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ACTUAL DATA REGARDING PHOTOBIOMODULATION IN PRESSURE ULCERS USING PULSED AND CONTINUOUS WAVE LASER THERAPY – A SYSTEMATIC LITERATURE REVIEW

Mihai BĂILĂ[1,2] and Gelu ONOSE[1,2]

[1]Faculty of Medicine – „Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[2] Neuromuscular Clinic Division – Teaching Emergency Hospital „Bagdasar-Arseni", Bucharest, Romania

Corresponding author: Mihai Băilă, mihai.baila@drd.umfcd.ro

Accepted May 19, 2022

Introduction. Background. Pressure ulcers (PU) affect individuals within risks groups, such as elderly, sensory and motor impaired and nursing dependent, patients. A coordinated treatment needs be adopted in order to prevent pressure ulcer appearance or to avoid their complications. Conventional local treatment modalities include local wound care with dressings, debridement and depending on the developmental stage and complications, surgery. Non-invasive physical therapy interventions delivers energy vectors into the surface and depth of chronic wounds and has gained a great interest in for the therapy of such lesions. The aim of this study was to synthetize the taxonomy, mechanisms of actions and potential therapeutic effects of LASER therapy/ photobiomodulation, in treating of pressure ulcers.

Methods: A systematic literature review was performed thorugh the examination of six international medical databases: Elsevier, National Center for Biotechnology Information (NCBI)/PubMed) and NCBI/PubMed Central (PMC) , Cochrane Library, Physiotherapy Evidence Database – PEDro and, respectively, the Institute for Scientific Information (ISI) Web of Knowledge/Science Database (the latter for verifying if the articles potentially eligible have been published in ISI Thomson Reuters indexed journals) in the period 1st January, 2011–31 December 2021.

Results and discussion. Two articles passed the last eligibility step were included for this systematic review. Dual wavelength (808nm, 905 nm) NIR modulates the expression of pro-inflammatory COX-2, iNOS, PGE-2, extracellular matrix degradation and building proteins (collagen I and MMP-1) and is related to alterations in cellular cytoskeleton in human normal dermal fibroblasts. Continuous wave low-level laser therapy (658 nm) has shown superior effects than 808nm and 940 nm irradiation, on the decrease of pro-inflammatory cytokines IL-6, IL-2 and TNF-α and increase of anti-inflammatory TGF-β1 and VEGF in human patients with II-IV grade pressure ulcers receiving a 5 times / week intervention for 1 month in addition to conventional local treatment. The resolution of dysregulated inflammation and uncontrolled activation of reconstructive cells (fibroblasts) is necessary for effective healing of pressure ulcers. Impaired cellular, local, regional and sometimes systemic host responses can maintain the wound in an unresolved and continuous inflammatory, pro-oxydative and distructive, even extending, state explain on one hand the very difficult to be obtained healing progresses and on the other the multimodal therpaeutic capabilities required to be provided by related approaches. Unfortunately, by now, this is very difficult to be achieved.

Conclusions. Our the results draw the attention that the related LASER therapy protocols need to be carefully assessed in more fundamental and clinical research endeavours.

Keywords: Pressure ulcer, Pressure sore, Photobiomodulation; Low-level laser therapy, LLLT, Laser MLS Therapy.

INTRODUCTION

Pressure ulcers (PU) are also known as eschars, bedsores, pressure sores or decubitus ulcers. These skin and connective tissue lesions are caused by compression (pression-induces ischemia and hypoxia), shearing and frictional forces most commonly over bony superficial surfaces[1].

