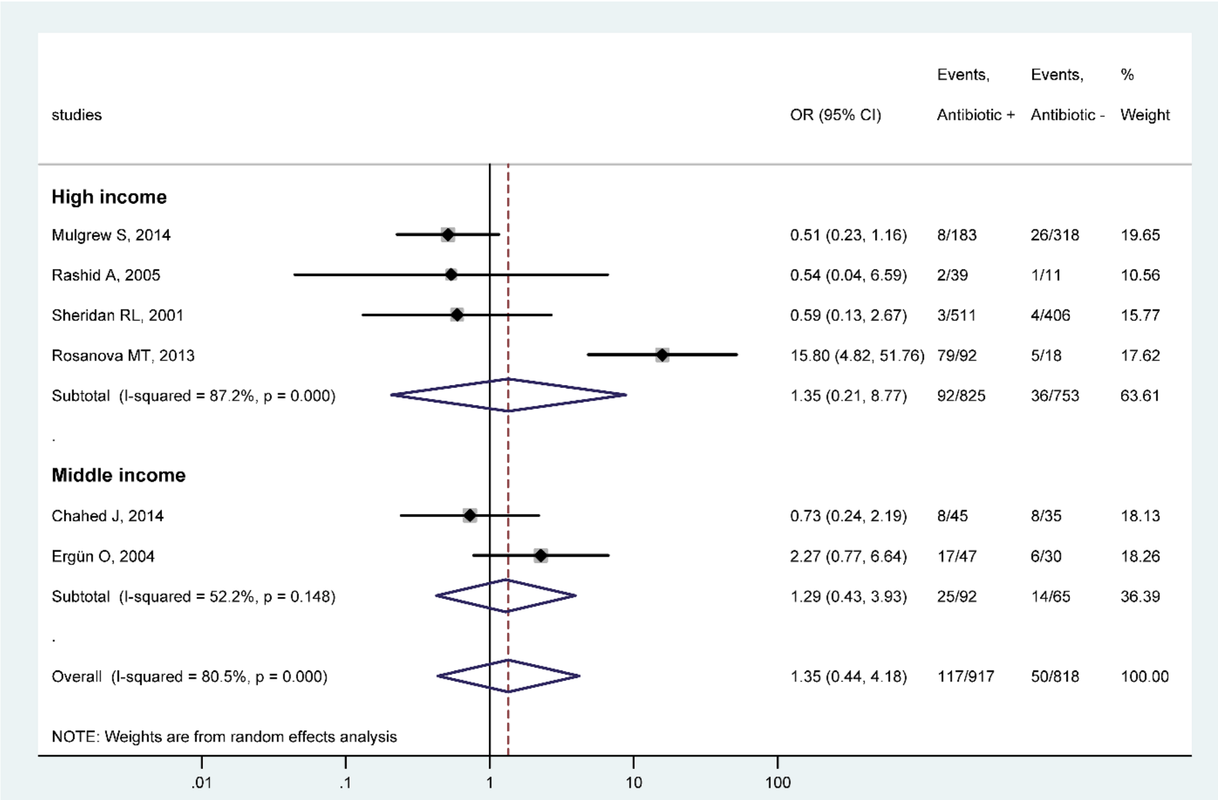


Figure 18. *Forest plot of the odds ratios (ORs) for all infectious complications in children with burn injuries who received versus those who did not receive systemic antibiotic prophylaxis in country subgroups of high income (top) and middle income (bottom) (Csenkey et al., 2019).*

Discussion

Our ultimate goal in this work was to study the characteristics of burn injuries using a multiple, translational research approach: experimental modelling, meta-analysis, and human trial. With the help of this complex approach, we were able to 1) develop a novel preclinical model for the study of partial thickness burn injuries and the comparison of therapeutic options of burns; 2) demonstrate the benefits of zinc-hyaluronan treatment in the new preclinical model and human patients with burn injuries; and 3) show with meta-analysis that the use of prophylactic antibiotic treatment does not provide benefits for the infectious outcome in children with burns.

In the first part of our investigation, we introduced a novel, easily accessible rat model of superficial partial-thickness burn injury and evaluation of wound healing, which can be used for the preclinical testing of different treatment options. In this model, we compared the effects of four treatments on different indicators of wound regeneration. We showed that the combination of zinc-hyaluronan gel with silver foam dressing was the most advantageous compared to the other treatments. In contrast, silver foam or zinc-hyaluronan alone was superior to silver-sulfadiazine cream. Different experimental designs were used to study the pathomechanism and therapeutic options in burns; however, an easily accessible and reproducible, cost-effective, in vivo animal model for preclinical studies remains to be established. In our study, we developed a rat model of superficial partial-thickness burns, which fulfils the listed criteria (Csenkey et al., 2022). We applied standardised preparations (adult male Wistar rats, nape skin, anaesthesia, shaving, disinfection), burning methods (commercially available soldering device with 4 × 4 mm flat surface on the iron tip, 130°C heating, 30 s contact time, and steady pressure), as well as post-intervention procedures (covering the wound with a cohesive conforming bandage and a perforated plastic sheet). Consequently, we were able to reliably reproduce histologically confirmed superficial partial-thickness burn wounds that penetrated the dermo-epidermal papillary region of the skin but did not extend to deeper layers.

The rat – as a widely available, affordable experimental model – was already used previously for the study of burns (Guo et al., 2007; Gurfinkel et al., 2010; Priya et al., 2002; Sakamoto et al., 2016; Tavares Pereira Ddos et al., 2012; Venter et al., 2015). Among those studies, only two reported the successful induction of superficial partial-thickness burns (Sakamoto et al., 2016; Venter et al., 2015). In contrast, in the others, the depth of the burn was deep partial-thickness (Guo et al., 2007; Tavares Pereira Ddos et al., 2012), full-thickness (Gurfinkel et al., 2010), or unknown (Priya et al., 2002). However, the authors manufactured or modified the device used for the induction of superficial partial-thickness burns in both earlier studies, which limits their widespread accessibility. In our study, for the first time to our knowledge, we used a commercially available soldering device without any modifications. Moreover, we described how it was used for the induction of burns, which enables its application for scientific research worldwide (Csenkey et al., 2022).

It should be noted, however, that although the rat skin is also composed of the primary layers (epidermis, dermis) as the human skin, it does not perfectly mimic the human skin architecture because of its unique skin morphology. Therefore, despite using rats for burn research in the present and previous studies, care should be taken when translating the results obtained in rats for human applications. Nevertheless, the developed model can be very well applied to study burn treatment options that are already available for human patients. However, to our knowledge, their parallel comparison under standardised circumstances (i.e., in a unified model) has not been reported.

In superficial partial-thickness burns, conservative therapy is the primary choice, while surgical interventions are usually unnecessary. In the case of conservative treatment, it is crucial to rinse the wound with a disinfecting agent prior to removing the dead tissue. This process, called as debridement, is considerably painful. Hence analgesic and anxiolytic drugs or general anaesthesia are often administered. During the healing of the wound, epithelial cells originating in the remaining epithelial appendages (e.g., the lining of sebaceous and sweat gland ducts) travel from the uninjured to the damaged areas to begin the healing process (Pastar et al., 2014). One of the major aims of conservative treatments is to facilitate the epithelialisation process and thereby promote the healing of the wound. Conservative treatments are very efficient in superficial partial-thickness burns because they cover the affected areas to maintain a moist environment. They can also deliver antimicrobial compounds to prevent the burn wounds' infection and progression (i.e., deeper penetration). Several conservative treatments are currently used (for a review, see Rowan et al., 2015), but their head-to-head comparisons under standardised conditions are scarce. Therefore, the results of different trials can be compared indirectly by meta-analyses. These are, however, suboptimal because of the methodological quality and heterogeneity of the analysed studies.

Our work compared four treatment options (see below) on different wound healing parameters in our novel rat model of superficial partial-thickness burns.

1) Silver-sulfadiazine (e.g., Dermazin) evokes antibacterial effects and promotes re-epithelialisation; its low cost and easy application contributes to its widespread use in clinical practice, which also explains why it could be used as a comparator treatment in previous studies. However, its use requires daily dressing changes and creates a yellowish plaque on the burn, which makes the assessment of burn depth difficult.

2) Hydrofiber (e.g., Aquacel Ag foam) is a newer dressing type, which contains an external polyurethane waterproof film layer that surrounds a multilayer absorbent surface with a silver ion content of 1.2 %. The multilayer cushion contains a foam sheet and a plate with hydrofiber technology. Absorption of the wound discharge leads to the gelification of the hydrofiber layer, which helps keep the wound moist and promotes wound healing while preventing infections. The bandage is comfortable, and its removal is painless without requiring anaesthesia.

3) Zinc-hyaluronan gel (e.g., Curiosa) helps maintain a moist environment due to the considerable molecular weight and negative charge of the hyaluronan content, which facilitates the healing process and reduces pain in second-degree burn injury. Furthermore, adding zinc contributes to an anti-inflammatory and antimicrobial effect, making it a suitable alternative for topical wound care therapy.

4) The combination of 2) and 3), which was found to perform better than other conservative methods in previous clinical trials (Blanchard et al., 2016; Borges Rosa de Moura et al., 2022; Eldad et al., 1991; Hernandez, 2011; Jozsa et al., 2017; 2018; Juhasz et al., 2012; Markiewicz-Gospodarek et al., 2022; Mehta et al., 2019; Wasiak et al., 2013).

In our experiments, we found that silver-sulfadiazine was less beneficial than the combination treatment at all three evaluation points, than zinc-hyaluronan on days 5 and 10, and silver foam on day 10. The combination treatment performed better than the other three interventions on day 10. It was the only method that caused a significant decrease in scar formation on day 22 compared to silver-sulfadiazine. These results are in accordance with previous studies that question the routine use of silver-sulfadiazine in the modern treatment of burn injuries (Blanchard et al., 2016; Borges Rosa de Moura et al., 2022; Jozsa et al., 2017; 2018; Mehta et al., 2019; Wasiak et al., 2013). Moreover, our findings highlight that newer treatment options such as silver foam dressing and zinc-hyaluronan or the combination of them can result in improved burn wound healing compared to silver-sulfadiazine. The mechanism by which the combination treatment was superior compared to silver foam dressing or zinc-hyaluronan alone remains subject to future studies. However, it can be suggested that the simultaneous presence of silver and zinc ions in the dressing exerts additional advantageous effects on wound healing as compared to the two components alone. Indeed, the combination of silver and zinc resulted in enhanced antibacterial effects combined with anti-inflammatory and antioxidant responses. Moreover, it was associated with improved wound healing, re-epithelialisation, and collagen deposition when used in vivo as a dressing for mechanical (not burn) wounds (Borges Rosa de Moura et al., 2022; Kyomuhimbo et al., 2019; Lu et al., 2017).

In the second part of our work, we showed that the application of zinc-hyaluronan is a very efficient treatment in paediatric patients with burn injuries in a single-centre clinical trial (Jozsa et al., 2018). We conducted a prospective study for four years in Hungary, in which we treated 37 children with superficial and mixed-typed second-degree hand burns with Aquacel Ag foam and Zn-hyaluronan gel applied simultaneously. Children with deep second-degree hand burns (II/B or II/2) were excluded from the study. The limitations of our study are that it was conducted in one centre only and involved only one method. The patients were not control-matched and not randomised. Because of the study design, we did not include children with II/B degree burn depth in this investigation; whether the same combined treatment would be beneficial in such cases, too, remains a subject for future studies. All the patients diagnosed with a burn injury <5% TBSA were treated with this method. Because of the modern dressings, epithelialisation generally occurred on the sixth day, as in a previous study by an independent group. According to our results, in our experience, there were no cases of infection.

Conservative treatment of hand burns with the widely used local remedy, silver-sulfadiazine ointment, creates a heavy, oozing fatty layer that is difficult to tolerate. This thick, adherent layer also makes the proper burn depth determination very difficult. Before this study, silver-sulfadiazine was the gold standard for treating superficial burns in our centre. The disadvantages of that treatment consisted of the need for daily dressing changes and difficulties with assessing burn depth.

In the children treated with traditional methods, anaesthesia had to be used daily or every other day to change the dressing. While the foam dressing containing silver could be used until the wound healed; thus, repeated anaesthesia was not needed.

Treatment of mixed-type burns remains a considerable challenge. It has been widely debated as of whether conservative treatment is sufficiently compelling or not. Treatment for a coherent and deep second-degree burn wound is a surgical intervention, whereas mixed-type second-degree burns can also be effectively treated with conservative methods. In nearly 90% of the children, we used Aquacel Ag foam dressing with Curiosa (Zn-hyaluronan) gel at the first intervention. We checked the dressing on the second day and removed it on the sixth or seventh day. Second-day control was essential to check the dressing condition. If the bandage was clean, changing the dressing was unnecessary. In those cases, when we found that the dressing was contaminated, we changed it, which explains the rationale for why we checked the wound on the second day.

