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Increased serum levels of proNGF, mature NGF and interleukins in burn-injured children

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Abstract. – OBJECTIVE: Burns are among the most common injuries in children. In burns of more than 20% of the total body surface area, a systemic inflammatory response involving several chemical mediators occurs. Among them, nerve growth factor (NGF) regulates the inflammatory response related to wound healing and promotes keratinocyte proliferation and angiogenesis. The aim of our study was to investigate the physiological response to injury in children with moderate-severe burns, assaying proNGF, mature NGF (mNGF), interleukins (IL)-1β, and IL-10 serum levels.

PATIENTS AND METHODS: This is a prospective observational study, including twelve children hospitalized for moderate-severe burns at the Gemelli Hospital (Rome). Their laboratory features were compared to those of patients with obstructive hydrocephalus who underwent surgery.

RESULTS: Our results showed an increase in proNGF and mNGF serum levels. In burn patients, proNGF levels increased before mNGF, and serum concentrations of both were not correlated with burn extension and depth. The most significant levels of mNGF and proNGF were reported in scalds involving the face. Serum IL-1β and IL-10 peak levels were reached with a time-course pattern similar to proNGF.

CONCLUSIONS: Our preliminary results validate the hypothesis that serum levels of proNGF and mNGF may represent inflammatory biomarkers useful for monitoring burn patients and defining new strategies for their treatment.

ClinicalTrials.gov: NCT05751174.

Key Words:
Burns, Nerve growth factor, Children.

Introduction

Burns are among the most frequent injuries in children under five years old worldwide[1]. They are mainly caused by hot liquid scalding, but in some cases also by contact and flame burns[2-5]. They occur mainly in children between 6 and 24 months of age when they learn to move independently, but they do not yet recognize the hazards[4-7]. In the UK, 97% of burn injuries affecting children under five years of age involve <10% of the Total Body Surface Area (TBSA)[6]. These conditions are typically not severe and do not require hospitalization, while the remaining percentage of burnt children may need admission to the Pediatric Intensive Care Unit (PICU), with appropriate management and treatment by a specialized team[8,9].

Despite advances in the last years, extensive burns can still cause damage to organs, resulting in serious complications or even death[10]. The outcome and survival of these children are related to the quality of management and the treatment carried out. In all children with burns of more than 20% TBSA, a systemic inflammatory response to burn injury occurs[11,12]. It is usually investigated with the dosage of inflammatory markers, such as C-reactive protein (CRP) and procalcitonin (PCT), which also increase in case of infection of the burned surface, mainly related to *Staphylococcus aureus* and *Pseudomonas aeruginosa* in children and adults as well[13-15]. New predictive tools of sepsis potentially applicable in burns are being developed but still need to be validated in this setting[16].

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Therefore, understanding the pathophysiological response to burn injury by assaying other biomarkers would help clinicians in decisions regarding the use of antibiotics in the management of these children.

Several studies[17] suggested that neurotrophins, particularly Nerve Growth Factor (NGF) and its receptors, are involved in wound healing. Physiologically, NGF is expressed by various cells, such as keratinocytes, fibroblasts, and mast cells, all of which are involved in maintaining skin homeostasis[17-20].

Previous studies[21,22], performed in animal models, showed that full-thickness skin wounds increased serum and skin levels of NGF released from peripheral nerve endings. Interestingly, it was demonstrated that the salivary glands of mice can store NGF and release it into the bloodstream after injury.

NGF promotes dose-dependent proliferation of keratinocytes and fibroblasts, regulates the immune inflammatory response related to the wound healing process, and promotes angiogenesis and myofibroblast differentiation[17-20].

Different studies[20,23-25] showed that exogenous NGF can be used as a therapeutic agent to restore and maintain neuronal function in the peripheral and central nervous system. In addition, the topical application of exogenous NGF plays an important role in promoting the healing and repair of diabetic ulcers, corneal ulcers, oral wounds, and in crush syndrome[26,27].

The hypothesis of this study is based on the possibility that skin burns may be related to an increased expression of circulating NGF in the blood. Moreover, burns are also associated with an enhanced release of both pro-inflammatory and anti-inflammatory cytokines in order to maintain homeostasis. These proteins are released by innate immune cells and act as a bridge between the innate and the acquired branches of the immune system. Interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ) are the major pro-inflammatory cytokines involved[28]. In particular, IL-1β is not only released by damaged keratinocytes and endothelial cells but by neutrophils, macrophages, and monocytes as well, acting as a T cell chemoattractant[29]. On the other hand, IL-10 is a major component of the anti-inflammatory response: indeed, it has inhibitory effects on the release of IL-1, IL-6, TNF-α, and IFN-γ[30].

