

Research Article

Brown Spiders' Venom Ulcer Necrosis Local Insulin Micro Doses Therapeutic Essay. Literature Review.

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Abstract

Background: Brown spiders' bites ulcers heal in about 3 months. Intralesional insulin micro multiples doses injections accelerate healing ulcers. In 3,5% of brown spiders' accidents occur hemolysis, intravascular coagulation, renal failure and, in 3%, death. *Loxosceles* venom transforms sphingomyelin into ceramides. Insulin inhibition by ceramides, may be responsible by necrosis, hemolysis, intravascular coagulation, and thrombosis. Relationship between insulin inhibition and lesion development isn't appreciated in literature about loxoscelism.

Objectives: 2-Evaluate healing acceleration by intralesional insulin in cutaneous ulcers by brown spiders' bites in 13 patients, (between 2015-2023) and it's efficacy preventing serious sequelae. Review diagnosis, physiopathology and therapeutic. Discuss ceramides' relationships with insulin resistance and hemolysis. Demonstrate intralesional insulin multiple micro-dosis injections efficacy as efficient treatment. Exhibit lesions photographic aspects, helping earlier diagnosis and treatment, preventing serious sequelae and death, especially, at distant places from serum therapy hospitals.

Methods: 3-Intralesional and perilesional applications sections of multiples 0,01ml of insulin NPH solution with 3/5 UI in 1ml of anesthetic or saline, with variable frequency (Gaspar N.K. 2020) and doxycycline. Photographic control.

Results: Healing tendency was observed since first applications. All ulcers healed in less than 36days. Authors refer ulcers healing take more than 90days. No patient required reconstructive surgery. No patient developed hypoglycemia symptoms during treatment.

Study Limitations: Small sampling; control group absence; different evolutionary stages injuries treatment; insulin data related in patient's absence.

Conclusions: This method proved to accelerate ulcers healing. It's necessary to clarify insulin resistance aspects in loxoscelism. Healing necessary sections were wound size related.

Keywords: brown spiders' ulcers; necrotic ulcers; venom; ceramides; insulin resistance; wounds difficult to heal; insulin healing induction.

INTRODUCTION

Found globally *Loxosceles* genus, includes small araneus, with 1-5cm body length, fine long legs, surviving 1-2 years in 8-43°C, with nocturnal habits, laying about 50 eggs^{1,2}.

Living in dry, dark, quiet places, caves, under rocks, in temperate and tropical regions. At home, they choose attics, basements, cupboards, drawers, boxes, sheets, furniture, shoes, under papers, dry leaves etc.²⁻³. They survive 50 days with no food or water². Accidents happened 86% at home⁴.

In Brazil, there are 50 species⁵. *L. Gaucho*, *L. intermedia* and *L. laeta* are blamed for most accidents^{1,2,6,7}. They were 39.409 between 2017 and 2021 in Brazil, most in south region(53%), mainly in Paraná state⁸. Cutaneous-systemic loxoscelism occurred in Chile, in around 5,5% of 200 accidents⁹.

Severity is influenced by geographic region¹⁰, injected venom amount and composition¹¹⁻¹⁴.

There is no laboratory method capable of diagnosing cutaneous or systemic loxoscelism. Most patients never see the spider. An isothermal amplification of DNA samples obtained from experimentally envenomed rabbit, through colorimetric lamp was related as diagnosis alternative¹⁵.

Cutaneous Loxoscelism

Bite is painless. After hours or days, the local becomes pale. May appear itching, pain, and bullae^{11,16}. Over days, occurs induration, erythema, edema¹⁷. Central ulcer comes up that can spread gravitationally¹⁸. Usually single, smaller than 10cm.¹⁹, evolves with a dark sunken necrotic center, with well-defined raised edges, intense inflammatory reaction, without

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typical purulent secretion but with mummified crust and hardened peripheral area¹⁷. This aspect aids diagnosis¹¹.

Healing usually takes three months^{20-21;17;19;22-23} leaving deep scar that can require excision and grafting^{24;16;22}.

Mixed inflammatory cell infiltrate, tissue aseptic coagulative necrosis and vasculitis occur after venom injection²⁵.