In addition, urinary, fecal incontinence, skin perspiration and/or poor hygiene predispose to such lesions of the somatic integument fivefold[2]. Advanced age, poor nutrition state, chronic comorbidites (such as diabetes, peripheral arterial

occlusive disease, cancer and immunosuppresion conditions), or the chronic use of medications with such properties (glucocorticoids, immunomodulatory drugs, etc), smoking, affect the skin's quality (loss elastic properites and resilience) and are risk factors for pressure ulcer development[3]. These etiologic factors often impact individuals within risks groups, such as elderly (who account for half of the population affected by this pathology), immobilized, including paralysed, and severely ill, bedridden patients[3]. A history of the number, positioning, size, depth, stage of development, color and complications of the pressure ulcers – such as odor and bacterial colonization, debris, fistulas/pockets – should be also performed[2], toghether with a risk assessment instrument, because the clinican needs to comprehend the general health problems as well as the biological resources of patients[3]. Additionally, higher risks for pressure ulcers development have been shown to be connected with larger or slower-healing wounds, which impose additional care to certain populations[4]. A possible solution to counteract the development of pressure ulcers is to put „patients at the centre of extensive medical surveillance, and to adopt social and economic measures in order to prevent both late medical complications and psychological, socio-professional, family and/or economic effects."[1]. Patients need to be assesed early in terms of specific risk factors for pressure ulcer development and a coordinated treatment should be adopted in order in order to prevent the appearance of pressure sores. In purpose to carry out such monitoring endeavour, such evaluation instruments are used in clinical practice: Braden[5], Waterlow[6] and Norton[7] scales or the Bates-Jensen Wound Assessment Tool[8]. Other neccesary related-evaluations that should be performed are the assessment of cognition and nutritional states[1], and respectively functioning in activities of daily living.

One international systematic review and meta-analysis targeting to estimate the global burden of pressure sores concluded that one in five patients with spinal cord injury had pressure ulcers[9]. Such patients, together with the ones suffering from other life-long neurological disabilities, such as traumatic brain injuries, tumors, ischemic and/or hemorrhagic stokes, are at very high risk for developing pressure ulcers[1]. Other populations at risk of developing pressure sores are: hospitalized patients in acute care facilities for severe illnesses, multiple trauma, burns and people depending on nursing[1,3]. To be emphasized that old aged who account for half of the population affected by this pathology. Pressure injuries are an important health care and socio-economic problem, associating a high rate of hospital lenght of stay and readmission and a high economic burden for the patients, their families and even communities (considering possible financial charges given to the caregivers) and reflected also on hospitals and insurance systems[10]. Once a pressure ulcer develops, spontaneous healing can take months or even longer, requiring the use of continuous pressure reduction devices, special dressings, adequate skin care and nutrition (which can include also intravenous infusion of aminoacids, derived blood – albumin, plasma factor XIII – products). Patients require frequent in-bed repositioning and the limitation of certain movements which may add overload to the affected area. This discomfort is further strenghtend by the fact that pressure wounds often have prolonged evolutions and tend to reccur, which creates also phychological symptoms, mainly: anxiety and depression. Conventional local treatment modalities for pressure ulcers include local wound care with dressings, debridement and depending on the developmental stage and complications of such lesions. Using natural light for the treatment of diseases is a continuum from the antique times to the present days and it has been researched and validated in the treatment of systemic and skin diseases, including chronic ulcers that healed inefficiently and/or difficult or extensive skin burns predisposed to inbalanced scarring. The use of modern phototherapy started in the twentieth century, with the discovery of the electric arc lamp and by using artificial light sources to treat skin and systemic diseases, such as vitiligo, lupus vulgaris, local infections, tuberculosis and rickets[11]. LASERs (acronym for "Light amplification by the stimulated emission of radiation") were built starting with the 6th decade of the XXIst century, by the principles of microwave amplification systems[12, 13]. High reactive-level LASER therapy or "HLLT" (term found in[14]) was the first form of this intervention to be used in ophthalmological and dermatological diseases, for its photothermal and photodestructive effects.The non-destructive,

photoactivating and biostimulation effects of LASERs have been inially regarded as secondary effects of HLLT and included analgesia, edema resorption and acceleration of tegument healing. It was named low reactive-lever LASER therapy[14] and later „photobiomodulation"[15]. This type of light radiation has some particular basic properties: monochromaticity, coherence, directionality, brightness/superior radiance over artificial light sources, resulting from the physical intimate – at atomic level – mechanism of generation/ production. These specific features differentiate LASER beams from other light sources, enabling for superior propagation and tissue-penetration effects[13].