Cytokines such as IL-1β, TNF-α, and IL-6 promote NGF synthesis in various cell types, such as neurons, glial, epithelial, endothelial, muscle, and connective tissue cells, thereby upregulating NGF production and release during the inflammatory response[31]. At the same time, NGF receptors are expressed on the membrane of immune system cells, enabling NGF to directly modulate their activity.

The main aim of our study is to assess NGF, pro-NGF, IL-1β, and IL-10 values in moderate and severe burned patients and compare them to those of a control group. The secondary objective is to analyze possible correlations between the serum expression of these biomarkers and burn severity in terms of depth and extension.

Patients and Methods

This is a prospective observational study, including all children hospitalized for moderate and severe burns at the Pediatric Intensive Care Unit (PICU) and the Pediatric Trauma Center of "Policlinico Gemelli" University Hospital of Rome. The study period was from April 2021 to May 2022. Participants were recruited among patients primarily presenting to the hospital within 12 hours of the accident with burns in 5-98% TBSA. All the patients belonged to moderate or severe burns according to the American Burns Association[32]: patients with moderate burns n=2 (5-10% TBSA burn, 2-5% full-thickness burn), and patients with severe burns n=10 (>10% TBSA burn, >5% full-thickness burn). In all but one case, the cause of the burns was hot fluids. Exclusion criteria were: antibiotic treatment 48 hours prior to admission or severe pre-existing infections, norepinephrine infusion >0.5 mcg/kg/min, comorbidities associated with liver disease, kidney disease, or immunological or cardiovascular diseases that required long-term medication, lack of informed consent from parents or legal caregivers. All parents of our patients gave written informed consent for both clinical and biochemical follow-ups. The study was approved by the Catholic University of the Sacred Heart Ethics Committee (No. 34249/21).

The following clinical parameters were recorded in an electronic database: age, sex, weight, TBSA involved, degree and severity of the burn, involvement of specific surfaces (face, genitals, palms of hands, and soles of feet), other organ failures, need of inotropic and vasoconstrictor drugs, need for intubation and mechanical ventilation, need for surgery with eventual homologous/autologous skin graft.

The laboratory features of these patients were compared to those of a control group, consisting of 10 children with obstructive hydrocephalus who had undergone elective surgery for the lengthening of their ventriculoperitoneal shunt, given the lack of possibility to compare these biomarkers in healthy control patients. These patients were under normal neurological conditions and well-controlled intracranial pressure (ICP) dynamics. Furthermore, they did not present a clinical picture characterized by systemic inflammation. Controls were matched for age, sex, and weight.

For all patients included, venous blood samples (7.5 ml) were drawn after 2-6 h from the admission and on days 2, 7, 14, and 30 after the burn injury for clinical follow-up as per internal protocol. A small aliquot of blood (5 ml) was dedicated separately for the assay of circulating biomarker levels. The children were not subjected to any invasive investigations for research purposes beyond the normal sampling required by the care protocol. Whole blood was collected in a Paxgene Collection Tube (Milan, Italy) for standard genomic analysis, with a volume of 2.5 mL. Additionally, an aliquot of 5 mL of whole blood was collected in dedicated vacutainers to obtain 1.5 mL of serum, which was then stored in standard tubes at -80°C. Enzyme-linked immunosorbent assays (ELISAs) were used to evaluate possible serum variations in proNGF, mNGF, IL-1β and IL-10. Total proNGF and mNGF levels were measured according to what was previously reported[33]. IL-1β and IL-10 were measured by Duoset® human ELISA (cat. DY201 and DY217B, respectively, R&D Systems, Milan, Italy), according to the manufacturer's instructions.

We chose to dose proNGF/mNGF serum levels as possible innovative biomarkers of diffuse inflammatory state[31,33-35] in burn injury patients, together with two well-established inflammatory cytokines, whose serum levels are known to be altered after burn injury[36-38].

Results

Twelve children hospitalized for moderate/severe burns were enrolled, three girls and nine boys, aged between 11 months and 15 years. Six patients (50%) had II-III-degree burns, while the remaining six (50%) had II-degree burns. Two children had a burn extension of <10%, six between 10 and 30%, and four had a 30% degree burn or greater, including one patient with a 98% total body surface area involved. The affected areas were the face district in seven patients, the thoracic one in seven patients, the abdomen in six, the genital area in two, the superior limbs in nine, and the inferior limbs in five. All patients' data are reported in Table I.

In the burned patients' group, the time-course analysis did not reveal significant variations in the serum levels of all four biomarkers up to 14 days after admission to the PICU (Figure 1).