Systemic Loxoscelism

Suspicion should be high, especially in children with cutaneous ulcer, hemolysis, or coagulopathy. Lesion is frequently misdiagnosed as "cellulitis". May occur pruritus, rash, headache, vomiting, fever, arthralgia²⁶ and coagulopathy^{4;27;22;26}. Hemoglobinuria, acute renal failure, rhabdomyolysis, thrombocytopenia, and jaundice may develop within 1-10 days²⁶. Bilirubin (BT) and LDH levels^{27;24} had 91% specificity and 94% sensibility in a 275 patient series²⁸. In 53.5% of patients, D-dimer levels were increased after 1 week and in 25%, after 2 weeks¹⁰. Acute kidney injury^{29-31;24;13;32;10}, occurred with massive hemolysis noticed in 2 of 81 patients²⁷.

High serum D-dimer level, by endothelial cell activation and intravascular coagulation, total bilirubin (TB), and LDH levels and leukocytosis with neutrophilia indicate necrosis, systemic involvement^{27;24;28;33;10} and patient condition. Coombs' test may be positive³³. Mortality was 3.5% (2/57 individuals)³³

The Venom

Spider evolution had natural selection and increase in noxiousness³⁴⁻³⁶. *L. laeta* venom revealing superior activity in Brazil¹⁴.

In 1978, Forrester D. et al³⁷, demonstrated direct lytic action of phospholipase D (referred to as sphingomyelinase D). Phospholipase D metabolizes sphingomyelin into ceramide and phosphocholine^{39-40;36}. Ceramides alter immune functions of NK cells, T CD8+ T cells, activating complement and recruiting polymorphonuclear infiltrate^{7;13;36}.

Venom is a complex of biological toxins especially phospholipase D, metalloproteases, serineproteases, hialuronidase and Inhibitor Cystine Knot (ICK) peptides³¹⁻³², induces rapid coagulation, capillaries occlusion and necrosis¹⁷. Proteomic analysis of *Loxosceles gaucho* venom showed 12 different phospholipases, Astacin-Metalloprotease (ASM) and insecticidal toxin Sicaritin-Lia³⁸.

Ceramides and Insulin Resistance

Ceramides are crucial actors in the development of insulin resistance and type 2 diabetes, and negatively affect mitochondrial respiratory chain, hallmark of type 2 diabetes (T2-D)⁵⁶.

The insulin inhibition by ceramides, through the protein kinase mTOR (target of rapamycin) has been evaluated by different authors^{45-48;39-40;49-54;36}. In addition, insulin production

is impaired by potent proinflammatory lactate hydrogenase overexpression^{55;36}.

Sphingomyelin and phosphatidylcholines constitute more than 50% of membranous phospholipids, exerting important impact on cell signaling, through their catabolic products, ceramides and glucoceramides⁴¹, controlling growth, differentiation, apoptosis^{42-43;31}, and immuno-inflammatory events^{17;44;10}.

Insulin has pivotal role in maintaining mitochondrial proteome abundance and quality by enhancing your biogenesis and preventing proteome degradation. Insulin resistance is characterized by inefficient mitochondrial coupling and excessive production of reactive oxygen species⁵⁷⁻⁵⁸. Hypoglycemia impairs fibrinolytic balance, increases pro-inflammatory responses, platelet activation, coagulation biomarkers, and reduces function endothelial mediated by nitric oxide⁵⁹.

The venom depletes serum hemolytic complement, clotting factors VIII, IX, XI, and XII, prolongs the activated partial thromboplastin time, induces rapid coagulation, occlusion of capillaries, necrosis^{17;60;13}.

Therapy

The specific treatment is serum therapy, (produced through *L. intermedia* venom) applied until for up to 12h after bite. Pauli I. demonstrated beneficial results reducing the size of necrotic area until 48hs⁶¹. Adverse reactions are described⁶². Systemic treatment involves aggressive supportive care, wound management, blood transfusions, intravenous fluid replacement and antibiotics.

In a mouse model of systemic loxoscelism, venom-induced kidney failure was abrogated by administration of tetracycline, that reduces kidney damage induced by venom⁶³, the progression of dermonecrotic lesion, the expression of MMP-2 and the induction of MMP-9⁶⁷. Tetracyclines action on MMPs is thought to be mediated by chelation of structural metals within the enzyme⁶⁴. The strength of tetracycline-metal interaction is dependent on metal ion present. Tetracycline's affinity for different divalent zinc differs and is highly dependent on pH and the presence of other metal ions. Doxycycline action, a zinc dependent enzyme, has a relative superiority over tetracycline and minocycline due to its increased affinity for Zn²⁺ compared with tetracycline or minocycline⁶⁵⁻⁶⁶.