This systemtic review aims at synthesizing the principle concepts regarding the releated: taxonomy, mechanisms of actions and potential therapeutic effects of LASER therapy/ photobiomodulation, in treating of pressure ulcers. Furthermore, this technology has enormously advanced in terms of the respective beams' characteristics and modulation options, as well as possiblities to irradiate a greater tissue surfaces. Secondly, our study has aimed to deliver data on new LASER equipment and protocols, i.e. continuous and pulsed waves LASER emission. Thereby, the Multiwave Locked System LASER (MLS), allows the simultaneous and synchronous radiations of two different wavelengths emitted in near infrared spectrum ($\lambda 1$= 808 nm in continuous emission and λ 2 = 905 nm in pulsed emission), thus enabling the energy from beams to be uniformly distributed over the about 5 mm diameter – larger then the usally spot dimension of classical LASERS – irradiated surface, with robotized controled sweeps/ scanning facility and the use of a multidiodic optic group[16]. The pulsed high intensity beam permits a true verticalization of the energy and an increased in-depth stimulation of the tissue.

METHOD

In order to achieve this systematic literature review we performed the examination of six international medical databases: Elsevier[17], National Center for Biotechnology Information (NCBI)/ PubMed)[18] and NCBI/PubMed Central (PMC)[18], Cochrane Library[19], Physiotherapy Evidence Database – PEDro[20] and, respectively, the Institute for Scientific Information (ISI) Web of Knowledge/ Science Database[21] (the latter for verifying if the articles potentially eligible have been published in ISI Thomson Reuters indexed journals) in the period 1st January, 2011–31 December 2021. The search was limited by allowing only open-access/free full-text articles written in English. Ten sets of keywords were used contextually, as follows: "Laser MLS therapy", "Multiwave Locked System Laser therapy", "MLS Photobiomodulation", "Photobiomodulation MLS", "Multiwave Locked System Photobiomodulation", "Photobiomodulation Multiwave Locked System", "Pulsed low level laser therapy", "Photomodulation superpulsed laser", "Photobiomodulation super-pulsed laser", "Photobiomodulation dual wavelenght laser therapy", AND, "pressure sore", "pressure sores", "pressure ulcer", "pressure ulcers", "eschar", "eschars", "bed sore", "bed sores", "wound healing" (Table 1). As a particular methodological detail related to the interrogation of the Cochrane library database, this was done by a medical term-based search (MeSh) as follows: 1. Search of the term "Pressure ulcers" and selecting only article with titles containing the keyword "Laser-therapy" and 2. Search of the term "Low-Level Light Therapy" and the selection of article titles including the keyword "Pressure ulcer".

The steps needed for the inclusion of the final articles were fulfilled using the method of Preffered Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[22]. After the first records were identified, other three systematic and rigorous stages were performed, as detailed below. The same articles (duplicates), those written in other languages than english (Stage 2), and the articles not found in Thomson Reuters master journals list[21] (Stage 3) were removed. The remaining articles were carefully analyzed in full-text, and consecutively, if argued, some of them have been excluded with reasons (Stage 4). This steps are pictured in a tailored to our endeavour form of a PRISMA type flow-chart[22] in Figure 1. Two articles which passed the last eligibility step were included for this systematic review – Table 2. Especially considering the paucity of the availabe scientific data as found according to the rigorously methology we have applied in this respect, in order to enhance/ consolidate our related knowledge base we have also used additional bibliographic resources, freely found in the literature.

Table 1

Keyword/ „syntaxes" selected for our article and the number of records found in each database.
The total number of records found listed on each column are related to the LASER-related term (set)

Keyword / "syntaxes" sets	**Elsevier**	**Pubmed**	**PMC**	**PEDro**
"Laser therapy MLS"	0	1	0	1
"Multiwave Locked System Laser therapy"	0	0	0	1
"MLS Photobiomodulation"	0	0	0	0
"Photobiomodulation MLS"	0	0	0	0
"Multiwave Locked System Photobiomodulation"	0	0	0	0
"Photobiomodulation Multiwave Locked System"	0	0	0	0
"Pulsed low level laser therapy"	0	1	0	21
"Photobiomodulation superpulsed laser"	0	0	0	1
"Photobiomodulation super-pulsed laser"	0	0	0	4
"Photobiomodulation dual wavelength laser therapy"	0	1	0	1
	"Pressure sore", "Pressure sores", "Pressure ulcer", "Pressure ulcers", "Bed sore", "Bed sores", "Eschar", "Eschars", "Wound healing"			