Hyaluronan gel containing zinc combined with hydrofiber dressing containing silver tends to be effective against infections and promotes wound healing. The combined dressing is comfortable and can be applied easily to the hand. It also creates an appropriate environment for proper wound healing (Lau et al., 2016). In contrast to traditional treatments, applying, changing, and removing a combination of Aquacel Ag foam with Zn-hyaluronan dressing is painless. A crucial aspect of this new method is that the physical strain and stress of the child are reduced because of fewer control check-ups and dressing changes. On average, 2.5 dressings were used per case. Because of the reduced number of dressings and anaesthesia required, the approximate cost of the treatment per child was cut by half. Currently, only a few clinical studies are available in the literature about applying Aquacel Ag foam dressing in paediatric patients with partial-thickness burns, however, in these studies, the length of hospital stay was significantly shorter in the Aquacel Ag group (Brown et al., 2016; Paddock et al., 2007; Saba et al., 2009). Moreover, dressing frequency was 3 to 4 times lower in the Aquacel Ag group than in the standard dressing group (Lau et al., 2016).

In addition to analysing the effectiveness of dressings in the novel animal model and clinical settings, we wanted to see if axillary treatments like prophylactic antibiotics are helpful in preventing infectious complications associated with partial-thickness burn injury in children (Csenkey et al., 2019). We used standard meta-analysis methods to answer that question and showed that systemic antibiotic prophylaxis has no beneficial effects on the risk for infectious complications in paediatric burn injuries. In particular, by analysing data from a total of 1,735 patients, we found that no patient subgroup (based on age, injured TBSA or country income) benefited (in regards to odds for infectious complications) from receiving prophylactic antibiotic treatment, as compared to burn patients without antibiotic treatment.

Infectious complications are often feared as threats after burn injuries. Superficial burns (traditionally named first-degree burns), which affect only the epidermis, usually do not require specialised medical care. On the contrary, in deeper burns, which penetrate the dermis (i.e., partial-thickness, formerly second-degree) or damage the entire dermis and potentially even deeper tissues (i.e., full-thickness, formerly third- and higher-degree burns), the chance of infectious complications is proportionally increasing with the depth of the burn (Church et al., 2006). Deeper burns usually require complex conventional and surgical interventions; among them, partial-thickness burns are the most common type in children. As part of the primary treatment of deeper burns, systemic antibiotic prophylaxis is occasionally initiated, even though there is no clinical evidence for such indication of anti-microbial treatment. In fact, The International Society for Burn Injuries recommends avoiding prophylactic systemic antibiotics in acute burns (ISBI Practice Guidelines Committee, 2016), which guideline is based in part on meta-analyses of data obtained in adult burn patients (Avni et al., 2010; Barajas-Nava et al., 2013). In paediatric burns, however, no meta-analysis has been performed, and to the best of our knowledge, only a systematic review was published (Lee et al., 2009), which lacked quantitative statistical analysis. Our work aimed at filling this gap by conducting a meta-analysis of six articles identified based on an extensive literature search. We showed that systemic antibiotic prophylaxis did not decrease the chance of systemic and all infectious complications. As a matter of fact, when we included the rates of all infectious complications from all six eligible studies in our analysis, we found that the overall chance of developing an infection tended to be 35% higher in antibiotic-treated patients (n = 917) than in patients without antibiotic prophylaxis (n = 818), as indicated by the overall OR of 1.35 (95% CI, 0.44, 4.18) (Figures 11–13). Although, the difference did not reach the level of statistical significance.

Nevertheless, two of the analysed studies reported a higher rate of infectious complications in children with burn injuries who received antibiotic prophylaxis (Ergun et al., 2004; Rosanova et al., 2013). It was thought to be due to the overgrowth of resistant microorganisms, thereby resulting in infections by opportunistic pathogens in the urinary tract, airways, and middle ear. Antibiotic prophylaxis did not prevent wound infection or potential lethal consequences in the study in 80 paediatric patients with burn injuries conducted by Chahed et al. (2014), which was, to our knowledge, the only randomised clinical trial designed to investigate the necessity of systemic antibiotic prophylaxis. Similarly, two other studies concluded antibiotic prophylaxis was unnecessary (Mulgrew et al., 2014; Sheridan et al.,2001). Whereas yet another suggested that prophylactic antibiotics may prevent toxic shock syndrome based on data obtained from 50 paediatric patients with burn injuries (Rashid et al., 2005). However, it has to be noted that in the latter study, only three patients became septic in the entire study population: two (of thirty-nine) in the antibiotic-treated group and one (of eleven) in the group without prophylaxis. Due to the low numbers, these results should be interpreted cautiously, as the authors noted.

The results of our meta-analysis regarding the lack of efficacy of prophylactic antibiotics on the overall infection rate in paediatric burns are in harmony with the conclusions drawn in the majority of previous human studies, a systematic review, and recent guidelines. Moreover, by quantitative synthesis of the data reported in the identified articles, our results strengthen the body of evidence for the avoidance of systemic antibiotic prophylaxis in paediatric burns. However, pooling all reported data together and analysing only the overall infection rate may mask a potentially beneficial effect of antibiotics in a specific subset of paediatric patients. Therefore, we also performed the meta-analysis in different sub-groups, which were defined based on known risk factors reported in the identified studies. We found three parameters that were reported in sufficient detail for subgroup analysis: age, affected TBSA and country income. Therefore, we assigned patients to subgroups based on these parameters. Remarkably, there was no statistical difference in the chance of infections between paediatric patients with and without systemic antibiotic prophylaxis in any of the three subgroups. These results suggest that systemic antibiotic prophylaxis should be avoided in paediatric burns independently of the age of paediatric patients, the injured TBSA, or the country's economic status.

Certain limitations of our meta-analysis must also be mentioned. Despite the extensive database search, only six studies could be included in the final analysis. This was sufficient for quantitative synthesis, but when we divided the studies into subgroups, in some cases, only two studies per group remained. Although a review of the Cochrane Library revealed that numerous meta-analyses are conducted with two studies, firm conclusions should not be drawn from the meta-analysis of such small subgroups. All of the studies included in our meta-analysis were single-centre studies, ranging from retrospective to prospective to randomised clinical trials. According to our quality assessment, only three studies were considered good quality, while two were fair, and one was poor quality [for further details, see Csenkey et al. (2019)]. The depths of the burns in the patient populations were not reported in sufficient detail to allow for subgroup analysis of the infectious outcome separately in partial- and full-thickness burns. Neither could we extract sufficient data about the latency from the time of the burn injury till initiation and the duration of the systemic antibiotic prophylaxis. Infectious comorbidities (or the lack of such), already present in the children before they suffered burn injuries, could not be assessed from the studies. Finally, the antibiotics administered to the children varied among the studies. While penicillins were used most commonly for prophylaxis, cephalosporins and macrolides were also used in some cases, while, in one study, the antibiotic was not identified. All these factors could influence infectious outcomes (whether systemic or local) in paediatric patients with burn injuries. However, we could not account for these factors in the present meta-analysis due to data unavailability. The mentioned statistical, methodological, and medical differences in study design can explain the considerably high between-study heterogeneity (indicated by an I^2 of ~80%), as observed in our analysis (Figures 14-16). To account for the presence of heterogeneity, we used the random-effects model in all forest plots of our meta-analyses. We also performed a leave-one-out sensitivity analysis to confirm that any single study did not drive our findings. However, it is still possible that, despite all of our approaches to reduce methodological errors, the low number, different design and quality, and high heterogeneity of the analysed studies may have negatively impacted our results.

Conclusions

We developed an easily accessible rat model for the study of superficial partial-thickness burn injury. We showed that this model is feasible for the preclinical testing of different treatment options by comparing four treatment methods. Among the studied treatments, the combination of silver foam dressing and zinc-hyaluronan was superior compared to the other methods based on various parameters of wound healing.

In a clinical trial including paediatric patients with burn injuries, we showed that a change in the paradigm of conservative treatments might be timely, as new teatment options such as zinc-hyaluronan was more beneficial than traditional treatments (e.g., silver-sulfadiazine) from different aspects.

Furthermore, our meta-analysis examining the usefulness of prophylactic antibiotic treatment in paediatric patients with burn injury showed that routine antibiotic prophylaxis has no benefit in preventing infectious complications in childhood burn patients. The meta-analysis of the data available in the literature quantitatively supported the position that the routine use of systemic antibiotic prophylaxis should be avoided in case of childhood burns. In addition to the quantitative synthesis of the available data, which to our knowledge, is the first in its field, we point out certain limitations of study design and data provision, which, however, can also be addressed during the planning of future clinical studies. Multinational, randomised controlled trials are warranted to confirm our findings and clearly demonstrate that routine systemic antibiotic prophylaxis is not indicated in paediatric burns.

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Publications and presentations

Sum of all impact factors (IF): 29.533

Sum of IF from publications related to the topic of PhD thesis: 8.596

Total number of citations: 122

Publications related to the topic of the PhD thesis:

Alexandra Csenkey, Emma Hargitai, Eszter Pákai, Bela Kajtar, Livia Vida, Aba Lőrincz, Marin Gergics, Péter Vajda, Gergő Józsa, András Garami.
Effectiveness of four topical treatment methods in a rat model of superficial partial-thickness burn injury: the advantages of combining zinc-hyaluronan gel with silver foam dressing.
Injury 53, 3912-19, 2022
doi: 10.1016/j.injury.2022.09.062

IF: 2.687 Q1

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Experimental Study of the Effectiveness of Different Treatment Methods in a Rat Model of Superficial Partial-Thickness Burn Injury
19th European Burns Association Congress Turin, Italy, 7–10 September 2022

Keringer, P; Rumbus, Z; Miko, A; **Csenkey A**; Gáspár, P; Pakai, E; Oláh, E; Khidhir, N; Füredi, N; Horváth-Szalai, Z; Zsiboras, Cs; Solymár, M; Polyák, É; Gaszner, B; Garami, A
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Effectiveness of four topical treatment methods in a rat model of superficial partial-thickness burn injury: the advantages of combining zinc-hyaluronan gel with silver foam dressing

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ABSTRACT

Background: There are several options available for conservative treatment of partial-thickness burns, however, reliable, affordable, and easily obtainable animal testing models are hard to find for the comparison of the different treatment methods. We aimed at developing a preclinical testing model and at comparing four treatment methods for superficial partial-thickness burns.
Methods: Burn injury was induced in 90 adult male Wistar rats by placing the 130°C hot tip of a commercially obtainable soldering device for 30 s on the clipped skin of the interscapular region at a steady pressure. Skin histology was studied on days 5, 10, and 22 after the induction of the burn injury, on which days, respectively, the ratio of the not epithelialized wound (%), the extent of re-epithelialization (score), and the scar thickness (μm) were assessed. We compared 4 groups: silver-sulfadiazine cream, zinc-hyaluronan gel, silver foam dressing, and the combination of zinc-hyaluronan gel with a silver foam dressing.
Results: On day 5, the induction of superficial partial-thickness burn injury was confirmed histologically in the rats. The zinc-hyaluronan gel and the combination treatment resulted in a markedly smaller ratio of the non-epithelialized area (29 ± 10% and 28 ± 13%, respectively) than silver-sulfadiazine cream (69 ± 4%; $p < 0.01$). On day 10, the extent of re-epithelialization was the lowest (~0.2) in the silver-sulfadiazine cream group, while the other 3 treatments performed significantly better. The combination treatment lead to the maximal score of 2 in all rats, which was higher than in the other 3 treatment groups. On day 22, the scar thickness was the smallest in the combination treatment group (560 ± 42 μm), which was significantly less than in the silver-sulfadiazine cream group (712 ± 38 μm; $p < 0.05$).
Conclusions: We designed and histologically confirmed a reproducible method for induction of superficial partial-thickness burns in rats for preclinical testing. In our model, the combination of zinc-hyaluronan gel with silver foam dressing was more effective than either of its components alone or than silver-sulfadiazine cream.

Introduction

A burn wound is an injury to the skin or other organic tissue primarily caused by heat or due to radiation, friction, radioactivity, electricity or contact with chemicals [1,2]. The severity of the burn is influenced by several factors such as the mechanism of the injury, the contact time, the depth and extent of the burn, the age and general condition of the injured person, as well as, by regional and socioeconomic factors [1–4].

Nowadays, annually 6 million patients seek medical treatment for burns worldwide. In the UK (with a population of more than 60 million), around 250,000 people suffer from burns each year and 300 people die because of the burn injury, which constitutes

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a serious burden on the healthcare system and economy [2–4]. In the United States (with a population of ~314 million), 1.25 million people suffer from burns every year. Of those, 450,000 receive medical treatment and 5,500 die from burns [2–4]. A systematic review found that the mortality rate of burns ranges from 1.4% to 18% across Europe [5].