Animals, inoculated with venom, treated twice a day for 48 hours with cream containing tetracycline or doxycycline (50 µg/ml) had significant reduction in lesions evolution. Similarly, topical, or oral tetracycline or doxycycline, could control progression of the dermonecrotic lesion in animals injected with venom⁶⁷.

The recombinant protein containing β -cell epitopes of different *Loxosceles* toxins, that generated neutralizing

antibodies in immunized rabbits can be candidate for anti-venom production⁶⁸.

Monoclonal antibodies targeting acid SMase inhibitors can be used as therapeutic tools⁶⁹.

Imipramine and zoledronic acid capable of reducing acid SMase activity in animals, may be useful in reducing ceramides⁷⁰.

Glucocorticoids and immunoglobulins may have benefits treating severe loxoscelism²⁴ and may trigger rapid recovery of platelets^{24;10}.

Dapsone was used by its activity to reduce polymorphonuclear leukocyte function^{11;17} but, should be avoided because it can trigger serious adverse reactions⁷²⁻⁷³.

The magnitude of hyperbaric therapy results in necrosis reduction was insignificant⁷⁴.

In vitro topic diethylazelat, a plasma membrane fluidizer, with a broad range of immune modulatory activities, inhibit hemolysis cause by the venom and suppressed phospholipase A2 activity in a dose dependent manner⁷⁵.

Marbled necrotic skin lesions require surgical removal of the necrotic area^{21;11;76;24}.

Complications as hyperkalemia (NR $\geq$ 2.0), metabolic acidosis requiring bicarbonate. Hypotension requiring vasopressors, and hypoxia requiring intubation, occurred only in patients with profound hemolytic anemia^{22;26}.

INTRALESIONAL INSULIN THERAPEUTIC ESSAY

Background

Insulin has metabolic and growth-stimulating actions, both through its own receptors and of its homologous factors (IGFs)⁷⁷⁻⁸⁰, having pivotal role in mitochondrial proteome preservation⁵⁸. Insulin has been used in humans to recover cutaneous and subcutaneous atrophies⁸¹ and was effective to accelerate healing experimental wounds in Wistar rats. Fibroblasts trans-differentiation in myofibroblasts occurred precociously⁸². Healing acceleration was obtained in difficult healing cutaneous wounds by multiple, simultaneous, intralesional and perilesional injections, of 0.04-0.05UI NPH* mono component insulin, without adverse effects⁸³.

Method

a- Multiples micro doses of intralesional and perilesional applications, of 4-5UI of NPH *Novolin Nordisk mono component Insulin NPH 100 UI, diluted in 1ml of lidocaine in multiple simultaneous injections of 0.01 ml (0.04-0.05UI), in various lesions planes and points, repeated at variable time intervals. Debridement of necrotic tissues on the first day treatment.

b- Doxycycline 100mg administration every 12hours in recent cases.

Patients

Pat.	Sex- Age	Days Evolution	Clinical aspects	Insulin sections	Doxycycline	Debridment	Healing/days
1	M-21	136	6 cm necrotic dark crust leg ulcer, reaching adipose tissue.(fig1)	5	No	Yes	36
2	M-23	57	2 days after bite, bullae that evolved to 6x8cm. gluteal ulcer with black mummified crust.(fig.2)	16	No	No	40
3	M-22	126	3/4cm left thigh, elevated cyanotic edges ulcer with marble consistence margins.	4	No	Yes	35
4	F-52	14	Necrotic, 1,5cm., dry ulcer with raised marble consistence edges, on right breast.	5	Yes		
	Yes	16					
5	F-40	6	Well-defined edges right leg ulceration. (1o episode)	6	Yes	Yes	22
6	F-40	6	Right leg ulcer. (2o episode)	7	Yes	Yes	18
7	F-40	40	Dry raised indurated edges, 3/5cm., leg ulcer.	6	Yes	No	30
8	M-79	7	Erythematous elevated borders, 2/3cm., leg ulcer.	6	Yes	Yes	29
9	F-42	30	Necrotic marble consistence, 3x2,5cm., dry crust, raised, irregular edges, ulcer on 2 nd finger.(fig-3)	18 (pos split use)	Yes	Yes	27 (ankylosis)