The blood assay of selected biomarkers (proNGF, mNGF, IL-1β, and IL-10) indicated that their pooled mean serum level was significantly higher in patients with burn injuries compared to those with obstructive hydrocephalus. The comparison made on a time-course level also revealed that the serum levels of proNGF, mNGF, IL-1β, and IL-10 were far higher in burn injury patients than in hydrocephalus patients at every time point examined.

In particular, results showed that in burn patients, serum concentrations of proNGF rose before the mature NGF concentrations (mean time day 2 *vs.* day 7 after admission in PICU). IL-1β and IL-10 reached their peak values at day 2 as well. Therefore, mNGF was the last biomarker to reach its peak level.

Analyzing our data, we found that the serum concentrations of mNGF and proNGF were not correlated with the extension of the burn lesions or the burn depth. Focusing on the burn site, the most significant levels of both mNGF and proNGF were reported in scalds involving the facial region. An exception to this is represented by patient 5, whose proNGF and mNGF levels were very low. This patient had a massive burn injury with almost 99% of the BSA burned and rapidly evolved into a multiorgan failure and died.

Discussion

Our results validate the hypothesis that the serum levels of proNGF and mNGF may represent possible inflammatory biomarkers in burn injury patients. Besides, we showed that in the burn injury patients, the proNGF levels increased before the mNGF.

Since its discovery, NGF has been identified as a neurotrophic factor increasing the growth and survival of cholinergic neurons in addition to protection, repair, and plasticity of the brain[39]. By this time, additional potential therapeutic applica-

Table I. Patients included in the study and their clinical features.

Sample ID	Sex	Age (years)	Burn degree	TBSA (%)	Site	Mechanical ventilation	Need of inotropic or vasoconstrictor	Need for surgery	Multi organ failure	Antibiotics	Days of hospitalization	Outcome
Patient 1	M	2 years	II-III	30-39%	Face-neck, scalp, hemithorax and left hemiabdomen, left arm and part of the left thigh, right eye cornea	None	None	Dermabrasion and autologous grafting	No	Cefepime	47	Positive
Patient 2	M	11 months	II	25%	Face, thorax, left upper limb, right shoulder, upper dorsal surface	8 days	Noradrenaline for 7 days	None	No	Cephazol in Ampicillin	17	Positive
Patient 3	M	1 year	II-III	15%	Upper limbs, trunk, neck and face	2 days	None	Superficial escharotomy	No	Cefepime Teicoplanin	23	Positive
Patient 4	F	8 years	II-III	30%	Both thighs circumferentially, right upper limb circumferentially, lower abdomen, right hemithorax, face	None	None	Autologous grafting	No	Cefepime	28	Positive
Patient 5	M	15 years	II-III	98%	Almost the whole-body surface area	2 days	None	None	Yes	None	2	Dead
Patient 6	M	1 year	II	2-3%	Left half face	None	Yes	None	No	None	4	Positive
Patient 7	F	3 years	II	7%	Left upper limb, right arm, face, one finger	None	None	None	No	None	9	Positive
Patient 8	M	6 years	II-III	15%	Thorax, abdomen	None	None	None	No	Piperacillin-Tazobactam Vancomycin	6	Positive
Patient 9	M	9 years	II	20%	Abdomen, thighs, penis, finger	12 hours	None	Autologous skin graft surgery	No	Vancomycin	18	Positive
Patient 10	F	5 years	II	30%	Thorax, thighs, right upper limb	None	None	None	No	None	16	Positive
Patient 11	M	1 years	II	14%	Right leg, both feet, upper limb	None	None	Escharotomy and autologous grafting	No	None	17	Positive
Patient 12	M	12 years	II-III	15%	Thorax, abdomen, upper limbs	None	None	Surgical toilet	No	Amoxicillin-clavulanate	14	Positive

Total Body Surface Area (TBSA).