Burn injuries are often classified based on the depth of the wound (i.e., superficial, partial-thickness, and full-thickness burns) and the size of the affected skin area, assessed as the percentage of total body surface area (TBSA) [6–8]. Superficial burns usually do not require hospital admission and special medical treatment, while surgical intervention is always needed for the most severe, full-thickness burns [9]. The therapeutic options and recommendations are more complex in case of partial-thickness (also called as second-degree) burns. Superficial partial-thickness (II/A or II/1) burns affect the epithelium and penetrate the papillary layer of the skin. They are characterized by moist and red surfaces, fluid-filled blisters, and severe pain upon touching. Deep partial-thickness (II/B or II/2) burns affect the deeper, reticular layer of the skin. In such injuries, the skin is usually dry, white or dull red in color, blisters may also be present, and it is relatively less painful [6,10]. While deep burns occasionally need surgery for skin grafting, superficial partial-thickness burns generally do not require surgical intervention, however, several different topical treatment options are available, which include silver-sulfadiazine cream, silver foam dressing, and zinc-hyaluronan-containing gel [11–13]. Currently, there is no gold standard topical treatment in case of partial-thickness burns. The treatment of choice is mainly based on individual experience and institutional recommendations [4,8,14]. While the different treatment options were all shown to have certain benefits for the healing of the wound, a tightly-controlled comparison of the effects of several dressings at different time points of the wound healing has not been performed, which may hinder their evidence-based recommendation. Besides the beneficial effects on wound healing, when choosing the topical treatment the need for anesthesia during dressing changes should be also taken into account. Repeated anesthesia (e.g., during regular dressing changes of a burn treatment), especially in childhood, can be associated with the impairment of cognitive functions. Neonates and infants (less than 6 months of age) who were anesthetized multiple times developed impaired cognitive functions compared to their peers who were anesthetized two times or less [15].

In the present study, our goal was to develop an adequate testing model and to compare the effects of four common topical treatment methods on superficial partial-thickness burns at three different time points (viz., 5, 10, and 22 days post-injury) of the wound healing.

Materials and methods

Animals

The experiments were performed in 90 adult male Wistar rats. The animals were housed in standard plastic cages kept in a room with ambient temperature maintained at 21–23°C and humidity at 30–40%. The room was on a 12 h light-dark cycle (lights on at 5:00 a.m.). Standard rodent chow and tap water were available ad libitum. At the time of the experiments, the rats weighed 298-466 g. All procedures were conducted under protocols approved by the Institutional Animal Use and Care Committee of the University of Pecs (registration no.: BA02/2000–15/2018, approved on 18 April 2018) and were in accordance with the directives of the National Ethical Council for Animal Research and those of the European Communities Council (86/609/EEC).

Induction of superficial partial-thickness burn injury

Rats were anesthetized with the intraperitoneal administration of a ketamine-xylazine cocktail [78 mg/kg (Calypsol; Gedeon Richter Plc., Budapest, Hungary) and 13 mg/kg (Sedaxylan; Eurovet Animal Health B.V., Bladel, The Netherlands), respectively]. The fur on the nape was clipped in a 3 × 3 cm area, and then the rat was placed in a ventral position on a surgery board.

The burn injury was induced with a soldering device (model Industa HF-5100; Stannol Inc., Velbert, Germany) in the center of the clipped skin area. The handpiece of the device weighed 46 g and it had a wedge-shaped iron tip with a 4 × 4 mm flat surface on each side (model M-4,2-HF; Stannol Inc., Velbert, Germany). Based on our literature search, contact burn injuries were induced in experimental models by heating the tip of the device to 60-200°C and keeping it in direct contact with the skin for 2-60 seconds [16–27]. In our experiments, we heated the device to 130°C and touched it to the skin of the rats for 30 seconds. To minimize the variability of the impact, the full (4 × 4 mm) flat side of the tip of the handpiece was steadily held by hand in direct contact with the skin without applying extra pushing or pulling force.

Treatment groups

The rats were assigned to one of four treatment groups: silver-sulfadiazine cream (Dermazin; LEK Pharmaceuticals, Ljubljana, Slovenia); silver foam dressing (Aquacel Ag; ConvaTec Ltd., Deeside, UK); zinc-hyaluronan gel (Curiosa; Gedeon Richter Plc., Budapest, Hungary); and the combination of zinc-hyaluronan gel with silver foam dressing. In all groups, the wound was covered with a cohesive conforming bandage (Peha-haft; Paul Hartmann AG, Heidenheim, Germany) and a perforated plastic sheet. The latter was needed to prevent the animal from scratching the wound and removing the bandage, while also maintaining the ventilation of the wound.

Tissue sample collection and histology

The sample collection was conducted 5, 10, and 22 days after the induction of the burn injury. On the day of the sample collection, the rat was anesthetized with intraperitoneal administration of ketamine and xylazine (for doses, see section 2.2). The bandage was removed from the nape, and then the wound was excised in the center of a 2 cm × 2 cm area (in toto). The excised tissue samples contained all layers of the skin and part of the underlying muscular layer. After the removal of the tissue sample, the rat was euthanized with an intraperitoneal injection of sodium thiopental [400 mg/kg (Tiobarbital; B. Braun Medical S.A., Barcelona, Spain)].

The removed tissue samples were immediately placed in 10% buffered formalin. After 48 hours of fixation, the biopsy has been serially sectioned and submitted entirely for histopathological examination (5-8 slices). The tissue samples were dehydrated in a graded series of ethanol solutions, embedded in paraffin, and cut into approximately 3-μm sections. The hematoxylin and eosin staining was performed according to routine procedure by a Leica ST 4040 linear automatic stainer (Leica Microsystems GmbH, Wetzlar, Germany). Histological changes were evaluated under a light microscope (DM500; Leica Microsystems GmbH, Wetzlar, Germany). The sections were evaluated by an expert pathologist blinded to the treatment of the rat.

Histological evaluations included the assessment of the degree of re-epithelialization and final wound contraction. On day 5, the ratio of the unhealed burned surface was calculated as the percentage of the not epithelialized distance compared to the total length of the wound (Fig. 1). On day 10, the re-epithelialization

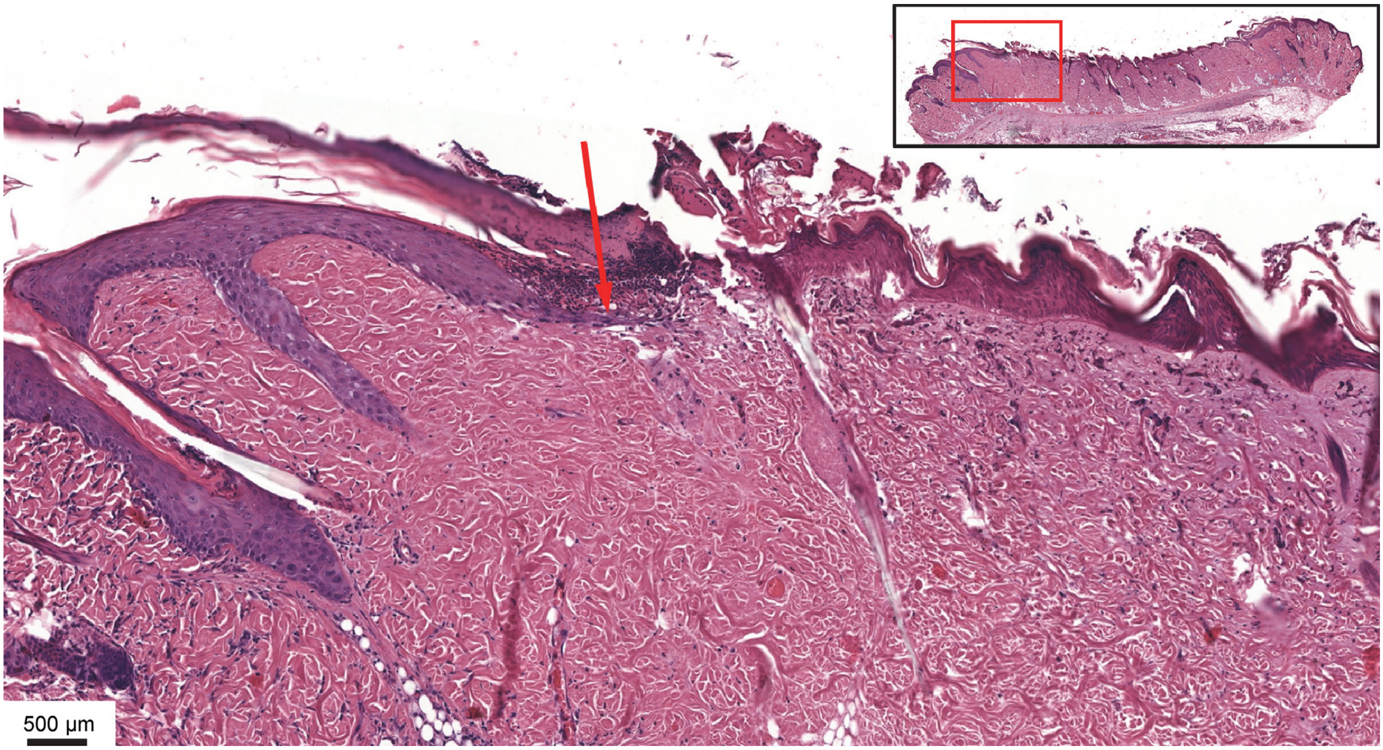

Fig. 1. Representative photomicrograph of the excised skin tissue on day 5 post-burn injury. The extent of the re-epithelialization was assessed by measuring the distance from the edge of the wound to the furthest newly formed keratinocyte (arrow). For orientation purposes, the insert on top right shows the whole tissue section with a red box indicating the magnified area.

of the wound was evaluated by a 3-score system (0: no re-epithelialization; 1: partial re-epithelialization; and 2: complete re-epithelialization that is multiple epithelial layers over the entire length of the wound) (Fig. 2). On day 22, the scar thickness was measured as the distance between the basal cell layer of the epidermis and the lowest cell layer of the dermis (Fig. 3).

Statistical analysis

Data on unhealed wound percentage, scores of re-epithelialization, and scar thickness were compared by one-way ANOVA. As in previous studies [28,29], ANOVA was followed by the Fisher's LSD post hoc test. Sigmaplot 11.0 (Systat Software, San Jose, CA, USA) software was used for statistical analyses. Differences were considered significant when $p < 0.05$. All data are reported as mean ± standrard error (SE).

Results

Percentage of the non-epithelialized wound area on day 5 post-burn injury

Five days after the induction of the burn injury, the depth of the burn and the ratio of the not epithelialized wound surface to the whole wound diameter was analyzed histologically. In all of the studied rats, it was confirmed that the applied method for burn induction resulted in a superficial partial-thickness burn injury (also see Fig. 4, for a representative photo).

When we compared the four used treatment options (viz., silver-sulfadiazine cream, silver foam dressing, zinc-hyaluronan gel, and the combination of zinc-hyaluronan gel with silver foam dressing), we found that the treatment had a significant effect on the wound healing [ANOVA, $F_{(3,26)} = 4.837$, $p = 0.08$], as assessed on day 5 by the percentage of the not epithelialized surface to the whole diameter of the burn (for an example, see Fig. 4). With post hoc analysis, we found that the zinc-hyaluronan gel and the combination treatment resulted in significantly smaller ratio of the not epithelialized area (29 ± 10% and 28 ± 13%, respectively) than the silver-sulfadiazine cream (69 ± 4%; $p < 0.01$ compared to both) (Fig. 5). The not epithelialized area tended to be decreased (47 ± 8%) also with silver foam treatment as compared to silver-sulfadiazine, but the difference did not reach the level of significance ($p = 0.080$).

Extent of wound re-epithelializatzion on day 10 post-burn injury

By day 10 after the induction of the burn injury, the whole wound diameter was to some extent epithelialized in the majority of the rats (in 20 of the 28 animals), therefore we used a simple scoring system to assess the healing of the wound. If at least some part of the wound was not re-epithelialized the score was 0; if the entire wound was re-epithelialized but only partially (i.e., in a single layer) the score was 1; if the wound was closed completely in multiple layers then the score was 2.

The effect of the treatment was significant on the re-epithelialization score [ANOVA, $F_{(3,24)} = 13.868$, $p < 0.001$]. We found that the extent of re-epithelialization was the lowest (0.2 ± 0.2) in the silver-sulfadiazine cream group, while the other 3 treatments performed significantly better than that with scores of 1.0 ± 0.2 for silver foam ($p = 0.008$), 1.0 ± 0.4 for zinc-hyaluronan (0.012), and 2.0 ± 0.0 for the combination treatment ($p < 0.001$) (Fig. 6). It should be noted that the combination treatment lead to the maximal score of 2 in all rats, which was higher than the scores in the other treatment groups ($p < 0.001$ vs. silver foam and $p = 0.002$ vs. zinc-hyaluronan).