Cochrane library MeSh-term search (11)	**Cochrane library**
Mesh : "Low-Level Light Therapy" and "Laser-therapy"	2
Total	34

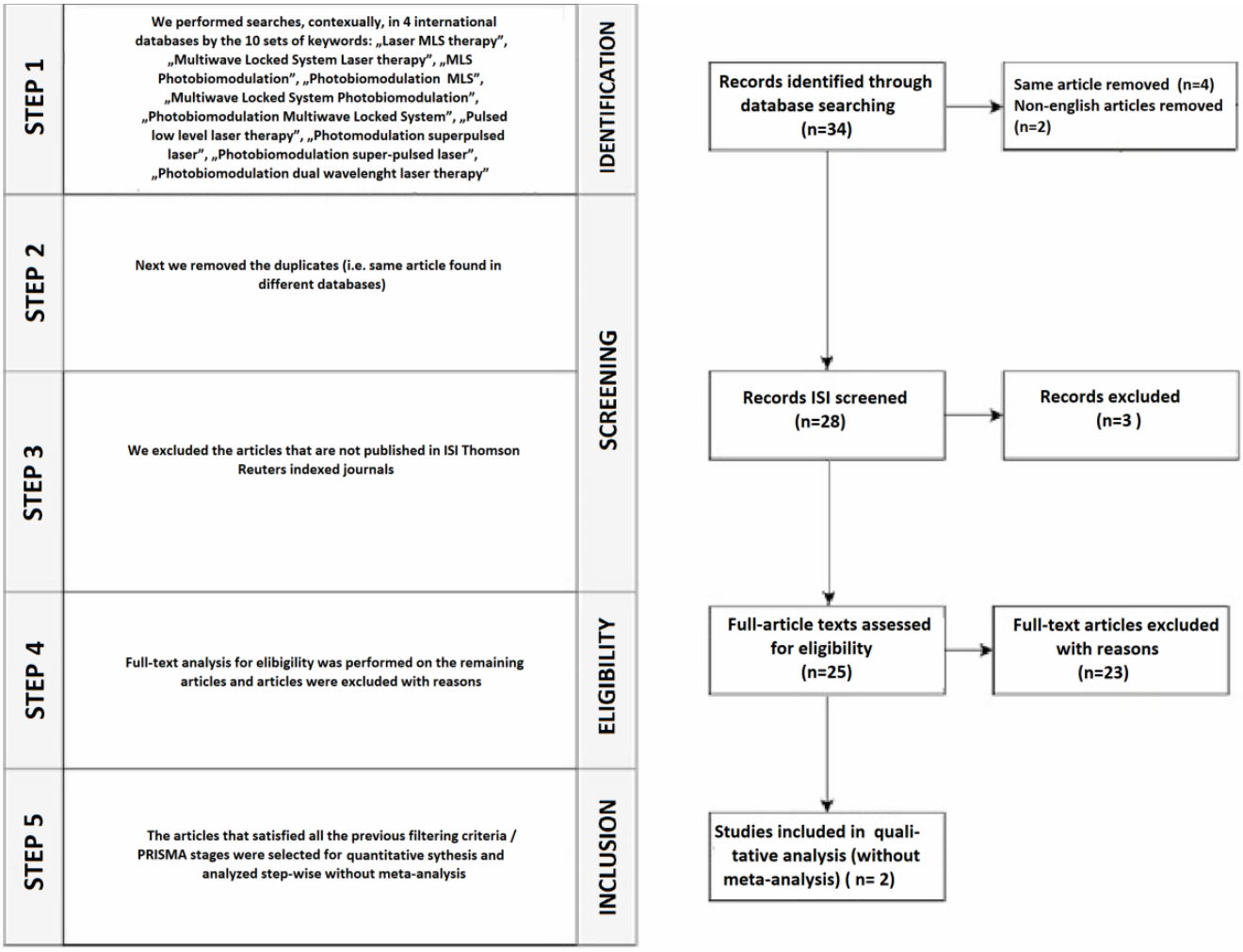

Figure 1. The step by step selection of the final articles to be included in our study,
adapted after the Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram[22].