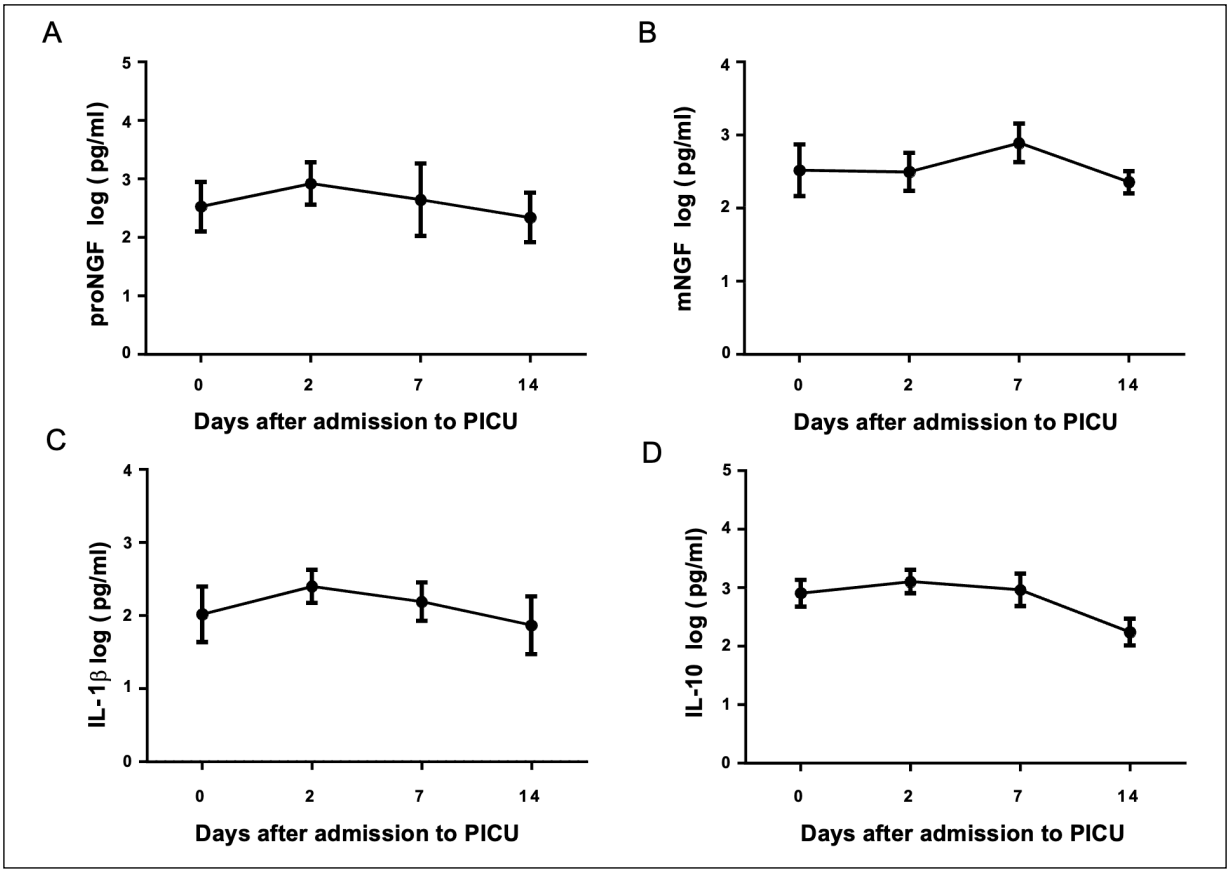

Figure 1. **A**, The time-course analysis of proNGF. **B**, The time-course analysis of mNGF. **C**, The time-course analysis of IL-1β. **D**, The time-course analysis of IL-10.

tions have been explored, including skin ulcers, wounds, ocular diseases, myocardial infarction, Alzheimer's disease, spinal cord damage, and a wide range of neuronal injuries[40-42]. NGF carries out its biological effects by binding a tyrosine kinase receptor, TrkA, and a pan-neurotrophin receptor, p75[43]. Recent studies[44] focused on the crucial role of NGF in burns and its interaction with skin cells in different stages of burn wound healing. In the first phase, macrophages and mast cells secrete NGF, which contributes remarkably to inflammation. During the following proliferative phase, NGF boosts angiogenesis through vascular endothelial growth factor (VEGF)[45] and promotes keratinocyte proliferation *via* the TrkA-PI3K/Akt pathway. In the last remodeling phase, NGF induces fibroblast differentiation into myofibroblasts, leading to wound contracture[46]. NGF trophic and regenerative properties have been thoroughly described in the literature[47]. Indeed, this protein can induce not only neural tissue and cutaneous regeneration but also seems to have a role in neoangiogenesis in experimental animal models[45-49]. The angiogenic effects are mediated through direct action on vascular endothelial cells or through indirect action on VEGF[50]. Tissue regeneration is induced by enhanced perfusion and metabolism and induction of an anti-inflammatory response. Indeed, NGF possesses regulatory effects on cytokine expression[31,] and it induces a switch towards the anti-inflammatory macrophage phenotype[51]. Topical delivery of NGF has been investigated in different ophthalmic diseases, such as neurotrophic keratitis[52], retinitis pigmentosa[53], glaucoma[54], and dry eye disease[55]. *In vitro* models[56] have also shown that NGF takes part in all stages of wound healing in the oral cavity since it is produced by keratinocytes, endothelial cells, leukocytes, acinar and ductal cells in the salivary glands, and it is also secreted into saliva. In the case of wounding, NGF and proNGF can bind to their receptors on keratinocytes on the wound edges and induce regeneration. These properties may be exploited to target new therapeutic strategies also for wound healing of the burns.