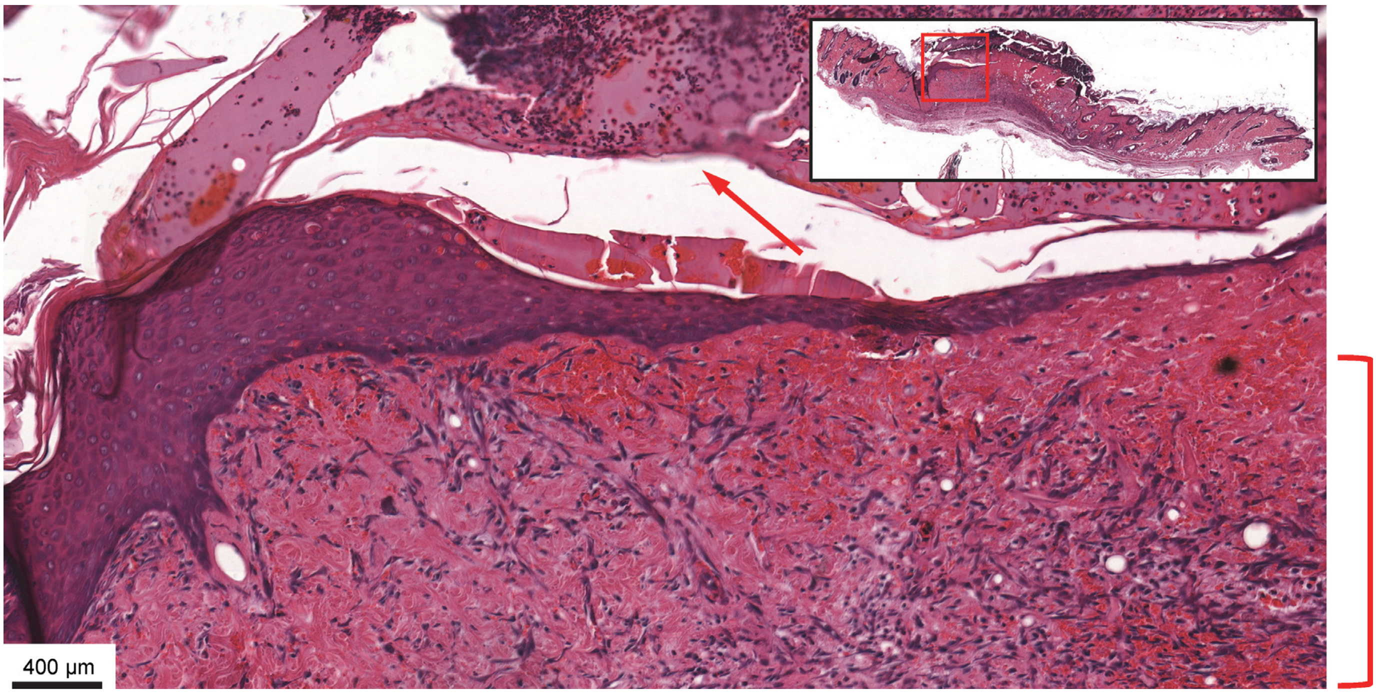

Fig. 2. Representative photomicrograph of the partially re-epithelialized skin tissue on day 10 post-burn injury. Loose granulation tissue (indicated by the red bracket) is present beneath the re-epithelialized surface, which contains newly-forming capillaries and plump fibroblasts. Scab is attached to the re-epithelialized surface from the top (arrow). For orientation purposes, the insert on top right shows the whole tissue section with a red box indicating the magnified area.

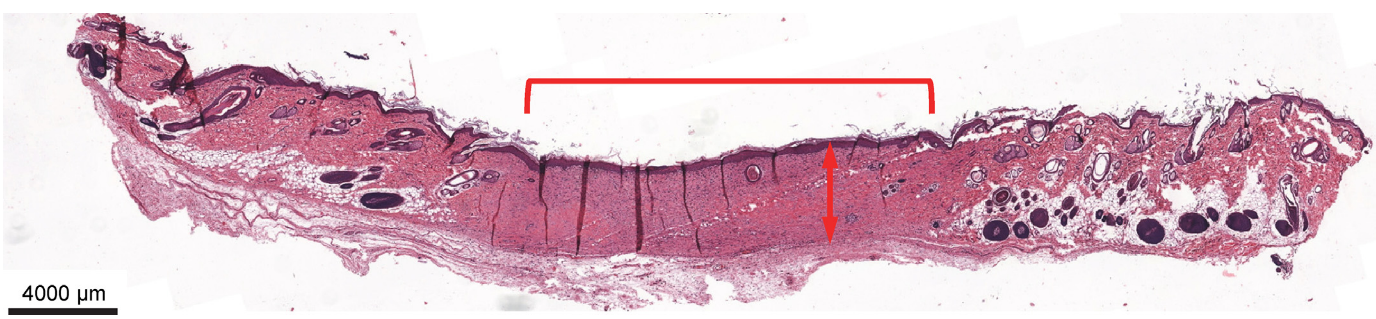

Fig. 3. Representative photomicrograph of the completely healed skin tissue on day 22 post-burn injury. A slightly more cellular dermis and the lack of adnexal structures are present in the center of the skin section (indicated by the red bracket) as remnants of the prevoius burn. The scar thickness of the burn wound is shown by the arrow.

Scar thickness of the wound on day 22 post-burn injury

On day 22, the burn wounds were already completely re-epithelialized in all of the studied rats. Therefore, to further evaluate the healing process, we analyzed if there is a difference in the scar thickness among the treatment groups, because an increased scar thickness can be an indicator of hyperthrophic scarring, which remains a major challenge following burn injury [30].

We found that the scar thickness was the smallest in the combination treatment group (560 ± 42 μm), which was significantly less than in the silver-sulfadiazine cream group (712 ± 38 μm; $p = 0.024$) (Fig. 7).

For macroscopic visualization of the burned skin, digital photographs of the burn wound in randomly selected rats at the beginning and at the end of the experiment are shown in Fig. 8.

Discussion

In the present study, we introduce a novel, accessible rat model of superficial partial-thickness burn injury and evaluation of the wound healing, which can be used for the preclinical testing of different treatment options. In this model, we compared the effects of four treatments on different indicators of the wound healing and showed that the combination of zinc-hyaluronan gel with silver foam dressing was the most advantageous compared to the other treatments, while silver foam or zinc-hyaluronan alone was superior to silver-sulfadiazine cream.

Different experimental designs were used to study the pathomechanism and therapeutic options in burns [31,32], however an easily accessible and reproducible, cost-effective, in vivo animal model for preclinical studies remains to be established. In the current study, we developed a rat model of superficial partial-thickness burns, which fulfills the listed criteria. By applying standardized preparations (adult male Wistar rats, nape skin, anesthesia, shaving, disinfection), burning methods (commercially available soldering device with 4 × 4 mm flat surface on the iron tip, 130°C heating, 30 s contact time, and steady pressure), as well as post-intervention procedures (covering the wound with a cohesive conforming bandage and a perforated plastic sheet), we were able to reliably reproduce histologically confirmed superficial partial-thickness burn wounds that penetrated into the dermo-epidermal papillary region of the skin but did not extend to deeper layers.

The rat – as a widely available, affordable experimental model – was already used previously for the study of burns [16,17,19–

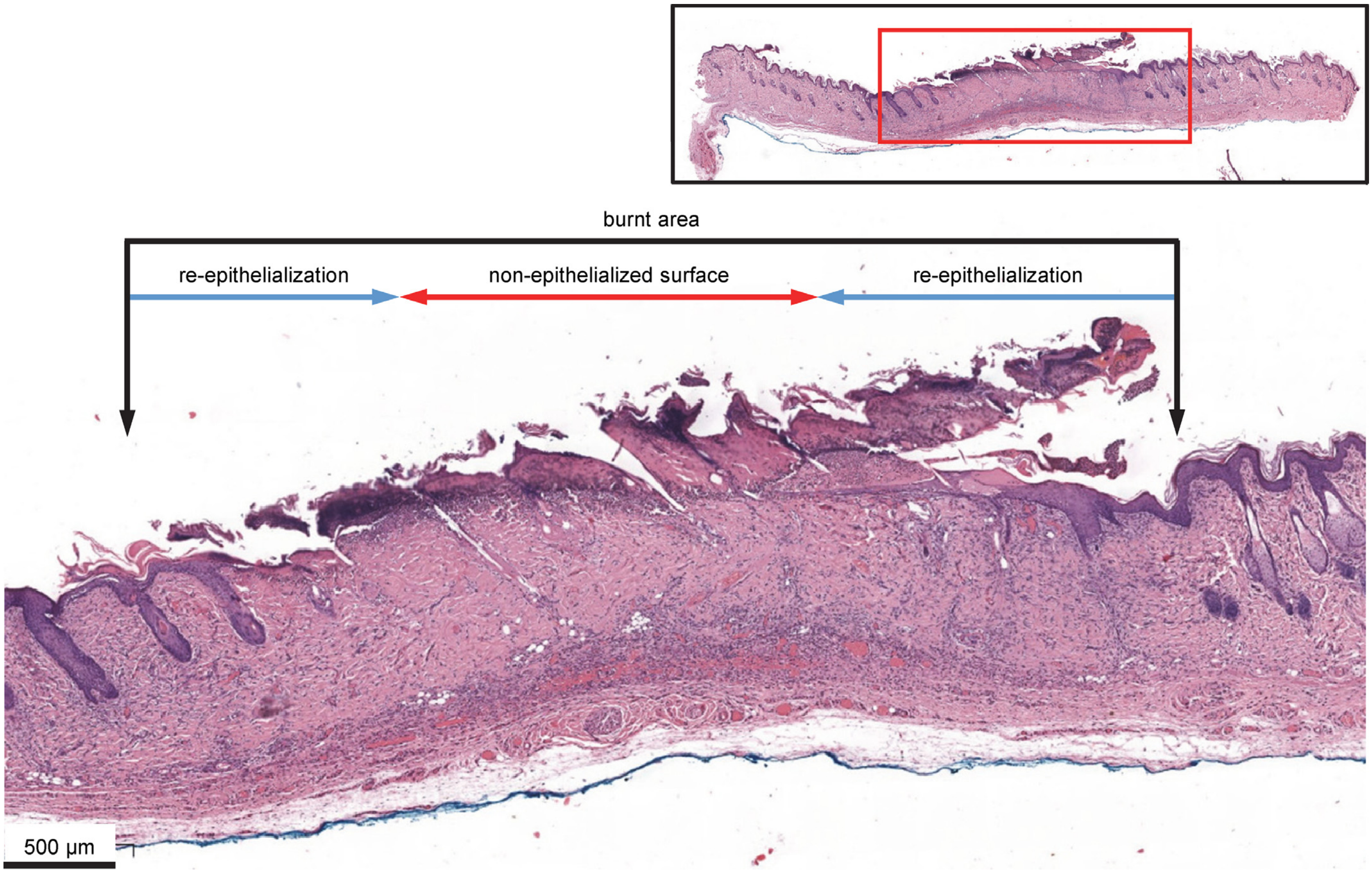

Fig. 4. The ratio of the not epithelialized surface and the burn wound diameter was calculatued to assess the healing of the burn on day 5 post-injury. The diameter of the entire burn wound, as well as, the epithelial invasion from both sides, and the not epithelialized surface are marked with black, blue, and red arrows, respectively. For orientation purposes, the insert on top right shows the whole tissue section with a red box indicating the magnified area.

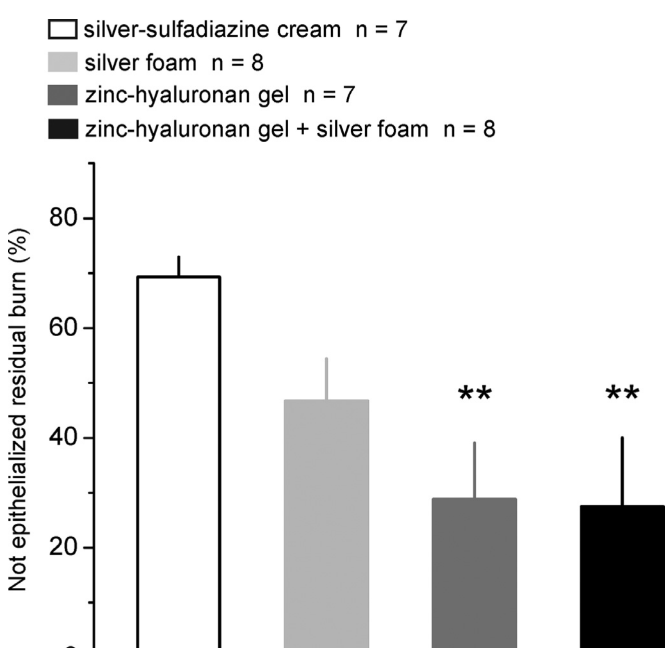

Fig. 5. The percentage of the open (not epithelialized) surface compared to the whole diameter of the burn wound 5 days after the induction of the burn injury in rats (treatment groups and number of animals are indicated). $^{**}p < 0.01$ vs. silver-sulfadiazine cream as determined by one-way ANOVA followed with Fisher LSD test. Data are presented as mean ± SE.