Table 2

Two articles were included for qualitive synthesis without meta-analysis

No.	Article	Publication year	Citation count
1	Genah S, Cialdai F, Ciccone V, Sereni E, Morbidelli L, Monici M. Effect of NIR Laser Therapy by MLS-MiS Source on Fibroblast Activation by Inflammatory Cytokines in Relation to Wound Healing. Biomedicines. 2021 Mar 16;9(3):307. doi: 10.3390/biomedicines9030307	2021	3
2	Taradaj J, Shay B, Dymarek R, et al. Effect of laser therapy on expression of angio- and fibrogenic factors, and cytokine concentrations during the healing process of human pressure ulcers. Int J Med Sci. 2018;15(11):1105-1112. Published 2018 Jul 13. doi:10.7150/ijms.25651	2018	30

RESULTS

In the first analyzed study[25] human normal dermal fibroblast (HDNF) were activated by exposure to a pro-inflammatory cytokine mix with tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) for 48 h and the effects of a dual wavelength (808nm, 905 nm) NIR LASER emission (MLS-MiS) exposure were researched. Primary outcomes were the expression levels of many regulatory molecules with known activity in fibroblast function and wound healing: nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), microsomal prostaglandin G synthase-1 (mPGES) and its final product prostaglandin E-2 (PGE-2), inducible nitric oxid syntase (iNOS), vascular endothelial growth factor (VEGF), tubulin, α actin, α smooth muscle actin (α-SMA), fibronectin (FN), α5β1 integrin, matrix metalloproteinases (MMPs). Additionally, fibroblasts were also stained and evaluated by immunofluorescence in order to visualize the cellular distribution of tubulin, α actin, α-SMA, fibronectin, collagen I, MMP-1 and nuclear localization of NF-kB. The used LASER protocol included 3 consecutive days exposure to the abovementioned laser source, with the following parameters: continuous wave with 10 Hz repetition rate, 50% int (mean power 1840 mW) and pulsed wave with peak power 1 kW ± 20%; total fluence measured 5.19 J/cm2. The LASER beam irradiated single cell wells having 13 mm diameter (spot size). The work included three study groups: Group 1 (cytokine stimulated and LASER exposed cells), Group 2 (cytokine stimulated and LASER unexposed cells), Group 3 (cytokine unstimulated and LASER unexposed cells), Group 4 (cytokine unstimulated and LASER exposed cells). As results, the fibroblasts exposed to the LASER emissions (group 1) were prevented from developing an exacerbated inflammatory reaction and were similar to the control group in terms of the level of pro-inflammatory molecules (COX-2, iNOS, PGE-2) and VEGF expression. LASER therapy limited NF-κB translocation into the nucleus. Another consequence of LASER irradiation was the modulation of Fibroblasts stimulated with IL-1β and TNF-α and exposed to NIR laser radiation (Group 1) recovered morphological features and cytoskeleton-protein expressions (tubulin, α actin, α-SMA, α5β1 integrin, fibronectin) that resembled those of controls (Group 3) and which were near to a basal physiological state. Also collagen I and MMP-1 expressions, two proteins which have key roles in extracellular matrix degradation and building, according to the authors, had similar values in group 1 and control group. More intense expression of pro-inflammatory molecules (COX-2, iNOS, PGE-2), alterations in cytosketon-protein synthesis (increase of tubulin density, formation of extracellular fibrils of fibronectin, increased expression of fibronectin receptor α5β1-integrin) were shown in cytokine stimulated fibroblasts without LASER interventions (Group 2). Also, additional accumulation of intracellular collagen I and increased MMP-1 synthesis, were shown for this group.