The results of our study correlate with the last molecular updates in the literature. The comparison made on a time-course level showed that the serum levels of proNGF, NGF, IL-1β, and IL-10 were altogether far higher in burn injury patients than in control patients at every time-point examined. Analyzing the single trends on the overall timeline of proNGF, mNGF, IL-1β, and IL-10, some differences arise. IL-1β and IL-10 had similar tendencies, with a peak on the 2nd day after admission to PICU and a gradually decreasing trend. This data shows that the upregulation is not limited to pro-inflammatory mediators (IL-1β) but involves anti-inflammatory mediators as well, such as IL-10. Our finding suggests that some sort of system of checks and balances in the cytokine network is maintained in these patients, according to what some authors showed in their studies[36-38]. The timeline trends with a peak on the 2nd day are similar to the ones shown in these previous studies[36-38]. In addition, proNGF had a similar time-course pattern, whereas NGF had a peak on the 7th day after admission to PICU. This could be due to the biological role of proNGF. As a matter of fact, proNGF is a precursor that has to be cleaved in the Golgi apparatus[57]. By the time the proteolytic cleavage of this large precursor protein boots NGF serum levels, reaching its peak later. As we already mentioned, the serum concentrations of NGF and proNGF were not correlated with the extension of the burn lesions or the burn depth. Focusing on the burn site, the most significant level of both NGF and proNGF was reported in scalds involving the facial region. This aspect is probably due to its specific anatomy, which is rich in blood vessels and nerves. During the inflammatory phase, macrophages and mast cells secrete a wide range of cytokines and growth factors, including NGF, which participate in the inflammatory reaction and induction of other cells, targeting a homeostatic state[46]. This specific anatomical aspect could lead to higher proNGF and mNGF production in patients with this kind of injury. Furthermore, the patients with the highest serum levels were two and three years old. In this age group, the head has a main role in determining the body surface area compared to older children[58].

To the best of our knowledge, this is the first pilot study to show an association between NGF and the burn site involved. Certainly, this correlation should be investigated in future larger cohort trials.

Our study shows several limitations. First of all, the single-center design of the study and the limited number of patients involved limit some assessments. Secondly, burned patients are complicated patients that need intensive management in the PICU. Therefore, in this setting, it becomes more challenging to establish if there is a direct correlation between the burns and the secretion of NGF. The intensive care setting, the severe clinical conditions and the generalized systemic inflammation are some of the biases that could have influenced the outcome. Thirdly, controls were not healthy subjects but obstructive hydrocephalus patients admitted to the PICU. Although they were not septic patients, their outcomes could be affected by the intensive care conditions they experienced.

Conclusions

Overall, our results validate the hypothesis that the serum levels of proNGF and mNGF may represent possible inflammatory biomarkers that are useful for monitoring burn-injured patients. Due to the short duration of our follow-up, the small sample of patients, and the difficulty of enrolling a sample of healthy age-matched controls, further studies, including a wider range of patients, will be necessary to develop standardized diagnostic protocols.

The definition and comprehension of NGF expression mechanisms in burn-injured patients could allow the individualization of new strategies for the treatment of children suffering from burn injuries.

Conflict of Interest

The authors declare no conflict of interest.

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Authors' Contributions

LDS and GE researched the literature and wrote the initial draft. AC, LM, OG, MS, and AC revised the manuscript. TCM, AG, MP, GS, and VP supplemented the materials. AC and GC developed the study design and conceptualization. LDS and GE collected and processed data. All authors reviewed and agreed on the final manuscript.

Ethics Approval
The study was approved by the Ethics Committee of the Catholic University of the Sacred Heart (No. 34249/21). No. 34249/21.

Informed Consent
All parents of our patients gave written informed consent for both clinical and biochemical follow-up.

Data Availability
The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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References