10	M-67	1	2mm clear lesion on 12cm. indurated erythematous plaque, that evolved to dark, crust, 1,5cm leg ulcer.	7	Yes	Yes	29
11	F-50	4	Erythematous-violaceous plaque, necrosis(4cm.) with raised edges, in right instep.	9	Yes	Yes	23
12**	F-58	13	1-day after bite pain, bullae, great induration. After 10days 1.5cm., elevated edges gluteal ulcer and left leg edema (fig.4).	11	Yes	Yes	20
13	F-31	4	Pain 4hs after. After 4 days painful erythematous-edematous, 10cm. right tight plaque, with central, 1,7cm., hemorrhagic crust ulcer.	4	Yes	No	8
14	F-35	8	A 5cm. gluteal ulceration with erythematous induration.	7	Yes	Yes	30

Figure 1. Pat.1 (A) First day after “debridment” (leg ulcer with well-defined raise edges) and insulin micro doses treatment. (B) After 5 insulin micro doses injection, healing with good cicatricial aspect.

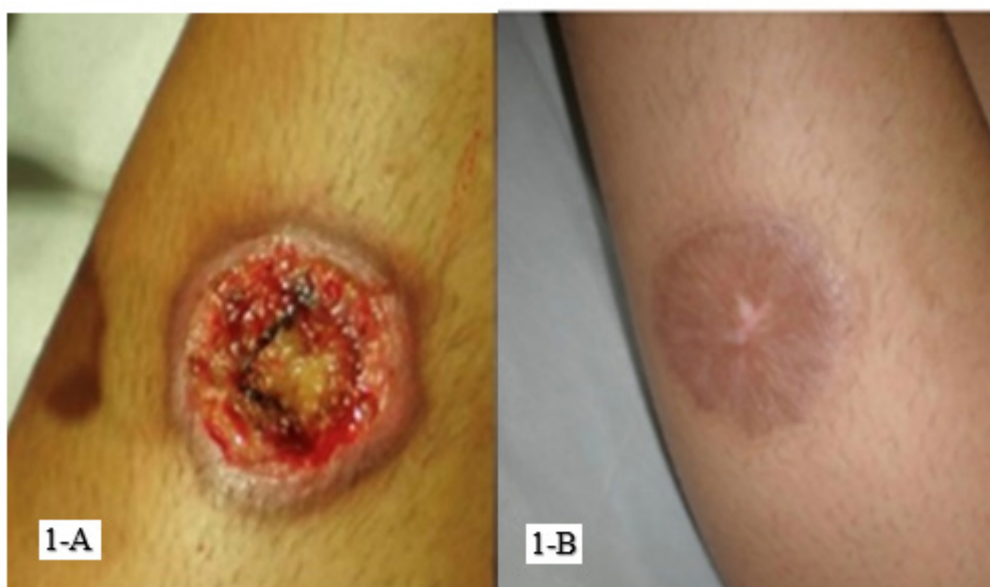


Figure 2. Pat.2 (A) Two days after bite, gluteal ulcer with black, mummified, 6x8cm. crust and elevated borders. First day micro doses insulin treatment at lesion edges. (B) Fiftieth six day, after 11th day insulin treatment and 1st peripheral “debridement”. (C) After 18th insulin microdoses injections on the 122th evolution day. (D) Five years later without hipertrophic scar.