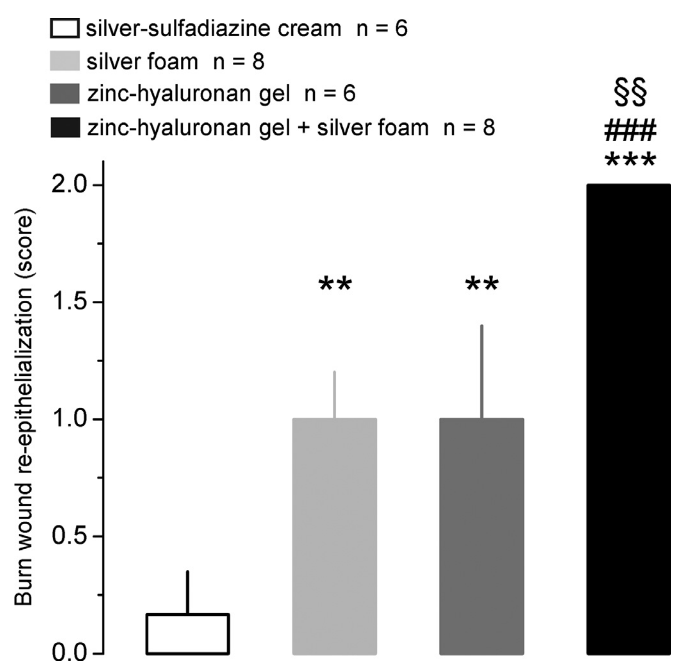

Fig. 6. The score for the re-epithelialization of the burn wound 10 days after the induction of the burn injury in rats (treatment groups and number of animals are indicated). 0: no re-epithelialization; 1: partial re-epithelialization; and 2: complete re-epithelialization in multiple layers over the entire length of the wound. $^{**}p < 0.01$ and $^{***}p < 0.001$ vs. silver-sulfadiazine cream; $^{###}p < 0.001$ vs. silver foam dressing; $^{§§}p < 0.01$ vs. zinc-hyaluronan gel as determined by one-way ANOVA followed with Fisher LSD test. Data are presented as mean ± SE.

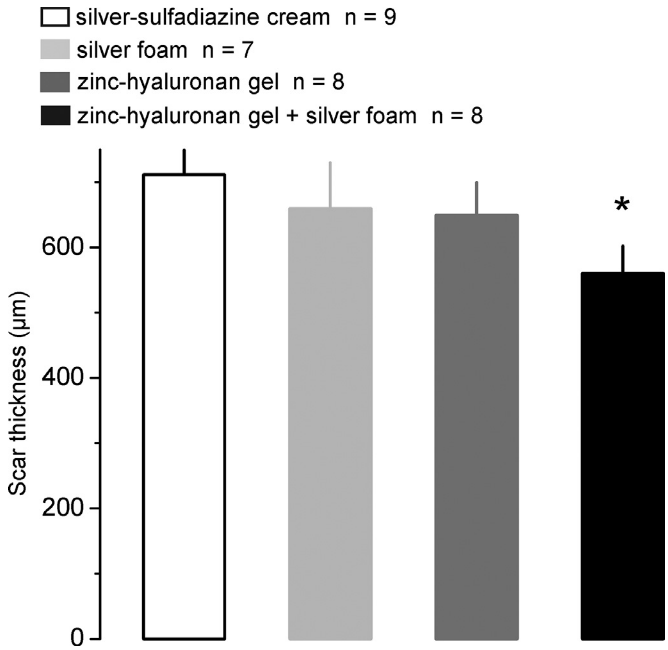

Fig. 7. The scar thickness of the burn wound 22 days after the induction of the burn injury in rats (treatment groups and number of animals are indicated). $^{*}p < 0.05$ vs. silver-sulfadiazine cream as determined by one-way ANOVA followed with Fisher LSD test. Data are presented as mean ± SE.

21,23]. Among those studies, only two reported the successful induction of superficial partial-thickness burns [21,23], while in the others the depth of the burn was deep partial-thickness [16,19], full-thickness [17], or unknown [20]. However, the device used for the induction of superficial partial-thickness burns in both earlier studies was manufactured or modified by the authors [21,23], which limits their widespread accessibility. In our current study, for the first time to our knowledge, we used a commercially available soldering device without any modifications, and described how it was used for the induction of burns, which enables its application for scientific research worldwide. It should be noted, however, that although the rat skin is also composed of the major layers (epidermis, dermis) as the human skin, it does not perfectly mimic the human skin architecture because of its unique skin morphology [31]. Therefore, despite the use of rats for burn research in the present and in previous studies [16,17,19–21,23], care should be taken when translating the results obtained in rats for human applications. Nevertheless, the developed model can be very well applied to study burn treatment options that are already available for human patients, but, to our knowledge, their parallel comparison under standardized circumstances (i.e., in a unified model) has not been reported.

In superficial partial-thickness burns, conservative therapy is the primary choice, while surgical interventions are usually not required [13]. In the case of conservative treatment, it is crucial to rinse the wound with a disinfecting agent prior to removing the dead tissue. This process, called as debridement, is considerably painful, hence analgesic and anxiolytic drugs or general anesthesia are often administered. During the healing of the wound, epithelial cells originating in the remaining epithelial appendages (e.g., the lining of sebaceous and sweat gland ducts) travel from the uninjured to the damaged areas to begin the healing process [33]. One of the major aims of conservative treatments is to facilitate the epithelialization process and thereby promote the healing of the wound [13]. Conservative treatments can be used effectively in superficial partial-thickness burns, as they involve the covering of the affected areas in order to maintain a moist environment, as well as, the delivery of antimicrobial compounds, which prevent the infection and progression (i.e., deeper penetration) of the burn wounds. Several conservative treatment methods can be used [11,12], but direct, parallel comparisons of the treatments under standardized conditions are scarce, hence the results of different trials can be compared indirectly by meta-analyses, which are, however, hindered by the methodological quality and heterogeneity of the analyzed studies [34,35].

In the present study we selected four treatment options (see below) and compared their effects on different parameters of wound healing in our newly developed rat model of superficial partial-thickness burns.

I) Silver-sulfadiazine (e.g., Dermazin) evokes antibacterial effects and promotes re-epithelialization; its low cost and easy application contributes to its widespread use in clinical practice, which also explains why it could be used as a comparator treatment in previous studies [35–37]. However, its use requires daily dressing changes and creates a yellowish plaque on the burn, which makes the assessement of burn depth difficult [36,38,39].

II) Hydrofiber (e.g., Aquacel Ag foam) is a newer dressing type, which contains an external polyurethane waterproof film layer that surrounds a multilayer absorbent surface with a silver ion content of 1.2 %. The multilayer cushion contains a foam sheet and a plate with hydrofiber technology. Absorption of the wound discharge leads to the gelification of the hydrofiber layer, which helps to keep the wound moist, and promotes wound healing while also preventing infections. The bandage is comfortable and its removal painless, without requiring anesthesia [37,40].

III) Zinc-hyaluronan gel (e.g., Curiosa) helps to maintain a moist environment due to the large molecular weight and negative charge of the hyaluronan content, which also facilitates the healing process and reduces pain in second-degree burn injury [41]. The addition of zinc contributes with an anti-inflammatory and antimicrobial effect, which makes it a suitable alternative for topical wound care therapy [42].

IV) The combination of of zinc-hyaluronan gel with silver foam dressing, which was found to perform better than other conservative methods in previous clinical trials [6,10].

In our experiments, we found that silver-sulfadiazine was less beneficial than the combination treatment at all 3 evaluation points, than zinc-hyaluronan on days 5 and 10, and than silver foam on day 10. The combination treatment performed better than the other 3 interventions on day 10 and it was the only method that caused a significant decrease in scar formation on day 22 compared to silver-sulfadiazine. These results are in accordance with previous studies that question the routine use of silver-sulfadiazine in the modern treatment of burn injuries [34,35]. Moreover, our findings highlight that newer treatment options such as silver foam dressing and zinc-hyaluronan or the combination of them can results in improved burn wound healing compared to silver-sulfadiazine. The mechanism by which the combination treatment was superior compared to silver foam dressing or zinc-hyaluronan alone remains subject to future studies, but it can be suggested that the simultaneous presence of silver and zinc ions in the dressing exerts additional advantageous effects on wound healing as compared to the two components alone. Indeed, the combination of silver and zinc resulted in enhanced antibacterial effect [43–45], anti-inflammatory and antioxidant responses [46], as well as improved wound healing, re-epithelialization, and collagen deposition when used in vivo as a dressing for mechanical (not burn) wounds [44,46].

In conclusion, we developed an easily accessible rat model for the study of superficial partial-thickness burn injuries. We showed

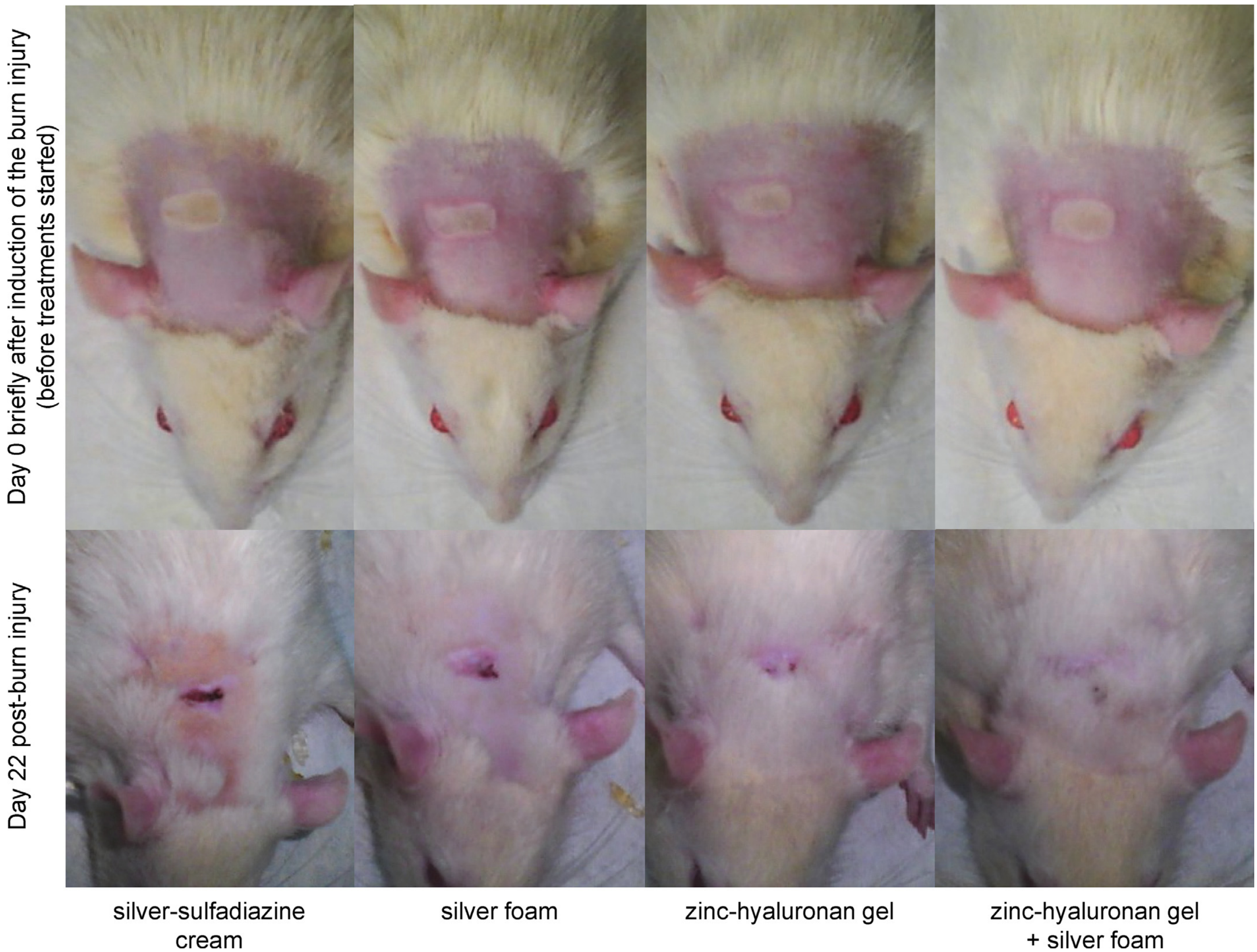

Fig. 8. Digital photographs of four randomly selected rats on day 0 briefly after the induction of the burn wound before any of the treatments were applied (top row) and a picture of one randomly selected rat from each treatment group at the end of the experiment on day 22 post-burn injury (bottom row).

that this model is feasible for the preclinical testing of different treatment options by comparing four treatment methods. Among the studied treatments, the combination of silver foam dressing and zinc-hyaluronan was superior compared to the other methods as assessed by different parameters of wound healing.

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Conflict of interest

The authors do not have any conflict of interest to declare.

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Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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RESEARCH ARTICLE

Systemic antibiotic prophylaxis does not affect infectious complications in pediatric burn injury: A meta-analysis

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Abstract

In pediatric burns the use of systemic antibiotic prophylaxis is a standard procedure in some burn centers, though its beneficial effect on the infectious complications is debated. The present meta-analysis aimed at determining whether systemic antibiotic prophylaxis prevents infectious complications in pediatric patients with burn injuries. We searched the PubMed, EMBASE, and Cochrane Library databases from inception to August 2019. We included 6 studies, in which event rates of infectious complications were reported in children with burn injuries receiving or not receiving systemic antibiotic prophylaxis. We found that the overall odds ratio (OR) of developing an infection (including local and systemic) was not different between the groups (OR = 1.35; 95% CI, 0.44, 4.18). The chances for systemic infectious complications alone were also not different between antibiotic-treated and non-treated patients (OR = 0.74; 95% CI, 0.38, 1.45). Based on the age, affected total body surface area, and country income level, we did not find any subgroup that benefited from the prophylaxis. Our findings provide quantitative evidence for the inefficacy of systemic antibiotic prophylaxis in preventing infections in pediatric burns. To validate our conclusion, multinational, randomized trials in a diverse population of children with burn injuries are warranted.