The second analyzed study[26] was a randomized control trial aiming to determine the serum- and wound expression level of pro-inflammatory cytokines (TNF-α, IL-2, IL-6) and growth factors (VEGF, TGF-β1) in 72 human patients with II-IV grade pressure ulcers, undergoing three wavelenght specific LASER therapy exposures for 5 times a week / 1 month: Group 1 – 940 nm; Group 2 – 808 nm; Group 3 – 658 nm and Group 4 – sham LASER therapy. The patients were supplimentary treated: they had a stabilized and unified diet one month before the start of the study and benefited from repositioning and mobilization, air pressure mattress, bed support surfaces, wound cleansing (0.9% normal saline and with solutions two solutions types:Propanolum 45%; 1-Propanolum 10%; 2-Biphenylol, 0.2%; hydrogen peroxide; purified water; ethanol 46%; isopropyl alcohol

27%; benzyl alcohol 1%; hydrogen peroxide; purified water), and dressing change (1–2 times a day depending on local exudation). According to the results, only the irradiation with a wavelength of 658 nm was associated with the reduction of pro-inflammatory cytokines IL-6, IL-2 and TNF-α within two weeks from irradiation and with increasing of the anti-inflammatory TGF-β1 and of the growth factor VEGF, the later result being similar with the first analyzed study[25].

DISCUSSION

In the case of pressure sores, prevention is better than cure. Dressings made up of new materials (polyacrylate, alginate, hydrocolloid, hydrogels, foams) aid the healing process by providing a moist environment or eliminating exudates, removing necrotic tissues and fighting/ diminishing infection. Non-invasive interventions, of physical therapy type, which deliver energy vectors into the surface and depth of chronic wounds, respectively : electrotherapy, ultrasound and phototherapy/photobiomodulation, negative pressure wound therapy, have gained a greater interest at present. Biological photoreactivity of LASER beams appears from the interaction between light energy and the living structures and depends both on the properties of the energy source (wavelength = λ measured in nanometers- nm; frequency in Herz – Hz or nr. of cycles/ second; energy = E measured in Joules- J; duration in seconds(s); intensity/ power P measured in Watt – J/sec, power density = ρP measured in W/cm^2, and type: continuous wave/ pulsed), the exposure (angle of incidence or collimation, distance to the targeted tissue and its surface size), and of those of the tissue exposed. By modulating dosage parameters of LASERS, as well as the exposure protocol, the biostimulatory LASER interventions elicit a large amount of biological effects, based on „reception and absorption”[23]. These range from photodistructive/ photothermal effects (protein denaturation, coagulation and/or vaporization) to photoactivating actions. LASER energy is absorbed by cells due to the chromophores they contain. Such intracellular elements are: specific proteins within the respiratory chain in mitochondria called cytochromes, co-factors for pro-oxidative enzymatic systems (nicotinamide adenine dinucleotide-NAD), water molecules, amino acids, nucleic acids and chromatin (a complex of DNA and histones that formes chromosome,), cell pigments (such as melanin) and haemoglobin. The receptory effects of LASER comprise the increase of cellular metabolic and biotrophic activities, including perfusion, nutrition and tissue clearance, and include: DNA and protein synthesis increase, cell division enhancement, enzymes activity and regenerative proccesses stimulation[23], acceleration of transmembrane transport, improving of the membrane's permeability and reestablishing the cell's resting potential, modulation of the systemic immunological response, increase of oxyhaemoglobin dissociation and tissue oxygen absorbtion[4,13,15,16,26].

The superficial layer of the skin, called epidermis, contains cells involved in metabolic reactions, immunological processing of external stimuli, tactile perception, temperature regulation. The underlying dermis is a supportive structure which contains excretory glands, blood and lymphatic vessels. Below the reticular layer of the dermis lies the hypodermis[24]. This abundent connective tissue supports the deep biological structures and includes various cells within a extracellular matrix (ECM). The EMC is heterogenous mass of fibrous proteins, such as collagen, proteoglycans, glycosaminoglycans, and fundamental substance. It can become tighlty bound or adopt a dispersed form, thus limiting or amplifying the movement of macromolecules and cells, which also impacts the dynamics of wound healing. In the normal healing processes, the initial reaction of the injured integument and/or subcutaneus layers is to limit the aggresion and to confine the damaged area. Local migration of neutrophils, moncytes (activated to M1-phenotype macrophages), T CD4 + and T CD8 + lymphocytes takes place under the action of the master regulator NF-kB and locally produced cytokines such as IFN-γ, TNF-α, IL-12, IL-1, IL-2, IL-6[4]. The formation of fibrin bonds, i.e. hemostasis, vasodilatation, and the migration of leukocytes and lymphocytes to the injured site, in order to escalate an effective inflammatory response, are the first steps of wound closure. As inflammation progresses, the increase of protein synthesis and removal of cell debris is concomitantly achieved, leading to the weakeaning of the primary pro-coagulant, pro-edematous and pro-oxidative actions. This marks the beginning of the third step of the normal healing process, called tissue granulation. Important signaling proteins in this phase are the cytokines, IL-4, IL-5, IL-10, IL-13, as well as the growth factors VEGF and TGF-1β - the latter being the most potent factor involved in