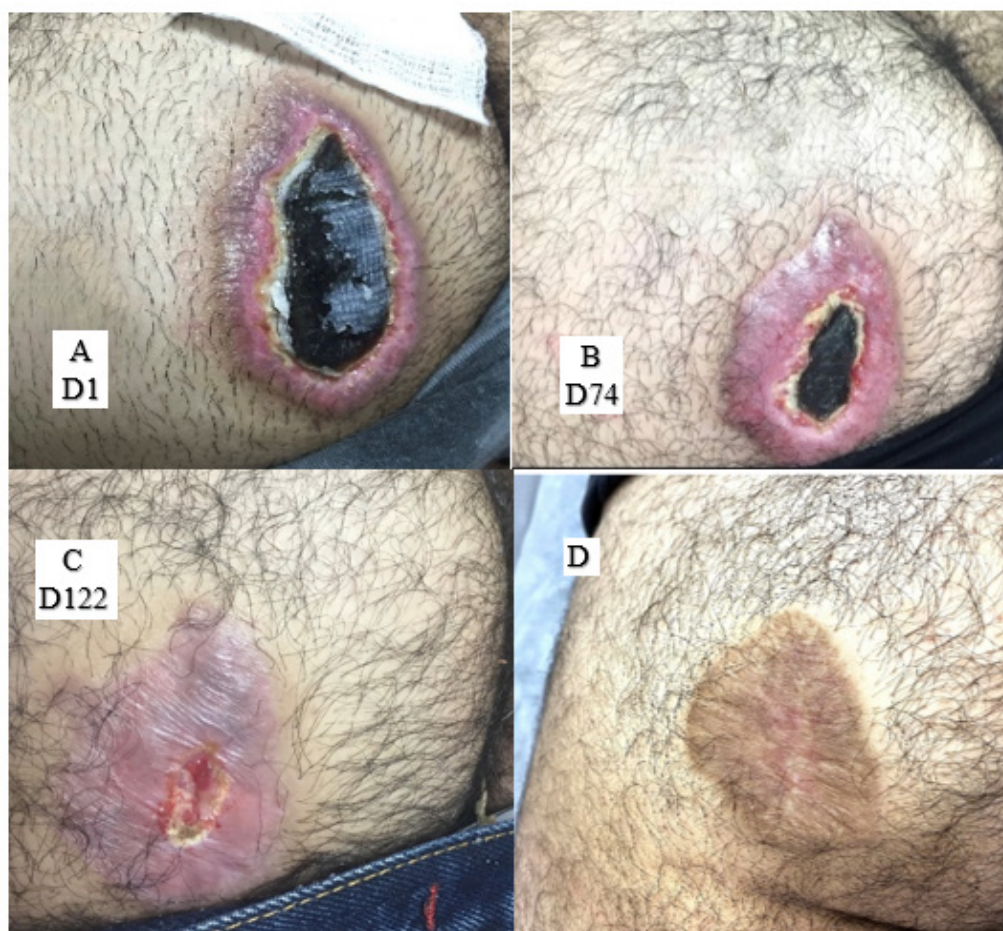


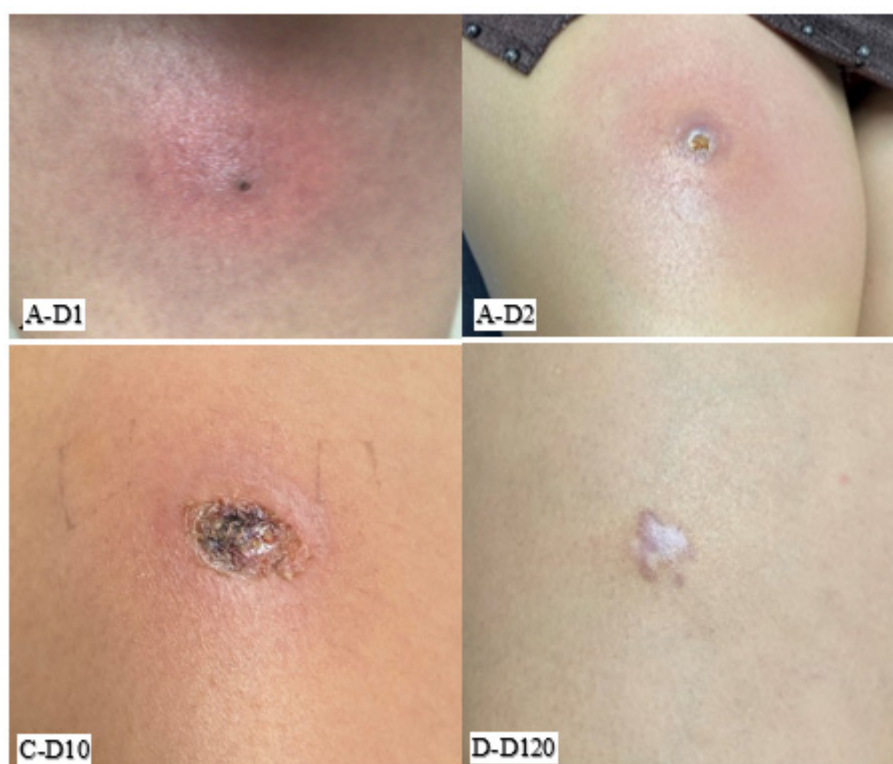
Figure 3. Pat.9 (A and B) First day treatment. (A) Necrotic ulcer with mummified crust, that spread gravitationally18. (B) After “debridement” and insulin treatment. (C) Thirty days after, was proceeded a surgical incision on the 2nd finger and immobilization with a splint, on extension, during insulin treatment, permitting anatomic position healing, in order to reduce the ankyloses. (D) Forty tree days after insulin treatment, occurs cicatricial ankyloses with minimal defect and perfect function.