1) Preston D, Ambardekar A. The Pediatric Burn: Current Trends and Future Directions. Anesthesiol Clin 2020; 38: 517-530.
2) Godson R. Reducing unintentional injuries in and around the home among children under five years. Community Pract 2014; 87: 12.
3) Kemp AM, Jones S, Lawson Z, Maguire SA. Patterns of burns and scalds in children. Arch Dis Child 2014; 99: 316-321.
4) Peden M, Oyegbite K, Ozanne-Smith, Hyder AA, Branche C, Fazlur Rahman AKM, Rivara F, Bartolomeos K, editors. World Report on Child Injury Prevention. Geneva: World Health Organization, 2008.
5) Sarginson JH, Hollén L, Emond A, Mackie I, Young AE. Multicentre observational study describing the systemic response to small-area burns in children. Burns 2021; 47: 560-568.
6) Agran PF, Anderson C, Winn D, Trent R, Walton-Haynes L, Thayer S. Rates of pediatric injuries by 3-month intervals for children 0 to 3 years of age. Pediatrics 2003; 111: e683-e692.
7) Flavin MP, Dostaler SM, Simpson K, Brison RJ, Pickett W. Stages of development and injury patterns in the early years: a population-based analysis. BMC Public Health 2006; 6: 187.
8) Stewart S, Juang D, Aguayo P. Pediatric burn review. Semin Pediatr Surg 2022; 31: 151217.
9) Partain KP, Fabia R, Thakkar RK. Pediatric burn care: new techniques and outcomes. Curr Opin Pediatr 2020; 32: 405-410.
10) Kraft R, Herndon DN, Al-Mousawi AM, Williams FN, Finnerty CC, Jeschke MG. Burn size and survival probability in pediatric patients in modern burn care: a prospective observational cohort study. Lancet 2012; 379: 1013-1021.
11) Jeschke MG, Chinkes DL, Finnerty CC, Kulp G, Suman OE, Norbury WB, Branski LK, Gauglitz GG, Mlcak RP, Herndon DN. Pathophysiologic response to severe burn injury. Ann Surg 2008; 248: 387-401.
12) Jeschke MG, Gauglitz GG, Kulp GA, Finnerty CC, Williams FN, Kraft R, Suman OE, Mlcak RP, Herndon DN. Long-term persistance of the pathophysiologic response to severe burn injury. PLoS One 2011; 6: e21245.
13) Steinvall I, Karlsson M, Elmasry M. C-reactive protein response patterns after antibiotic treatment among children with scalds. Burns 2018; 44: 718-723.
14) Viola L, Langer A, Pulitanò S, Chiaretti A, Piastra M, Polidori G. Serious Pseudomonas aeruginosa infection in healthy children: case report and review of the literature. Pediatr Int 2006; 48: 330-333.
15) Zhang YJ, Guo ZH, Ming ZG, Hao ZM, Duan P. Meta-analysis of the diagnostic value of serum procalcitonin for burn sepsis in adults. Eur Rev Med Pharmacol Sci 2023; 27: 7188-7200.
16) Capossela L, Margiotta G, Ferretti S, Curatola A, Bertolaso C, Pansini V, Di Sarno L, Gatto A. Presepsin as a diagnostic marker of sepsis in children and adolescents: a short critical update. Acta Biomed 2023; 94: e2023062.
17) Liu Z, Wu H, Huang S. Role of NGF and its receptors in wound healing (Review). Exp Ther Med 2021; 21: 599.
18) Marconi A, Terracina M, Fila C, Franchi J, Bontè F, Romagnoli G, Maurelli R, Failla CM, Dumas M, Pincelli C. Expression and function of neurotrophins and their receptors in cultured human keratinocytes. J Invest Dermatol 2003; 121: 1515-1521.
19) Murase K, Murakami Y, Takayanagi K, Furukawa Y, Hayashi K. Human fibroblast cells synthesize and secrete nerve growth factor in culture. Biochem Biophys Res Commun 1992; 184: 373-379.
20) Manni L, Rocco ML, Bianchi P, Soligo M, Guadagna M, Paparo Barbaro, Aloe L. Nerve growth factor: basic studies and possible therapeutic applications. Growth Factors 2013; 31: 115-122.
21) Matsuda H, Koyama H, Sato H, Sawada J, Itakura A, Tanaka A, Matsumoto M, Konno K, Ushio H, Matsuda K. Role of nerve growth factor in cutaneous wound healing: Accelerating effects in normal and healing impaired diabetic mice. J Exp Med 1998; 187: 297-306.

22) Li AK, Koroly MJ, Schattenkerk ME, Malt RA, Young M. Nerve growth factor: Acceleration of the rate of wound healing in mice. Proc Natl Acad Sci 1980; 77: 4379-4381.

23) Rocco ML, Soligo M, Manni L, Aloe L. Nerve Growth Factor: Early Studies and Recent Clinical Trials. Curr Neuropharmacol 2018; 16: 1455-1465.

24) Chiaretti A, Barone G, Riccardi R, Antonelli A, Pezzotti P, Genovese, Tortorolo L, Conti G. NGF, DCX, and NSE upregulation correlates with severity and outcome of head trauma in children. Neurology 2009; 72: 609-616.