Introduction

Burn injuries in children constitute a major challenge for health care. The incidence and mortality rates of burns show a declining trend worldwide, mainly due to the decreased rates in highly developed countries [1], but several reports indicate an increasing incidence rate of

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burns in children in well-developed countries like Finland [2], the Netherlands [3], and the Czech Republic [4]. Children accounted for nearly 50% of the population with severe burn injuries in an analysis of studies from 22 European countries, which included data from more than 186,500 patients [5]. The majority of childhood burns occurred in children younger than 5 years of age [5]. In the US, burns were the third leading cause of unintentional injury and death for 1 to 9 year-old children in 2006 [6]. Of 1,559 injured children in low income countries in 2007, burns were also the third most frequent (13%) cause of injuries and had the highest (79%) admission rate among all types of unintentional injuries [7]. According to a recent global estimate, the overall child burn mortality is 2.5 per 100,000, and it is negatively correlated with the economic level of the country, being as high as 9.5 per 100,000 in low income countries such as Mongolia, Rwanda, and Togo [8]. The highest fire-related death rates occur in children younger than four years of age [6].

Burns can be caused by extreme heat (e.g., hot surfaces, fluids, and flame), chemicals, electricity, friction or radiation. Scald burns are the most frequent type of thermal injuries in children under the age of 5 years [5, 6], while between 5 and 16 years of age flame burns are most common [6]. The severity of the burn injury is influenced by several factors, including the nature and duration of the exposure, age and premorbid health and wealth conditions of the child, as well as regional and socioeconomic factors [6, 9]. Burns are classified based on the extent of the damage to the skin layers (depth of burns) and the size of affected skin area, assessed as percentage of total body surface area (TBSA) [10].

Infections, including wound, respiratory, and urinary tract infections, as well as those associated with sepsis, are among the most common complications of burns [11]. In pediatric patients, sepsis is a leading cause of mortality after burn injury, accounting for up to 54% of deaths [12, 13]. Burn wound infections and subsequently sepsis can occur in patients with partial-thickness or full-thickness burn injuries [11]; deeper burns present higher risk for infections [11, 14]. Despite these alarming data, there are no firm rules or guidelines for prophylactic, systemic antibiotic administration in pediatric patients with burn injury. It is recommended that systemic antibiotic administration should be reserved for cases with clear evidence of infection [10], but about 60% of the burn centers in the UK did not have a formal policy on the use of antibiotics, and there was no consensus on antibiotic prophylaxis, according to a study published in 1995 [15]. A more current survey revealed that standard operating procedures were implemented in less than half of UK burn units [16], and a recent study showed notable variations in guideline use for diagnosing and managing infections in pediatric burns [17]. Inappropriate use of antibiotics in burn injuries can increase the chance for complications [18] and result in antibiotic resistance [19], hence it can raise the cost of healthcare to both patients and the community [20]. The present meta-analysis of published clinical trials aims at determining whether systemic antibiotic prophylaxis improves the outcome of pediatric burn injuries. Similar analyses were performed in adult burn patients [21, 22] and helped to form guidelines [23].

Materials and methods

Search strategy

The meta-analysis was conducted as described in our recent studies [24, 25]. In brief, we followed the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols [26] (Table A in S1 File). The question of our analysis was formulated with the Participants, Intervention, Comparison, Outcome (PICO) model: in children with burn injury, we aimed to assess the effect of systemic antibiotic prophylaxis on infectious complications. This meta-analysis has been registered with PROSPERO International Prospective Register of Systematic Reviews (registration number: CRD42018102498).

We searched the PubMed, EMBASE, and Cochrane Library databases for relevant articles from inception to August 2019 with the following query: "(antibiotic* OR antimicrobial*) AND (prophylaxis OR prophylactic) AND (burn* OR scald OR flame) AND (pediatric* OR child*)". Search results were filtered for human studies. The search was conducted separately by two authors (AC, GJ), who also assessed study eligibility and extracted data from the selected studies independently. Disagreements were resolved by consensus, if needed, with the help of a third party (AG). As a specific example for the search, in the EMBASE database, which identified the highest number of articles, the term "(antibiotic* OR antimicrobial*) AND (prophylaxis OR prophylactic) AND (burn* OR scald OR flame) AND (pediatric* OR child*)" was entered and retrieved 230 records, which decreased to 213 studies after the "humans" filter was selected.

Study selection and data extraction

After screening of the titles and abstracts of the publications identified with the literature search, the full texts of potentially eligible articles were obtained. We included studies which compared event rates of systemic and local complications of burns between children receiving and not receiving systemic antibiotic prophylaxis. Antibiotic prophylaxis was defined as systemic antibacterial drug administration to patients without confirmed infection and systemic inflammatory signs. Wound infection was considered as local complication, while systemic complications included sepsis and suspected toxic shock syndrome.

From the included studies, we extracted the country of origin, characteristics of the patient population (sample size, age, TBSA), and complication events in the different treatment groups (i.e., with or without systemic antibiotic prophylaxis) of children with burn injuries.

To evaluate the quality of the studies included in the meta-analysis, two independent reviewers (AC and BT) assessed the bias of a randomized controlled trial according to the Cochrane Risk of Bias Tool for Randomized Controlled studies [27] (Table B in S1 File), while the quality of other study types was assessed by using the Newcastle-Ottawa Scale [28] (Table C in S1 File).

Statistical analysis

The statistical analysis was performed according to the standard methods of meta-analysis. Patients were grouped as either treated with systemic antibiotic prophylaxis or not. Pooled odds ratio (OR) with 95% confidence intervals (CI) for infectious complications in pediatric patients with burn injuries were calculated for the dichotomous outcomes. In all forest plots, we applied the random-effect model with DerSimonian-Laird estimation. The OR was calculated by dividing the ratio of events to no events in the antibiotic-treated group with the same ratio in the group without systemic antibiotic prophylaxis. Statistical heterogeneity was determined by the I^2 statistical test ($P<0.1$ indicating significant heterogeneity), while publication bias was assessed by the visual inspection of funnel plots (Figs A and B in S1 File), as described elsewhere [24, 25]. Heterogeneity in clinical outcomes was explored by creating different subgroups (age, income, TBSA, type of complication). Sensitivity analysis (i.e., iteratively removing one study from the analyses and recalculating OR to investigate the impact of each individual study on the summary estimate) showed no difference in the final pooled results. The analyses were performed using the Stata 11 SE software (StataCorp LLC, College Station, TX, USA).

Results

Study selection

Fig 1 presents the flow chart of the study selection. Until August 2019 the electronic literature search identified altogether 432 human studies from the PubMed, EMBASE, and Cochrane Library databases. After removing duplicates, 349 articles remained, which were screened on title and abstract for inclusion criteria. Full texts of 41 articles were reviewed and, in the end, 6 publications were found eligible for statistical analysis [18, 29–33], which included data from a total of 1,735 patients. The descriptive characteristics of these studies are shown in Table D in S1 File.

Effects of systemic antibiotic prophylaxis on local and systemic infectious complications in children with burn injuries

First, we analyzed whether systemic antibiotic prophylaxis has an effect on the OR for either local or systemic infectious complications. Studies which separately reported the event rates of local [18, 33] or systemic complications [18, 30, 31] were included in the forest plot (Fig 2). Prophylactic administration of systemic antibiotics did not cause a significant change in the odds for systemic infections (OR = 0.74; 95% CI, 0.38, 1.45). With regards to local complications, the use of antibiotics did not have a significant effect in either of the two included studies, however, their averaged result (OR = 0.99; 95% CI, 0.40, 2.47) should be taken with scrutiny due to the low number of studies in this subgroup. The odds of all infectious complications (i.e., both systemic and local) was also not significantly different between the antibiotic-treated and non-treated groups (OR = 0.82; 95% CI, 0.48, 1.40) (Fig 2).

Chance for infectious complications in different subgroups of pediatric burn patients treated or not treated with systemic antibiotic prophylaxis

Next, we divided the studies into different subgroups according to the known risk factors of the outcome of burns and data availability. Unlike in the first forest plot (Fig 2), where systemic and local complications were distinguished from each other, in the remaining part of our meta-analysis we considered all (i.e., both local and systemic) complications together as the outcome. Using the combined rate of complications allowed us to include two studies in the analysis, in which the separate event rates of local and systemic complications were not reported [29, 32]. Merging the rates of local and systemic complications looked rational, for we did not find a significant difference in the OR between systemic and local complications (Fig 2).

Based on the age range of the patient populations, the studies were assigned to either of two subgroups: limited to children only, viz., under 10 years of age [29, 30, 32], or also including adolescents up to the age of 16 years [18, 31, 33]. It should be mentioned that if the electronic search was expanded to children and adolescents, the number of eligible studies for quantitative analysis did not increase. Systemic antibiotic prophylaxis did not change the chance for complications in either of the age groups (Fig 3). The OR in the younger (children only) group was 1.75 (95% CI, 0.24, 13.09), while in the older group, which also included adolescents, it was 1.19 (95% CI, 0.44, 3.19). Antibiotic administration did not have any effect on the odds for infections when all 6 studies in the forest plot were combined (OR = 1.35, 95% CI, 0.44, 4.18) (Fig 3).

Based on the mean TBSA affected by the burns, the studies were divided into subgroups of less than 20% [18, 30, 31, 33] and more than 20% of injured TBSA [29, 32]. The reported values of TBSA are included in Table D in S1 File for each study. We did not find a significant effect

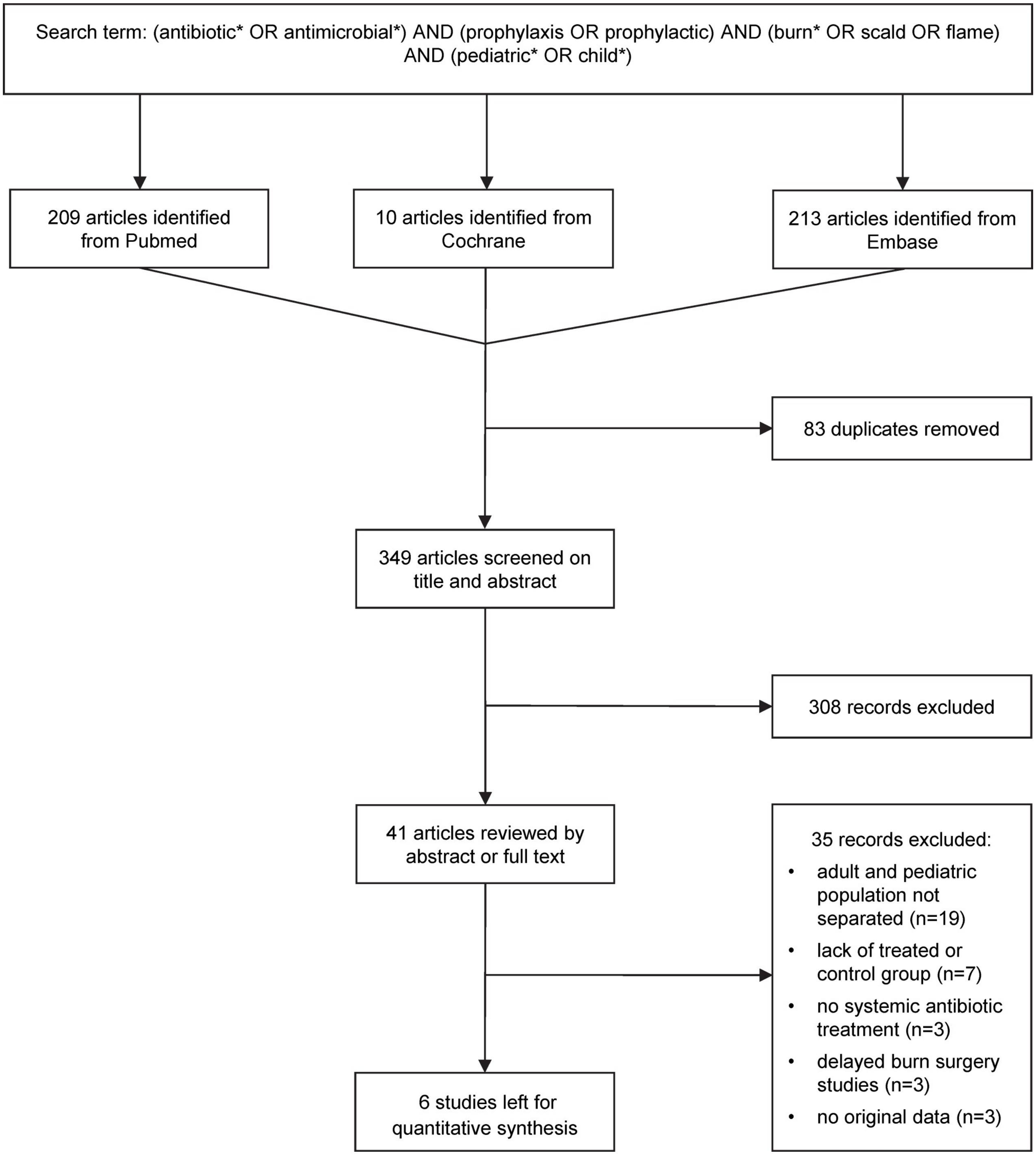

Fig 1. Flow chart of study selection and inclusion.