Figure 4.Pat.12 (A) One day after spider bite and 1st day insulin multiple micro doses treatment. (B) Eight days of daily insulin micro doses injections. (C) Twentieth day of insulin micro doses treatment when ulcer healing occurs.



Figure 5.Pat.13 (A) Forth day after bite and 1st insulin micro doses treatment. Lesion with remarkable target aspect. (B) Second day insulin micro doses injection with pale halo (C) Tenth day after bite with crust and 4th insulin application. (D) Four months later, cicatricial aspect after abandoning treatment at 4th section.



COMMENTS

- 1- Systemic suspicious loxoscelism should be high, especially in children and younger adults, who presents cutaneous ulcer, hemolysis and coagulopathy.
- 2- The edges sharply defined and elevated, without purulent secretion aid diagnosis.
- 3- Debridement of necrotic lesions can accelerate healing.
- 4- Tetracyclines, especially doxycycline, given early, can limit the aggressive proteases. Topical applications may offer advantages, especially in infants⁶⁷.
- 5- The only case taking more than 30days to heal, was n.2, who presented enormous crust that wasn't debrided. In all our cases, cosmetics' cicatricial aspects were good.
- 6- In case n.9, after occurs cicatricial ankylosis, was proceeded a surgical incision on the 2nd finger and immobilization with a splint, on extension, during insulin treatment, permitting anatomic position healing, in order to reduce the ankyloses with minimal defect and perfect function.
- 7- Healing time and applications number were related to lesions size and severity. Patients 1 and 3 with long intervals between applications, could have achieved faster healing.
- 9- The speed and time of wound resolution was dependent on the frequency and number of sessions.

CONCLUSION

- 1-Healing ulcers by *Loxoscelles* venom takes 3 months. With insulin in intralesional micro multiple doses, healing occurs in about 1 month.
- 2-Healing response onset begins in 24-48 hours, with final resolution in less than 1 month in our patients, indicating that this therapy may help.
- 3-Different intervals between applications did not change the results.
- 4-The method proved to be effective and without adverse effects.
- 5-Regenerative activity onset in all lesions was observed between 1 or 2 days after first application, and can be attributed to its stimulating healing activity, as demonstrated by Gaspar and Gaspar, and the insulin's reduction of ceramides' resistance.
- 6-Advantages obtained indicate that the process can be successfully used to accelerate healing of brown spider's bites ulcers.
- 7-According to previous experience (Gaspar 2020)⁸³, applications performed using this method don't determine glycemic changes in adult patients.
- 8-Raw (or open) wounds can heal with undiluted insulin in topical applications⁸³.
- 9-Despite a significant correlation between sphingolipids, mitochondrial dysfunction, insulin resistance and T2 D, this

subject hasn't received much attention compared to direct sphingolipids effect on insulin signaling pathway⁸⁴.

10-Insulin's availability and safety use, indicates the method as an effective therapeutic option for difficult-to-heal wounds, without adverse effects.

Conflicts of Interest

None declared.

Authors' contributions

Neide Kalil Gaspar: The study concept and design, writing of the manuscript or critical review of important intellectual content, data collection, effective participation in the research guidance, intellectual participation in the propaedeutic and/or therapeutic conduct of the studied cases, critical review of the literature, approval of the final version of the manuscript. Marilia Nunes Nogueira: Data collection, or analysis and interpretation of data, writing of the manuscript or critical review of important intellectual content, data collection, analysis and interpretation, effective participation in the research guidance, intellectual participation in the propaedeutic and/or therapeutic conduct of the studied cases, critical review of the literature, approval of the final version of the manuscript.

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