25) Gatto A, Capossela L, Conti G, Eftimiadi G, Ferretti S, Manni L, Curatola A, Graglia B, Di Sarno L, Calcagni ML, Di Giuda D, Cecere S, Romeo DM, Soligo M, Picconi E, Piastra M, Della Marca G, Staccioli S, Ruggiero A, Cocciolillo F, Pulitanò S, Chiaretti A. Intranasal human-recombinant NGF administration improves outcome in children with post-traumatic unresponsive wakefulness syndrome. Biol Direct 2023; 18: 61.

26) Lambiase A, Manni L, Rama P, Bonini S. Clinical application of nerve growth factor on human corneal ulcer. Arch Ital Biol 2003; 141: 141-148.

27) Generini S, Tuveri MA, Matucci Cerinic M, Mastinu F, Manni L, Aloe L. Topical application of nerve growth factor in human diabetic foot ulcers. A study of three cases. Exp Clin Endocrinol Diabetes 2004; 112: 542-544.

28) Sierawska O, Małkowska P, Taskin C, Hrynkiewicz R, Mertowska P, Grywalska E, Korzeniowski T, Torres K, Surowiecka A, Niedźwiedzka-Rystwej P, Strużyna J. Innate Immune System Response to Burn Damage—Focus on Cytokine Alteration. Int J Mol Sci 2022; 23: 716.

29) Blears E, Sommerhalder C, Toliver-Kinsky T, Finnerty CC, Herndon DN. Current Problems in Burn Immunology. Curr Probl Surg 2020; 57: 100779.

30) Dehne MG, Sablotzki A, Hoffmann A, Muhling J, Dietrich FE, Hempelmann G. Alterations of Acute Phase Reaction and Cytokine Production in Covers Following Severe Burn Injury. Burns 2002; 28: 535-542.

31) Minnone G, De Benedetti F, Bracci-Laudiero L. NGF and Its Receptors in the Regulation of Inflammatory Response. Int J Mol Sci 2017; 18: 1028.

32) Singer AJ, Dagum AB. Current management of acute cutaneous wounds. N Engl J Med 2008; 359: 1037-1046.

33) Soligo M, Protto V, Florenzano F, Bracci-Laudiero L, De Benedetti F, Chiaretti A, Manni L. The mature/pro nerve growth factor ratio is decreased in the brain of diabetic rats: Analysis by ELISA methods. Brain Res 2015; 1624: 455-468.

34) Aalto K, Korhonen L, Lahdenne P, Pelkonen P, Lindholm D. Nerve growth factor in serum of children with systemic lupus erythematosus is correlated with disease activity. Cytokine 2002; 20: 136-139.

35) Farina L, Minnone G, Alivernini S, Caiello I, MacDonald L, Soligo M, Manni L, Tolusso B, Coppola S, Zara E, Conti LA, Aquilani A, Magni-Manzoni S, Kurowska-Stolarska M, Gremese E, De Benedetti F, Bracci-Laudiero L. Pro Nerve Growth Factor and Its Receptor p75NTR Activate Inflammatory Responses in Synovial Fibroblasts: A Novel Targetable Mechanism in Arthritis. Front Immunol 2022; 13: 818630.

36) Hur J, Yang HT, Chun W, Kim JH, Shin SH, Kang HJ, Kim HS. Inflammatory cytokines and their prognostic ability in cases of major burn injury. Ann Lab Med 2015; 35: 105-110.

37) Kupper TS, Deitch EA, Baker CC, Wong WC. The human burn wound as a primary source of interleukin-1 activity. Surgery 1986; 100: 409-415.

38) Pileri D, Accardo Palombo A, D'Amelio L, D'Arpa N, Amato G, Masellis A, Cataldo V, Mogavero R, Napoli B, Lombardo C, Conte C. Concentrations of cytokines IL-6 and IL-10 in plasma of burn patients: their relationship to sepsis and outcome. Ann Burns Fire Disasters 2008; 21: 182-185.

39) Xhima K, Markham-Coultes K, Hahn Kofoed R, Saragovi HU, Hynynen K, Aubert I. Ultrasound delivery of a TrkA agonist confers neuroprotection to Alzheimer-associated pathologies. Brain 2022; 145: 2806-2822.

40) Kaye DM, Vaddadi G, Gruskin SL, Du XJ, Esler MD. Reduced myocardial nerve growth factor expression in human and experimental heart failure. Circ Res 2000; 86: E80-E84.

41) Falsini B, Chiaretti A, Rizzo D, Piccardi M, Ruggiero A, Manni L, Soligo M, Dickmann A, Federici M, Salerni A, Timelli L, Guglielmi G, Lazzareschi I, Caldarelli M, Galli-Resta L, Colosimo C, Riccardi R. Nerve growth factor improves visual loss in childhood optic gliomas: a randomized, double-blind, phase II clinical trial. Brain 2016; 139: 404-414.