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of systemic antibiotic prophylaxis on the chance of infectious complications in the subgroup consisting of studies with less than 20% affected TBSA (OR = 0.84, 95% CI, 0.37, 1.91) (Fig 4). Though the OR was also not significant in the subgroup with more than 20% of injured TBSA, this group included only 2 studies, which is not sufficient for proper meta-analysis.

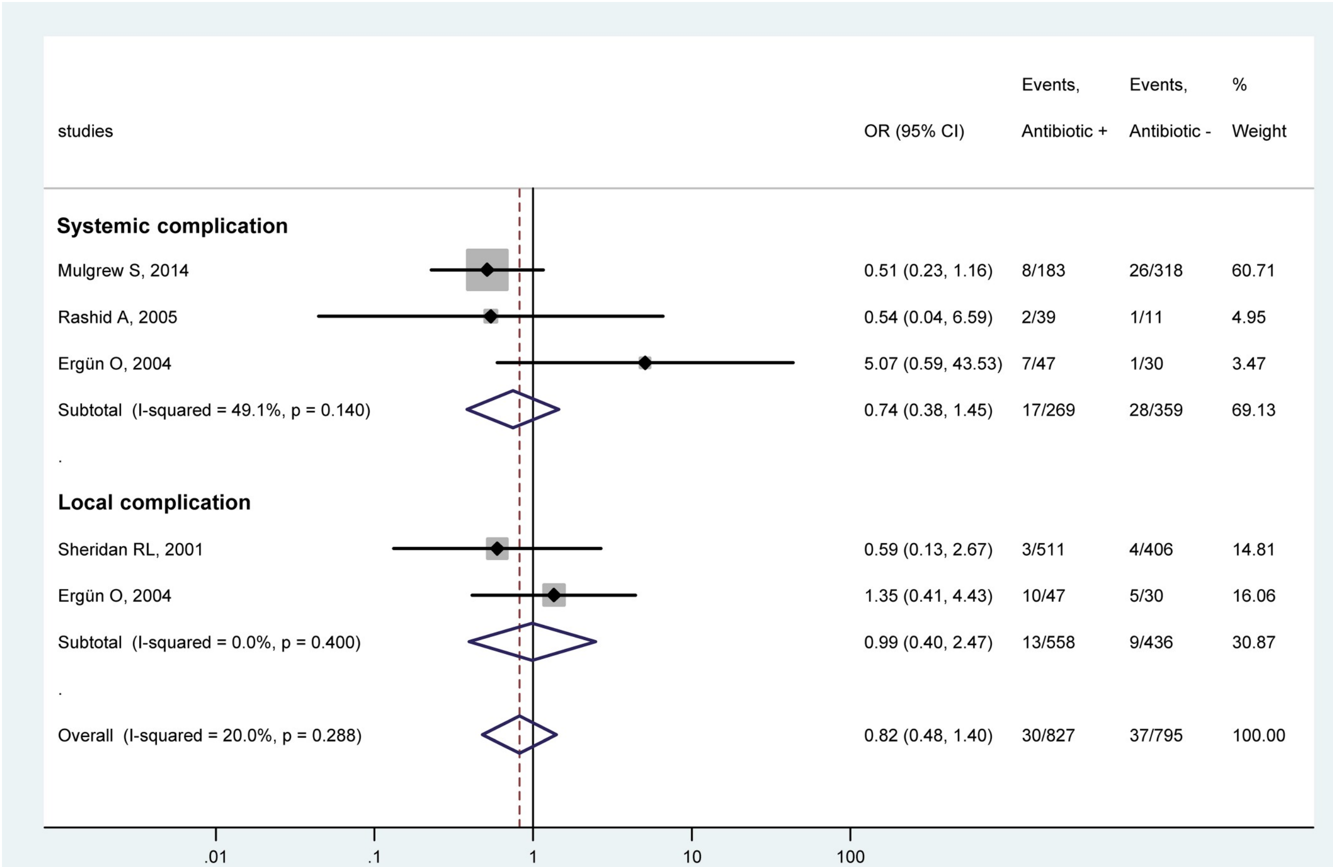

Fig 2. Forest plot of the odds ratios (ORs) for systemic and local subgroups of infectious complications in pediatric patients with burn injuries who received systemic antibiotic prophylaxis compared to those who did not. Here, and in Figs 3–5, black circles represent the OR for each study, while the left and right horizontal arms of the circles indicate the corresponding 95% confidence intervals (CI) for the OR. The size of the gray box is proportional to the sample size of the study; bigger box represents larger sample size, thus bigger relative weight of the study, and vice versa. The diamonds represent the average OR calculated from the ORs of the individual studies in a subgroup (top and middle) and in all studies (bottom). The left and right vertices of the diamonds represent the 95% CI of the average ORs. The vertical dashed line is determined by the low and top vertices of the bottom diamond and indicates the value of the average OR of all studies in the forest plot. An OR lesser than 1 indicates that the use of systemic antibiotic prophylaxis decreased the chance for infectious complications, whereas an OR higher than 1 indicates an increased chance for infections in the antibiotic-treated children.

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Regarding the economic status of the country of the studies, the studies were divided into high-income [30–33] and middle-income subgroups [18, 29] according to classification of the countries in the World Bank Data. Our analysis showed no significant effect of antibiotic prophylaxis on the chance for infections in either of the subgroups. The OR in the high-income subgroup was 1.35 (95% CI, 0.21, 8.77) (Fig 5). The use of antibiotics was also without an effect in either of 2 studies in middle-income countries (Fig 5), but caution is needed regarding their averaged OR due to the low number of studies in this subgroup.

Discussion

In the present study, we show that systemic antibiotic prophylaxis has no beneficial effects on the risk for infectious complications in pediatric burn injuries. By analyzing data of a total of 1,735 patients, we found that no patient subgroup (based on the age, injured TBSA or country income) benefited (in regards to odds for infectious complications) from receiving prophylactic antibiotic treatment, as compared to burn patients without antibiotic treatment.

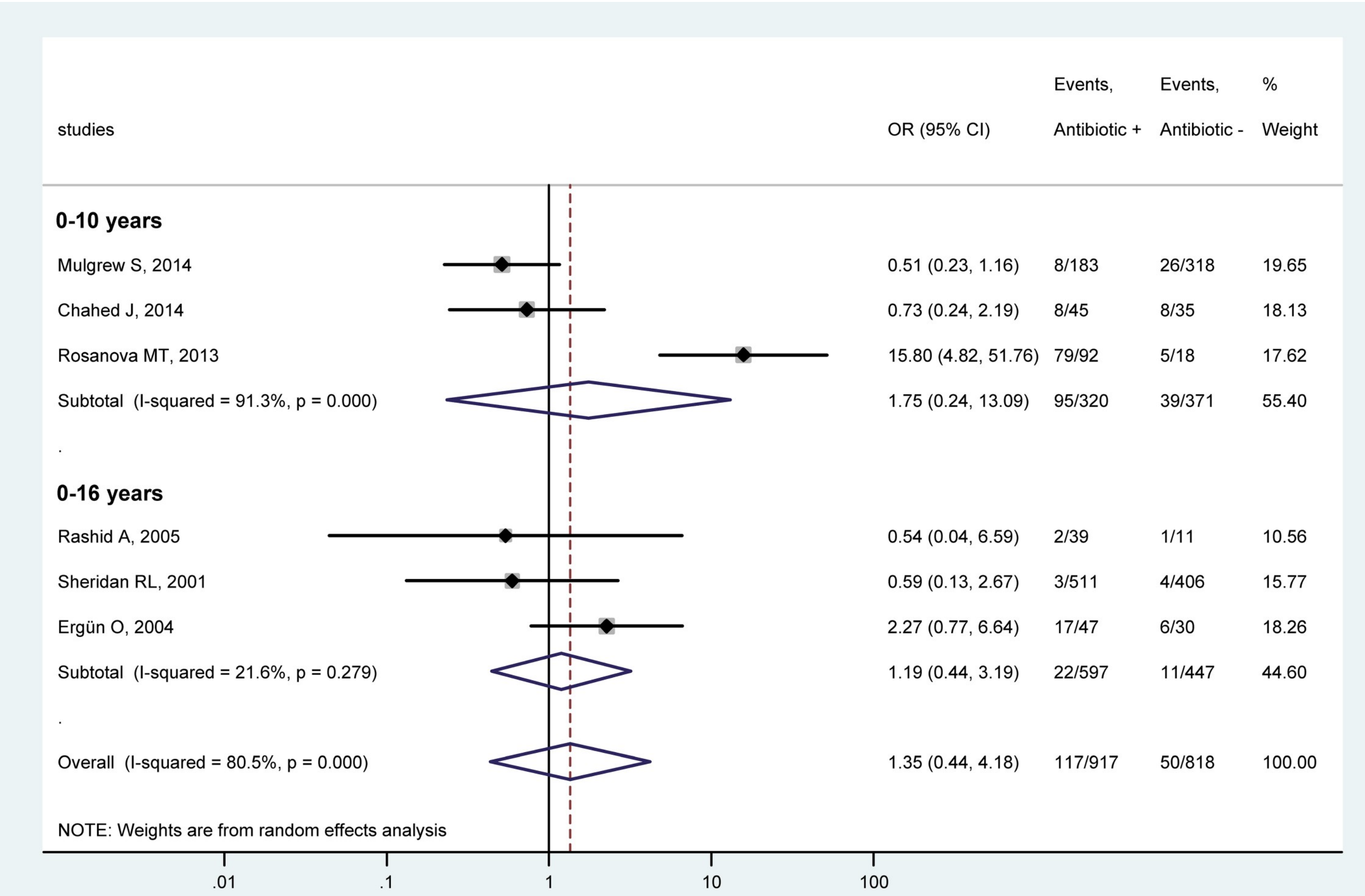

Fig 3. Forest plot of the odds ratios (ORs) for all infectious complications in pediatric patients of 0–10 years (top) and 0–16 years (bottom) with burn injuries who received versus those who did not receive systemic antibiotic prophylaxis.

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Infectious complications are often feared as threats after burn injuries. Superficial burns (traditionally named as first-degree burn), which affect only the epidermis, usually do not require specialized medical care. On the contrary, in deeper burns, which penetrate into the dermis (i.e., partial-thickness, formerly second-degree) or damage the entire dermis and potentially even deeper tissues (i.e., full-thickness, formerly third- and higher-degree burns), the chance of infectious complications is proportionally increasing with the depth of the burn [11]. Deeper burns usually require complex conventional and surgical interventions [10, 34], among them partial-thickness burns are the most common type in children [9, 35, 36].

As part of the primary treatment of deeper burns, systemic antibiotic prophylaxis is occasionally initiated [15, 17], even though there is no clinical evidence for such indication of antimicrobial treatment. In fact, The International Society for Burn Injuries recommends to avoid prophylactic systemic antibiotics in acute burns [23], which guideline is based in part on meta-analyses of data obtained in adult burn patients [21, 22]. In pediatric burns, however, no meta-analysis has been performed, to the best of our knowledge, only a systematic review was published which lacked quantitative statistical analysis [37]. The present work aimed at filling this gap by conducting a meta-analysis of 6 articles [18, 29–33] identified based on an extensive literature search. We showed that systemic antibiotic prophylaxis did not decrease the chance