wound healing[27]. Endothelial cells proliferate and migrate to the affected area and produce new blood vessels. Activated fibroblasts differentiate to myofibroblasts, cells that synthesize and destroy collagen fibers and can also produce contractile proteins (actin α / SMA α - found in smooth muscle fiber). Their role is to restore the structure of the extracellular matrix, including by reorganizing mechanical stress lines, through traction forces, a process called wound contraction[25–27]. Alternatively, activated macrophages (M1) support the reduction of inflammation, cell debris removal and angioneogenesis[25–27]. The reepithelialization of the skin surface is achieved by keratinocytes, the cells of basal layer of the epidermis, which divide intensely on the surface and beneath the wound's crust. The final product of wound healing is the formation of scar tissue that covers the site of injury[24].

Pressure ulcers often have large and separate edges, with significant loss of skin and subcutaneous tissue, and could require surgical interventions for covering the damaged and lost area with skin and/or muscle flaps, or sometimes, when possible, simple excision and suturing. If the pressure sore closes spontaneosly, it does so by connective/ granulation tissue deposition.The deeper and/or larger the pressure sore, the more reduced are the chances of spontaneous healing[29], due to the long time that is needed for the integument and subcutaneous tissue to reform and the higher risk of complications by self-sustaining noxious agents: repeted trauma, moisture imbalance, necrosis and infection[31]. These events generate impaired cellular, local, regional and sometimes systemic host responses to stress and maintain the wound in an unresolved and continuous inflammatory, pro-oxydative and distructive, even extending, state, further lowering the affect tissue's renewing resources[4,26,27,29]. The resolution of dysregulated inflammation and uncontrolled activation of reconstructive cells (fibroblasts) is necessary for effective healing[25]. All these, and many of them in vicious circle perpetuated/ augmented patology pathways explain on one hand the very difficult to be obtained healing progresses and on the other the multimodal therpaeutic capabilities required to be provided by related approaches and unfortunately, by now, this is very difficult to be achieved.

CONCLUSION

Pressure ulcers appear in risk populations, such as: difficult to mobilize/immobilized, nursing dependent, sensory and motor impaired, severley ill, (including burned, immune depressed and very old) patients. Prevention is cure and stars „at the first patient contact"[3] and it entails a comprehensive, complete risk and/or chronic wound assessment and, respectively, sustained nursing interventions to keep the sensitive skin areas dry, clean and pressure-free. The lesion's dimensions/ surface area and depth[31] togehter with limited mobility, poor nutrition, low hemoglobin levels, low arterial pressure[32], consumptive comorbidities and/or advanced age impact the healing potential of pressure ulcers. Photobiomodulation, including/with emphasis on MLS laser therapy, could to be an efficient non-invasive and risk-free option to interrupt the vicious circle of non-healing pressure sores, yet the simultaneos conservative wound care : cleansing, debridement, dressing, and topical therapy with biological agents, and, respecively adapted to the stage of the pressure ulcers, surgical approaches, remains a mandatory part of the overall specific therapy. Our the results draw the attention that the related LASER therapy protocols need to be carefully assessed in more fundamental and clinical research endeavours.

Aknowledgment: *We warmly thank the distinguished Assist. Prof. Eng. Vlad Ciobanu, for his valuable help in automatizing and IT underpinning our quest related to the systematic literature review.*

Conflict of interests: The authors declare no conflicts of interests.

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