42) Chiaretti A, Eftimiadi G, Buonsenso D, Rendeli C, Staccioli S, Conti G. Intranasal nerve growth factor administration improves neurological outcome after GBS meningitis. Childs Nerv Syst 2020; 36: 2083-2088.

43) Huang EJ, Reichardt LF. Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci 2001; 24: 677-736.

44) Korzeniowski T, Mertowska P, Mertowski S, Podgajna M, Grywalska E, Struzyna J, Torres K. The Role of the Immune System in Pediatric Burns: A Systematic Review. J Clin Med 2022; 11: 2262.

45) Chiaretti A, Piastra M, Caresta E, Nanni L, Aloe L. Improving ischaemic skin revascularisation by nerve growth factor in a child with crush syndrome. Arch Dis Child 2002; 87: 446-448.

46) El Baassiri MG, Dosh L, Haidar H, Gerges A, Baassiri S, Leone A, Rappa F, Jurjus A. Nerve growth factor and burn wound healing: Update of molecular interactions with skin cells. Burns 2023; 49: 989-1002.

47) Eftimiadi G, Soligo M, Manni L, Di Giuda D, Calcagni ML, Chiaretti A. Topical delivery of nerve growth factor for treatment of ocular and brain disorders. Neural Regen Res 2021; 16: 1740-1750.

48) Li X, Li F, Ling L, Li C, Zhong Y. Intranasal administration of nerve growth factor promotes angiogenesis via activation of PI3K/Akt signaling following cerebral infarction in rats. Am J Transl Res 2018; 10: 3481-3492.

49) Troullinaki M, Alexaki VI, Mitroulis I, Witt A, Klotzsche-von Ameln A, Chung KJ, Chavakis T, Economopoulou M. Nerve growth factor regulates endothelial cell survival and pathological retinal angiogenesis. J Cell Mol Med 2019; 23: 2362-2371.

50) Asanome A, Kawabe J, Matsuki M, Kabara M, Hira Y, Bochimoto H, Yamauchi A, Aonuma T, Takehara N, Watanabe T, Hasebe N. Nerve growth factor stimulates regeneration of perivascular nerve, and induces the maturation of microvessels around the injured artery. Biochem Biophys Res Commun 2014; 443: 150-155.

51) Prencipe G, Minnone G, Strippoli R, De Pasquale L, Petrini S, Caiello I, Manni L, De Benedetti F, Bracci-Laudiero L. Nerve growth factor downregulates inflammatory response in human monocytes through TrkA. J Immunol 2014; 192: 3345-3354.

52) Pflugfelder SC, Massaro-Giordano M, Perez VL, Hamrah P, Deng SX, Espandar L, Foster CS, Affeldt J, Seedor JA, Afshari NA, Chao W, Allegretti M, Mantelli F, Dana R. Topical Recombinant Human Nerve Growth Factor (Cenegermin) for Neurotrophic Keratopathy: A Multicenter Randomized Vehicle-Controlled Pivotal Trial. Ophthalmology 2020; 127: 14-26.

53) Falsini B, Iarossi G, Chiaretti A, Ruggiero A, Manni L, Galli-Resta L, Corbo G, Abed E. NGF eyedrops topical administration in patients with retinitis pigmentosa, a pilot study. J Transl Med 2016; 14: 8.

54) Lambiase A, Aloe L, Centofanti M, Parisi V, Bao SN, Mantelli F, Colafrancesco V, Manni GL, Bucci MG, Bonini S, Levi-Montalcini R. Experimental and clinical evidence of neuroprotection by nerve growth factor eye drops: Implications for glaucoma. Proc Natl Acad Sci USA 2009; 106: 13469-13474.

55) Sacchetti M, Lambiase A, Schmidl D, Schmetterer L, Ferrari M, Mantelli F, Allegretti M, Garhoefer G. Effect of recombinant human nerve growth factor eye drops in patients with dry eye: a phase IIa, open label, multiple-dose study. Br J Ophthalmol 2020; 104: 127-135.

56) Schenck K, Schreurs O, Hayashi K, Helgeland K. The Role of Nerve Growth Factor (NGF) and Its Precursor Forms in Oral Wound Healing. Int J Mol Sci 2017; 18: 386.

57) Howard L, Wyatt S, Nagappan G, Davies AM. ProNGF promotes neurite growth from a subset of NGF-dependent neurons by a p75NTR-dependent mechanism. Development 2013; 140: 2108-2117.

58) Moore RA, Waheed A, Burns B. Rule of Nines. Treasure Island (FL): StatPearls Publishing, 2023.