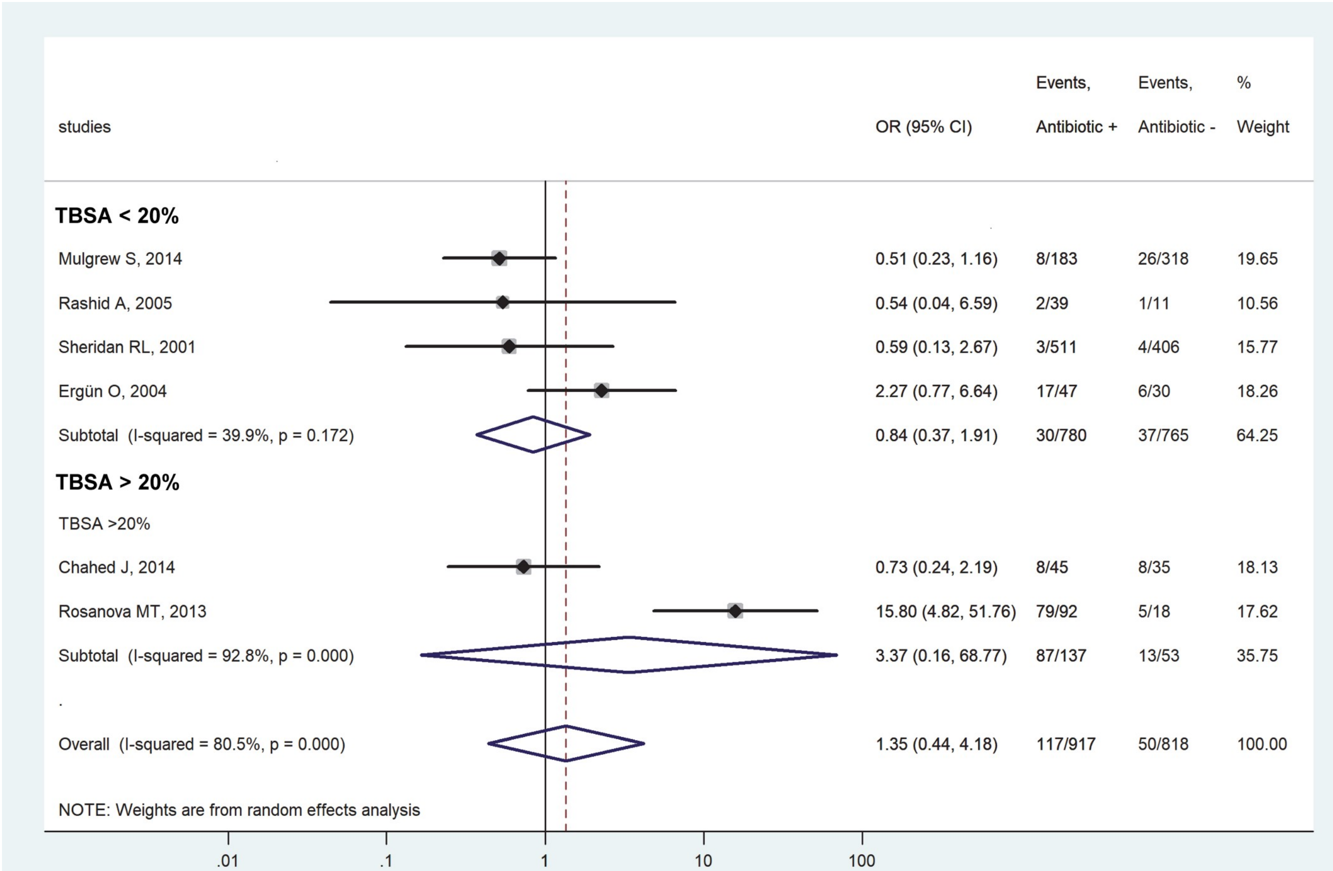

Fig 4. Forest plot of the odds ratios (ORs) for all infectious complications in pediatric patients with burn injuries who received versus those who did not receive systemic antibiotic prophylaxis in subgroups of less than 20% (top) and more than 20% (bottom) mean extent of injury as related to the total body surface area (TBSA).

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for systemic and all infectious complications. As a matter of fact, when we included the rates of all infectious complications from all 6 eligible studies in our analysis, we found that the overall chance for developing an infection tended to be 35% higher in antibiotic-treated patients (n = 917) than in patients without antibiotic prophylaxis (n = 818), as indicated by the overall OR of 1.35 (95% CI, 0.44, 4.18) (Figs 3–5), although the difference did not reach the level of statistical significance. In accordance, a higher rate of infectious complications was reported in children with burn injuries who received antibiotic prophylaxis in two of the analyzed studies [18, 32]. It was thought to be due to the overgrowth of resistant microorganisms, thereby resulting in infections by opportunistic pathogens in the urinary tract, airways, and middle ear [18]. Antibiotic prophylaxis did not prevent wound infection or potential lethal consequences in the study in 80 pediatric patients with burn injuries conducted by Chahed et al. [29], which was, to our knowledge, the only randomized clinical trial designed to investigate the necessity of systemic antibiotic prophylaxis. Similarly, antibiotic prophylaxis was concluded to be unnecessary in two other studies [30, 33], whereas yet another suggested that prophylactic antibiotics may prevent toxic shock syndrome, based on data obtained from 50 pediatric patients with burn injuries [31]. It has to be noted, however, that in the latter study only 3 patients became septic in the entire study population: 2 (of 39) in the antibiotic-treated group and 1 (of 11) in the group without prophylaxis [31]. Due to the low numbers, these results should be interpreted with caution, as also noted by the authors.

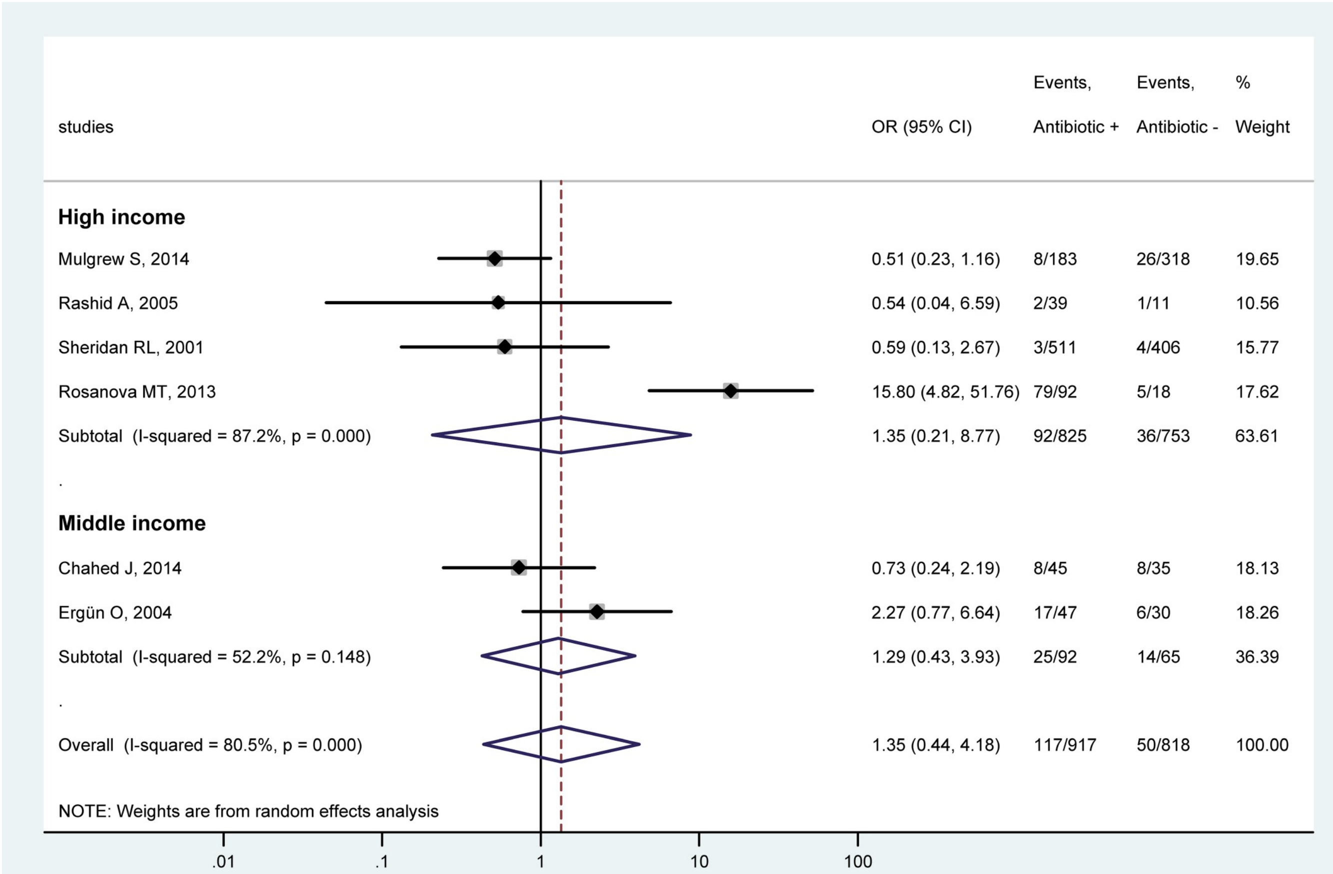

Fig 5. Forest plot of the odds ratios (ORs) for all infectious complications in pediatric patients with burn injuries who received versus those who did not receive systemic antibiotic prophylaxis in country subgroups of high income (top) and middle income (bottom).

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The results of our meta-analysis regarding the lack of efficacy of prophylactic antibiotics on the overall infection rate in pediatric burns are in harmony with the conclusions drawn in the majority of previous human studies [18, 29, 30, 32, 33], a systematic review [37], and recent guidelines [23]. Moreover, by quantitative synthesis of the data reported in the identified articles, our results strengthen the body of evidence for the avoidance of systemic antibiotic prophylaxis in pediatric burns. However, pooling all reported data together and analyzing only the overall infection rate may mask a potentially beneficial effect of antibiotics in a specific subset of pediatric patients. Therefore, we also performed the meta-analysis in different subgroups, which were defined based on known risk factors reported in the identified studies. We found 3 parameters that were reported in sufficient details for subgroup analysis: age, affected TBSA, and country income. We assigned patients to subgroups based on these parameters. Remarkably, there was no statistical difference in the chance of infections between pediatric patients with and without systemic antibiotic prophylaxis in any of the three subgroups. These results suggest that systemic antibiotic prophylaxis should be avoided in pediatric burns independently of the age of pediatric patients, the injured TBSA, or the economic status of the country.

Certain limitations of our study must be also mentioned. Despite the extensive database search, only 6 studies could be included in the final analysis. This was sufficient for quantitative synthesis, but when we divided the studies into subgroups, in some cases only 2 studies per group remained. Although a review of the Cochrane Library revealed that numerous meta-analyses are conducted with two studies [38], firm conclusions should not be drawn from the meta-analysis of such small subgroups. All of the studies included in our meta-analysis were single-center studies, ranging from retrospective [18, 30, 33] to prospective [31, 32] to randomized clinical trials [29]. According to our quality assessment, only 3 studies were considered as good quality [18, 30, 32], while 2 studies as fair [31, 33], and 1 study as poor quality [29]. Based on visual inspection of the funnel plots (Figs A and B in S1 File), some asymmetry may be present, indicating the possible existence of publication bias, but statistical tests could not be performed, because for those at least 10 studies are required according to the Cochrane Handbook [39]. The depths of the burns in the patient populations were not reported in sufficient details to allow for subgroup analysis of the infectious outcome separately in partial- and full-thickness burns. Neither could we extract sufficient data about the latency from the time of the burn injury till initiation and the duration of the systemic antibiotic prophylaxis. Infectious comorbidities (or the lack of such) which had been already present in the children before they suffered burn injury could not be assessed from the studies. Finally, the antibiotics administered to the children varied among the studies. While penicillins were used most commonly for the prophylaxis [18, 29–31, 33], cephalosporins [18, 33] and macrolides [18, 30, 31] were also used in some cases, while, in one study [32], the antibiotic was not identified. All these factors could influence infectious outcomes (whether systemic or local) in pediatric patients with burn injuries, but, due to data unavailability, we could not account for these factors in the present meta-analysis. The mentioned statistical, methodological, and medical differences in study design can explain the considerably high between-study heterogeneity (indicated by an I^2 of ~80%), as observed in our analysis (Figs 3–5). To account for the presence of heterogeneity, we used the random-effects model in all forest plots of our meta-analyses. We also performed leave-one-out sensitivity analysis to confirm that our findings were not driven by any single study. However, it is still possible that, despite all of our approaches to reduce methodological errors, the low number, different design and quality, and high heterogeneity of the analyzed studies may have negatively impacted our results.

In conclusion, the present study shows that systemic antibiotic prophylaxis as a routine has no benefits for the prevention of infectious complications in pediatric patients with burn injuries. Our meta-analysis of the data available in literature provides quantitative support to the position of avoiding routine use of the systemic antibiotic prophylaxis in pediatric burns. In addition to the quantitative synthesis of the available data, which to our knowledge, is the first in its field, we point out certain limitations in study design and data reporting, which, however, can also be addressed in the design of future clinical trials. Multinational, randomized controlled trials are warranted to validate our findings and prove unequivocally that routine systemic antibiotic prophylaxis is not indicated in pediatric patients with burn injuries.

Supporting information

S1 File. Supporting information including Tables A-D and Figures A and B.
Table A. PRISMA 2009 checklist. **Table B**. Risk of bias assessment of a randomized controlled trial included in the meta-analysis using the Cochrane Risk of Bias Tool for Randomized Controlled Trials. **Table C**. Quality assessment of the studies included in the meta-analysis using the Newcastle-Ottawa Scale. **Table D**. Summary of study characteristics for publications included in the meta-analyses. **Figure A**. Funnel plot of the studies that were included in the forest plot of the odds ratios (ORs) for systemic and local subgroups of infectious complications in children with burn injuries who received systemic antibiotic prophylaxis compared to those who did not. **Figure B**. Funnel plot of the studies that were included in the forest plot of

the odds ratios (ORs) for all infectious complications in children with burn injuries who received versus those who did not receive systemic antibiotic prophylaxis in the age, TBSA, and country income level subgroups.
(PDF)

Author Contributions

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